



21-22 November, 2024

Investigation of the Impact of Sequence Mutations on the Structural Properties and Stability of P-glycoprotein

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- Abstract:** *P-glycoprotein, an energy-dependent efflux pump in the ABC transporter family, exports xenobiotics from cells and is encoded by the ABCB1 gene. It has 1280 amino acids, with 27 identified missense mutations affecting hydrophilicity, flexibility, and stability. Analyses using ProtScale, PDB data (7A65), AlphaFold (AF-P08183), and DynaMut revealed that these mutations generally increase hydrophilicity and flexibility while causing more destabilizing than stabilizing effects. Such changes can impact drug transport, complicate medication dosing, and compromise physiological functions.*

Introduction

P-glycoprotein is an energy-dependent efflux pump in the ATP-binding cassette (ABC) transporter family, encoded by the ABCB1 gene. It consists of 1280 amino acids, including an active site of 60 amino acids, with the N- and C-terminal regions being 43% identical. It translocates xenobiotics from the inside to the outside of cells and is expressed in tissues like the nerve, kidney, liver, intestines, testes, and placenta, playing a key role in reducing drug accumulation in multidrug-resistant cells.

Material and method

Within the 1280 amino acid sequence of P-glycoprotein, 27 missense mutations have been identified. Analyses of hydrophilicity, flexibility, and structural stability were conducted using ProtScale, PDB data (code 7A65), and the AlphaFold model (AF-P08183). Mutation effects on stability were assessed with DynaMut.

Aminoacid nativ	N	F	N	N	A	M	F	N	I	S	R	R	A	L
Pozitia mutatiei	15	17	21	44	80	89	103	183	261	400	492	593	599	662
Aminoacid alelic	D	L	D	S	G	T	L	S	V	N	C	C	T	R
Aminoacid nativ	R	V	I	I	I	M	A	P	Q	W	S	V	T	
Pozitia mutatiei	669	801	829	836	849	986	999	1051	1107	1108	1141	1251	1256	
Aminoacid alelic	C	M	V	V	M	V	T	A	P	R	T	I	K	

Fig. 1: Table containing the amino acid mutations and their corresponding positions

Results and discussions

Following an analysis of the regional hydrophilicity of P-glycoprotein as influenced by observed mutations, it was determined that these mutations lead to an increase in hydrophilicity and enhanced flexibility. In the evaluation of stability variations induced by amino acid mutations in the P-glycoprotein structure, both stabilizing and destabilizing effects were noted. However, it was observed that destabilizing mutations predominantly outweigh stabilizing ones, as evidenced both in the experimentally determined structure and in the AlphaFold model.

Conclusions

P-glycoprotein is essential for absorbing, transporting, and eliminating xenobiotics. Amino acid mutations that change its hydrophilicity and flexibility can affect molecule interactions and compromise structural stability, potentially impacting drug dosing and its physiological functions.

Acknowledgement

This work was funded by the project PN-IV-P8-8.3-PM-RO-FR-2024-0056, Modulation of membrane transporters by therapeutic molecules.

Amino acid position	14	15	16	17	18	20	21	22	43	44	45	79	80	81	88	89	90
Wild type	0.467	0.413	0.360	0.363	0.383	0.430	0.463	0.480	0.477	0.380	0.377	0.277	0.383	0.383	0.383	0.423	
Allelic form	0.463	0.430	0.397	0.383	0.403	0.447	0.480	0.487	0.480	0.443	0.397	0.437	0.437	0.453	0.440	0.440	0.470
Amino acid position	102	103	104	182	183	184	260	261	262	399	400	401	491	492	493	595	596
Wild type	0.430	0.387	0.307	0.463	0.473	0.500	0.393	0.450	0.477	0.380	0.413	0.480	0.497	0.523	0.487	0.503	0.457
Allelic form	0.450	0.497	0.327	0.480	0.490	0.517	0.370	0.427	0.453	0.363	0.397	0.463	0.437	0.463	0.437	0.493	0.447
Amino acid position	592	593	594	598	599	600	601	662	663	668	669	670	800	801	802	828	829
Wild type	0.453	0.460	0.450	0.403	0.453	0.403	0.463	0.447	0.453	0.483	0.500	0.523	0.483	0.470	0.403	0.453	0.503
Allelic form	0.393	0.490	0.390	0.430	0.480	0.430	0.430	0.517	0.500	0.507	0.433	0.440	0.463	0.433	0.440	0.430	0.480
Amino acid position	835	836	837	848	849	850	892	893	894	985	986	987	988	999	1000	1050	1051
Wild type	0.438	0.438	0.462	0.480	0.477	0.427	0.517	0.530	0.507	0.480	0.340	0.350	0.480	0.417	0.397	0.483	0.423
Allelic form	0.380	0.407	0.440	0.407	0.423	0.375	0.493	0.507	0.483	0.430	0.370	0.380	0.457	0.443	0.423	0.463	0.423
Amino acid position	1108	1109	1110	1109	1140	1141	1142	1250	1251	1252	1253	1256	1257				
Wild type	0.447	0.397	0.390	0.403	0.430	0.463	0.500	0.487	0.463	0.453	0.433	0.433	0.417				
Allelic form	0.453	0.477	0.470	0.477	0.407	0.440	0.477	0.510	0.487	0.477	0.443	0.443	0.427				

Fig. 2: Tables containing data on flexibility profile changes

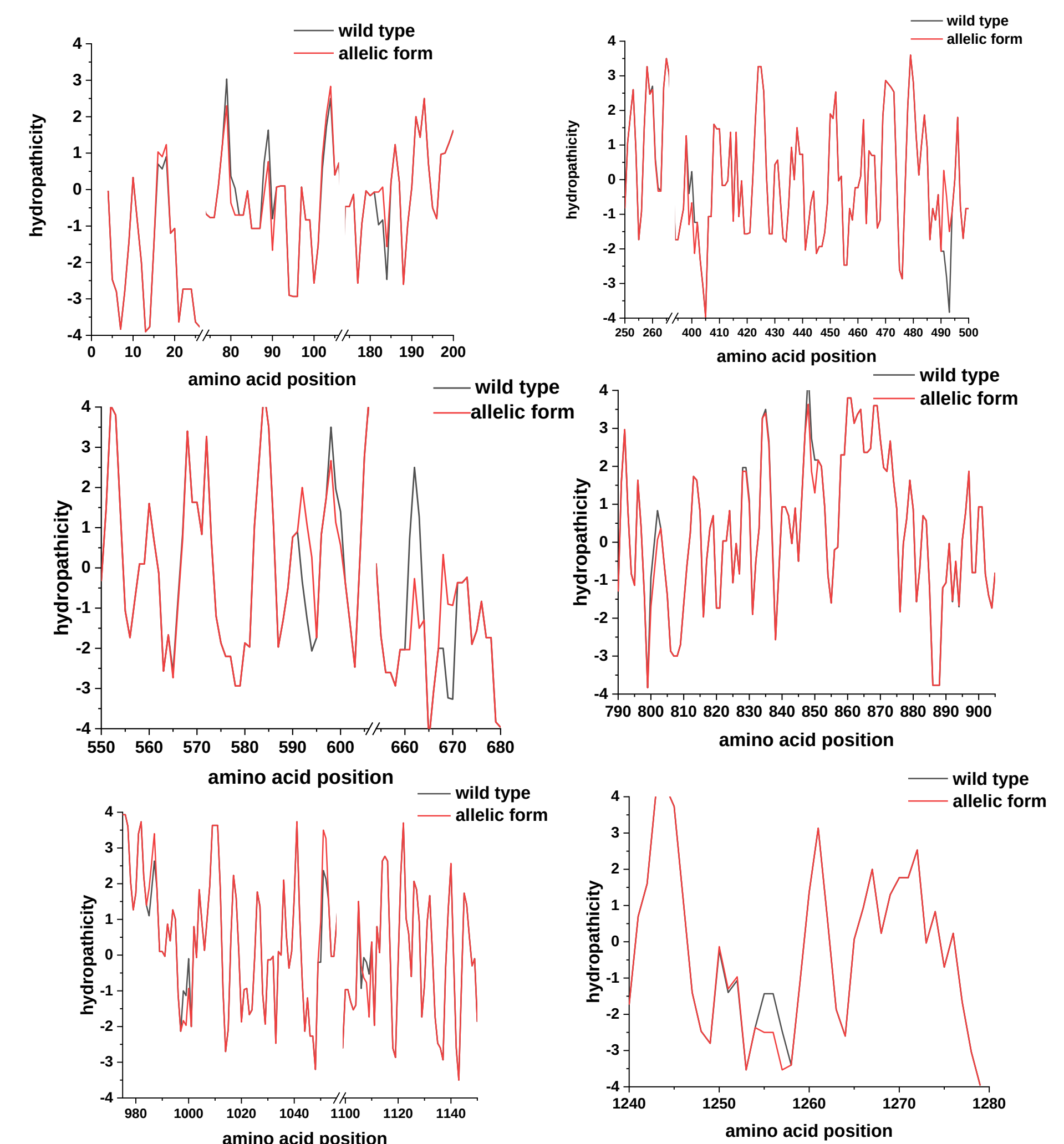


Fig. 3: Graphs corresponding to the hydrophilic profile

Mutația aminoacizilor	ΔΔG (kcal/mol)	
	Structura experimentală	Model AlphaFold
N15D	indisponibil	0,21
F17L	indisponibil	-0,34
N21D	indisponibil	0,07
N44S	0,08	0,33
A80G	-1,33	-1,51
M89T	indisponibil	0,3
F103L	indisponibil	-0,09
N183S	0,3	0,11
I261V	-0,76	-1,11
S400N	-0,56	-0,53
R492C	0,55	-0,18
R593C	0,58	0,59
A599T	-0,88	-0,82
L662R	indisponibil	0,37
R669C	indisponibil	0,47
V801M	0,09	0,24
I829V	-1,07	-0,99
I836V	0,03	-0,52
I849M	0,03	-0,04
M986V	-1,47	-0,42
A999T	-0,38	-0,66
P1051A	0,15	-0,3
Q1107P	-0,15	-0,13
W1108R	-0,33	-1,49
S1141T	-0,28	-0,62
V1251I	-0,45	-0,49
T1256K	-0,06	-0,28

Fig. 4: Table containing stability changes of P-glycoprotein