

## Computational and functional analyses of angiogenin mutations identified in Hungarian patients with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of motor neurons leading to paralysis and death 3–5 years after disease onset. Mutations in the *angiogenin* (*ANG*) gene are known to be associated with both familial and sporadic amyotrophic lateral sclerosis (ALS). The angiogenin protein belongs to the pancreatic ribonuclease superfamily, and it plays an important role in rRNA biogenesis and cellular proliferation in addition to its crucial role in inhibiting protein translation by cleaving tRNA. The majority of disease-causing mutations of *ANG* result in loss of either ribonucleolytic activity, nuclear translocation activity or both. We sequenced *ANG* gene from a total of 136 sporadic ALS patients and 112 healthy controls of Hungarian origin. Mutation screening revealed a mutation located in the signal peptide (M-24I) and two mutations that affect the mature protein (R33W, V103I). The R33W mutation, which has not been previously detected in ALS patients, affects the key amino acid of the nuclear translocation signal of the angiogenin protein. To elucidate the role of the R33W mutation in the disease mechanism, computational and functional analyses were performed. Molecular dynamics simulation of the R33W protein was carried out using AMBER 14 software to model structural and dynamics changes in the functional sites compared to the wild-type protein. Ribonucleolytic assay and nuclear translocation assay of the R33W angiogenin protein were performed to investigate the enzymatic and nuclear translocational activity of the mutant protein. Molecular dynamics simulations suggested that the R33W mutation results in partial loss of ribonucleolytic activity and reduced nuclear translocation activity. The functional assays of the R33W angiogenin protein

confirmed the molecular dynamics results. In the Hungarian ALS population, the observed frequency of *ANG* mutations was 2.9%, which is higher than previously reported for sporadic cohorts. Our findings confirm the relevance of *ANG* mutations in ALS and points out the variability among populations of different ethnic origin.