Symposium 1: Amyotrophic Lateral Sclerosis (Wed July 28, 9-11AM JST)
Chair: Seung Hyun Kim (Hanyang University Hosp, Seoul, Korea)

9:00-9:40 Seung Hyun Kim (Hanyang University Hosp, Seoul, Korea)
WES based identification of novel pathogenic mutations and their roles in neuronal cell death mechanisms in ALS and FTD-ALS spectral diseases

9:40-10:20 Shinsuke Ishigaki/Gen Sobue (Nagoya Univ Sch of Med, Nagoya, Japan)
Aberrant FUS-SFPQ interactions in the neuronal nuclei in ALS and FTLD spectrum diseases, a possible pathogenesis

10:20-11:00 Shuo-Chien Ling (Natl Univ Singapore, Singapore)
TDP-43 mediates SREBF2-regulated gene expression required for oligodendrocyte myelination

Symposium 1 Speaker 1:
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WES based identification of novel pathogenic mutations and their roles in neuronal cell death mechanisms in ALS and FTD-ALS spectral diseases

The recent identification of novel mutant genes in ALS and the emerging concept of multisystem proteinopathies have changed the previous concept that ALS affects only the motor neuron system. Heterogeneic presentations in ALS may relate to underlying genetic susceptibilities, including de novo mutations or oligogenic or polygenic genetic roles in unresolved sporadic ALS.

After introducing the ALS genetic characteristics of Korean and Asian populations, recently identified novel pathogenic variants found in Whole Exome Sequencing and TRIO [case-unaffected parents] and their pathogenic role will be presented.

In WES analysis of 500 Korean cases with sporadic ALS, nine *Annexin A11 (ANXA11)* variants in 13 patients were identified, and pathogenic mechanisms of dysfunctional ANXA11 on motoneuron(MN) cell death were investigated. Mutations in the *ANXA11* gene contribute to MN degeneration by disrupting cellular calcium ion homeostasis and impaired SG assembly/disassembly dynamics. Among these mutations, two amino-terminal variants in the low-complexity domain of *ANXA11* were shown to enhance SG aggregation propensity. In contrast, two carboxyl-terminal ANX domain variants altered calcium ion responses. Moreover, all four *ANXA11* variants were seen to undergo abnormal liquid-liquid phase separation to form droplets with aggregates, which led to the alteration of the biophysical properties of ANXA11. We found that ANXA11 can be considered one of the RNA binding protein families by demonstrating colocalized with FUS and hnRNP protein in aggregated SG of brain tissue. In 30 TRIO studies of the Korean ALS population, candidates of de novo variants (*FUS, CLEC4C, ATP1A3, RabGef26,* etc.) and functional study data related to cell death mechanisms using iNeuron models and animal models will be discussed.

Identification of novel genes, gene modifiers, and the pathogenic mechanisms caused by the aberrant genes has expanded our understanding of ALS. With networks of genetic-clinical dB and Biobank, personalized/stratified prospective therapeutic strategies based on new ALS genes can help us conquer the intractable disease, ALS.

Symposium 1 Speaker 2:
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Aberrant FUS-SFPQ interactions in the neuronal nuclei in ALS and FTLD spectrum diseases, a possible pathogenesis

Fused in sarcoma (FUS) is genetically and clinicopathologically linked to frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). We have previously reported that intranuclear interactions of FUS and Splicing factor, proline- and glutamine-rich (SFPQ) contribute to neuronal homeostasis. Using a mouse model, we reported that FUS regulates alternative splicing of tau proteins in coordination with SFPQ. Under physiological conditions, the two proteins form a high-molecular-weight complex in the nucleus. Disease-associated mutations in FUS gene, however, disrupt formation of the complex resulting in unregulated alternative splicing of tau, a disproportional increase in the 4R-tau/3R-tau ratio, and eventually neurodegeneration. Disruption of the FUS-SFPQ interaction leads to an increase in the ratio of 4-repeat tau (4R-tau)/3-repeat tau (3R-tau), which manifests in FTLD-like phenotypes in mice. Here, we examined FUS-SFPQ interactions in 142 autopsied individuals with FUS-related ALS/FTLD (ALS/FTLD-FUS), TDP-43-related ALS/FTLD (ALS/FTLD-TDP), progressive supranuclear palsy (PSP), cortico-basal degeneration (CBD), Alzheimer disease (AD), or Pick disease (PiD) as well as controls. Immunofluorescent imaging showed impaired intranuclear colocalization of FUS and SFPQ in neurons of ALS/FTLD-FUS, ALS/FTLD-TDP, PSP, and CBD cases, but not in AD and PiD cases. Immunoprecipitation analyses of FUS and SFPQ revealed reduced interactions between the two proteins in ALS/FTLD-TDP and PSP cases, but not in those with AD. Furthermore, the ratio of 4R/3R-tau was elevated in cases with ALS/FTLD-TDP and PSP, but was largely unaffected in cases with AD. Thus, impaired interactions between intranuclear FUS and SFPQ and the subsequent increase in the ratio of 4R/3R-tau constitute a common pathogenesis pathway in FTLD spectrum diseases.

Symposium 1 Speaker 3:

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TDP-43 mediates SREBF2-regulated gene expression required for oligodendrocyte myelination

Cholesterol metabolism operates autonomously within the central nervous system (CNS), where the majority of cholesterol resides in myelin. We demonstrate that TDP-43, the pathological signature protein for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), regulates cholesterol metabolism in oligodendrocytes. TDP-43 binds directly to mRNA of SREBF2, the master transcription regulator for cholesterol metabolism, and multiple mRNAs encoding proteins responsible for cholesterol biosynthesis and uptake, including *HMGCR*, *HMGCS1*, and *LDLR*. TDP-43 depletion leads to reduced SREBF2 and LDLR expression, and cholesterol levels *in vitro* and *in vivo*. TDP-43-mediated cholesterol reduction can be restored by reintroducing SREBF2 or LDLR. Additionally, cholesterol supplementation rescues demyelination caused by TDP-43 deletion. Furthermore, oligodendrocytes harboring TDP-43 pathology from FTD patients show reduced HMGCR and HMGCS1, and co-aggregation of LDLR and TDP-43. Collectively, our results indicate that TDP-43 is required for cholesterol homeostasis in oligodendrocytes, and cholesterol dysmetabolism may be implicated in TDP-43 proteinopathies-related diseases.