Capturing VCP: Another Molecular Piece in the ALS Jigsaw Puzzle

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TDP-43 mislocalization and aggregation are implicated in the pathogenesis of ALS and FTLD-U. Valosin containing protein (VCP) mutations also lead to TDP-43 deposition, resulting in Inclusion Body Myopathy, Paget disease, and Frontotemporal Dementia (IBMPFD). In this issue of *Neuron*, Johnson et al. used whole-exome capture to identify VCP mutations in familial ALS. This extends the VCP phenotype to include motor neuron degeneration and provides another molecular tool to explore neurodegeneration disease mechanisms underlying the TDP-43 proteinopathies.

Amyotrophic lateral sclerosis (ALS) is one of the most feared adult-onset illnesses in man (reviewed in Leigh and Wijesekera, 2010). Previously known as "the creeping paralysis," it declares its presence with focal muscular weakness, most commonly in one limb, and relentlessly spreads to affect all muscle groups, depriving patients of the ability to walk, talk, and care for themselves. No therapies significantly alter the disease course, and death due to respiratory failure occurs on average within 3 years of symptom onset. As a result, ALS is the most common reason that people seek euthanasia. ALS affects all populations, and the life-time risk in people of European ancestry is around 1 in 400. Ninety-five percent of cases are sporadic (SALS), but ~5% of cases have an affected first- or second-degree relative and are considered familial (FALS). The clinical diagnosis of ALS is based on signs of progressive upper and lower motor neuron dysfunction. Evidence of mild frontal lobe involvement can be detected in 30% of cases, and in ~3% of cases ALS is accompanied by marked behavioral changes and language deficits indicative of frontotemporal lobar dementia (FTLD). The phenotypes of SALS and FALS cases are clinically indistinguishable, and the link to FTLD has been made through many neurophysiological, imaging, and genetic studies.

Molecular proof that ALS and FTLD represent two ends of a phenotypic spectrum came from pathological studies that identified transactivation response DNA binding protein (TDP-43) as the major constituent of ubiquitinated neuronal cytoplasmic inclusions (NCIs) in > 90% of ALS and 60% of FTLD cases (Neumann et al., 2006), subsequently labeled as the "TDP-43 proteinopathies" (reviewed in Mackenzie et al., 2010). TDP-43 is a predominantly nuclear protein that regulates RNA transcription, splicing, and transport as well as micro-RNA biogenesis. In FTLD and ALS cases, TDP-43 protein is lost from the nucleus and accumulates in massive amounts in the cytoplasm, forming detergent-resistant inclusions that are heavily phosphorylated and ubiquitinated. It is not yet clear whether neurodegeneration is initiated by a loss of nuclear TDP-43 function, a toxic gain of function in the cytoplasm, or a combination of the two.

Although many genetic variants have been associated with sporadic ALS, mutations in only three genes have been firmly linked in autosomal-dominant classical ALS kindreds: *Cu/Zn superoxide dismutatse* (*SOD1* 20% of FALS), *TARDBP* (1%–4%), and *fused in sarcoma* (*FUS,* 2%–4%) (reviewed in Lagier-Tourenne et al., 2010). Interestingly, although TDP-43 deposition is the hallmark of ALS pathology in 90% of cases, it is absent from *SOD1* and *FUS* mutant cases, indicating that their pathogenic pathways appear distinct. This may explain why therapies effective in mutant *SOD1* cellular and animal models did not work in ALS patients who predominantly have a TDP-43 proteinopathy. Mutations in the *TARDBP* that are known to be neurotoxic in vivo and are associated with TDP-43 pathology in ALS have been identified in sporadic and familial ALS (Sreedharan et al., 2008). The identification of FUS mutations is particularly important as FUS is a functional homolog of TDP-43, playing a key role in RNA processing and micro-RNA biogenesis. Similar to TDP-43, ALS patients with *FUS* mutations also develop large cytoplasmic aggregates within spinal motor neurons and lose nuclear FUS (Vance et al., 2009; Kwiatkowski et al., 2009).

In retrospect, *VCP* was a very good candidate gene for screening in ALS but it was not biology that led the authors to discover the mutations, it was two emerging technologies—exon capture and massive parallel sequencing (Johnson et al., 2010). Most pathogenic mutations are within the 1% of the genome that encodes exons and result in changes to the amino acid sequence of proteins. Using commercially available CGH oligomers, they were able to capture, sequence, and align 76%–80% of the known exome. The Illumina sequencing platform exploits a powerful microscope and computer that is able to observe millions of short DNA fragments being synthesized, base by base, in real time. These short DNA sequences can be aligned to the reference human genome, allowing variants to be identified.

The authors started by sequencing the exome in two members of an autosomal-dominant Italian ALS kindred. After filtering for known SNPs from the 1000 genome project and dbSNP databases,
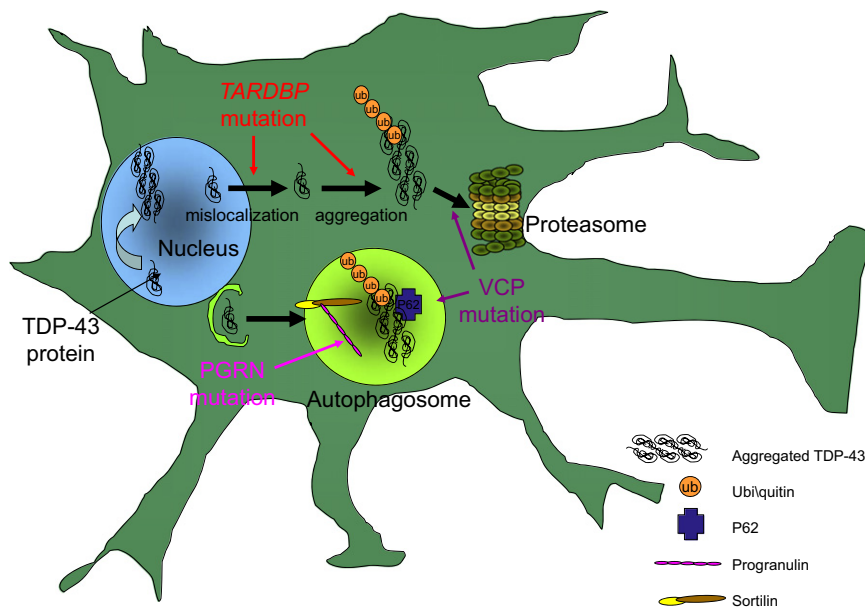
Figure 1. Ubiquitinated TDP-43 Inclusions in the Neuronal Cytoplasm and Nucleus Are the Pathological Hallmark of ALS and FTLD-U

Defective clearance of TDP-43 aggregates can arise due to mutation in three genes: *TARDBP* mutation may increase cytoplasmic levels of TDP-43 and promote aggregation, *PGRN* mutations inhibit maturation of the autophagosome, while VCP mutations inhibit TDP-43 degradation by the autophagy and proteasomal pathways. The common pathology and clinical phenotype of ALS and FTLD-U clearly implicates TDP-43 misaccumulation in their pathogenesis.

they identified 75 coding SNPs and 13 insertions and deletions that were shared by both affected individuals. Sanger sequencing these variants in an additional affected family member and comparing them to 200 controls narrowed the search down to just four variants within regions of genomic linkage. The most obvious candidate was the heterozygous c.961G>A nucleotide change, predicted to substitute an arginine for glutamine at position 191 (R191Q) in the valosin-containing protein.

The authors proceeded to sequence all exons of *VCP* in 210 index FALS and 78 pathologically proven cases detecting four more cases with *VCP* mutations (another R191Q, R159G, D592N, and R155H) that were not present in 1589 controls. All of the mutations were detected in familial cases and none in the 73 sporadic cases sequenced. One ALS patient with a R155H mutation had TDP-43 and ubiquitin-positive NCIs, confirming the pathological diagnosis of ALS. They conclude that *VCP* mutations cause the mislocalization of TDP-43 within motor neurons in the spinal cord that results in neurodegeneration.

Muscle wasting due to IBM can be mistaken for ALS. Electromyography in IBM cases can suggest denervation, so is this a case of mistaken identity? Several of the authors are highly experienced neurologists who elicited signs of upper and lower motor neuron degeneration. The rapidly progressive disease course leading to respiratory failure is not seen with IBM. Following up the Italian R191Q family, it was discovered that one parent of the index case did have IBM, Paget's, and FTLD. One ALS patient from the R159H kindred also had FTD and another had Paget's, which might have raised suspicions about *VCP*. Finally the R155H mutation was detected in a known IBMPFD kindred. Four out of the five kindreds, however, had individuals with a pure ALS phenotype, which suggests that features of IBM, FTD, or Paget's (and serum Alkaline Phosphatase measurements) should be sought in all patients and their relatives.

The question remains as to whether TDP-43 mislocalization and aggregation is mechanistic or just a bystander phenomenon? TDP-43 is predominantly nuclear in healthy myocytes; however, large TDP-43 and ubiquitin immunoreac-

tive cytoplasmic inclusions were abundant in all five muscle biopsies of *VCP* mutation-positive IBMPFD cases and in 21/27 sporadic IBM cases (Weihl et al., 2008). Western blots of muscle tissue revealed much higher levels of TDP-43 deposition and a higher-molecular-weight band (as seen in FTLD) that was absent from controls.

Striking cytoplasmic mislocalization and aggregation of TDP-43 was also described in the frontal and temporal lobes of patients with *VCP* mutations and IBMPFD (Neumann et al., 2007). Detergent-resistant phosphorylated and ubiquitinated forms of TDP-43 were identified on western blot, characteristic of other FTLD-U cases, but the 25 kDa C-terminal fragment was much less evident. Intriguingly, TDP-43 and ubiquitin-positive lentiform (lens shaped) intranuclear inclusions were identified that are very similar to those described in patients with mutations in PGRN that are also known to cause FTLD-U (Mackenzie, 2007). Lastly, mice overexpressing two different human VCP mutations developed progressive muscle weakness and pathology typical of IBM (Custer et al., 2010). They also developed behavioral abnormalities and brain pathology similar to FTLD and multiple bony lesions similar to Paget's disease. Striking evidence of cytoplasmic TDP-43 aggregation was evident in myocytes and nuclear clearing in neurons in the frontal cortex and motor neurons in the spinal cord. In all of these studies, there is no evidence that VCP itself accumulates or is directly toxic. Instead the mutations appear to cause a dominant-negative effect on the wild-type protein.

VCP is a highly conserved member of the AAA+ ATPase family as knockout is lethal from yeast to mice (reviewed in Ju and Weihl, 2010). It is highly expressed in all tissues where it has a variety of cellular functions including protein homeostasis through the ubiquitin proteasome system (UPS) and autophagy pathways. Previously, it was thought that these two pathways worked independently but VCP appears to be integral to both. Overexpression of IBMPFD-related *VCP* mutants leads to TDP-43 mislocalization from the nucleus to the cytoplasm of transfected cells and transgenic mice (Ju et al., 2009). Similar events are observed when

autophagosome maturation is inhibited by the chronic administration of bafilomycin A or chloroquine in cells and in mice. This led the authors to conclude that the pathology observed in IBMPFD is due to disruption of autophagy and that TDP-43 is a major substrate. One of the markers of proteins targeted for degradation by the autophagy pathway is p62, and it is interesting to note that p62-positive inclusions are more abundant than ubiquitinated inclusions in ALS. So is TDP-43 merely a sticky protein that fails to be degraded by the UPS and autophagy pathways, or might it be mechanistic in initiating neurodegeneration?

More than 38 mutations have been identified in *TARDBP* in FALS and SALS cases (Lagier-Tourenne et al., 2010). Overexpression of wild-type TDP-43 and many mutants or knockdown of endogenous protein leads to neurodegeneration. The fact that TDP-43 mislocalization of TDP-43 is seen following *VCP* and *PGRN* mutations adds further evidence that TDP-43 is mechanistic in ALS and FTLD (see Figure 1). In addition to *FUS*, motor neurons appear to be particularly susceptible to defects in other RNA-processing genes as evidenced by several autosomal-recessive syndromes (reviewed in van Blitterswijk and Landers, 2010). Homozygous deletions in *SMN1*, encoding a protein involved in RNA splicing and trafficking, cause spinal muscular atrophy (SMA), while point mutations in *GLE1*, essential for the nuclear export of mRNA, cause a fetal motor neuronopathy called lethal congenital contracture syndrome (Nousiainen et al., 2008). Muta-

tions in *IGHMBP2*, which is involved in translation, cause SMA and respiratory distress (SMARD1). Furthermore, variants in *ELP3*, which is involved in RNA transcription and processing, and *ANG*, which can act as a tRNA RNase, have been associated with susceptibility to sporadic ALS.

The frequency of *VCP* mutations in the study by Johnson et al. (2010) is around 2% of FALS, which is similar to that seen for *TARDBP* and *FUS*. The number of patients with mutations is still small, and screening should be undertaken in larger FALS and SALS cohorts in order to replicate this finding and establish the frequency in different populations. The clinical message is that features of IBMPFD should be sought in familial ALS patients and *VCP* screening conducted whether or not serum alkaline phosphatase is raised. A detailed comparative exploration of TDP-43 pathology and the role of autophagy in cellular and animal models exploiting *TARDBP*, *VCP*, and *PGRN* mutations may help identify a common pathway linking neurodegeneration in ALS and FTLD.

REFERENCES

Custer, S.K., Neumann, M., Lu, H., Wright, A.C., and Taylor, J.P. (2010). Hum. Mol. Genet. *19*, 1741–1755.

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., et al. (2010). Neuron *68*, this issue, 857–864.

Ju, S.J., and Weihl, C.C. (2010). Hum. Mol. Genet. *19*, R38–R45.

Ju, J.-S., Fuentealba, R.A., Miller, S.E., Jackson, E., Piwnica-Worms, D., Baloh, R.H., and Weihl, C.C. (2009). J. Cell Biol. *187*, 875–888.

Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Munsat, T., et al. (2009). Science *323*, 1205–1208.

Lagier-Tourenne, C., Polymenidou, M., and Cleveland, D.W. (2010). Hum. Mol. Genet. *19*, R46–R64.

Leigh, P.N., and Wijesekera, L.C. (2010). Nat. Rev. Neurol. *6*, 191–192.

Mackenzie, I.R. (2007). Acta Neuropathol. *114*, 49–54.

Mackenzie, I.R., Rademakers, R., and Neumann, M. (2010). Lancet Neurol. *9*, 995–1007.

Neumann, M., Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., et al. (2006). Science *314*, 130–133.

Neumann, M., Mackenzie, I.R., Cairns, N.J., Boyer, P.J., Markesbery, W.R., Smith, C.D., Taylor, J.P., Kretzschmar, H.A., Kimonis, V.E., and Forman, M.S. (2007). J. Neuropathol. Exp. Neurol. *66*, 152–157.

Nousiainen, H.O., Kestilä, M., Pakkasjärvi, N., Honkala, H., Kuure, S., Tallila, J., Vuopala, K., Ignatius, J., Herva, R., and Peltonen, L. (2008). Nat. Genet. *40*, 155–157.

Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J.C., Williams, K.L., Buratti, E., et al. (2008). Science *319*, 1668–1672.

van Blitterswijk, M., and Landers, J.E. (2010). Neurogenetics *11*, 275–290.

Vance, C., Rogelj, B., Hortobágyi, T., De Vos, K.J., Nishimura, A.L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P., et al. (2009). Science *323*, 1208–1211.

Weihl, C.C., Temiz, P., Miller, S.E., Watts, G., Smith, C., Forman, M., Hanson, P.I., Mimonis, V., and Pestronk, A. (2008). J. Neurol. Neurosurg. Psychiatry *79*, 1186–1189.