Homology model of Abcc6 provides insight into the function of mutations causing cardiovascular phenotype

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Rare mutations in the *ABCC6* gene have been demonstrated to cause pseudoxanthoma elasticum in humans as well as dystrophic cardiovascular calcification (DCC) in mice. A case-control study indicates that frequent DNA variants (SNPs) in the *ABCC6* gene are not rare in the general population and contribute to an increased propensity toward premature atherosclerotic vascular disease. ABCC6 abbreviates for multidrug resistance-associated protein 6. It is a member of the ATP-binding cassette sub-family C and as such involved in the transport of molecules using ATP. The exact biological function of ABCC6 is presently still unknown, same as the functional relationship of this transmembrane transporter to the pathogenesis of atherosclerosis or calcification.

In this study we aim to get insights into the molecular effect of the Aa substitutions on protein function by molecular and computational methods. C3H/He and NZB mice are predisposed to DCC, whereas the mice C57BL/6 strain remains resistant. Seven base pair exchanges leading to Aa substitutions were found in C3H/He strain (S3R, L166F, A706V, I927T, H1401Q, L1448V, N1476S) and only two Aa substitutions were found in NZB strain (A706V, I927T). We functionally investigated the effect of both C3H (Mut-1) and NZB (Mut-2) mutants on protein integration and stability and observed demonstrated that the seven mutations in Mut-1 lead to unstable and deficient protein. On the other hand the two mutations in DCC-predisposed NZB mice lead to stable protein. Thus, single mutations in *Abcc6* in NZB mice may affect the protein function and not the protein stability.

We next build a homology model of the Abcc6 transporter, and analyzed the position of the previously reported mutations in mice within the predicted structure. It is well known that the ABC domains associate and disassociate during the ATP-hydrolysis cycle. Clustering the mutations at these domain-domain contacts, mutations from NZB (A706V and I927T) are located near or within the domain-domain contact region. 2009, Fülöp *et al.* suggested the importance of this region in context of functionality. Four of the mutations (A706V, H1401Q, L1448 and N1477S) are located within or near the ATP-interface region. H1401Q has the closest distance to ATP-interface region and is a strong conserved Aa. A trajectory of a first short dynamic simulation shows a movement of Q1401 and a loss of hydrogen bond interaction between two Aa of the ATP-interface region (S1389 and V1399, close to Q1401) and ATP in contrast to the *wt*Abcc6.

The experimental observations in loss of function in NZB are consistent with the computational results. In vitro experiments are on going to further confirm the importance of the H1401Q mutation. In conclusion the predicted structural model of Abcc6 allows us to generate and improve hypotheses in a very short time compared to the classical NMR analysis. Also the structural model help us for better planning of future *in-vitro*/*in-vivo* experimental work.