THE NEW ROLE OF CONNECTIVE PEPTIDE 1 IN HUMAN TYROSYL-tRNA SYNTHETASE AND ITS MUTANT FORMS, ASSOCIATED WITH DI-CMTC NEUROPATHY STUDIED BY MOLECULAR MODELING AND MD SIMULATIONS TECHNIQUES

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Background. Aminoacyl-tRNA synthetases are key enzymes of protein biosynthesis which are also implicated in other cellular processes. The application of novel methods in molecular genetics of hereditary diseases revealed unexpected connections between some neurodegenerative diseases and mutations in aminoacyl-tRNA synthetases. **Charcot-Marie-Tooth disease** (**CMT**), known also as Hereditary Sensorimotor Neuropathy (HSMN), is a group of heterogeneous inherited disorders, that is characterized by degeneration of peripheral nerve fibers, loss of muscle tissue and touch sensation. *Homo sapiens* TyrRS (*Hs*TyrRS) is α_2-dimer (2x59 kDa), that catalyze the aminoacylation of cognate tRNA by L-tyrosine. The enzyme consists of catalytic N-terminal module (mini-TyrRS) and non-catalytic C-terminal module; for both isolated modules were revealed cytokine-like activities.

Two heterozygous missense mutations (G41R, E196K) and one *de novo* deletion (153-156delVKQV) in *Hs*TyrRS were identified in different families of patients with DI-CMTC. There are several possible mechanisms: loss of charging function, aggregation, mischarging, nucleolar dysfunction, dimerization, non-canonical functions, novel interactions resulting from mutations, mitochondrial toxicity or dysfunction, impaired axonal transport that leads to deficits in local translation (Motley, 2009). G41R and 153-156delVKQV mutations could block L-tyrosine binding (decrease rate of catalytic activity >100-fold) (Froelich, 2011). The common mechanism is still unknown. An attractive hypothesis is that mutant tRNA synthetases may hamper translation elongation, as an appropriate supply of charged aminoacyl-tRNAs to the ribosome is essential for efficient translation elongation. It is tempting to speculate that CMT-mutant tRNA synthetases may cause defects downstream of tRNA aminoacylation, such as defective transfer of aminoacylated tRNAs to eEF1A, the elongation factor that transports charged tRNAs to the ribosome (Niehues, 2015).

Methods. All three mutants of *Hs*TyrRS were constructed *in silico* using mutate scripts for Modeller 9.7 software. Molecular dynamics (MD) simulations were carried out for all 3 *Hs*TyrRS mutants for 100 ns each using GROMACS package with GROMOS 53A6 force field. All MD simulations and trajectories analysis were performed in Ukrainian National Grid (UNG) and European Grid Infrastructure (EGI) environment using the resources of MolDynGrid virtual laboratory (***http://moldyngrid.org***).

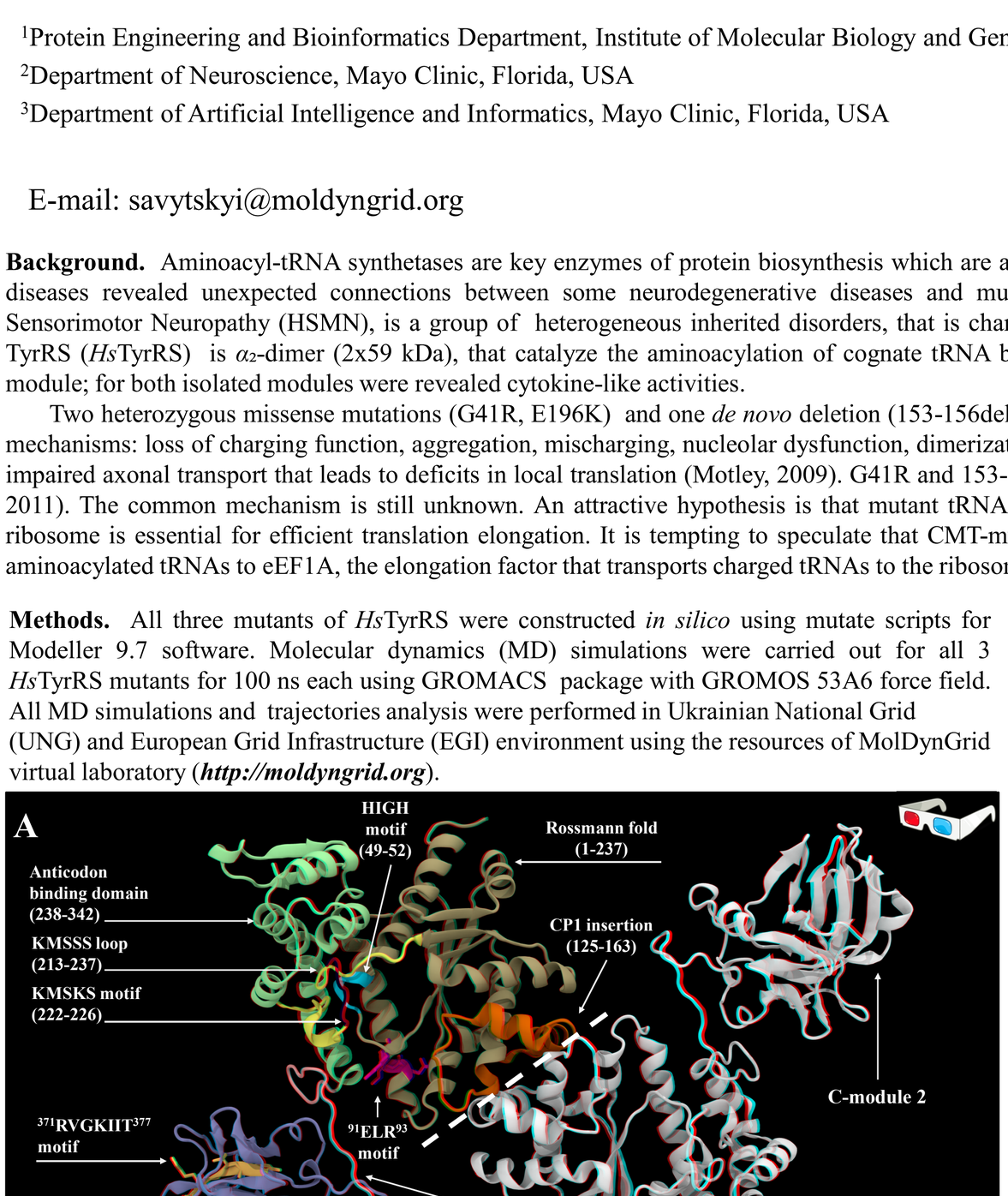

Figure 1. The structural elements of the modeled full-length *H.sapiens* TyrRS **(A)**. Locations of two point mutations (G41R, E196K) and deletion 153-156delVKQV in 3D structure of human mini-TyrRS dimer in modeled complex with $tRNA^{Tyr}$ and translation elongation factor eEF1A2 **(B)**.

Results. The 3D structure of G41R, E196K and 153-156delVKQV *Hs*TyrRS mutants are still unknown and its structural models with $tRNA^{Tyr}$ and translation elongation factor eEF1A2 were constructed *in silico* using Modeller v9.7 software (Figs. 1, 2).

Molecular dynamics of wild-type and G41R, 153-156delVKQV mutants were simulated for 100 ns each (Figs. 3-6). Obtained MD trajectories show stable behavior after approximately 40-60 ns.

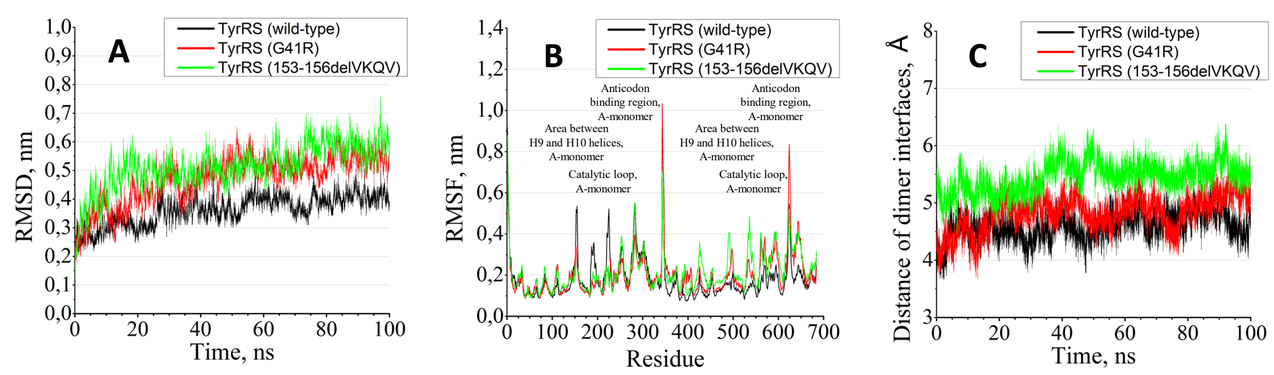

Figure 3. Mutant structure revealed less relaxed states with higher values of Root Mean Square Deviation (RMSD) **(A)**, Root Mean Square Fluctuations (RMSF) of the wild-type and mutants *Hs*TyrRS structures (for 60-100 ns**) (B)**, Distance of dimer interfaces in *H.sapiens* TyrRS **(C)**.

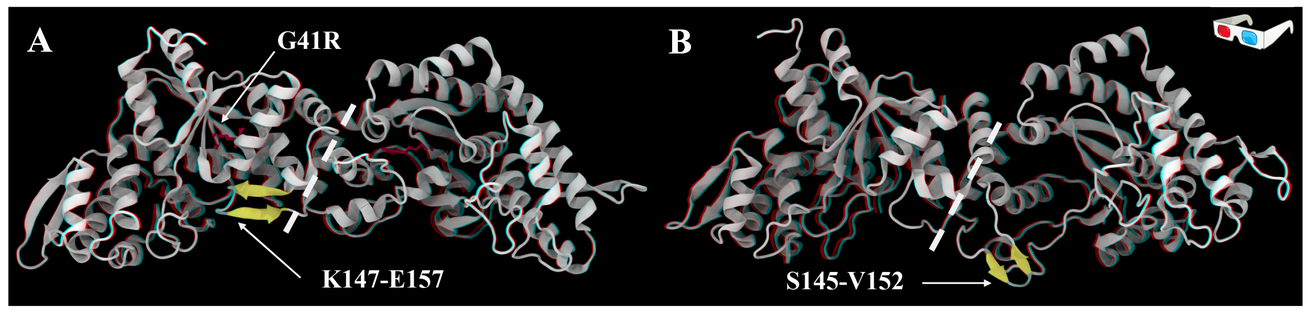

Figure 4. Local β-sheet formation in K147-E157 region in G41R (monomer A) **(A)** and in S145-V152 region in 153-156delVKQV (monomer B) **(B)** mutants of *Hs*TyrRS.

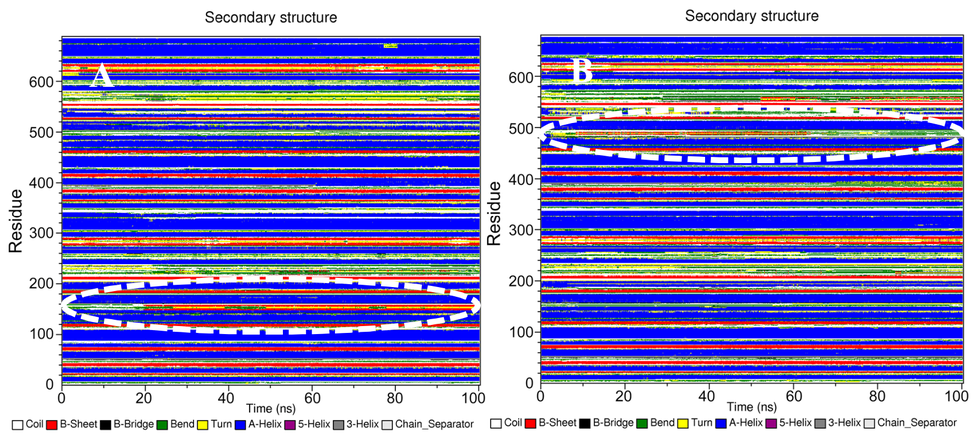

Figure 5. DSSP analysis of secondary structure changes during 100 ns for G41R **(A)** and 153-156delVKQV **(B)** mutants. Antiparallel β-sheet formation in K147-E157 (monomer A) region for 20-100 ns time interval (~80 % of time). Anti- parallel β-sheet formation in S145-V152 (monomer B) region for 5-65 ns time interval (~60 % of time).

Figure 6. MolDynGrid Virtual Laboratory (http://moldyngrid.org) is a part of Ukrainian National Grid-infrastructure (UNG) and EGI. It was established for interdisciplinary research in computational structural biology. MolDynGrid usually utilizes computing elements (CE) of 9 clusters and storage elements (SE) of 2 clusters that correspond to ~2500 CPUs and ~100 TBytes of disk space, respectively.

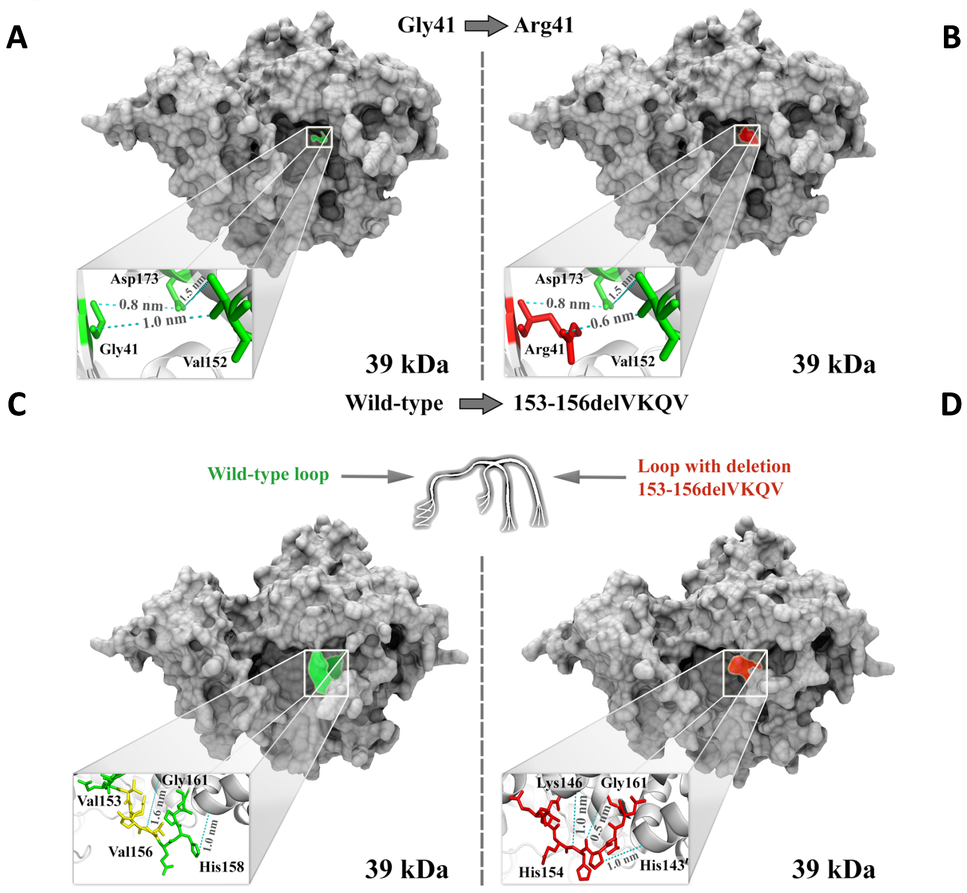

Figure 2. Computational mutagenesis of G41R mutant **(A, B)** and 153-156delVKQV mutant **(C, D)** of *Homo sapiens* TyrRS. Localization of G41R mutation in catalytic domain, β-strand 2 (Lys37-Thr42). Localization 153-156delVKQV mutation between H9 and H10 alpha helices.

Conclusions. The G41R and 153-156delVKQV mutants of *Hs*TyrRS revealed significant changes of their conformational dynamics. In general, mutants protein revealed less relaxed states with higher values of root-mean square fluctuations. Analysis of spatial contacts between 2 monomers in enzyme revealed the expanse dimer interface in *Hs*TyrRS. The melting of H9 helix (T141-A148) and subsequent partial melting of H11 helix were observed in 153-156delVKQV mutant of TyrRS. A novel β-sheet formation was observed in K147-E157 region (monomer A) in G41R mutant for 20-100 ns and in S145-V152 region (monomer B) for 5-65 ns time interval of MD simulation of Rossmann fold in CP1 region. Calculation of hydrogen bonds for region K147-E157 (WT) with $tRNA^{Tyr}$ shows (lifetime >10%): E151:C75 – 39%, Q155:A76 – 13%, K147:G72 – 13%, K147:G71 – 10%. Moreover, the interaction between K154 and gamma-phosphate of ATP (77.8% hydrogen bond lifetime) suggests its new important role in tyrosylation reaction step. Our results support the idea, that defects between intermolecular interfaces between mutant forms of TyrRS with $tRNA^{Tyr}$ and/or eEF1A2 may be common mechanism of disorder for mentioned mutational forms.

Acknowledgments.
This work was partly supported by NASU. O.S. was partly supported by travel grant from Mayo Clinic and ISCoC.