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A Molecular Dynamics Simulation study of the Arg206Cys variant in DNASE1L3 enzyme

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INTRODUCTION

- Studies have shown the association of the DNASE1L3 rs35677470 Caucasian-specific single nucleotide polymorphism (SNP) **[1]** with Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) and Systemic Sclerosis (SSc) **[2-5]** and structural investigation on the topic has been undertaken **[6]**.
- Deoxyribonuclease I-*like* 3 (DNASE1L3) is a member of the human DNase I family, representing a nuclease that cleaves double-stranded DNA during apoptosis **[7]**.

Purpose of the study

The present study aims to assess the potential role of the rs35677470 variant at the *DNASE1L3* gene and the resultant Arg206Cys substitution in the enzyme structure. To this end:

1. Structural differences after performing Molecular Dynamics simulations (MDs) on both wild type (wt) DNASE1L3 and Arg206Cys variant were traced.
2. The mechanism underlying the DNASE1L3 loss of activity was investigated.

EXPERIMENTAL DESIGN

Homology modeling of the DNASE1L3 protein based on the crystal structure of human Deoxyribonuclease-1 (PDB ID: 4awn, **[8]**) using the SWISS-MODEL server

→ Arg206Cys mutation using PyMOL → Molecular Dynamics simulations of the wt and mutated proteins using AMBER 12 **[9]**

→ Data analysis using Pymol, VMD, R & cpptraj module in AMBER → Result comparison between wt and mutated proteins

RESULTS

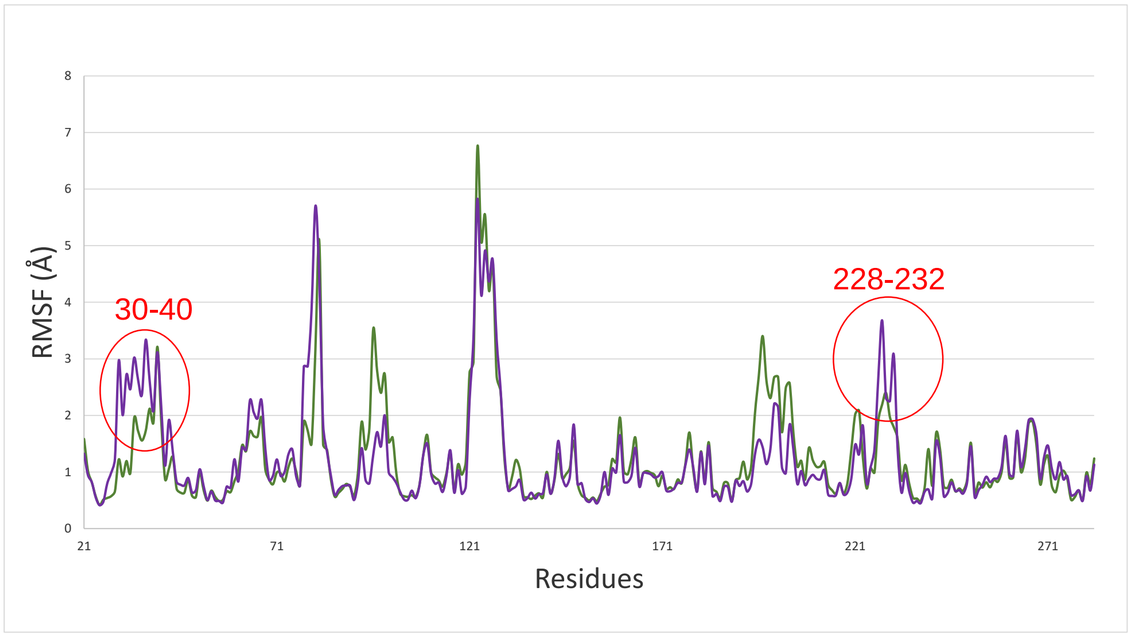

Figure 1. RMSF vs residue number plots reveal an augment fluctuation for residues 30-40 and 228-232 in the mutated protein (purple) in comparison to the wt (green).

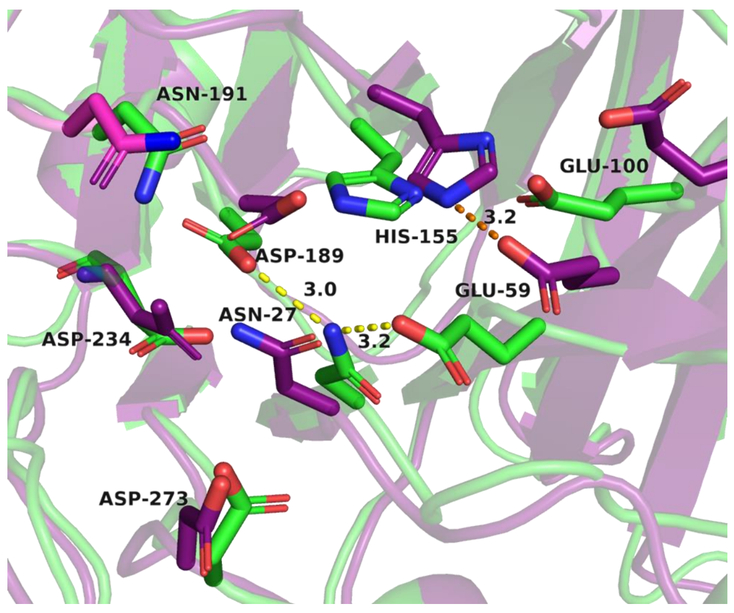

Table 1. Results are summarized.

Residue number	Changes in DNASE1L3 structure	
	Wild type Protein	Mutated Protein
30-40 & 228-232	Active site	Larger fluctuation
30-40	Nuclear localization signal	Larger fluctuation
95-99	Small DNA groove binding	Altered structure
193-196	DNA binding	Altered structure and less positive charge
27,59,100,155, 189,191,234,273	Active site	Altered structure and less positive charge
170-206	Salt bridge	Lost
208-219	Salt bridge	Less frequent

Figure 2. Superposition of the active sites of wt and mutated proteins. The wt is shown in green and the mutated in purple. Residues of the active site that move away from their initial positions in the mutated protein resulting in spatial changes of the site, are shown in stick representation and interactions are depicted as yellow and orange dashed lines for the wt and mutated structures, respectively. Figure was generated using PyMOL Molecular Graphics System, Version 1.8.0.5, Schrödinger, LLC, 2017.

CONCLUSIONS

- Large fluctuation of regions 30-40 and 228-232 of the active site.
- Structural changes in residues of the active site.
- Altered local charge distribution in regions interacting with DNA
- The Arg206Cys variant on the DNASE1L3 enzyme, seems to interfere with DNA binding through structural and local charge distribution changes on the enzyme's active site and DNA binding loops, leading to accumulation of uncleaved apoptotic DNA and symptoms of autoimmunity.
- MDs are an indispensable tool for structural studies resulting from mutations, shedding light on the underlying function mechanisms.

REFERENCES

[1] Ueki, *et al.*, *Clin. Chim. Acta* 407: 20–4 (2009). **[2]** Harley, *et al.*, *Nat Genet.* 40:204–10 (2008). **[3]** Gateva, *et al., Nat Genet.* 41:1228–33 (2009). **[4]** Westra, *et al.*, *Nat Genet.* 50:1366–74 (2018). **[5]** Zochling, *et al., Arthritis Res Ther.* 16:438 (2014). **[6]** Zervou, *et al.*, *Mol. Med. Rep.* 22: 4492–8 (2020). **[7]** Rodriguez, *et al., Genomics.* 42:507–13 (1997). **[8]** Parsiegla, *et al.*, *Biochemistry* 51: 10250–8 (2012). **[9]** Salomon-Ferrer *et al., WIREs Comput. Mol. Sci.* 3: 198-210 (2013).

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