Adverse structural and biochemical effects of Alport syndrome-related missense mutations on collagen IV

***Jingjie Yeo[1], Yimin Qiu[2], GangSeob Jung[3], Markus J. Buehler[3] and †David L. Kaplan[2]**

[1]Institute of High Performance Computing, A*STAR, Singapore.
[2]Department of Biomedical Engineering, Tufts University, USA.
[3]Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, USA.

*Presenting author: yeojj@ihpc.a-star.edu.sg
†Corresponding author: david.kaplan@tufts.edu

Abstract

Alport syndrome (AS) is a rare, debilitating genetic disorder where patients manifest symptoms of blood and high levels of protein in their urine, inflamed kidneys and progressive loss of kidney functions that lead to end-stage renal disease, loss of hearing, and visual abnormalities. Approximately 80% of Alport Syndrome cases are caused by mutations in the COL4A5 gene encoding the α5 chain of type IV collagen. Human type IV collagen constitutes a major component of extracellular scaffolds for the assembly and mechanical stability of certain types of tissues, especially that of glomerular basement membranes. Type IV collagen is also a vital component for interacting with cells, which is crucial for cell adhesion and differentiation. Even though the hierarchical structures of wild-type collagen IV were characterized extensively, the molecular reasons underlying how AS-related mutations perturb the structure and function of type IV collagen is not fully elucidated yet. We combined fully atomistic molecular dynamics (MD) simulations and recombinant collagen experiments to investigate the structural and biochemical effects of Gly missense mutation within and adjacent to the predicted integrin binding. The introduction of any reported Gly substitution in Alport syndrome (Gly→ Glu, Gly→Val and Gly→Asp) in the recombinant collagen greatly reduced their thermal stability in terms of their calorimetric enthalpies and melting temperatures. MD simulations also showed large decreases in both the tensile and rotational energies needed to unfold the mutant peptides. Replica exchange MD simulations of the bound structures of collagen with integrin also showed extensive structural kinking and unfolding of the mutant peptides near the sites of mutation. These kinks and misfolds likely led to increased protease susceptibility and impaired binding functions. Wild-type collagen peptides were resistant to trypsin and chymotrypsin digestion, whereas mutant peptides had markedly increased susceptibility. Molecular docking of trypsin to each collagen construct showed that the increased enzymatic susceptibility correlated with a large increase in the number of contacts between the enzyme and the mutated collagen peptides. The increased numbers of contacts might be due to enhanced accessibility of the degrading enzymes as they docked onto the structural distortions that were localized around the mutated regions which unwound the triple helices. Using integrin binding assays, the mutations also abolished collagen's integrin binding affinity, even if the mutation site was not located within the essential integrin binding site. MD sampling with adaptive biasing forces showed considerable reductions in integrin binding energies compared to the wild-type peptides. *In vitro* cell adhesion studies were consistent with the integrin binding assay. No cells adhered to the mutant peptides, thereby confirming the poor binding to integrin.

Keywords: Collagen IV missense mutations, Alport syndrome, integrin binding, molecular dynamics simulations, recombinant peptides