Supporting Information

Aggregation of M3 (E376D) variant of alpha1 antitrypsin

Arif Bashir[1], Younis M. Hazari[1], Debnath Pal[3], Dibyajyoti Maity[3], Samirul Bashir[1], Laishram R. Singh[4], Naveed N. Shah[2], and Khalid M. Fazili[1*]

[1]Department of Biotechnology, University of Kashmir, Srinagar 190006, India,
[2]Department of Chest Medicine, Government Medical College, Srinagar 190006, India,
[3]Computational Data Sciences, Indian Institute of Science, Bengaluru 560012, India,
[4]Dr B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007, India

Running title: Aggregation of M3-α1AT

*To whom correspondence should be addressed

Khalid Majid Fazili
Professor
Department of Biotechnology
University of Kashmir-190006
fazili@kashmiruniversity.ac.in
bashir.aarif@gmail.com
Tel. (+91) 9419003881
Fax. (+91) 1942403610

Keywords: COPD: chronic obstructive pulmonary disease, α1AT: alpha1 antitrypsin, CD (circular dichroism) spectroscopy, molecular dynamics (MD) simulations, DLS: dynamic light scattering

Figure S1. Native-PAGE of all α1AT proteins showing aggregation behaviour over 7 days of time.

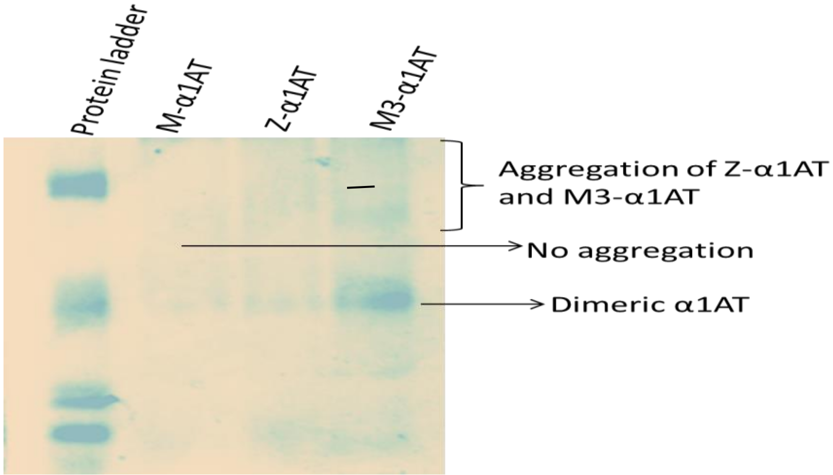

Figure S1

Figure S2. All α1AT proteins behave differently upon trypsin treatment.

(A-C). 12% SDS-PAGE of 0.54μM α1ATs (M-α1AT, Z-α1AT and M3-α1AT) without and with 0.06 μM trypsin incubated at 37°C for two minutes. Lane 1 represents multicolor protein ladder. Lane 2 represents a mixture with only 0.06 μM trypsin. Lane 3–5 represents undigested M-α1AT, Z-α1AT, and M3-α1AT. Lane 6–8 represents 0.06 μM trypsin treated M-α1AT, Zα1AT, and M3-α1AT.

(D). Western blot of 0.54μM α1ATs (M-α1AT, Z-α1AT and M3-α1AT) without and with 0.06 μM trypsin incubated at 370C for two minutes. Lane 1 represents multicolor protein ladder.
Lane 2 represents a mixture with only 0.06 μM trypsin. Lane 3-5 represents undigested M-α1AT, Z-α1AT, and M3-α1AT. Lane 6-8 represents 0.06 μM trypsin treated M-α1AT, Z-α1AT, and M3-α1AT.

(E). The inhibitory properties of α1AT proteins tested against trypsin on 12% SDS-PAGE. Lane 1 represents multicolor protein ladder. Lane 2 is loaded with 0.54μM M-α1AT + 0.010 μM trypsin, Lane 3 with 0.54μM M-α1AT+ 0.015 μM trypsin, Lane 4 with 0.54μM Z-α1AT+ 0.010 μM trypsin, Lane 5 with 0.54μM Z-α1AT+ 0.015 μM trypsin, Lane 6 with 0.54μM M3-α1AT+ 0.010 μM trypsin, and lane 7 with 0.54μM M3-α1AT+ 0.015 μM trypsin.

Supporting Information

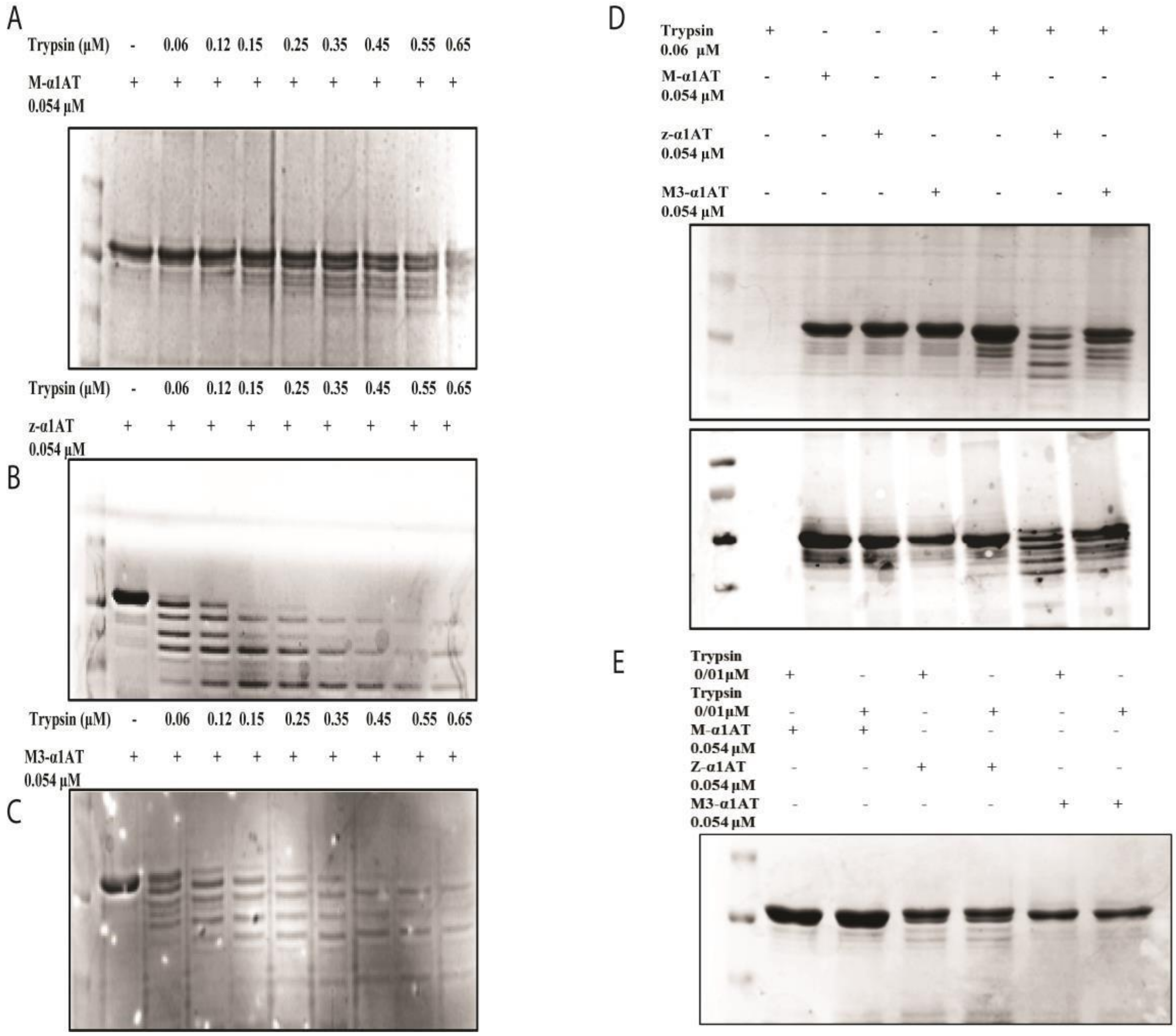

Figure S2

Supporting Information

Table S1. Association of 376 A/C single nucleotide polymorphism with various demographic variables, environmental factors, clinicopathological parameters, and family history in COPD cases.

Characteristics	**N=70**	**AA** **14 (20.00%)**	**AC** **41 (58.57%)**	**CC** **15 (21.43%)**	**Odds ratio 95%CI, P value**	**χ2; P value (Overall)***
Gender						
Male	49 (70.00%)	10 (71.42%)	27 (65.85%)	12 (80.00%)	AC 1.29 (0.34 – 4.88); p = 0.7551	1.0635;
Female	21 (30.00%)	04 (28.57%)	14 (34.14%)	03 (20.00%)	CC 0.62 (0.11 – 3.47); p = 0.6816	0.5875
Dwelling						
Rural	45 (64.28%)	10 (71.42%)	22 (53.65%)	13 (86.66%)	AC 2.15 (0.58 – 8.01); p =0.3497	5.660;
Urban	25 (35.71%)	04 (28.57%)	19 (46.34%)	02 (13.33%)	CC 0.38 (0.05 – 2.53); p = 0.3898	0.0607
Smoking status						
Ever	42 (60.00%)	09 (64.28%)	22 (53.65%)	11 (73.33%)	AC 1.55 (0.44 – 5.44); p =0.5470	1.905;
Never	28 (40.00%)	05 (35.71%)	19 (46.34%)	04 (26.66%)	CC 0.65(0.13 – 3.19); p = 0.6999	0.3857
Hemoptysis						
Yes	25 (35.71%)	04 (28.57%)	18 (43.90%)	03 (20.00%)	AC 0.51(0.13 – 1.90); p = 0.3614	3.121;
No	45 (64.28%)	10 (71.42%)	23 (56.09%)	12 (80.00%)	CC 1.60(0.28 – 8.90); p = 0.6816	0.2099
PFT** =70%	52 (74.28%)	12 (85.71%)	28 (68.29%)	12 (80.00%)	AC 2.79(0.54 – 14.30); p = 0.3040	1.984;
<70%	18 (25.71%)	02 (14.28%)	13 (31.70%)	03 (20.00%)	CC 1.50 (0.21 – 10.65); p = 1.000	0.3707
Obstructive jaundice						
Yes	29 (41.42%)	03 (21.42%)	19 (46.34%)	07 (46.66%)	AC 0.29(0.07 – 1.30); p = 0.1245	2.885;
No	41 (58.57%)	11 (78.57%)	22 (53.65%)	08 (53.33%)	CC 0.31(0.06 – 1.59); p = 0.2450	0.2363
Family history						
Yes	44 (62.85%)	09 (64.28%)	28 (68.29%)	07 (46.66%)	AC 0.83 (0.23– 2.99); p = 1.0000	2.215;
No	26 (37.14%)	05 (35.71%)	13 (31.70%)	08 (53.33%)	CC 2.05 (0.46– 9.14); p = 0.4621	0.3303

[*] The values in bold indicate significant results. CI: confidence interval.

** PFT (pulmonary function test) done before the salbutamol nebulization to COPD patients.

Table S2. Secondary structure distribution from DSSP calculation on the trajectory between 700 ns and 1000 ns.

	M-α1AT	**Z-α1AT**	**M3-α1AT**
Structured	0.66	0.63	0.63
Helix	0.30	0.27	0.29
β-Sheet	0.28	0.28	0.28
Turn + Bend	0.2	0.2	0.19
Coil	0.22	0.25	0.24

Table S3. Residues with significantly different mean electrostatic potential in the 700–1000 ns molecular dynamics trajectory.

Residue position	**Percentage overlap in mean electrostatic potential distribution**		
M-α1AT	**M-α1AT-Z-α1AT**	**M-α1AT-M3α1AT**	**Zα1AT- M3-α1AT**
60-Ala*	91.7	0.0**	0.0**
351-Met*	20.7	2.0	81.6
113-Thr	76.8	5.3	0.0**
188-Ile	88.0	17.1	4.1
335-Lys	92.8	0.0**	19.8
223-Arg	0.0**	0.0**	99.9
227-Phe	3.5	97.5	6.1
228-Asn	0.0	67.2	32.5
290-Lys	28.3	48.3	0.0**
341-Asp	0.0**	76.1	0.0**
342-Glu	0.0**	75.3	0.0**

* Residues shown in groups are contiguous to each other except for 60-Ala and 351-Met.

**Residues with zero overlap of mean electrostatic potential mean significant perturbation.