High frequency of the expanded C9orf72 hexanucleotide repeat in familial and sporadic Greek ALS patients

Kin Y. Moka, Georgios Koutsis, Lucia V. Schottlaender, James Polke, Marios Panas, Henry Houlden

Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK
Neurogenetics Unit, Department of Neurology, University of Athens Medical School, Eginitio Hospital, Athens, Greece
MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, UK

Received 29 January 2012; received in revised form 19 February 2012; accepted 19 February 2012

Abstract

An intronic expansion of a hexanucleotide GGGGCC repeat in the C9orf72 gene has recently been shown to be an important cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in familial and sporadic cases. The frequency has only been defined in a small number of populations where the highest sporadic rate was identified in Finland (21.1%) and the lowest in mainland Italy (4.1%). We examined the C9orf72 expansion in a series of 146 Greek ALS cases, 10.95% (n = 16) of cases carried the pathological expansion defined as greater than 30 repeats. In the 10 familial ALS probands, 50% (n = 5) of them carried a pathologically large expansion. In the remaining 136 sporadic ALS cases, 11 were carriers (8.2%). None of the 228 Greek controls carried an expanded repeat. The phenotype of our cases was spinal (13/16) or bulbar (3/16) ALS, the familial cases were all spinal ALS and none of our cases had behavioral frontotemporal dementia. Expansions in the C9orf72 gene therefore represent a common cause of ALS in Greece and this test will be diagnostically very important to implement in the Greek population. The frequency is higher than other populations with the exception of Finland and this may be due to Greece being a relatively isolated population.

© 2012 Elsevier Inc. Open access under CC BY license.

Keywords: ALS; C9orf72; Expansion; Hexanucleotide; Greek population

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by degeneration of the brain and spinal cord leading to weakness, wasting, bulbar problems, and usually death within 3 years (Clarke et al., 2009). Originally noted in an essay in 1830 by Charles Bell the disorder has been described throughout the world with an ALS incidence of between 1.7 and 8.2 people per 100,000 in populations in Europe and the United States, the lowest incidence being in Italy (Turin) (Leone et al., 1983) and highest in Finland (Beghi et al., 2011; Cronin et al., 2007; Logroscino et al., 2010; Murros and Fogelholm, 1983). Approximately 10% of ALS cases are familial, and genetic defects have been identified in approximately a third of families (Rohrer and Warren, 2011; Valdmanis and Rouleau, 2008; Vance et al., 2006) and a small percentage of sporadic cases, in an ever-increasing number of genes that include: superoxide dismutase 1 (SOD1) (Rosen et al., 1993), transactivation response (TAR) DNA binding protein (TARDBP) (Sreedharan et al., 2008), fused in sarcoma (FUS) (Kwiatkowski et al., 2009), valosin-containing protein (VCP) (Johnson et al., 2010), factor-induced gene 4 (FIG4) (Chow et al., 2009), angiogenin (ANG) (Greenway et al., 2006), ubiquilin 2 (UBQLN2) (Deng et al., 2011), and optineurin (OPTN) (Maruyama et al., 2010).
More than 10 years ago, a locus for autosomal dominant familial ALS and frontotemporal dementia was identified on chromosome 9p21 (Morita et al., 2006). This region was refined to a common 140 kb haplotype containing 3 genes, MOBKL2B, C9orf72, and IFNK that were extensively investigated (Laaksovirta et al., 2010; van Es et al., 2009). Two research groups identified a pathogenic GGGGCC hexanucleotide repeat expansion in intron-1 of the C9ORF72 gene as an important cause for both familial and sporadic ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011). They found the expansion (greater than 30 repeats) was present in 46.4% of familial ALS and 21% of sporadic ALS cases from Finland and between 28.5% and 38% familial ALS in series from Italy, Germany, and the United States (Orr, 2011). The expanded repeat was absent in US, Italian, and German controls but present at a rate of 0.4% in Finnish controls. In the UK, pathological expansions accounted for 50% (26/52) of familial and 7% (35/496) of sporadic ALS (Cooper-Knock, 2012). Further analysis of the incidence in sporadic ALS suggests a marked difference across Europe (north to south), the highest prevalence being in Finland (21.1%), then the UK (7%), then Germany (5.2%), Belgium (5%), and the lowest in Italy at 4.1% (Cooper-Knock, 2012; DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011; Traynor, B.J., personal communication, C9ORF72 hexanucleotide repeat expansion in sporadic ALS and FTD around the world, 2012). Sardinia is likely to have a higher prevalence, but this is an unusual isolated population which also contains a high frequency of TARDBP mutations (Chiô et al., 2011). The overall incidence of ALS may suggest a founder expansion, disseminated by a traveling population such as the Vikings or it could be accounted for by population-specific effects such as isolation. Clinically, the C9ORF72 expansion causes a frontotemporal dementia (FTD), and an ALS with phenotype is often associated with cognitive problems compared with unexpanded patients. Neuropathologically, abnormal subcellular localization and aggregation of TAR DNA binding protein 43 (TDP-43) is found widely distributed in most patients with ALS and FTD, cortical lesions are TDP-43 positive but cerebellar lesions are negative (Al-Sarraj et al., 2011; Ince et al., 2011; Stewart et al., 2012). These findings further highlight the importance of TDP-43 in FTD and ALS.

There are no further data available on the frequency and phenotype of C9ORF72 expansions in other European or Mediterranean populations. We therefore examined the frequency, spectrum, and phenotype of patients with an expanded C9ORF72 hexanucleotide repeat in a well-defined series of ALS cases and controls from Greece.

2. Methods

DNA was extracted from 146 ALS cases and 228 controls consecutively collected from the Neurology Depart-
a cohort of Greek ALS patients revealing a relatively high prevalence of expanded repeats in the Greek sporadic ALS population (8.2%). This is the highest sporadic figure outside of Finland reported to date. These data support the pathological expansion as an important cause of ALS that extends to Mediterranean populations. A further interesting point is that the majority of Greek cases with expansions have a spinal onset, much higher than in the previous reports where the proportion of bulbar onset cases was much higher (DeJesus-Hernandez et al., 2011; Renton et al., 2011).

The 50% prevalence of the expansion within Greek familial ALS is congruent with previous reports. Previously we have reported the likely common founder effect originating from Scandinavia. Haplotypes in the ALS cases from other populations (USA and Italy) also carry part of the Finnish haplotype and it would be extremely interesting to further define the haplotype that harbors the mutation in the Greek population. The high frequency of expansions in the Greek population is not consistent with the reported decreasing frequency of expanded repeats in ALS when traveling south across Europe where frequencies consist of Finland (21.1%), UK (7%), Germany (5.2%), Belgium (5%), and Italy at 4.1% (Cooper-Knock, 2012; DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011; Traynor, B.J., personal communication, C9ORF72 hexanucleotide repeat expansion in sporadic ALS and FTD around the world, 2012). These frequencies are similar to the overall distribution of ALS in these countries. It has been suggested the ALS haplotype could be accounted for by the Vikings, but Viking influence was limited in Greece. A more plausible explanation is the relatively isolated nature of the Greek population over recent centuries with low values of past and present racial admixture. This explanation may underlie the high frequency of the ALS haplotype in Finland, which also has a relatively isolated population and a high frequency of ALS and the C9ORF72 expansion. The high frequency of expansions in apparently sporadic individuals is difficult to explain but is likely due to lack of family history information or previous generations dying early before the expansion caused disease. Another explanation is reduced penetrance of the expansion, and a further question is whether this pathogenic expansion goes through an intermediate expansion/anticipation phase as in other repeat expansion disorders such as Friedreich’s ataxia (Orr

### Table 1
Demographic and C9ORF72 repeat data from the Greek ALS cases

|                      | Total cohort | Familial | Sporadic | Expanded | Nonexpanded | Expanded (familial) | Expanded (sporadic) |
|----------------------|--------------|----------|----------|----------|-------------|--------------------|---------------------|
| n (%)                | 146          | 10 (6.8) | 136 (93.2)| 16 (11.0)| 130 (89.0)  | 5 (50.0)           | 11 (8.1)            |
| Age as of 01/01/12 (y ± SD) | 59.1 ± 13.0  | 50.8 ± 12.2 | 59.7 ± 12.9 | 56.4 ± 12.2 | 59.5 ± 13.1 | 57.2 ± 12.6 | 56.1 ± 12.6 |
| Male                 | 108 (74.0)   | 4 (40.0)  | 104 (76.5) | 12 (75.0) | 96 (73.8)   | 2 (40.0)           | 10 (90.9)           |
| Female               | 38 (26.0)    | 6 (60.0)  | 32 (23.5)  | 4 (25.0)  | 34 (26.2)   | 3 (60.0)           | 1 (9.1)             |
| Age at onset of ALS (y ± SD) | 57.3 ± 13.0  | 49.7 ± 11.1 | 57.9 ± 12.9 | 55.1 ± 12.1 | 57.6 ± 13.1 | 55.6 ± 12.0 | 54.8 ± 12.7 |
| Mode of onset (%)    |              |          |          |          |             |                    |                     |
| Spinal               | 106 (75.2)   | 10 (100.0)| 96 (73.3)  | 13 (81.2) | 93 (74.4)   | 5 (100.0)          | 8 (72.7)            |
| Bulbar               | 35 (24.8)    | 0 (0.0)   | 35 (26.7)  | 3 (18.8)  | 32 (25.6)   | 0                  | 3 (27.3)            |
| C9ORF72 repeat (%)   |              |          |          |          |             |                    |                     |
| Expanded             | 16 (11.0)    | 5 (50.0)  | 11 (8.1)  | 0.067 b   | 3 (18.8)    | 0                  | 3 (27.3)            |
| Nonexpanded          | 130 (89.0)   | 5 (50.0)  | 125 (91.9) | 0.002 b   | 32 (25.6)   | 0                  | 3 (27.3)            |

Missing data from 5 sporadic patients.

Key: ALS, amyotrophic lateral sclerosis.

* t test, familial versus sporadic.

* Fisher exact test, 2-sided p value, familial versus sporadic; using a t test there was no significant difference seen between expanded versus nonexpanded age at onset.

---

Fig. 1. Cumulative frequency of the age of onset in C9ORF72 expansion carriers.
and Zoghbi, 2007). Although we detected a small number of cases with 20–24 repeats (4/228 = 1.8%) in the control Greek population this frequency is not significantly different when compared with 2 UK control series (unpublished 1/85 = 1.2% and 6/361 = 1.7%).

In summary, we have shown that the pathogenic GGGGCC hexanucleotide repeat expansion in C9ORF72 is an important cause of ALS in the Mediterranean Greek population in the familial and sporadic forms of disease. The frequency is high, second only to the Scandinavian population and this suggests the repeat prevalence in Greece, and likely in the Scandinavian countries is a consequence of population isolation and little racial admixture. The frequency also highlights the importance of establishing C9ORF72 as a diagnostic test in familial and sporadic patients.

Disclosure statement
The authors report no conflicts of interest.

Acknowledgements
The authors thank the patients and families for their essential help with this project, and The Medical Research Council (MRC), The Wellcome Trust, the National Organization for Rare Disorders (NORD), The Brain Research Trust (BRT), and the National Institute for Health Research (NIHR) UCL/UCLH biomedical research center for grant support.

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2012.02.021.

References
Al-Sarraj, S., King, A., Troakes, C., Smith, B., Maekawa, S., Bodí, I., Rogelj, B., Al-Chalabi, A., Hortobágyi, T., Shaw, C.E., 2011. 962 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol. 122, 691–702.

Beghi, E., Chio, A., Courratier, P., Esteban, J., Hardiman, O., Logroscino, G., Millat, A., Mitchell, D., Preux, P.M., Pupillo, E., Stiev, Z., Swingler, R., Traynor, B.J., Van der Berg, L.H., Veldink, J.H., Zoccolera, S., 2011. The epidemiology and treatment of ALS: focus on the heterogeneity of the disease and critical appraisal of therapeutic trials. Amyotroph. Lateral Scler. 12, 1–10.

Brooks, B.R., Miller, R.G., Swash, M., Munsat, T.L., 2000. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Other Mot. Neuron Disord. 1, 293–299.

Chió, A., Borghero, G., Pugliatti, M., Ticca, A., Calvo, A., Moglia, C., Mutani, R., Brunetti, M., Ossola, I., Marrosu, M.G., Murru, M.R., Floris, G., Cannas, A., Parish, L.D., Cossu, P., Abramzon, Y., Johnson, J.O., Nalls, M.A., Arepalli, S., Chong, S., Hernandez, D.G., Traynor, B.J., Restagno, G., Italian Amyotrophic Lateral Sclerosis Genetic (ITALSGEN) Consortium, 2011. Large proportion of amyotrophic lateral sclerosis cases in Sardinia due to a single founder mutation of the TARDBP gene. Arch. Neurol. 68, 594–598.

Chow, C.Y., Landers, J.E., Bergren, S.K., Sapp, P.C., Grant, A.E., Jones, J.M., Everett, L., Lenk, G.M., McKenna-Yasek, D.M., Weissman, L.S., Figlewicz, D., Brown, R.H., Meisler, M.H., 2009. Deletious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS. Am. J. Hum. Genet. 84, 85–88.

Clarke, C., Howard, R., Rossoe, M., Shorvon, S.E., 2009. Neurology—A Queen Square Textbook. Wiley-Blackwell, Oxford.

Cooper-Knock, J., Hewett, C., Highley, J.R., Brockington, A., Milano, A., Man, S., Martindale, J., Hartley, J., Walsh, T., Ghelsthorpe, C., Baxter, L., Forster, G., Fox, M., Bury, J., Mok, K., McDermott, C.J., Traynor, B.J., Kirby, J., Wharton, S.B., Ince, P.G., Hardy, J., Shaw, P.J., 2012. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. Brain, 135, 751–764.

Cronin, S., Hardiman, O., Traynor, B.J., 2007. Ethnic variation in the incidence of ALS: a systematic review. Neurology 68, 1002–1007.

DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adams, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knoopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

Deng, H.X., Chen, W., Song, S., Boycott, K.M., Gorrie, G.H., Siddique, N., Yang, Y., Fecto, F., Shi, Y., Zhai, H., Jiang, H., Hirano, M., Rampersaud, E., Jansen, G.H., Donkervoort, S., Bigio, E.H., Brooks, B.R., Ajoud, K., Suft, R.L., Haines, J.L., Magi, E., Pergolizzi, V., Siddique, N., Dix, S., 2009. 9p expansions in C9ORF72-linked FTLD and MND/ALS. Acta Neuropathol. 122, 657–671.

DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adams, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knoopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

Gijselinck, I., Van Langenhove, T., van der Zee, J., Sleevers, K., Philtjens, S., Kleinberger, G., Janssens, J., Betens, K., Van Cauwenberghe, C., Peres, C., Engelborghs, S., Sieben, A., De Jonghe, P., Vandenberghhe, R., Santens, P., De Bleecker, J., Maes, G., Bäumer, V., Dillen, L., Joris, G., Cuijts, I., Corsmit, E., Elinck, E., Van Dongen, J., Vermeulen, S., Van den Broeck, M., Waerencent, C., Matteijjensens, M., Peeters, K., Robberecht, W., Cras, P., Martin, J.J., De Buyse, D., Cruts, M., Van Broeckhoven, C., 2012. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. Lancet Neurol. 11, 54–65.

Greenway, M.J., Andersen, P.M., Russ, C., Ennis, S., Cashman, S., Donaghy, C., Patterson, V., Swingler, R., Kieran, D., Prehn, J., Morrison, K.E., Green, A., Acharya, K.R., Brown, R.H., Jr., Hardiman, O., 2006. ANG mutations segregate with familial and “sporadic” amyotrophic lateral sclerosis. Nat. Genet. 38, 411–413.

Ince, P.G., Highley, J.R., Kirby, J., Wharton, S.B., Takahashi, H., Strong, M.J., Shaw, P.J., 2011. Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. Acta Neuropathol. 122, 657–671.

Johnson, J.O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V.M., Trojanowski, J.Q., Gibbs, J.R., Brunetti, M., Gronka, S., Wuu, J., Ding, J., McCluskey, L., Martinez-Lage, M., Falcone, D., Hernandez, D.G., Arepalli, S., Chong, S., Schimmick, J.C., Rothstein, J., Landi, F., Wang, Y.D., Calvo, A., Mora, G., Sabatelli, M., Monsurro, M.R., Battistini, S., Salvi, F., Spatara, R., Sola, P., Borghero, G., Galassi, G., Scholz, S.W., Taylor, J.P., Restagno, G., Chio, A., Traynor, B.J., 2010. Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron 68, 857–864.
Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vandenberg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Mun-sat, T., Valdmanis, P., Rouleau, G.A., Hosler, B.A., Cortelli, P., de Jong, P.J., Yoshinaga, Y., Haines, J.L., Pericak-Vance, M.A., Yan, J., Ticozzi, N., Siddique, T., McKenna-Yasek, D., Sapp, P.C., Horvitz, H.R., Landers, J.E., Brown, R.H., Jr., 2009. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323, 1205–1208.

Laaksovirta, H., Peuralinna, T., Schymick, J.C., Scholz, S.W., Lai, S.L., Mylllykangas, L., Sulkava, R., Jansson, L., Hernandez, D.G., Gibbs, J.R., Nalls, M.A., Heckerman, D., Tienari, P.J., Traynor, B.J., 2010. Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: a genome-wide association study. Lancet Neurol. 9, 978–985.

Leone, M., Chio, A., Mortara, P., Rosso, M.G., Schiffer, D., 1983. Motor neuron disease in the Province of Turin, Italy, 1971–1980. Acta Neurol. Scand. 67, 316–327.

Logroscino, G., Traynor, B.J., Hardiman, O., Chio, A., Mitchell, D., Swingler, R.J., Millul, A., Benn, E., Beghi, E., 2010. Incidence of amyotrophic lateral sclerosis in Europe. J. Neurol. Neurosurg. Psychiatry 81, 385–390.

Maruyama, H., Morino, H., Ito, H., Izuhi, Y., Kato, H., Watanabe, Y., Kinoshita, Y., Kamada, M., Nodera, H., Suzuki, H., Komure, O., Matsuura, S., Kojita, K., Morimoto, N., Abe, K., Suzuki, N., Aoki, M., Kawata, A., Hirai, T., Kato, T., Ogasa, K., Hiran, A., Takanami, T., Usaka, H., Higawara, K., Kaji, R., Kawakami, H., 2010. Mutations of optineurin in amyotrophic lateral sclerosis. Nature 465, 223–226.

Morita, M., Al-Chalabi, A., Andersen, P.M., Hosler, B., Sapp, P., Englund, E., Mitchell, J.E., Habgood, J.J., de Belleroce, J., Xi, J., Tongaronprasert, W., Horvitz, H.R., Gunnarsson, L.G., Brown, R.H., Jr., 2006. A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. Neurology 67, 51–57.

Murros, K., Fogelholm, R., 1983. Amyotrophic lateral sclerosis in childhood and adult onset dystonia-parkinsonism cases with PLA2G6 mutations. Neurobiol. Aging 3, 232–237.

Paisán-Ruiz, C., Leclerc, A.L., Tamrazian, E., Van-denberg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Mun-sat, T., Valdmanis, P., Rouleau, G.A., Hosler, B.A., Cortelli, P., de Jong, P.J., Yoshinaga, Y., Haines, J.L., Pericak-Vance, M.A., Yan, J., Ticozzi, N., Siddique, T., McKenna-Yasek, D., Sapp, P.C., Horvitz, H.R., Landers, J.E., Brown, R.H., Jr., 2009. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323, 1205–1208.

Rademakers, R., Mackenzie, I.R., 2012. Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. Acta Neuropathol. 123, 409–417.

Valdmanis, P.N., Rouleau, G.A., 2008. Genetics of familial amyotrophic lateral sclerosis. Neurology 70, 144–152.

van Es, M.A., Veldink, J.H., Saris, C.G., Blauw, H.M., van Vught, P.W., Birve, A., Lemmens, R., Schelhaas, H.J., Groen, E.J., Huizinga, W.M., van der Kooi, A.J., de Visser, M., Dahlberg, C., Estrada, K., Rivade- neira, F., Hofman, A., Zwarts, M.J., van Doorn, P.T., Rujescu, D., Strengman, E., Giegling, I., Miglia, P., Tomik, B., Slowik, A., Uitter-linden, A.G., Hendrich, C., Waibel, S., Meyer, T., Ludolph, A.C., Glass, J.D., Purcell, S., Cichon, S., Nöthen, M.M., Wichmann, H.E., Schreiber, S., Vermeulen, S.H., Kiemern, L.A., Wokke, J.H., Cronin, S., McLaughlin, R.L., Hardiman, O., Fumoto, K., Kruker, K., Meineker, V., Melki, J., Leigh, P.N., Shaw, C.E., Landers, J.E., Al-Chalabi, A., Brown, R.H., Jr., Robberecht, W., Andersen, P.M., Ophoff, R.A., van den Berg, L.H., 2009. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672.

Sward, H., Rutherford, N.J., Brieremore, H., Krieger, C., Cashman, N., Fabros, M., Baker, M., Fok, A., Dejesus-Hernandez, M., Eisen, A., Rademakers, R., Mackenzie, I.R., 2012. Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. Acta Neuropathol. 123, 409–417.

Valdmanis, P.N., Rouleau, G.A., 2008. Genetics of familial amyotrophic lateral sclerosis. Neurology 70, 144–152.

van Es, M.A., Veldink, J.H., Saris, C.G., Blauw, H.M., van Vught, P.W., Birve, A., Lemmens, R., Schelhaas, H.J., Groen, E.J., Huizinga, W.M., van der Kooi, A.J., de Visser, M., Dahlberg, C., Estrada, K., Rivade- neira, F., Hofman, A., Zwarts, M.J., van Doorn, P.T., Rujescu, D., Strengman, E., Giegling, I., Miglia, P., Tomik, B., Slowik, A., Uitter-linden, A.G., Hendrich, C., Waibel, S., Meyer, T., Ludolph, A.C., Glass, J.D., Purcell, S., Cichon, S., Nöthen, M.M., Wichmann, H.E., Schreiber, S., Vermeulen, S.H., Kiemern, L.A., Wokke, J.H., Cronin, S., McLaughlin, R.L., Hardiman, O., Fumoto, K., Kruker, K., Meineker, V., Melki, J., Leigh, P.N., Shaw, C.E., Landers, J.E., Al-Chalabi, A., Brown, R.H., Jr., Robberecht, W., Andersen, P.M., Ophoff, R.A., van den Berg, L.H., 2009. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. Nature 465, 364–362.