Common Variants in Signal Peptide Sequences; Modelling Effects and Relationship to Function

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Background. It has been proposed that the orientation and angle of insertion of a signal peptide (SP), at the hydrophobic-hydrophilic interface, is of critical importance for its function. We have examined this from both theoretical and experimental points of view.

Methods. A number of previous studies reported that site-directed mutations in the yeast invertase SP affected invertase activity and using this as a model system, we correlated the modeled SPs with invertase secretion. Using the PC-TAMMO, PC-PROT and PC-MGM programs we analysed the SPs hydrophobicity and the orientation of the molecule at a modeled hydrophobic-hydrophilic interface and correlated this to enzyme activity. We have applied this approach to naturally occurring SP variants in apolipoproteins.

Results. While the predicted angle of insertion of the yeast invertase SP correlated with the measured extent of invertase secretion, with an optimum angle of 45°, mutations that changed the angle of orientation reduced the extent of invertase secretion. We applied these same molecular modeling principles to the naturally occurring variants of the human apolipoprotein B (apoB) and apoAV signal peptides, to test their functionality. These variants have been associated with effects on lipid parameters *in vivo*. For apoB, three common SP variants occur, a common 27 amino acid (aa) SP (SP27), a rarer 24aa (SP24) variant, resulting from 3aa deletion in the hydrophobic core, and a very rare insertion variant of 29aa (SP29). Compared to SP27, both SP24 and SP29 confer secretion defective phenotypes when fused to yeast invertase and expressed in yeast. Modeling of these SP showed that while the SP27 has a near optimal angle of insertion of 54° to the interface, SP29 has an angle of 23°. SP24 has an optimal angle of 45°, but had insufficient hydrophobicity to penetrate the interface efficiently. A common apoAV signal peptide variant, S19W, is associated with increased plasma triglyceride (TG) levels in carriers. We have modeled the W19 SP and compared this to the S19 SP, using IMPALA minimisation. Compared to the wildtype apoAV SP, S19, the W19 SP shows increased destabilisation, which suggests that the W19 apoAV favours lipid insertion which would result in increased secretory activity. This agrees with our *in vitro* studies examining the effect of the apoAV S19W variants on secretory alkaline phosphatase activity.

Conclusion. Using this modeling approach, we initially were able to identify a strong correlation between the predicted angle of insertion of a SP into the membrane and its ability to direct secretion in the yeast invertase system. *APOB* SP24 is associated with effects on plasma triglyceride (TG) levels and its allele frequency of 30% suggests it may have an impact on plasma TG levels at the population level. Similarly carriers of the *APOA5* W19 variant have higher plasma TG levels than S19 carriers. Thus the analysis of functionality of SP variant has wider implication, in our case to apolipoproteins and their relation to plasma lipids and coronary heart disease risk.