COMMENTARY

Aberrant stress granule network is involved in CMT2 neuropathies

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Charcot-Marie-Tooth (CMT) diseases are the most common inherited peripheral neuropathies with a population prevalence of about 1:2500.[1] CMT diseases are usually characterized with distal weakness, sensory loss and foot deformity, and the symptoms progress slowly, which bring the patients lots of sufferings. CMT patients are classified into different groups, including CMT type 1 (CMT1; demyelinating form), CMT type 2 (CMT2; axonal form) and intermediate CMT form.[2] And the diversity of CMT causative genes leads to the further CMT subtype classification. According to reports, over 30 out of over 100 pathogenic genes that cause CMT diseases are associated with CMT2. Although similar clinical presentation among different CMT2 subtypes, their causal proteins are highly varied in their cellular localizations and functions. So, whether there exists a molecular link among various CMT2-causal mutant proteins?

As is known, environmental factors are also the important reasons affecting the pathogenesis of peripheral neuropathies except genetic factors. Some translationally silent ribonucleoproteins will be aggregated upon stress in stress granules (SGs), a membrane-less organelle assembling by liquid-liquid phase separation (LLPS),[3] mediating cell response to the stress and protecting the cell from further damage. The core SG network consists of about 36 proteins with RasGAP SH3 domain specific binding protein (G3BP) as the central node. Evidences showed that pathogenic proteins form protein aggregates in SGs, disturbing the SG dynamic and driving the pathogenesis of neurodegenerative disease. However, pathogenic proteins of CMT2 do not form pathological protein aggregates in cells generally. And it's unknown whether the SG network is abnormal and participates in the pathogenesis of CMT2 neuropathies in response to environmental stress.

Recently, Cui and colleagues revealed an aberrant SG network as a common mechanism for different CMT2 subtype. Interestingly, they found that diverse CMT2 neuropathy-causing mutant proteins have different cell localization in normal physiological conditions but converged in SGs, where they aberrantly interacted with G3BP and perturbed the SG network upon environmental stress, thus disrupting the normal stress response of peripheral nerves to adverse stimuli, leading to CMT2 peripheral neuropathy. This study highlighted an abnormal SG-mediated molecular link among diverse

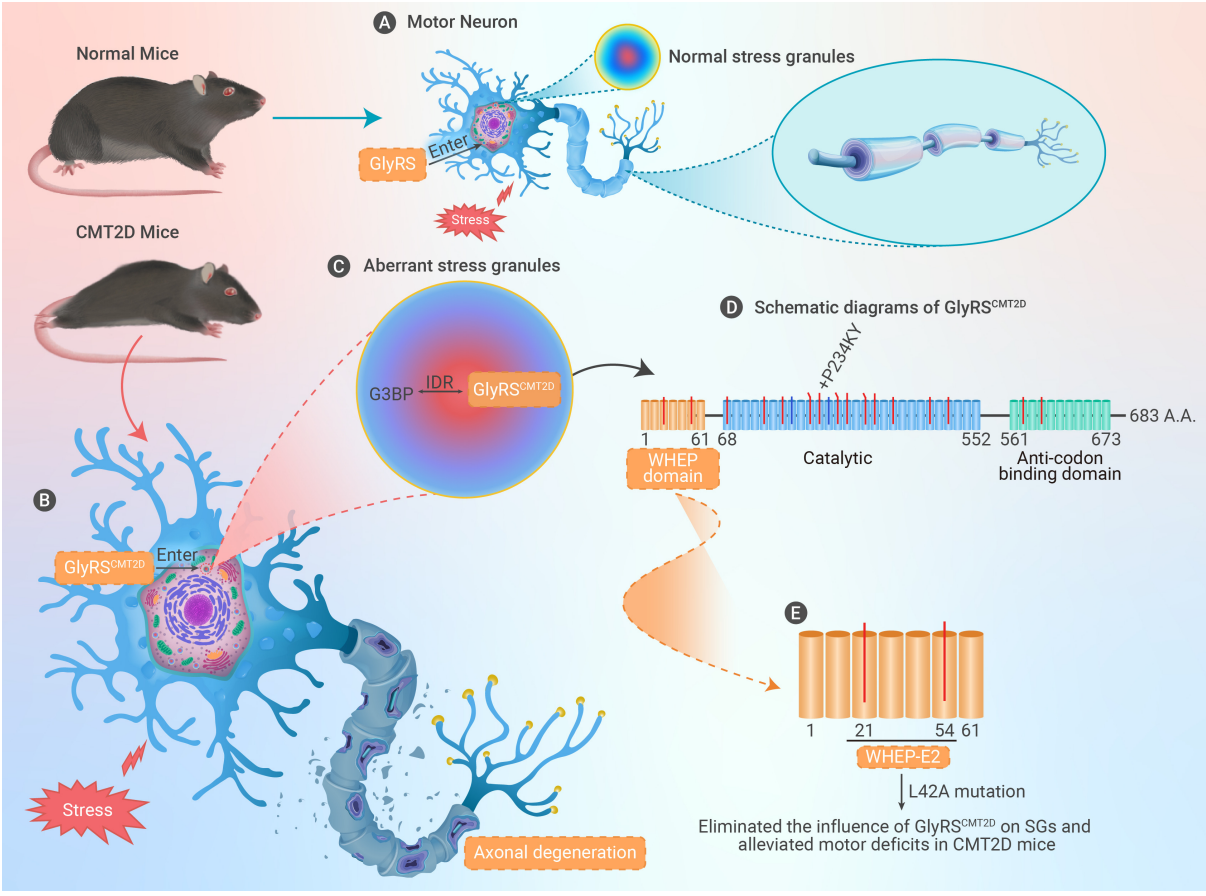

Figure 1. Aberrant stress granule network is involved in CMT2 neuropathies and aggravates the stress vulnerability of mouse motor function Under normal conditions, GlyRS and GlyRS^{CMT2D} are evenly distributed in the cytoplasm, whereas, under environmental stress, both of them translocate to SGs (A and B) and GlyRS^{CMT2D} binds abnormally to the central protein G3BP of the SG network with it's intrinsically disordered region (C), affecting the normal function of SG and thus leading to motor neuron axonal degeneration (B). GlyRS contains three domains: catalytic domain, anti-codon binding domain and WHEP domain (D). The WHEP domain with intrinsically disordered region is required for GlyRS^{CMT2D} to bind to G3BP. L42 mutation of WHEP can eliminate the effect of GlyRS^{CMT2D} on SGs, thus alleviate motor defects in CMT2D mice (E).

CMT2 mutants and the motor deficits of CMT2 mice can be alleviated by reducing such aberrant SG network.[4]

At the beginning of this research, researchers established GlyRS^{WT}-V5 (WT GlyRS (glycyl-tRNA synthetase encoded by *Gars*)) or GlyRS^{CMT2D}-V5 (CMT2D-associated GlyRS mutants, P234KY, L129P, or G240R)-expressing Hela cell and spinal motoneurons (MNs) of chick embryos, and found that both GlyRS^{WT} and GlyRS^{CMT2D} distributed evenly in cytoplasm (Figure 1A) but translocated into SGs upon stress treatment (Figure 1B). In addition, the endogenous GlyRS^{WT}-V5 or GlyRS^{P234KY}-V5 proteins were also found to co-localized with G3BP1-positive SGs in MNs after stressor AS ($NaAsO_2$) treatment, which was consistent with the phenotype described above, ruling out possible artifact of GlyRS overexpression.

Given that the translocation of GlyRS-to-SGs upon stress, researchers started to explore whether mutant GlyRS^{CMT2D} proteins interworked with SG proteins upon stress. TurboID-mediated proximity labeling assay showed that GlyRS^{CMT2D} exhibited aberrantly increased interaction with G3BP1 compared to GlyRS^{WT} upon AS treatment in HeLa cells. By mimicking the LLPS microenvironment of SGs[5] and then using *in vitro* pull-down assay, researchers found that such aberrant interaction was direct and also happened in CMT2D mice. However, the assembly and disassembly of SGs and the motility of G3BP1 were unaffected, but the G3BP-centric core SG network might be affected with GlyRS^{CMT2D}. By combining G3BP1-TurboID-based proximity labeling, quantitative MS and coIP, Cui and colleagues demonstrated an acutely enhanced SG network within GlyRS^{CMT2D} as SG core proteins were excessively interacted with G3BP1. What's more, increased molecular density of G3BP1, denser SGs accompanied with the expansion of SG core-foci, and stronger co-phase separation behavior between G3BP1 and GlyRS^{CMT2D} all suggested that GlyRS^{CMT2D} aberrantly bound to G3BP and perturbed the core SG network.

So how such changes in SG influence the function of SG and whether it is involved in CMT2 motor phenotype? Researchers first discovered that excessive accumulation of proteins involved in promoting cell survival and maintaining cell viability were sequestrated in GlyRS^{CMT2D} SGs, such as CORO1C, which has been found to be associated with resistance to MN degeneration. Then, they found that upon stress, GlyRS^{CMT2D}-expressing chicken MNs and primary MNs of CMT2D mice exhibited severe neurite dying-back compared to GlyRS^{WT} MNs, indicating their deficient stress response. Subsequently, high-intensity exercise (HIE, oxidative stress induced by hypoxia) was used and found that HIE significantly increased GlyRS^{CMT2D}-G3BP1 interaction. And not surprisingly, the motor function of CMT2D mice after HIE was severely impaired accompanied with notably loss of nerve terminals at neuromuscular junctions, which behaved worse than non-HIE-experienced CMT2D littermates. In addition, the accelerated motor deficits were due to SG-associated-stress but not non-SG-induced-stress. These findings indicated that CMT2D mice exhibited increased vulnerability to SG-related stress and thus promoting disease progression.

But how did the abnormal GlyRS^{CMT2D}-G3BP binding result in the increased stress vulnerability. By predictor of natural disordered regions (PONDR), researchers found that WHEP-E2 (Exon 2 in the *Gars* gene) showed an inherently disordered region (IDR) (Figure 1C), which was often related to LLPS-associated multivalent interactions. GFP-G3BP-TurboID proximity labeling experiment showed that ΔE2-coding sequence significantly reduced the aberrant GlyRS^{P234KY}-G3BP binding. And as expected, GlyRS$^{\Delta E2\text{-}P234KY}$ significantly reduced the effect of GlyRS^{CMT2D} on SGs, as well as relieved the motor neurite dying-back compared to GlyRS^{P234KY}. By individually mutating 9 highly conserved residues in the WHEP-E2 domain in GlyRS^{P234KY}, they further found that only L42A mutation could significantly reduce the aberrant GlyRS^{P234KY}-G3BP1 binding (Figure 1E). Besides, in *in vivo* assay, L42A-*Gars*^{P234KY} mice showed that L42A indeed significantly decreased the aberrant GlyRS^{P234KY}-G3BP interaction and failed to worsen the motor deficits under HIE stress and CMT-like pathology, suggesting that WHEP-E2 was responsible for the susceptibility to environmental stress and motor functions in CMT2D mice.

But whether the aberrant SG network involved in CMT2D was applied to other CMT2 subtypes? Researchers found that 14 out of the examined 25 CMT2-associated proteins originally located in different subcellular locations were translocated to SGs in HeLa cells when treated with AS. Same results were also found in primary cultured MEF cells isolated from CMT2P (*Lrsam1*^{C698Y}) mice. Furthermore, G3BP1-TurboID-based proximity labeling assay showed that 12 out of 14 CMT2-related proteins showed aberrant interaction with G3BP1 and suggested an enhanced core SG network. And this was associated with more severe neurite death in chicken MNs upon AS treatment. Primary cultured MNs isolated from CMT2P mice and CMT2W (*Lrsam1*^{D364Y}) mice also behaved increased susceptibility to SG-related stress. All these above findings indicated that the aberrant binding of CMT2 mutant proteins in SGs led the dysfunction of SGs, which may be a common mechanism of CMT2 diseases.

In summary, Cui and colleagues reported a stress-dependent molecular link shared with various CMT2 subtypes,[4] they found that CMT-causal proteins translocated from physiologically cytoplasmic localization to SGs upon stress, interacted abnormally with G3BP to damage the G3BP-centric core SG network, resulting in SG functional defect to environmental stimuli in MNs, thus motor deficiency of CMT2 neuropathies. However, there are still many unanswered issues. For instance, if SGs serve as the target for CMT treatment, when and how to interfere its aberrant core network is suitable? Then, the aberrant SG network caused by CMT2-causal proteins under stress was focused in this study, but is it stress-dependent? If without stress, what's the contribution of these CMT2 proteins in the progress of CMT2 neuropathies? In other words, the stressors may have unknown and additional effects for cells or animals in this study. Next, in CMT2D model, researchers found that WHEP-E2 mutation of GlyRS was the key for the aberrant GlyRS^{CMT2D}-G3BP interaction, but whether WHEP mutation also interferes the formation of normal SGs and thereby causing damage to cells by stress is not explored. If so, this will be a major obstacle to drug development aiming at WHEP-E2 or SGs. In addition, most CMT2-causing proteins detected in this study (14 out 25) were found to enter SGs under stress, there are still some proteins did not. Considering WHEP was identified for aberrant SG core network because it was first analyzed as an IDR, and researchers also mentioned that IDR-like sequence is almost in all CMT2 proteins in the discussion part, it seems that the IDR-containing CMT2-causal proteins is not a premise for involving in the formation of aberrant SG core network and the SG-unentered CMT2 proteins seem to have other effects on CMT2 under stress, which we think is a point to be explored too, like neurotransmission defects may also contribute to CMT disease. In addition, CMT are classified into different types and then various subtype.[2] In that, except for the CMT2 explored in this study, whether this common stress-dependent molecular link also applied to other subtypes for CMT1 and intermediate CMT is also an important issue needs comprehensively further exploration, as well as CMT development mechanism and CMT treatment direction.

Still, in the long run, this breakthrough with revelation of aberrant SG network that is the common pathogenic mechanism mediating various CMT subtypes will provide a theoretical basis for the development of broad-spectrum therapeutic drugs for multi-subtypes of CMT and provides new ideas for the mechanism study of genetic heterogeneity in other diseases.

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DECLARATION OF INTERESTS

The authors declare no competing interests.