Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Proposed association between the hexanucleotide repeat of *C9orf72* and opposability index of the thumb

ZHONGBO CHEN[1], KUANG LIN[1], JEFFREY D. MACKLIS[2] & AMMAR AL-CHALABI[1]

[1]*Department of Basic and Clinical Neurosciences, Maurice Wohl Clinical Neuroscience Institute, King's College London, London, UK and* [2]*Department of Stem Cell and Regenerative Biology, Center for Brain Science and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*

Abstract

Objective: Amyotrophic lateral sclerosis (ALS) is a fatal disease caused by motor neuron and sub-cerebral projection neuron degeneration. We sought to explore the particular susceptibility of humans to neurodegeneration and whether any characteristic human features might predispose to selective vulnerability of the critical motor circuitry in ALS. The pathophysiology of the *C9orf72* repeat is not yet understood, despite its role as a common cause of ALS and frontotemporal dementia. *Methods*: We examined the development of the monosynaptic cortico-motoneuronal system, key to skilled hand movements, measured by the thumb opposability index, and its relationship to the *C9orf72* hexanucleotide repeat expansion, a strong predisposing factor for neurodegeneration, using the genomic tool BLAST. *Results*: We found a statistically significant linear relationship between the *C9orf72* hexanucleotide bit score, a measure of genomic conservation of the aligned region across different species, and the thumb opposability index (Pearson's correlation coefficient of 0.78, *p* value 0.023). The *C9orf72* hexanucleotide repeat was only found in humans, chimpanzees and gorillas, species with higher opposability indices. *Conclusions*: This may support a role of the hexanucleotide repeat in the same developmental pathways in species with higher prehensility, which may be associated with the selective vulnerability of cortico-motoneuronal cells in humans, manifested most obviously as the 'split hand' syndrome in ALS.

Key words: *C9orf72, thumb opposability, cortico-motoneuronal vulnerability*

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease manifesting as a syndrome of unremitting progressive motor weakness, cognitive impairment, and ultimate respiratory failure. The central pathology involves the degeneration of both corticospinal and spinal motor neurons. The median duration of survival in ALS is poorer than for other neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (1–4). We sought to explore the particular vulnerability of humans to neurodegeneration in these central neuronal populations, and whether the evolutionary development of any characteristic human features might predispose to the selective vulnerability in ALS.

Previously, the development of ALS has been ascribed to a failure of neural networks inherent in complex functional adaptations in humans such as fine movements of the hand (5,6). Comparative analyses of the genomes of different species may reveal sequence changes in the human lineage that might have contributed to the evolution of human traits, and the pathological alteration of which causes disease. For example, Alzheimer's disease pathology is rare in great apes and neurofibrillary tangles are absent in other non-human primates (7,8). Likewise, the development of a monosynaptic cortico-motoneuronal system is a key contributor to the capacity for skilled finger movements (9,10). However, this system may also render an increased susceptibility to motor neuron degeneration (6), and an increase in human survival may also unmask selective connectome-based vulnerability.

A pathological hexanucleotide repeat expansion (G_4C_2) of the chromosome 9 open reading frame 72

(*C9orf72*) gene is a common risk factor for ALS and frontotemporal dementia (FTD) in both familial and apparently sporadic cases (11,12). In neuro-logically-normal European individuals, there are usually between two and 20 repeats, but in ALS the sequence can be repeated hundreds of times (11). The mechanism of neurodegeneration in *C9orf72* expansions still remains unclear. Previous studies have explored how the repeat expansion itself was introduced through a single founder effect in Northern Europe (13). Although the *C9orf72* gene itself is ancient, the repeat region is a recent evolutionary event, as is the development of a monosynaptic cortico-motoneuronal pathway for fine motor control, and this association suggests their evolutionary relationship may be important.

Therefore, we aimed to study the conservation of the *C9orf72* hexanucleotide repeat in different species to further understand its phylogenetic origins. We tested the association of the conservation of the hexanucleotide repeat with a measure of thumb opposability (14), a function key to complex motor tasks.

Materials and Methods

We tested our hypothesis using readily-available genomic data. The expansion affects a repeated sequence of four guanine and two cytosine nucleotides (G_4C_2). First, we searched for the sequence of the human hexanucleotide repeat of *C9orf72* in the UCSC Genome Browser (Feb. 2009 GRCh37/hg19 assembly) (15). We then compared this sequence across a track of multiple alignments of other species using MULTIZ, a process that identifies segments that remain similar among species (16).

Next, we used the Basic Local Alignment Search Tool (BLAST) (17–19) and entered input sequences of the hexanucleotide repeat region in FASTA format (via the NCBI Genomes [chromosome] database). This produced the multiple sequence alignment outputs of other vertebrates in FASTA format. We then used these multiple sequences as our input comparison files in Jalview (JalviewLite applet) in order to visualize the molecular phylogeny and genomic rearrangements of those species (20). We constructed the phylogenetic tree and distances of this hexanucleotide region using Clustal W2-Phylogeny (21,22).

Homology is the existence of shared ancestry between genes in different species. To assess whether a given alignment constitutes evidence for homology, we used bit scores and the E-value computed from BLAST as an estimate (23–25). These are local alignment statistics that give an indication of the strength of similarity between species. The E-value takes into account the extreme value distribution to give an indication of the expected homologous sequences (25). The bit score places the raw alignment score in context by taking into account the statistical essence of the scoring system and standardizing it (17,25).

The computed alignment scores were compared with the opposability indices of the thumb for each species (14,26,27).

$$\text{Opposability index} = \frac{\text{thumb length} \times 100}{\text{index ray length}} \quad [1]$$

We used SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corporation) for the association analyses.

Results

The results of the across-species aligned *C9orf72* hexanucleotide region bit scores and E-values, converted to the negative natural logarithmic scale for ease of presentation, are shown in Table 1 along with the opposability index for that species. The species were ones identified in the BLAST alignment. Figure 1(a) depicts the linear relationship between bit score of the *C9orf72* hexanucleotide region and the opposability index. Figure 1(b) depicts the relationship between -ln(E-value) and opposability index. The linear equations with R^2 scores (coefficients of determination) are shown. The Pearson's correlation coefficient for bit score and opposability index was 0.78, *p* value 0.023, with a significant chi-square test for linear-by-linear association *p* value of 0.04. The Pearson's correlation coefficient for -ln(E-value) and opposability index was 0.80, *p*-value 0.018, with a significant chi-square test for linear-by-linear association *p* value of 0.035.

Figure 2 shows the aligned hexanucleotide sequences across 35 vertebrate species (panel a) and these sequences aligned via Jalview showing nucleotide differences (panel b). The hexanucleotide sequence here is 'GGCCCC' as the reverse strand is presented. Only the gorilla and the chimpanzee have the three-repeat hexanucleotide sequence seen in humans.

Figure 3 shows a constructed cladogram and branch length distances based on the aligned *C9orf72* hexanucleotide repeat region. As can be seen, this region is conserved most recently in human, chimpanzee and gorilla, with the lowest evolutionary distance, indicating late divergence. The hexanucleotide regions of the other non-human primates diverged on separate phylogenetic paths from humans. Non-primates (African elephant and Weddell seal) showed divergence pertaining to the furthest distance away. The sequence alignment from Clustal W2 can be found in Supplementary Materials Figure 1.

Discussion

We report a statistically significant linear association between conservation of the *C9orf72* hexanucleotide

Table 1. Bit scores and -ln(E-value)s of the hexanucleotide repeat region, and relation to opposability indices in eight different species. [1]Opposability indices derived from mean of values from two sources (14,26). The opposability index of the two non-primate species (Leptonychotes weddellii and Loxodonta Africana) are taken to be zero based on the absence of a thumb.

| Species	Common name	Bit Score	-ln(E-value)	Opposability index[1]
Homo sapiens	Human	183	96	65
Pan troglodytes	Chimpanzee	174	90	42
Gorilla gorilla gorilla	Gorilla	168	86	48
Papio anubis	Baboon	98.7	38	54.9
Pongo abelii	Orangutan	125	56	39.5
Macaca mulatta	Rhesus macaque	96.9	42	53.6
Leptonychotes weddellii	Weddell seal	44.6	0	0
Loxodonta africana	African elephant	42.8	−1	0
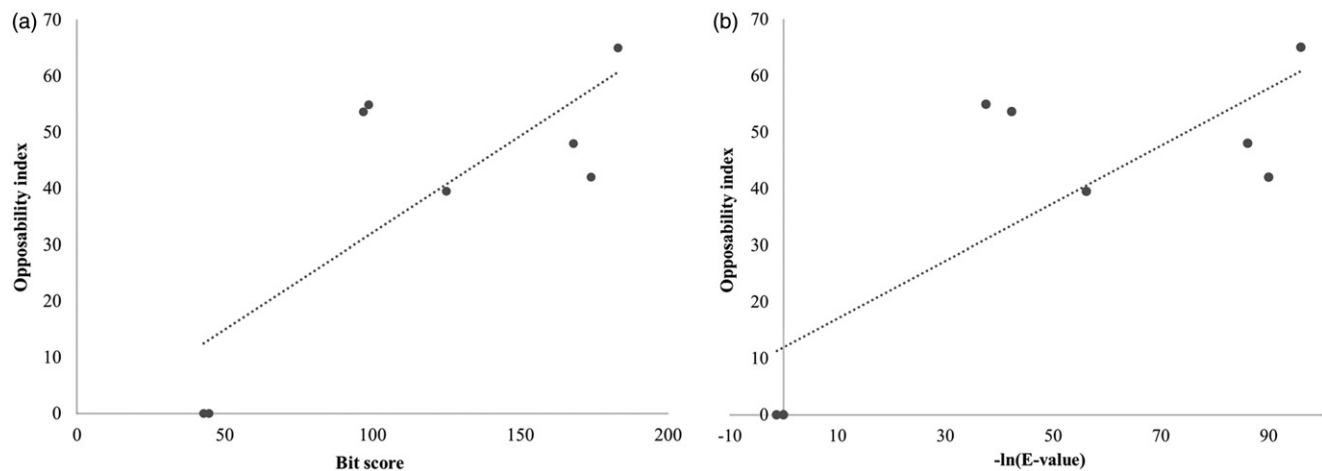
Figure 1. (a) Linear relationship between bit score of the *C9orf72* hexanucleotide region and opposability index ($y = 0.3438x - 2.2216$, $R^2 = 0.6031$). (b) Relationship between -ln(E-value) of the *C9orf72* hexanucleotide region and opposability index ($y = 0.509x + 11.981$, $R^2 = 0.6316$).

repeat region and the opposability index of the thumb. We find that the repeat region, pathological expansion of which is implicated in ALS and FTD, is present only in humans, gorillas and chimpanzees, and not in other vertebrates. This selective preservation is seen in these primates with higher opposability indices. The correlation suggests that the *C9orf72* hexanucleotide region may have a role in the same developmental pathways that are important in animals with higher opposability indices, for example in the development of the corticospinal motor neuron-to-spinal motor neuron circuitry (cortico-motoneuronal connections), for voluntary motor dexterity.

Although there could be other traits associated with the conservation of the hexanucleotide repeat, the heterogeneity of opposability in great apes suggests that there may be a direct link. For example, although orangutans share more genomic homology with humans than baboons, they are devoid of the hexanucleotide repeat. This may be due to the regression in opposability in orang-utans as they developed elongation of the second to fifth digits for brachiation without elongation of the thumb, giving a low opposability index despite being evolutionarily closer to modern humans (14). This shows that the association between

opposability and alignment is not simply based on the phyletic level of a species as a whole, and also independent of the degree of neurological similarity to mankind (10).

The cortico-motoneuronal pathway for hand control

In humans and higher order primates, extensive direct cortico-motoneuronal connections have superseded more primitive functions mediated by spinal interneurons (28,29). These cortico-motoneuronal connections are used for independent control of the digits (30). Repeat sequences may be involved in gene expression control. A plausible mechanism could be that the *C9orf72* repeat sequence is involved in the development of a direct monosynaptic input to hand motor neurons, which may be vulnerable to degeneration in ALS. Oligosynaptic corticospinal connections have been shown to exist in lower mammals such as mice and rats (31–33) and in New World monkeys such as the non-dextrous squirrel monkey (34). Cortico-motoneuronal connections are particularly well developed in great apes (35). This is in keeping with the trend seen in the introduction of the *C9orf72* repeat region in chimpanzees, gorillas, and also in humans. This also suggests that the cortico-motoneuronal

(a)

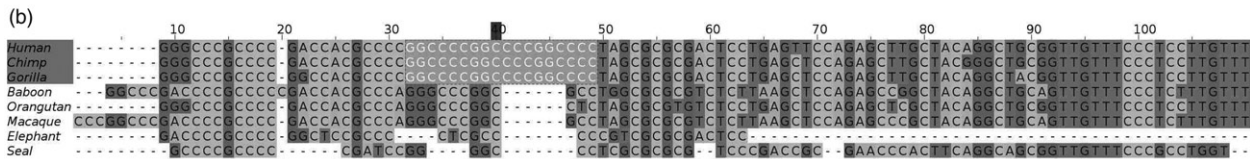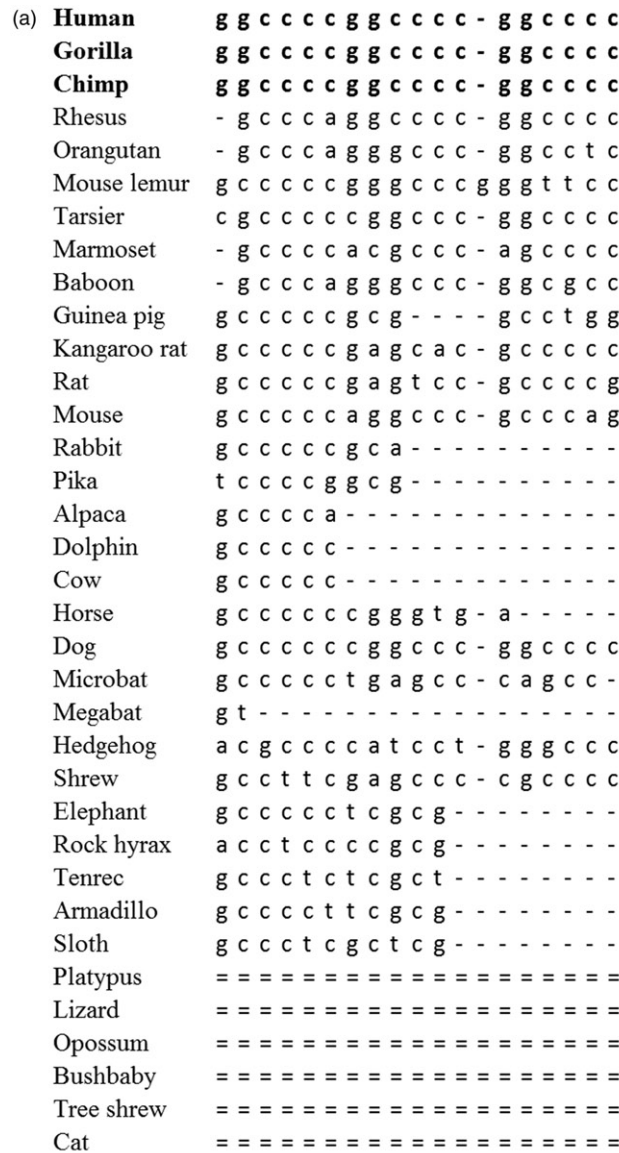|	
Human	g g c c c c c g g c c c c - g g c c c c
Gorilla	g g c c c c c g g c c c c - g g c c c c
Chimp	g g c c c c c g g c c c c - g g c c c c
Rhesus	- g c c c a g g c c c c - g g c c c c
Orangutan	- g c c c a g g g c c c - g g c c t c
Mouse lemur	g c c c c c g g g c c c g g g t t c c
Tarsier	c g c c c c c g g c c c - g g c c c c
Marmoset	- g c c c c a c g c c c - a g c c c c
Baboon	- g c c c a g g g c c c - g g c g c c
Guinea pig	g c c c c c g c g - - - - g c c t g g
Kangaroo rat	g c c c c c g a g c a c - g c c c c c
Rat	g c c c c c g a g t c c - g c c c c g
Mouse	g c c c c c a g g c c c - g c c c a g
Rabbit	g c c c c c g c a - - - - - - - - - -
Pika	t c c c c g g c g - - - - - - - - - -
Alpaca	g c c c c a - - - - - - - - - - - - -
Dolphin	g c c c c c - - - - - - - - - - - - -
Cow	g c c c c c - - - - - - - - - - - - -
Horse	g c c c c c c g g g t g - a - - - - -
Dog	g c c c c c c g g c c c - g g c c c c
Microbat	g c c c c c t g a g c c - c a g c c -
Megabat	g t - - - - - - - - - - - - - - - - -
Hedgehog	a c g c c c c a t c c t - g g g c c c
Shrew	g c c t t c g a g c c c - c g c c c c
Elephant	g c c c c c t c g c g - - - - - - - -
Rock hyrax	a c c t c c c c g c g - - - - - - - -
Tenrec	g c c c t c t c g c t - - - - - - - -
Armadillo	g c c c c t t c g c g - - - - - - - -
Sloth	g c c c t c g c t c g - - - - - - - -
Platypus	= = = = = = = = = = = = = = = = = = =
Lizard	= = = = = = = = = = = = = = = = = = =
Opossum	= = = = = = = = = = = = = = = = = = =
Bushbaby	= = = = = = = = = = = = = = = = = = =
Tree shrew	= = = = = = = = = = = = = = = = = = =
Cat	= = = = = = = = = = = = = = = = = = =

Figure 2. Alignment of the hexanucleotide repeat sequence of *C9orf72* (reverse strand) in the UCSC MULTIZ alignment configuration (16) (a) and on Jalview (20) (b).

system is a recently evolved feature, subserving new aspects of motor behaviour (35). The sequence is also absent in Old World monkeys, such as the macaque and baboon, and other species such as the racoon, which have a high level of dexterity and a greater index of opposability than either the chimpanzee or gorilla, but less than the human (36). It could be that the opposability in Old World monkeys is governed by another genetic association independent of the *C9orf72* sequence.

Thus, the cortico-motoneuronal system may predispose to selective vulnerability in the more dextrous primates. This susceptibility to motor neuron degeneration has been demonstrated in a ubiquitin carboxy-terminal hydrolase-L1 (*UCHL1*) knockout mouse model, revealing the vulnerability of these neurons to increased endoplasmic reticular stress (37). If similar vulnerability existed in humans, this might underlie their degeneration in ALS. Similar selective vulnerability of both corticospinal motor neurons or related cortico-brainstem subcerebral projection neurons and spinal motor neurons has also been shown in the transgenic G93AhSOD1 mouse model of ALS (38).

The tactile acuity of the hand, associated with opposability, is also reflected in the size of motor
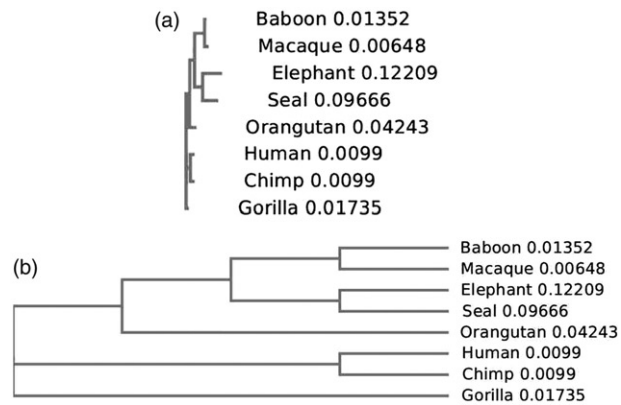
Figure 3. Constructed phylograms based on real branch distances (a) and cladogram (b). A smaller number represents more recent divergence.

and sensory cortical association areas and higher functional differentiation of the brain (14). This not only points to a cortical basis for motor degeneration in ALS as suggested previously (6), but also to the degenerative mechanism that leads to cognitive and behavioural impairment in ALS. The trait of opposability may have also come with the development of a phylogenetically recent reorganization of frontal cortical circuitry key to higher order social function and cognition (5), which may explain the bridging of FTD and ALS through the *C9orf72* pathological expansion.

ALS manifesting as the failure of thumb opposability

The thumb opposability in Cercopithecoidea and hominids is associated with a specialized differentiation of thenar muscles (14). There is preferential wasting of these muscle groups in the split hand syndrome, which is a typical clinical feature in ALS (39). Electrophysiological studies have demonstrated a stronger cortico-motoneuronal input to the thenar muscles, further supporting the notion that the cortico-motoneuronal pathway is particularly vulnerable in ALS, and that thumb opposability is a good measure of its function (39–41). As a higher opposability index ratio corresponds to a shorter index finger, it is interesting that a lower index-to-ring finger ratio was found to be associated with ALS, possibly from pre-defined motoneuronal vulnerability (42).

Most ALS is segmental and focal in onset (43), such as in bulbar-onset disease. Likewise, similarly direct monosynaptic connections of the corticobulbar tracts have evolved in humans, possibly subserving more complex communication (44). Extraocular muscles (45) and Onuf's nucleus (46) do not receive a direct cortical input, and in ALS there is relative sparing of eye movements (47) and a lack of sphincter disturbance (48), attributed to the absence of vulnerability to motoneuronal degeneration in these regions. Thus, ALS could be viewed as a disease that particularly involves the cortico-

motoneuronal system. It would be interesting to look at further measures of bipedalism and vocalization across species to support this argument.

Conclusions

The *C9orf72* hexanucleotide is present in many animals, in a partial form, but the repeated hexanucleotide sequence is only present in gorillas, chimpanzees, and humans. The conservation of this region is proportional to a measure of thumb opposability, which supports a role of the sequence in the same developmental pathways in species with higher prehensility. This may be associated with the selective vulnerability of the cortico-motoneuronal cells in humans, manifested most obviously as the split hand syndrome in ALS. These findings have implications for how we approach further functional studies of *C9orf72* and at the same time identifying phenotypic correlates of cortico-motoneuronal degeneration, which may also reflect the pathophysiology underlying FTD. We are starting to recognize the importance of cortico-motoneuronal health in neurodegeneration, and the *C9orf72* hexanucleotide repeat may represent a clue to the puzzle.

Declaration of interest

ORCID

AMMAR AL-CHALABI http://orcid.org/0000-0002-4924-7712

References

1. Chio A, Logroscino G, Hardiman O, Swingler R, Mitchell D, Beghi E, et al. Prognostic factors in ALS: a critical review. Amyotroph Lateral Scler. 2009;10:310–23.
2. Barclay LL, Zemcov A, Blass JP, Sansone J. Survival in Alzheimer's disease and vascular dementias. Neurology. 1985;35:834–40.
3. Mylne AQ, Griffiths C, Rooney C, Doyle P. Trends in Parkinson's disease related mortality in England and Wales, 1993-2006. Eur J Neurol. 2009;16:1010–16.
4. Norris F, Shepherd R, Denys E, U K, Mukai E, Elias L, et al. Onset, natural history and outcome in idiopathic adult motor neuron disease. J Neurol Sci. 1993;118:48–55.
5. Eisen A, Turner MR, Lemon R. Tools and talk: an evolutionary perspective on the functional deficits associated with amyotrophic lateral sclerosis. Muscle Nerve. 2014;49:469–77.
6. Eisen A, Turner MR. Does variation in neurodegenerative disease susceptibility and phenotype reflect cerebral differences at the network level? Amyotroph Lateral Scler Frontotemporal Degener. 2013;14:487–93.
7. Gearing M, Tigges J, Mori H, Mirra SS. Beta-Amyloid (A beta) deposition in the brains of aged orangutans. Neurobiol Aging. 1997;18:139–46.
8. Gearing M, Rebeck GW, Hyman BT, Tigges J, Mirra SS. Neuropathology and apolipoprotein E profile of aged chimpanzees: implications for Alzheimer disease. Proc Natl Acad Sci USA. 1994;91:9382–6.
9. Lemon RN, Kirkwood PA, Maier MA, Nakajima K, Nathan P. Direct and indirect pathways for corticospinal control of upper limb motoneurons in the primate. Prog Brain Res. 2004;143:263–79.
10. Heffner RS, Masterton RB. The role of the corticospinal tract in the evolution of human digital dexterity. Brain Behav Evol. 1983;23:165–83.
11. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011;72:257–68.
12. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011;72:245–56.
13. Smith BN, Newhouse S, Shatunov A, Vance C, Topp S, Johnson L, et al. The C9ORF72 expansion mutation is a common cause of ALS+/-FTD in Europe and has a single founder. Eur J Hum Genet. 2013;21:102–8.
14. Napier JR, Napier PH. A handbook of living primates: morphology, ecology and behaviour of nonhuman primates. London, New York: Academic Press; 1967:456.
15. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Res. 2002;12:996–1006.
16. Blanchette M, Kent WJ, Riemer C, Elnitski L, Smit AF, Roskin KM, et al. Aligning multiple genomic sequences with the threaded blockset aligner. Genome Res. 2004;14:708–15.
17. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.
18. Livingstone CD, Barton GJ. Protein sequence alignments: a strategy for the hierarchical analysis of residue conservation. Comput Appl Biosci. 1993;9:745–56.
19. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. NCBI BLAST: a better web interface. Nucleic Acids Res. 2008;36:W5–9.
20. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview version 2: a multiple sequence alignment editor and analysis workbench. Bioinformatics. 2009;25:1189–91.
21. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23:2947–8.
22. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.
23. Karlin S, Altschul SF. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc Natl Acad Sci USA. 1990;87:2264–8.
24. Pearson WR, Lipman DJ. Improved tools for biological sequence comparison. Proc Natl Acad Sci USA. 1988;85:2444–8.
25. Altschul SF, Boguski MS, Gish W, Wootton JC. Issues in searching molecular sequence databases. Nat Genet. 1994;6:119–29.
26. Etter HF. Terrestrial adaptations in the hands of Cercopithecinae. Folia Primatol. 1973;20:331–50.
27. Jones LA, Lederman SJ. Human Hand Function. Oxford: Oxford University Press; 2006.
28. Rathelot JA, Strick PL. Subdivisions of primary motor cortex based on cortico-motoneuronal cells. Proc Natl Acad Sci USA. 2009;106:918–23.
29. de Noordhout AM, Rapisarda G, Bogacz D, Gerard P, De Pasqua V, Pennisi G, et al. Corticomotoneuronal synaptic connections in normal man: an electrophysiological study. Brain. 1999;122:1327–40.
30. Davare M, Kraskov A, Rothwell JC, Lemon RN. Interactions between areas of the cortical grasping network. Curr Opin Neurobiol. 2011;21:565–70.
31. Alstermark B, Ogawa J, Isa T. Lack of monosynaptic corticomotoneuronal EPSPs in rats: disynaptic EPSPs mediated via reticulospinal neurons and polysynaptic EPSPs via segmental interneurons. J Neurophysiol. 2004;91:1832–9.
32. Yang HW, Lemon RN. An electron microscopic examination of the corticospinal projection to the cervical spinal cord in the rat: lack of evidence for cortico-motoneuronal synapses. Exp Brain Res. 2003;149:458–69.

33. Greig LC, Woodworth MB, Galazo MJ, Padmanabhan H, Macklis JD. Molecular logic of neocortical projection neuron specification, development and diversity. Nat Rev Neurosci. 2013;14:755–69.

34. Maier MA, Olivier E, Baker SN, Kirkwood PA, Morris T, Lemon RN. Direct and indirect corticospinal control of arm and hand motoneurons in the squirrel monkey (Saimiri sciureus). J Neurophysiol. 1997;78:721–33.

35. Lemon RN. Descending pathways in motor control. Annu Rev Neurosci. 2008;31:195–218.

36. Heffner R, Masterton B. Variation in form of the pyramidal tract and its relationship to digital dexterity. Brain Behav Evol. 1975;12:161–200.

37. Jara JH, Genc B, Cox GA, Bohn MC, Roos RP, Macklis JD, et al. Corticospinal motor neurons are susceptible to increased ER stress and display profound degeneration in the absence of UCHL1 function. Cereb Cortex. 2015;25:4259–72.

38. Ozdinler PH, Benn S, Yamamoto TH, Guzel M, Brown RH Jr, Macklis JD. Corticospinal motor neurons and related subcerebral projection neurons undergo early and specific neurodegeneration in hSOD1G(9)(3)A transgenic ALS mice. J Neurosci. 2011;31:4166–77.

39. Weber M, Eisen A, Stewart H, Hirota N. The split hand in ALS has a cortical basis. J Neurol Sci. 2000;180:66–70.

40. Menon P, Bae JS, Mioshi E, Kiernan MC, Vucic S. Split-hand plus sign in ALS: differential involvement of the flexor pollicis longus and intrinsic hand muscles. Amyotroph Lateral Scler Frontotemporal Degener. 2013;14:315–18.

41. Flament D, Goldsmith P, Buckley CJ, Lemon RN. Task dependence of responses in first dorsal interosseous muscle to magnetic brain stimulation in man. J Physiol (Lond). 1993;464:361–78.

42. Vivekananda U, Manjalay ZR, Ganesalingam J, Simms J, Shaw CE, Leigh PN, et al. Low index-to-ring finger length ratio in sporadic ALS supports prenatally defined motor neuronal vulnerability. J Neurol Neurosurg Psychiatry. 2011;82:635–7.

43. Ravits J, Paul P, Jorg C. Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. Neurology. 2007;68:1571–5.

44. Liscic RM, Zidar J, Mihelin M. Evidence of direct connection of corticobulbar fibers to orofacial muscles in man: electromyographic study of individual motor unit responses. Muscle Nerve. 1998;21:561–6.

45. Krauzlis RJ. The control of voluntary eye movements: new perspectives. Neuroscientist. 2005;11:124–37.

46. Fowler CJ, Griffiths D, de Groat WC. The neural control of micturition. Nat Rev Neurosci. 2008;9:453–66.

47. Gizzi M, DiRocco A, Sivak M, Cohen B. Ocular motor function in motor neuron disease. Neurology. 1992;42: 1037–46.

48. Konno H, Yamamoto T, Iwasaki Y, Iizuka H. Shy-Drager syndrome and amyotrophic lateral sclerosis. Cytoarchitectonic and morphometric studies of sacral autonomic neurons. J Neurol Sci. 1986;73:193–204.

Supplementary material available online