

The South African POIKTMP-associated *FAM111B* gene mutation affects its protein expression

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Introduction: The human FAM111B protein is a predictive serine/trypsin protease. Mutations in this protein's gene coding DNA are associated with POIKTMP, a rare, hereditary fibrosing disease characterised by mottled pigmentation, telangiectasia, epidermal, muscular atrophy, and pulmonary fibrosis. There is very little information on the biochemical or structural characterisation of the FAM111B protein nor the effect of the reported mutations on the structure and function of this protein. Hence this study aims to provide insight into the impact of the FAM111B Y621D mutation seen in the South African POIKTMP-affected family.

Methodology: Recombinant SNAP-tagged FAM111B protein was expressed in human embryonic (Hek293) cells. We created and expressed the South African POIKTMP FAM111B Y621D mutant protein through site-directed mutagenesis. We also created a catalytic domain mutant protein (S650A) and a double mutant of the catalytic domain, including the Y621D mutation (Y621D-S650A). The protein expression of wildtype and mutant proteins was then assessed by Western blot analysis.

Results: Though wildtype (wt.) FAM111B was successfully expressed; there was an indication of cleavage products at 55 kDa, 65 kDa and 25 kDa. We also saw that Y621D mutant protein expression was significantly low, suggesting the patient mutation affects the protein expression. However, the active site's serine residue mutation to alanine (S650A) abolished these cleavage protein products. Furthermore, introducing the S650A mutation alongside Y621D improved the protein expression.

Discussion and conclusion: Our results suggest that FAM111B is a self-cleaving protease, and Y621D enhances its catalytic property and hence rapid clearance of FAM111B protein. Altogether, Y621D affects the expression of FAM111B and proteolytic function.

Reference: (Khumalo *et al.*, 2006), (Mercier *et al.*, 2014), (Hoffman *et al.*, 2020)

Keywords: Fibrosis, POIKTMP, *FAM111B*

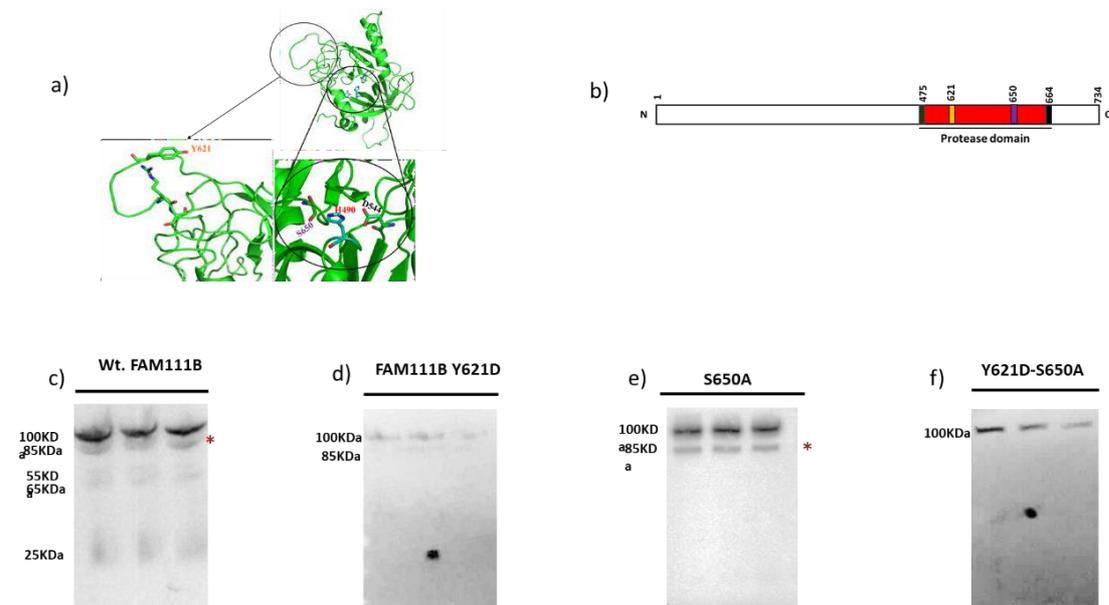


Fig: a) FAM111B protein model b) Schematic illustration of FAM111B protein c) Western blot SNAP-tagged wt. FAM111B d) Y621D e) Catalytic domain mutant S650A expression and f) Double mutation Y621D-S650A mutant protein expression. Protein band in red asterisks indicates endogenous FAM111B protein.