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Aggregation of M3 (E376D) variant of alpha1- antitrypsin

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Alpha1-antitrypsin (α1AT) is an abundant serine-protease inhibitor in circulation. It has an important role in neutralizing the neutrophil elastase activity. Different pathogenic point mutations like Z$^{(E342K)}$-α1AT have been implicated in the development of liver cirrhosis and Chronic Obstructive Pulmonary Disease (COPD), the latter being a cluster of progressive lung diseases including chronic bronchitis and emphysema. M3-α1AT (376Glu > Asp) is another variant of α1AT which so far is largely being considered as normal though increased frequency of the variant has been reported in many human diseases including COPD. We also observed increased frequency of M3-α1AT in COPD cases in Kashmiri population. The frequency of heterozygous (AC) genotype in cases and controls was 58.57% and 27.61% (odds-ratio 6.53 (2.27–15.21); $p < 0.0001$) respectively, while homozygous CC genotype was found to be 21.42% and 6.66% (odds-ratio 10.56 (3.63–18.64); $p < 0.0001$) respectively. Comparative *in vitro* investigations that include trypsin–antitrypsin assay, Circular Dichroism spectroscopy and dynamic light scattering performed on wild-type (M-α1AT), M3-α1AT, and Z-α1AT proteins along with the molecular dynamics simulations revealed that M3-α1AT has properties similar to Z-α1AT capable of forming aggregates of varied size. Our maiden observations suggest that M3-α1AT may contribute to the pathogenesis of COPD and other disorders by mechanisms that warrant further investigations.

Alpha1-antitrypsin (α1AT) is one of the most abundant circulating antiproteases. The serum levels of α1AT are raised secondary to activation of inflammatory-immune processes in humans[1]. α1AT is coded by a serine-protease inhibitor (*SERPIN*) A1. It is primarily expressed in hepatocytes and to some extent by lung tissue, macrophages, and monocytes. Among the different variants of α1AT, Z-α1AT (Glu342Lys) is the most pathogenic variant of α1AT and has been extensively studied. This variant has a distinctive capacity to form loop-sheet polymers due to the conformational change. The gain-of-toxic function of Z-α1AT in the hepatocytes leads to the manifestation of cirrhosis and hepatocellular carcinoma. On the contrary, reduced levels of serum α1AT lead to unregulated neutrophil elastase activity leading to the pathogenesis of a host of diseases including emphysema[1]. Owing to its pathogenicity, Z-α1AT is considered as a double-edged sword whose aggregation, on one hand leads to a pathological state of liver and loss-of-function on the other side results in emphysema[2]. The X-ray crystallography, *in silico* analysis, and kinetics of α1AT have provided valuable insights to understand its folding mechanism[3,4]. The conformational plasticity of serpins is not only important in terms of inhibitory activity but also unfolds a mechanistic understanding of their susceptibility towards misfolding and aggregation. During the folding process, α1AT is kinetically trapped in a metastable state. In this state, a patch of 15 amino acid residues (345–360) located near the C-terminus of α1AT, protruding out of its main body, is exposed to the polar solvent as a flexible loop connected between β-s5A and β-s1C that is called as reactive center loop (RCL). Native fold of α1AT is composed of three β-sheets (A–C) surrounded by 8–9 α-helices (hA–hI). The interaction of protease with the metastable α1AT gives rise to a marked conformational transition driven upon cleavage at P1′–P1 site in the RCL.

The 342 $Glu^{-} \rightarrow Lys^{+}$ substitution just above the top of s5A strand in Z-α1AT removes a salt bridge between Glu342 and Lys290, thereby driving an electrostatic repulsion between them. This promotes polymerization by delaying the already slow insertion of s5A that prolong the exposure of the C-terminal domain of Z-α1AT[4]. Z-α1AT is the commonest of all the deficient variant observed with serum levels 0.06–0.2 g/L among

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