UMassMemorial
University of Massachusetts Medical School

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Progress Report – ALS Investigations

U Mass Medical Center
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The ALS laboratories at the U Mass Medical Center have made progress on several fronts, including therapy development, discovering some new ALS genes, and generating new animal models of ALS based on ALS genes.

I. Therapeutic Trials

A. Silencing Genes

1. SOD1. We have continued our studies to find ways to silence genes whose mutations cause ALS. Our most intensive effort has focused on silencing the SOD1 gene. Over the years we and others have documented that this gene can be effectively silenced in a Petri dish. Late last year, we published a report of studies from the laboratory of Zuoshang Xu here showing that this gene can be silenced in adult ALS mice, significantly prolonging survival. More recently, we have worked closely with Loreli Stoica in the laboratory of Miguel Sena-Esteves to document that by treating the ALS mice within a day of birth, one can delay the appearance of motor neuron disease for several months.

In parallel with these mouse studies, we have begun to test efficacy of the same intervention in large animals. With Florey Borel in the laboratory of Chris Mueller, we have been able to deliver the silencing reagents to the spinal cord and brains of monkeys. The delivery is achieved using a virus (adeno-associated virus or AAV) that has a remarkable capacity to permeate the central nervous system. We are fortunate in this regard to collaborate with the UMass Gene Therapy laboratory, headed by Dr. Guangping Gao, who is an expert in the biology of AAV. Using AAV as a delivery vehicle, we find that in some monkeys we achieve excellent permeation into the tissues of the brain and spinal cord and thereby also induce excellent silencing of the SOD1 gene.

These data have encouraged us to outline a pathway to a human trial. Toward that end, we have begun to work closely with colleagues (James Berry, Merit Cudkowicz) at the Massachusetts General Hospital to design the format for such a human trial. The transition from laboratory-based studies to a human trial is lengthy and costly, but the steps are clearly defined. We are extremely grateful to the Angel Fund for its commitment to this program; Angel Fund support has been absolutely pivotal in moving us toward this human trial of gene silencing. It should be noted that if these proof-of-concept studies are successful with the SOD1 gene, the path is paved for analogous trials with many other ALS genes (and indeed for a variety of toxic mutant genes that cause other diseases).

2. Other approaches to gene silencing. Given the early promise of the SOD1 program, we are also embarking on two related programs. (1) We have now generated reagents that allow us to silence the most common ALS gene, known as C9orf72 or simply C9. Because the initial studies in cell cultures were promising, we have begun to use the AAV system to silence the C9 gene in mouse brain and spinal cord. This work is conducted by Gaby Toro with Chris Mueller. (2) We are continuing efforts to develop silencing reagents that do not require the use of a virus for delivery. These are built on small strands of DNA that target the genes we want to silence. These DNA-based molecules are chemically modified to enhance their stability and at the same time to facilitate their movement into the spinal cord. These studies, done by Havisha Karnam under the direction of Anastasia Khvorova, have produced a set of small DNA molecules that silence the target genes in cell culture and also show a capacity to permeate the nervous system. Testing underway now will ascertain whether or not the target gene is silenced in the spinal cord.

B. Stem cell therapy trial

We are now into the first year of a two year trial of humN stem cells in ALS patients. This study, coming from the Israeli company Brainstorm Cell Therapeutics, uses bone marrow derived stem cells. The patient's bone marrow is harvested, the stem cells are prepared over one month in a cell culture laboratory, and the cells are then injected back into patients, via both the spinal fluid and one arm muscle. This study is being conducted jointly with the Massachusetts General Hospital and the Mayo Clinic. The purpose of the study is to test safety of this approach. Of course, as in any safety study, we are hopeful that we WILL also see evidence of a clinical benefit. The full assessment, which will await follow-up of the last patients enrolled, will probably be available by mid-2016.

II. Gene Discovery in ALS

A. ***Profilin-1***. As reported previously, Dr. John Landers (a former Angel Fund fellow) identified a new ALS gene called *profilin-1*. This was identified in a large family in Israel and France, studied by Bob Brown along with clinicians from Israel. Dr. Landers and others have now documented that mutations in the profilin-1 gene can be detected in several families in the U.S. and Europe. Several other laboratories have now robustly confirmed John's observations.

B. ***Tubulin-4A.*** With Dr. Lander's team, we have also identified another ALS gene, tubulin-4A, which is mutated in a small subset of familial ALS cases. This is of interest because the gene, and the protein it makes, are implicated in processes that control how well a nerve extends its axonal process to connect with other neurons or muscle. We are intrigued that these biological processes implicated by the tubulin-4A finding are similar to those implicated by profilin-1.

C. ***TANK Binding Kinase-1 (TBK-1)*.** Over the several years, Dr. Brown and Landers have been involved in several large consortia combining large sets of DNA from ALS cases, including non-familial (sporadic) ALS, to use high throughput DNA sequencing methods to search for new ALS genes. One consortium, funded and coordinated Biogen, Inc., studied ~2,800 sporadic ALS cases and more than 6,000 controls, identifying a new ALS gene (TBK-1). The importance of this gene is still under investigation, but early studies indicated that it interrelates with other ALS genes and thus begins to define another network of ALS genes.

D. ***ERB4.*** As part of a collaboration headed by colleagues in Japan, we have identified a small subset of ALS patients whose disease reflects mutations in a gene that makes a protein known as receptor tyrosine kinase ERB4. The biological significance of this finding remains to be defined. However, it is already of interest that this gene is central importance in several fundamental cellular processes that can involve nerve cells, including differentiation.

III. New ALS Models – in Animals and Stem Cells

A. ***C9orf72.*** Among the most useful tools in studies of any disease are cell and animal models of the disease. These permit one to test hypothesis about how the disease evolves and how it can be treated. Several of us at UMMS have collaborated to generate a new mouse model of the C9orf72 gene defect that, as noted above, is the most commonly mutated ALS gene. Because these mice reproduce many of the molecular features of C9orf72-related ALS, we are hopeful that they will be valuable in the analysis of C9orf72. We already have begun preliminary treatment trials in mice using this model.

B. ***TDP43.*** We have also been engaged in studying models of another ALS gene known as TDP43. Zuoshang Xu has generated a line of mice in which the activity of the TDP43 is fractionally reduced. We were surprised to find that with no other molecular change these mice develop an adult-onset lethal paralysis, suggesting that one of the ways the TDP43 mutations cause ALS is by partially blunting the activity of this gene. In other studies, Jemeen Sreedharan has worked with Marc Freeman to develop a fruit-fly model of TDP43. They have then exploited the power of genetics in the fruit fly to discover new mutations that block this, thereby defining new pathways involved in TDP43 ALS. We are hopeful that knowledge of these pathways will provide new targets for therapy. This is particularly important because the TDP43 protein has been implicated in many cases of ALS and a related disorder, frontotemporal dementia (FTD).

IV. Related Studies of ALS Biology.

A. ***Axonal Transport in ALS.*** As described previously, we have continued to be interested in the possibility that mutant ALS genes may be toxic in part because they disturb the process of axonal transport in motor neurons. This is the mechanism whereby substances produced in the cell body of a motor neuron can be carried along the lengthy motor neuron process (the axon) to reach the distal terminal where the motor neuron contacts muscle and activates muscle contraction. In collaborations with Drs. Scott Brady and Gerardo Morfini, Dr. Daryl Bosco and I have found that mutant SOD1 protein is toxic to the molecular apparatus that governs axonal transport. These studies were performed at the Woods Hole Marine Biological Laboratories with Dr. Scott Brady, who has studied axonal transport in the squid giant axon for years. We are now attempting to find ways to relate these studies in squid to measuring and modifying axonal transport in humans.

B. ***Stem Cell Biology and ALS.*** Several of us are now using stem cells to study the biology of different aspects of ALS. It is striking that one can now generate stem cells from skin cells of a human. This has allowed investigators like Dr. Fen-Biao Gao at UMMS and Dr. Kevin Eggan at Harvard, to generate multiple lines of stem cells from a diversity of different ALS patients. With these, one can then generate motor neurons, meaning that one can now study in cell culture motor neurons from living patients. Dr. Gao has done

pivotal work using such stem cells to probe many aspects of ALS and fronto-temporal dementia; he was the first person to make a tissue culture model of C9orf72 ALS-FTD. He has used these cultures to study many phenomena including toxic proteins made by the C9orf72 gene and a type of regulatory RNA known as microRNA. Dr. Eggan has used cells provided from the UMMS ALS clinic and elsewhere to develop stem cell models of ALS with which he has demonstrated a propensity toward enhanced electrical activity, consistent with the longstanding view that motor nerve hyperexcitability is one of the underpinnings of ALS. We have studied approaches to diminishing this using a drug known as mexilitine. Dr. Eggan and collaborators have found that a different drug, retigabine, is also effective in suppressing motor neuron hyperexcitability. It is exciting that both mexilitine and retigabine are now being put into trials in human ALS.

As the above documents, the ALS laboratories at U Mass Medical School are making significant progress in understanding ALS. While we are fortunate to receive funding from several sources, both federal and private, the funding provided by the Angel Fund is truly a cornerstone for our investigations; this is particularly true for the gene therapy and gene silencing program. All of us in ALS research here are extremely grateful for the remarkably generous support provided by the Angel Fund; we are optimistic that this will have a transformative impact as we all work toward finding a cure for this disease.

Please contact me with any questions about this program.

Respectfully submitted,

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ALS Publications – U Mass Medical School 2013 through June 2015

1. Ahmeti KB, Ajroud-Driss S, Al-Chalabi A, Andersen PM, Armstrong J, Birve A, Blauw HM, Brown RH, Bruijn L, Chen W, Chio A, Comeau MC, Cronin S, Diekstra FP, Soraya Gkazi A, Glass JD, Grab JD, Groen EJ, Haines JL, Hardiman O, Heller S, Huang J, Hung WY; ITALSGEN consortium, Jaworski JM, Jones A, Khan H, Landers JE, Langefeld CD, Leigh PN, Marion MC, McLaughlin RL, Meininger V, Melki J, Miller, JW, Mora G, Pericak-Vance MA, Rampersaud E, Robberecht W, Russell LP, Salachas F, Saris CG, Shatunov A, Shaw CE, Siddique N, Siddique T, Smith BN, Sufit R, Topp S, Traynor BJ, Vance C, van Damme P, van den Berg LH, van Es MA, van Vught PW, Veldink JH, Yang Y, Zheng JG; ALSGEN Consortium. Age of onset of amyotrophic lateral sclerosis is modulated by a locus on 1p34.1. Neurobiol Aging. 2013 Jan;34(1):357.e7-19. doi: 10.1016/j.neurobiolaging.2012.07.017. Epub 2012 Sep 5. PubMed PMID: 22959728; PubMed Central PMCID: PMC3839234.

2. Akimoto C, Volk AE, van Blitterswijk M, Van den Broeck M, Leblond CS, Lumbroso S, Camu W, Neitzel B, Onodera O, van Rheenen W, Pinto S, Weber M, Smith, B, Proven M, Talbot K, Keagle P, Chesi A, Ratti A, van der Zee J, Alstermark H, Birve A, Calini D, Nordin A, Tradowsky DC, Just W, Daoud H, Angerbauer S, DeJesus-Hernandez M, Konno T, Lloyd-Jani A, de Carvalho M, Mouzat K, Landers JE, Veldink JH, Silani V, Gitler AD, Shaw CE, Rouleau GA, van den Berg LH, Van Broeckhoven C, Rademakers R, Andersen PM, Kubisch C. A blinded international study on the reliability of genetic testing for GGGGCC-repeat expansions in C9orf72 reveals marked differences in results among 14 laboratories. J Med Genet. 2014 Jun;51(6):419-24. doi: 10.1136/jmedgenet-2014-102360. Epub 2014 Apr 4. PubMed PMID: 24706941; PubMed Central PMCID: PMC4033024.

3. Almeida S, Gascon E, Tran H, Chou HJ, Gendron TF, Degroot S, Tapper AR, Sellier C, Charlet-Berguerand N, Karydas A, Seeley WW, Boxer AL, Petrucelli L, Miller BL, Gao FB. Modeling key pathological features of frontotemporal dementia with C9ORF72 repeat expansion in iPSC-derived human neurons. Acta Neuropathol. 2013 Sep;126(3):385-99. doi: 10.1007/s00401-013-1149-y. Epub 2013 Jul 9. Erratum in: Acta Neuropathol. 2014 Jun;127(6):941. PubMed PMID: 23836290; PubMed Central PMCID: PMC3753484.

4. ALSGEN Consortium, Ahmeti KB, Ajroud-Driss S, Al-Chalabi A, Andersen PM, Armstrong J, Birve A, Blauw HM, Brown RH, Bruijn L, Chen W, Chio A, Comeau MC, Cronin S, Diekstra FP, Soraya Gkazi A, Glass JD, Grab JD, Groen EJ, Haines JL, Hardiman O, Heller S, Huang J, Hung WY; ITALSGEN consortium, Jaworski JM, Jones A, Khan H, Landers JE, Langefeld CD, Leigh PN, Marion MC, McLaughlin RL, Meininger V, Melki J, Miller JW, Mora G, Pericak-Vance MA, Rampersaud E, Robberecht W, Russell LP, Salachas F, Saris CG, Shatunov A, Shaw CE, Siddique N, Siddique T, Smith BN, Sufit R, Topp S, Traynor BJ, Vance C, van Damme P, van den Berg LH, van Es MA, van Vught PW, Veldink JH, Yang Y, Zheng JG. *Age of onset of amyotrophic lateral sclerosis is modulated by a locus on 1p34.1.* Neurobiol Aging. 2013 Jan;34(1):357.e7-19

5. Auclair JR, Salisbury JP, Johnson JL, Petsko GA, Ringe D, Bosco DA, Agar NY,Santagata S, Durham HD, Agar JN. Artifacts to avoid while taking advantage of top-down mass spectrometry based detection of protein S-thiolation. Proteomics. 2014 May;14(10):1152-7. doi: 10.1002/pmic.201300450. Epub 2014 Apr 17. PubMed PMID: 24634066.

6. Auclair JR, Johnson JL, Liu Q, Salisbury JP, Rotunno MS, Petsko GA, Ringe D, Brown RH Jr, Bosco DA, Agar JN. Post-translational modification by cysteine protects Cu/Zn-superoxide dismutase from oxidative damage. Biochemistry. 2013 Sep 10;52(36):6137-44. doi: 10.1021/bi4006122. Epub 2013 Aug 26. PubMed PMID: 23927036; PubMed Central PMCID: PMC3859700.

7. Baron DM, Kaushansky LJ, Ward CL, Sama RR, Chian RJ, Boggio KJ, Quaresma AJ, Nickerson JA, Bosco DA. Amyotrophic lateral sclerosis-linked FUS/TLS alters stress granule assembly and dynamics. Mol Neurodegener. 2013 Aug 31;8:30. doi: 10.1186/1750-1326-8-30. PubMed PMID: 24090136; PubMed Central PMCID: PMC3766239.

8. Boopathy S, Silvas TV, Tischbein M, Jansen S, Shandilya SM, Zitzewitz JA, Landers JE, Goode BL, Schiffer CA, Bosco DA. Structural basis for mutation-induced destabilization of profilin 1 in ALS. Proc Natl Acad Sci U S A. 2015 Jun 30;112(26):7984-9. doi: 10.1073/pnas.1424108112. Epub 2015 Jun 8. PubMed PMID: 26056300.

9. Broering TJ, Wang H, Boatright NK, Wang Y, Baptista K, Shayan G, Garrity KA, Kayatekin C, Bosco DA, Matthews CR, Ambrosino DM, Xu Z, Babcock GJ. Identification of human monoclonal antibodies specific for human

SOD1 recognizing distinct epitopes and forms of SOD1. PLoS One. 2013 Apr 17;8(4):e61210. doi: 10.1371/journal.pone.0061210. Print 2013. PubMed PMID: 23613814; PubMed Central PMCID: PMC3629177.

10. Calini D, Corrado L, Del Bo R, Gagliardi S, Pensato V, Verde F, Corti S, Mazzini L, Milani P, Castellotti B, Bertolin C, Sorarù G, Cereda C, Comi GP, D'Alfonso S, Gellera C, Ticozzi N, Landers JE, Ratti A, Silani V; SLAGEN Consortium. Analysis of hnRNPA1, A2/B1, and A3 genes in patients with amyotrophic lateral sclerosis. Neurobiol Aging. 2013 Nov;34(11):2695.e11-2. doi: 10.1016/j.neurobiolaging.2013.05.025. Epub 2013 Jul 2. PubMed PMID: 23827524.

11. Cheever TR, Berkley D, Braun S, Brown RH, Byrne BJ, Chamberlain JS, Cwik V, Duan D, Federoff HJ, High KA, Kaspar BK, Klinger KW, Larkindale J, Lincecum J, Mavilio F, McDonald CL, McLaughlin J, Weiss McLeod B, Mendell JR, Nuckolls G, Stedman HH, Tagle DA, Vandenberghe LH, Wang H, Wernett PJ, Wilson JM, Porter JD, Gubitz AK. Perspectives on best practices for gene therapy programs. Hum Gene Ther. 2015 Mar;26(3):127-33. doi: 10.1089/hum.2014.147. Epub 2015 Mar 3. PubMed PMID: 25654329; PubMed Central PMCID: PMC4367233.

12. Cheruiyot A, Lee JA, Gao FB, Ahmad ST. Expression of mutant CHMP2B, an ESCRT-III component involved in frontotemporal dementia, causes eye deformities due to Notch misregulation in Drosophila. FASEB J. 2014 Feb;28(2):667-75. doi: 10.1096/fj.13-234138. Epub 2013 Oct 24. PubMed PMID: 24158394; PubMed Central PMCID: PMC3898657.

13. Convertini P, Zhang J, de la Grange P, Hayward LJ, Zhu H, Stamm S. Genome wide array analysis indicates that an amyotrophic lateral sclerosis mutation of FUS causes an early increase of CAMK2N2 in vitro. Biochim Biophys Acta. 2013 Aug;1832(8):1129-35. doi: 10.1016/j.bbadis.2013.03.015. Epub 2013 Mar 29. PubMed PMID: 23545117; PubMed Central PMCID: PMC3679306.

14. Deng M, Wei L, Zuo X, Tian Y, Xie F, Hu P, Zhu C, Yu F, Meng Y, Wang H, Zhang F, Ma H, Ye R, Cheng H, Du J, Dong W, Zhou S, Wang C, Wang Y, Wang J, Chen X, Sun Z, Zhou N, Jiang Y, Liu X, Li X, Zhang N, Liu N, Guan Y, Han Y, Han Y, Lv X, Fu Y, Yu H, Xi C, Xie D, Zhao Q, Xie P, Wang X, Zhang Z, Shen L, Cui Y, Yin X, Cheng H, Liang B, Zheng X, Lee TM, Chen G, Zhou F, Veldink JH, Robberecht W, Landers JE, Andersen PM, Al-Chalabi A, Shaw C, Liu C, Tang B, Xiao S, Robertson J, Zhang F, van den Berg LH, Sun L, Liu J, Yang S, Ju X, Wang K, Zhang X. Genome-wide association analyses in Han Chinese identify two new susceptibility loci for amyotrophic lateral sclerosis. Nat Genet. 2013 Jun;45(6):697-700. doi: 10.1038/ng.2627. Epub 2013 Apr 28. PubMed PMID: 23624525.

15. Diekstra FP, Van Deerlin VM, van Swieten JC, Al-Chalabi A, Ludolph AC, Weishaupt JH, Hardiman O, Landers JE, Brown RH Jr, van Es MA, Pasterkamp RJ, Koppers M, Andersen PM, Estrada K, Rivadeneira F, Hofman A, Uitterlinden AG, van Damme P, Melki J, Meininger V, Shatunov A, Shaw CE, Leigh PN, Shaw PJ, Morrison KE, Fogh I, Chiò A, Traynor BJ, Czell D, Weber M, Heutink P, de Bakker PI, Silani V, Robberecht W, van den Berg LH, Veldink JH. C9orf72 and UNC13A are shared risk loci for amyotrophic lateral sclerosis and frontotemporal dementia: a genome-wide meta-analysis. Ann Neurol. 2014 Jul;76(1):120-33. doi: 10.1002/ana.24198. Epub 2014 Jun 27. PubMed PMID: 24931836; PubMed Central PMCID: PMC4137231.

16. Fritz E, Izaurieta P, Weiss A, Mir FR, Rojas P, Gonzalez D, Rojas F, Brown RH, Madrid R, van Zundert B. *Mutant SOD1-expressing astrocytes release toxic factors that trigger motor neuron death by hyperexcitability*. J Neurophysiol. 2013 Mar 13. [Epub ahead of print].

17. Gonzalez-Perez P, Woehlbier U, Chian RJ, Sapp P, Rouleau GA, Leblond CS, Daoud H, Dion PA, Landers JE, Hetz C, Brown RH. Identification of rare protein disulfide isomerase gene variants in amyotrophic lateral sclerosis patients. Gene. 2015 Jul 25;566(2):158-65. doi: 10.1016/j.gene.2015.04.035. Epub 2015 Apr 22. PubMed PMID: 25913742.

18. Goris A, van Setten J, Diekstra F, Ripke S, Patsopoulos NA, Sawcer SJ; The International Multiple Sclerosis Genetics Consortium, van Es M; The Australia and New Zealand MS Genetics Consortium, Andersen PM, Melki J, Meininger V, Hardiman O, Landers JE, Brown RH, Shatunov A, Leigh N, Al-Chalabi A, Shaw CE, Traynor BJ, Chiò A, Restagno G, Mora G, Ophoff RA, Oksenberg JR, Van Damme P, Compston A, Robberecht W, Dubois B, van den

Berg LH, De Jager PL, Veldink JH, de Bakker PI. *No evidence for shared genetic basis of common variants in multiple sclerosis and amyotrophic lateral sclerosis*. Hum Mol Gen 2013.

19. Govindaraj P, Nalini A, Krishna N, Sharath A, Khan NA, Tamang R, Gourie-Devi M, Brown RH, Thangaraj K. Mitochondrial DNA variations in Madras motor neuron disease. Mitochondrion. 2013 Nov;13(6):721-8. doi: 10.1016/j.mito.2013.02.003. Epub 2013 Feb 16. PubMed PMID: 23419391.

20. Filiano AJ, Martens LH, Young AH, Warmus BA, Zhou P, Diaz-Ramirez G, Jiao J, Zhang Z, Huang EJ, Gao FB, Farese RV Jr, Roberson ED. Dissociation of frontotemporal dementia-related deficits and neuroinflammation in progranulin haploinsufficient mice. J Neurosci. 2013 Mar 20;33(12):5352-61. doi: 10.1523/JNEUROSCI.6103-11.2013. PubMed PMID: 23516300; PubMed Central PMCID: PMC3740510.

21. Fogh I, Ratti A, Gellera C, Lin K, Tiloca C, Moskvina V, Corrado L, Sorarù G, Cereda C, Corti S, Gentilini D, Calini D, Castellotti B, Mazzini L, Querin G, Gagliardi S, Del Bo R, Conforti FL, Siciliano G, Inghilleri M, Saccà F, Bongioanni P, Penco S, Corbo M, Sorbi S, Filosto M, Ferlini A, Di Blasio AM, Signorini S, Shatunov A, Jones A, Shaw PJ, Morrison KE, Farmer AE, Van Damme P, Robberecht W, Chiò A, Traynor BJ, Sendtner M, Melki J, Meininger V, Hardiman O, Andersen PM, Leigh NP, Glass JD, Overste D, Diekstra FP, Veldink JH, van Es MA, Shaw CE, Weale ME, Lewis CM, Williams J, Brown RH, Landers JE, Ticozzi N, Ceroni M, Pegoraro E, Comi GP, D'Alfonso S, van den Berg LH, Taroni F, Al-Chalabi A, Powell J, Silani V; SLAGEN Consortium and Collaborators. A genome-wide association meta-analysis identifies a novel locus at 17q11.2 associated with sporadic amyotrophic lateral sclerosis. Hum Mol Genet. 2014 Apr 15;23(8):2220-31. doi: 10.1093/hmg/ddt587. Epub 2013 Nov 20. PubMed PMID: 24256812; PubMed Central PMCID: PMC3959809.

22. Fritz E, Izaurieta P, Weiss A, Mir FR, Rojas P, Gonzalez D, Rojas F, Brown RH Jr, Madrid R, van Zundert B. Mutant SOD1-expressing astrocytes release toxic factors that trigger motoneuron death by inducing hyperexcitability. J Neurophysiol. 2013 Jun;109(11):2803-14. doi: 10.1152/jn.00500.2012. Epub 2013 Mar 13. PubMed PMID: 23486205; PubMed Central PMCID: PMC3680799.

23. Gao FB, Taylor JP. RNA metabolism in neurological disease. Brain Res. 2014 Oct 10;1584:1-2. doi: 10.1016/j.brainres.2014.09.011. PubMed PMID: 25248315; PubMed Central PMCID: PMC4196277.

24. Gascon E, Gao FB. The emerging roles of microRNAs in the pathogenesis of frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS) spectrum disorders. J Neurogenet. 2014 Mar-Jun;28(1-2):30-40. doi: 10.3109/01677063.2013.876021. Epub 2014 Feb 10. Review. PubMed PMID: 24506814; PubMed Central PMCID: PMC4199862.

25. Gascon E, Lynch K, Ruan H, Almeida S, Verheyden JM, Seeley WW, Dickson DW, Petrucelli L, Sun D, Jiao J, Zhou H, Jakovcevski M, Akbarian S, Yao WD, Gao FB. Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemporal dementia. Nat Med. 2014 Dec;20(12):1444-51. doi: 10.1038/nm.3717. Epub 2014 Nov 17. PubMed PMID: 25401692; PubMed Central PMCID: PMC4257887.

26. Gonzalez-Perez P, Woehlbier U, Chian RJ, Sapp P, Rouleau GA, Leblond CS, Daoud H, Dion PA, Landers JE, Hetz C, Brown RH. Identification of rare protein disulfide isomerase gene variants in amyotrophic lateral sclerosis patients. Gene. 2015 Jul 25;566(2):158-65. doi: 10.1016/j.gene.2015.04.035. Epub 2015 Apr 22. PubMed PMID: 25913742.

27. Goris A, van Setten J, Diekstra F, Ripke S, Patsopoulos NA, Sawcer SJ; International Multiple Sclerosis Genetics Consortium, van Es M; Australia and New Zealand MS Genetics Consortium, Andersen PM, Melki J, Meininger V, Hardiman O, Landers JE, Brown RH Jr, Shatunov A, Leigh N, Al-Chalabi A, Shaw CE, Traynor BJ, Chiò A, Restagno G, Mora G, Ophoff RA, Oksenberg JR, Van Damme P, Compston A, Robberecht W, Dubois B, van den Berg LH, De Jager PL, Veldink JH, de Bakker PI. No evidence for shared genetic basis of common variants in multiple sclerosis and amyotrophic lateral sclerosis. Hum Mol Genet. 2014 Apr 1;23(7):1916-22. doi: 10.1093/hmg/ddt574. Epub 2013 Nov 13. PubMed PMID: 24234648; PubMed Central PMCID: PMC3943520.

28. Ingre C, Landers JE, Rizik N, Volk AE, Akimoto C, Birve A, Hübers A, Keagle PJ, Piotrowska K, Press R, Andersen PM, Ludolph AC, Weishaupt JH. A novel phosphorylation site mutation in profilin 1 revealed in a large screen of US, Nordic, and German amyotrophic lateral sclerosis/frontotemporal dementia cohorts. Neurobiol Aging.

2013 Jun;34(6):1708.e1-6. doi: 10.1016/j.neurobiolaging. 2012.10.009. Epub 2012 Nov 8. PubMed PMID: 23141414.

29. Kiskinis E, Sandoe J, Williams LA, Boulting GL, Moccia R, Wainger BJ, Han S, Peng T, Thams S, Mikkilineni S, Mellin C, Merkle FT, Davis-Dusenbery BN, Ziller M, Oakley D, Ichida J, Di Costanzo S, Atwater N, Maeder ML, Goodwin MJ, Nemesh J, Handsaker RE, Paull D, Noggle S, McCarroll SA, Joung JK, Woolf CJ, Brown RH, Eggan K. Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant SOD1. Cell Stem Cell. 2014 Jun 5;14(6):781-95. doi: 10.1016/j.stem.2014.03.004. Epub 2014 Apr 3. PubMed PMID: 24704492.

30. Lee WC, Almeida S, Prudencio M, Caulfield TR, Zhang YJ, Tay WM, Bauer PO, Chew J, Sasaguri H, Jansen-West KR, Gendron TF, Stetler CT, Finch N, Mackenzie IR, Rademakers R, Gao FB, Petrucelli L. Targeted manipulation of the sortilin-progranulin axis rescues progranulin haploinsufficiency. Hum Mol Genet. 2014 Mar 15;23(6):1467-78. doi: 10.1093/hmg/ddt534. Epub 2013 Oct 26. PubMed PMID: 24163244; PubMed Central PMCID: PMC3929086.

31. Li S, Sheng J, Hu JK, Yu W, Kishikawa H, Hu MG, Shima K, Wu D, Xu Z, Xin W, Sims KB, Landers JE, Brown RH Jr, Hu GF. Ribonuclease 4 protects neuron degeneration by promoting angiogenesis, neurogenesis, and neuronal survival under stress. Angiogenesis. 2013 Apr;16(2):387-404. doi: 10.1007/s10456-012-9322-9. Epub 2012 Nov 10. PubMed PMID: 23143660; PubMed Central PMCID: PMC3582744.

32. Lu Y, Zhang Z, Sun D, Sweeney ST, Gao FB. Syntaxin 13, a genetic modifier of mutant CHMP2B in frontotemporal dementia, is required for autophagosome maturation. Mol Cell. 2013 Oct 24;52(2):264-71. doi: 10.1016/j.molcel.2013.08.041. Epub 2013 Oct 3. PubMed PMID: 24095276; PubMed Central PMCID: PMC3825790.

33. Matus S, Bosco DA, Hetz C. Autophagy meets fused in sarcoma-positive stress granules. Neurobiol Aging. 2014 Dec;35(12):2832-5. doi: 10.1016/j.neurobiolaging.2014.08.019. Epub 2014 Sep 28. PubMed PMID: 25444610; PubMed Central PMCID: PMC4324442.

34. Morfini GA, Bosco DA, Brown H, Gatto R, Kaminska A, Song Y, Molla L, Baker L, Marangoni MN, Berth S, Tavassoli E, Bagnato C, Tiwari A, Hayward LJ, Pigino GF, Watterson DM, Huang CF, Banker G, Brown RH Jr, Brady ST. Inhibition of fast axonal transport by pathogenic SOD1 involves activation of p38 MAP kinase. PLoS One. 2013 Jun 12;8(6):e65235. doi: 10.1371/journal.pone. 0065235. Print 2013. PubMed PMID: 23776455; PubMed Central PMCID: PMC3680447.

35. Özoğuz A, Uyan Ö, Birdal G, Iskender C, Kartal E, Lahut S, Ömür Ö, Agim ZS, Eken AG, Sen NE, Kavak P, Saygı C, Sapp PC, Keagle P, Parman Y, Tan E, Koç F, Deymeer F, Oflazer P, Hanağası H, Gürvit H, Bilgiç B, Durmuş H, Ertaş M, Kotan D, Akalın MA, Güllüoğlu H, Zarifoğlu M, Aysal F, Döşoğlu N, Bilguvar K, Günel M, Keskin Ö, Akgün T, Özçelik H, Landers JE, Brown RH, Başak AN. The distinct genetic pattern of ALS in Turkey and novel mutations. Neurobiol Aging. 2015 Apr;36(4):1764.e9-18. doi: 10.1016/j.neurobiolaging.2014.12.032. Epub 2015 Jan 10. PubMed PMID: 25681989.

36. Pensato V, Tiloca C, Corrado L, Bertolin C, Sardone V, Del Bo R, Calini D, Mandrioli J, Lauria G, Mazzini L, Querin G, Ceroni M, Cantello R, Corti S, Castellotti B, Soldà G, Duga S, Comi GP, Cereda C, Sorarù G, D'Alfonso S, Taroni F, Shaw CE, Landers JE, Ticozzi N, Ratti A, Gellera C, Silani V; SLAGEN Consortium. TUBA4A gene analysis in sporadic amyotrophic lateral sclerosis: identification of novel mutations. J Neurol. 2015 May;262(5):1376-8. doi: 10.1007/s00415-015-7739-y. Epub 2015 Apr 18. PubMed PMID: 25893256.

37. Peng L, Liu H, Ruan H, Tepp WH, Stoothoff WH, Brown RH, Johnson EA, Yao WD, Zhang SC, Dong M. Cytotoxicity of botulinum neurotoxins reveals a direct role of syntaxin 1 and SNAP-25 in neuron survival. Nat Commun. 2013;4:1472. doi: 10.1038/ncomms2462. PubMed PMID: 23403573; PubMed Central PMCID: PMC4052923.

38. Peters OM, Ghasemi M, Brown RH Jr. Emerging mechanisms of molecular pathology in ALS. J Clin Invest. 2015 May;125(5):1767-79. doi: 10.1172/JCI71601. Epub 2015 May 1. PubMed PMID: 25932674; PubMed Central PMCID: PMC4463186.

39. Pickles S, Destroismaisons L, Peyrard SL, Cadot S, Rouleau GA, Brown RH Jr, Julien JP, Arbour N, Vande Velde C. Mitochondrial damage revealed by immunoselection for ALS-linked misfolded SOD1. Hum Mol Genet. 2013 Oct 1;22(19):3947-59. doi: 10.1093/hmg/ddt249. Epub 2013 Jun 4. PubMed PMID: 23736301.

40. Rotunno MS, Auclair JR, Maniatis S, Shaffer SA, Agar J, Bosco DA. Identification of a misfolded region in superoxide dismutase 1 that is exposed in amyotrophic lateral sclerosis. J Biol Chem. 2014 Oct 10;289(41):28527-38. doi: 10.1074/jbc.M114.581801. Epub 2014 Aug 27. PubMed PMID: 25164820; PubMed Central PMCID: PMC4192504.

41. Rotunno MS, Bosco DA. An emerging role for misfolded wild-type SOD1 in sporadic ALS pathogenesis. Front Cell Neurosci. 2013 Dec 16;7:253. doi: 10.3389/fncel.2013.00253. Review. PubMed PMID: 24379756; PubMed Central PMCID: PMC3863749.

42. Sama RR, Ward CL, Kaushansky LJ, Lemay N, Ishigaki S, Urano F, Bosco DA. FUS/TLS assembles into stress granules and is a prosurvival factor during hyperosmolar stress. J Cell Physiol. 2013 Nov;228(11):2222-31. doi: 10.1002/jcp.24395. PubMed PMID: 23625794; PubMed Central PMCID: PMC4000275.

43. Sama RR, Ward CL, Bosco DA. Functions of FUS/TLS from DNA repair to stress response: implications for ALS. ASN Neuro. 2014 Jun 1;6(4). pii: 1759091414544472. doi: 10.1177/ 1759091414544472. Review. PubMed PMID: 25289647; PubMed Central PMCID: PMC4189536.

44. Seijffers R, Zhang J, Matthews JC, Chen A, Tamrazian E, Babaniyi O, Selig M, Hynynen M, Woolf CJ, Brown RH Jr. ATF3 expression improves motor function in the ALS mouse model by promoting motor neuron survival and retaining muscle innervation. Proc Natl Acad Sci U S A. 2014 Jan 28;111(4):1622-7. doi: 10.1073/pnas.1314826111. Epub 2014 Jan 13. PubMed PMID: 24474789; PubMed Central PMCID: PMC3910594.

45. Smith BN, Ticozzi N, Fallini C, Gkazi AS, Topp S, Kenna KP, Scotter EL, Kost J, Keagle P, Miller JW, Calini D, Vance C, Danielson EW, Troakes C, Tiloca C, Al-Sarraj S, Lewis EA, King A, Colombrita C, Pensato V, Castellotti B, de Belleroche J, Baas F, ten Asbroek AL, Sapp PC, McKenna-Yasek D, McLaughlin RL, Polak M, Asress S, Esteban-Pérez J, Muñoz-Blanco JL, Simpson M; SLAGEN Consortium, van Rheenen W, Diekstra FP, Lauria G, Duga S, Corti S, Cereda C, Corrado L, Sorarù G, Morrison KE, Williams KL, Nicholson GA, Blair IP, Dion PA, Leblond CS, Rouleau GA, Hardiman O, Veldink JH, van den Berg LH, Al-Chalabi A, Pall H, Shaw PJ, Turner MR, Talbot K, Taroni F, García-Redondo A, Wu Z, Glass JD, Gellera C, Ratti A, Brown RH Jr, Silani V, Shaw CE, Landers JE. Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. Neuron. 2014 Oct 22;84(2):324-31. doi: 10.1016/j.neuron.2014.09.027. Epub 2014 Oct 22. PubMed PMID: 25374358.

46. Smith BN, Vance C, Scotter EL, Troakes C, Wong CH, Topp S, Maekawa S, King A, Mitchell JC, Lund K, Al-Chalabi A, Ticozzi N, Silani V, Sapp P, Brown RH Jr, Landers JE, Al-Sarraj S, Shaw CE. Novel mutations support a role for Profilin 1 in the pathogenesis of ALS. Neurobiol Aging. 2015 Mar;36(3):1602.e17-27. doi: 10.1016/j.neurobiolaging.2014.10.032. Epub 2014 Oct 31. PubMed PMID: 25499087; PubMed Central PMCID: PMC4357530.

47. Sreedharan J, Brown RH Jr. Amyotrophic lateral sclerosis: Problems and prospects. Ann Neurol. 2013 Sep;74(3):309-16. doi: 10.1002/ana.24012. Review. PubMed PMID: 24038380.

48. Staats KA, Van Helleputte L, Jones AR, Bento-Abreu A, Van Hoecke A, Shatunov A, Simpson CL, Lemmens R, Jaspers T, Fukami K, Nakamura Y, Brown RH Jr, Van Damme P, Liston A, Robberecht W, Al-Chalabi A, Van Den Bosch L. Genetic ablation of phospholipase C delta 1 increases survival in SOD1(G93A) mice. Neurobiol Dis. 2013 Dec;60:11-7. doi: 10.1016/j.nbd.2013.08.006. Epub 2013 Aug 19. PubMed PMID: 23969236.

49. Su Z, Zhang Y, Gendron TF, Bauer PO, Chew J, Yang WY, Fostvedt E, Jansen-West K, Belzil VV, Desaro P, Johnston A, Overstreet K, Oh SY, Todd PK, Berry JD, Cudkowicz ME, Boeve BF, Dickson D, Floeter MK, Traynor BJ, Morelli C, Ratti A, Silani V, Rademakers R, Brown RH, Rothstein JD, Boylan KB, Petrucelli L, Disney MD. Discovery of a biomarker and lead small molecules to target r(GGGGCC)-associated defects in

c9FTD/ALS. Neuron. 2014 Sep 3;83(5):1043-50. doi: 10.1016/j.neuron.2014.07.041. Epub 2014 Aug 14. Erratum in: Neuron. 2014 Oct 1;84(1):239. PubMed PMID: 25132468; PubMed Central PMCID: PMC4232217.

50. Takahashi Y, Fukuda Y, Yoshimura J, Toyoda A, Kurppa K, Moritoyo H, Belzil VV, Dion PA, Higasa K, Doi K, Ishiura H, Mitsui J, Date H, Ahsan B, Matsukawa T, Ichikawa Y, Moritoyo T, Ikoma M, Hashimoto T, Kimura F, Murayama S, Onodera O, Nishizawa M, Yoshida M, Atsuta N, Sobue G; JaCALS, Fifita JA, Williams KL, Blair IP, Nicholson GA, Gonzalez-Perez P, Brown RH Jr, Nomoto M, Elenius K, Rouleau GA, Fujiyama A, Morishita S, Goto J, Tsuji S. *ERBB4 mutations that disrupt the neuregulin-ErbB4 pathway cause amyotrophic lateral sclerosis type 19*. Am J Hum Genet. 2013 Nov 7;93(5):900-5..

51. Tibshirani M, Tradewell ML, Mattina KR, Minotti S, Yang W, Zhou H, Strong MJ, Hayward LJ, Durham HD. Cytoplasmic sequestration of FUS/TLS associated with ALS alters histone marks through loss of nuclear protein arginine methyltransferase 1. Hum Mol Genet. 2015 Feb 1;24(3):773-86. doi: 10.1093/hmg/ddu494. Epub 2014 Sep 30. PubMed PMID: 25274782; PubMed Central PMCID: PMC4291251.

52. Tiloca C, Ticozzi N, Pensato V, Corrado L, Del Bo R, Bertolin C, Fenoglio C, Gagliardi S, Calini D, Lauria G, Castellotti B, Bagarotti A, Corti S, Galimberti D, Cagnin A, Gabelli C, Ranieri M, Ceroni M, Siciliano G, Mazzini L, Cereda C, Scarpini E, Sorarù G, Comi GP, D'Alfonso S, Gellera C, Ratti A, Landers JE, Silani V; SLAGEN Consortium. Screening of the PFN1 gene in sporadic amyotrophic lateral sclerosis and in frontotemporal dementia. Neurobiol Aging. 2013 May;34(5):1517.e9-10. doi: 10.1016/j.neurobiolaging.2012.09.016. Epub 2012 Oct 11. PubMed PMID: 23063648; PubMed Central PMCID: PMC3548975.

53. van Blitterswijk M, Wang ET, Friedman BA, Keagle PJ, Lowe P, Leclerc AL, van den Berg LH, Housman DE, Veldink JH, Landers JE. Characterization of FUS mutations in amyotrophic lateral sclerosis using RNA-Seq. PLoS One. 2013;8(4):e60788. doi: 10.1371/journal.pone.0060788. Epub 2013 Apr 8. PubMed PMID: 23577159; PubMed Central PMCID: PMC3620060.

54. van Doormaal PT, Ticozzi N, Gellera C, Ratti A, Taroni F, Chiò A, Calvo A, Mora G, Restagno G, Traynor BJ, Birve A, Lemmens R, van Es MA, Saris CG,Blauw HM, van Vught PW, Groen EJ, Corrado L, Mazzini L, Del Bo R, Corti S, Waibel S, Meyer T, Ludolph AC, Goris A, van Damme P, Robberecht W, Shatunov A, Fogh I, Andersen PM, D'Alfonso S, Hardiman O, Cronin S, Rujescu D, Al-Chalabi A, Landers JE, Silani V, van den Berg LH, Veldink JH. Analysis of the KIFAP3 gene in amyotrophic lateral sclerosis: a multicenter survival study. Neurobiol Aging. 2014 Oct;35(10):2420.e13-4. doi: 10.1016/j.neurobiolaging.2014.04.014. Epub 2014 Apr 19. PubMed PMID: 24838185.

55. Van Helleputte L, Jones AR, Bento-Abreu A, Van Hoecke A, Shatunov A, Simpson CL, Lemmens R, Jaspers T, Fukami K, Nakamura Y, Brown RH, Van Damme P, Liston A, Robberecht W, Al-Chalabi A, Van Den Bosch L. *Genetic ablation of phospholipase C delta 1 increases survival in SOD1G93A mice*. Neurobiol of Disease. 2013

56. Wainger BJ, Kiskinis E, Mellin C, Wiskow O, Han SS, Sandoe J, Perez NP, Williams LA, Lee S, Boulting G, Berry JD, Brown RH Jr, Cudkowicz ME, Bean BP, Eggan K, Woolf CJ. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep. 2014 Apr 10;7(1):1-11. doi: 10.1016/j.celrep.2014.03.019. Epub 2014 Apr 3. PubMed PMID: 24703839; PubMed Central PMCID: PMC4023477.

57. Wang H, Yang B, Qiu L, Yang C, Kramer J, Su Q, Guo Y, Brown RH Jr, Gao G, Xu Z. Widespread spinal cord transduction by intrathecal injection of rAAV delivers efficacious RNAi therapy for amyotrophic lateral sclerosis. Hum Mol Genet. 2014 Feb 1;23(3):668-81. doi: 10.1093/hmg/ddt454. Epub 2013 Sep 18. PubMed PMID: 24108104; PubMed Central PMCID: PMC3888258.

58. Ward CL, Boggio KJ, Johnson BN, Boyd JB, Douthwright S, Shaffer SA, Landers JE, Glicksman MA, Bosco DA. A loss of FUS/TLS function leads to impaired cellular proliferation. Cell Death Dis. 2014 Dec 11;5:e1572. doi: 10.1038/cddis.2014.508. PubMed PMID: 25501833.

59. West RJ, Lu Y, Marie B, Gao FB, Sweeney ST. Rab8, POSH, and TAK1 regulate synaptic growth in a Drosophila model of frontotemporal dementia. J Cell Biol. 2015 Mar 30;208(7):931-47. doi: 10.1083/jcb.201404066. Epub 2015 Mar 23. PubMed PMID: 25800055; PubMed Central PMCID: PMC4384727.

60. Wu CH, Fallini C, Ticozzi N, Keagle PJ, Sapp PC, Piotrowska K, Lowe P, Koppers M, McKenna-Yasek D, Baron DM, Kost JE, Gonzalez-Perez P, Fox AD, Adams J, Taroni F, Tiloca C, Leclerc AL, Chafe SC, Mangroo D, Moore MJ, Zitzewitz JA, Xu ZS, van den Berg LH, Glass JD, Siciliano G, Cirulli ET, Goldstein DB, Salachas F, Meininger V, Rossoll W, Ratti A, Gellera C, Bosco DA, Bassell GJ, Silani V, Drory VE, Brown RH Jr, Landers JE. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature. 2012 Aug 23;488(7412):499-503. doi: 10.1038/nature11280. PubMed PMID: 22801503; PubMed Central PMCID: PMC3575525.

61. Yang D, Abdallah A, Li Z, Lu Y, Almeida S, Gao FB. FTD/ALS-associated poly(GR) protein impairs the Notch pathway and is recruited by poly(GA) into cytoplasmic inclusions. Acta Neuropathol. 2015 Jun 2. [Epub ahead of print] PubMed PMID: 26031661.

62. Yang C, Wang H, Qiao T, Yang B, Aliaga L, Qiu L, Tan W, Salameh J, McKenna-Yasek DM, Smith T, Peng L, Moore MJ, Brown RH Jr, Cai H, Xu Z. Partial loss of TDP-43 function causes phenotypes of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2014 Mar 25;111(12):E1121-9. doi: 10.1073/pnas.1322641111. Epub 2014 Mar 10. PubMed PMID: 24616503; PubMed Central PMCID: PMC3970502.

63. Yuva-Aydemir Y, Xu XL, Aydemir O, Gascon E, Sayin S, Zhou W, Hong Y, Gao FB. Downregulation of the Host Gene jigr1 by miR-92 Is Essential for Neuroblast Self-Renewal in Drosophila. PLoS Genet. 2015 May 22;11(5):e1005264. doi: 10.1371/journal.pgen.1005264. eCollection 2015 May. PubMed PMID: 26000445; PubMed Central PMCID: PMC4441384.

64. Zhang Z, Almeida S, Lu Y, Nishimura AL, Peng L, Sun D, Wu B, Karydas AM, Tartaglia MC, Fong JC, Miller BL, Farese RV Jr, Moore MJ, Shaw CE, Gao FB. Downregulation of microRNA-9 in iPSC-derived neurons of FTD/ALS patients with TDP-43 mutations. PLoS One. 2013 Oct 15;8(10):e76055. doi: 10.1371/journal.pone.0076055. eCollection 2013. PubMed PMID: 24143176; PubMed Central PMCID: PMC3797144.