Defective ER retention signal due to mutation VKORC1:p.Arg98Trp results in VKCFD2

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Introduction and Objectives

Vitamin K Oxidoreductase Complex subunit 1 (VKORC1) is the key enzyme of the vitamin K-cycle and is located in the membrane of the endoplasmatic reticulum (ER). VKORC1 reduces vitamin K to vitamin K hydroquinone to sustain γ-carboxylation of the vitamin K dependent coagulation factors by the enzyme γ-Carboxylase (GGCX). The only reported mutation p.Arg98Trp in human VKORC1 (hVKORC1) gene resulting in reduced VKOR activity causes a combined deficiency of vitamin K dependent clotting factors type 2 (VKCFD2). However, the pathophysiological mechanism underlying this bleeding disorder is still unknown. Here, we demonstrate for the first time, that the hVKORC1 variant p.Arg98Trp disrupts a di-arginine ER retention signal leading to largely diminished VKORC1 concentration in the ER membrane.

Materials and Methods

The sequence of hVKORC1 was scanned for potential linear motifs around the region of interest on different motif databases. The pEGFP-N3 vectors containing the cDNA of wild-type hVKORC1, the variants Arg98Trp, Arg98Ala+Arg100Ala, and 98_100delRTR were transfected into HEK293T cells. The ER and nucleus were immunostained and the hVKORC1 variants were visually analysed by confocal microscopy. The degree of co-localization for hVKORC1 and its variants with ER was quantified using co-localization analysis plugins embedded in the ImageJ 1.43m software. The comparative percentage co-localization was reported as mean Pearson´s and Mander´s R coefficient.

Results

The structural and sequence analysis suggests the existence of a di-arginine ER retention motif on the p.Arg98Trp locus (RXR). All mutated variants of the di-arginine motif of hVKORC1 showed significantly lower degree of co-localization in the ER (all $p<0.01$) in comparison with the wild type hVKORC1 (Fig.1 and Tab.1). The variant p.Arg98Trp causing VKCFD2 yielded only 27% co-localization in the ER compared to the wild type (Tab.1).

Conclusion

The hVKORC1 variant p.Arg98Trp decreases the protein's ER retention capacity resulting in sub-cellular mislocalization and its subsequent removal, thereby causing the VKCFD2 phenotype.

References

1. Oldenburg J, von Brederlow B, Fregin A, Rost S, Wolz W, Eberl W, Eber S, Lenz E, Schwaab R, Brackmann HH *et al*: **Congenital deficiency of vitamin K dependent coagulation factors in two families presents as a genetic defect of the vitamin K-epoxide-reductase-complex**. *Thromb Haemost* 2000, **84**(6):937-941.
2. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortnagel K, Pelz HJ, Lappegard K, Seifried E, Scharrer I, Tuddenham EG *et al*: **Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2**. *Nature* 2004, **427**(6974):537-541.
3. Marchetti G, Caruso P, Lunghi B, Pinotti M, Lapecorella M, Napolitano M, Canella A, Mariani G, Bernardi F: **Vitamin K-induced modification of coagulation phenotype in VKORC1 homozygous deficiency**. *J Thromb Haemost* 2008, **6**(5):797-803.

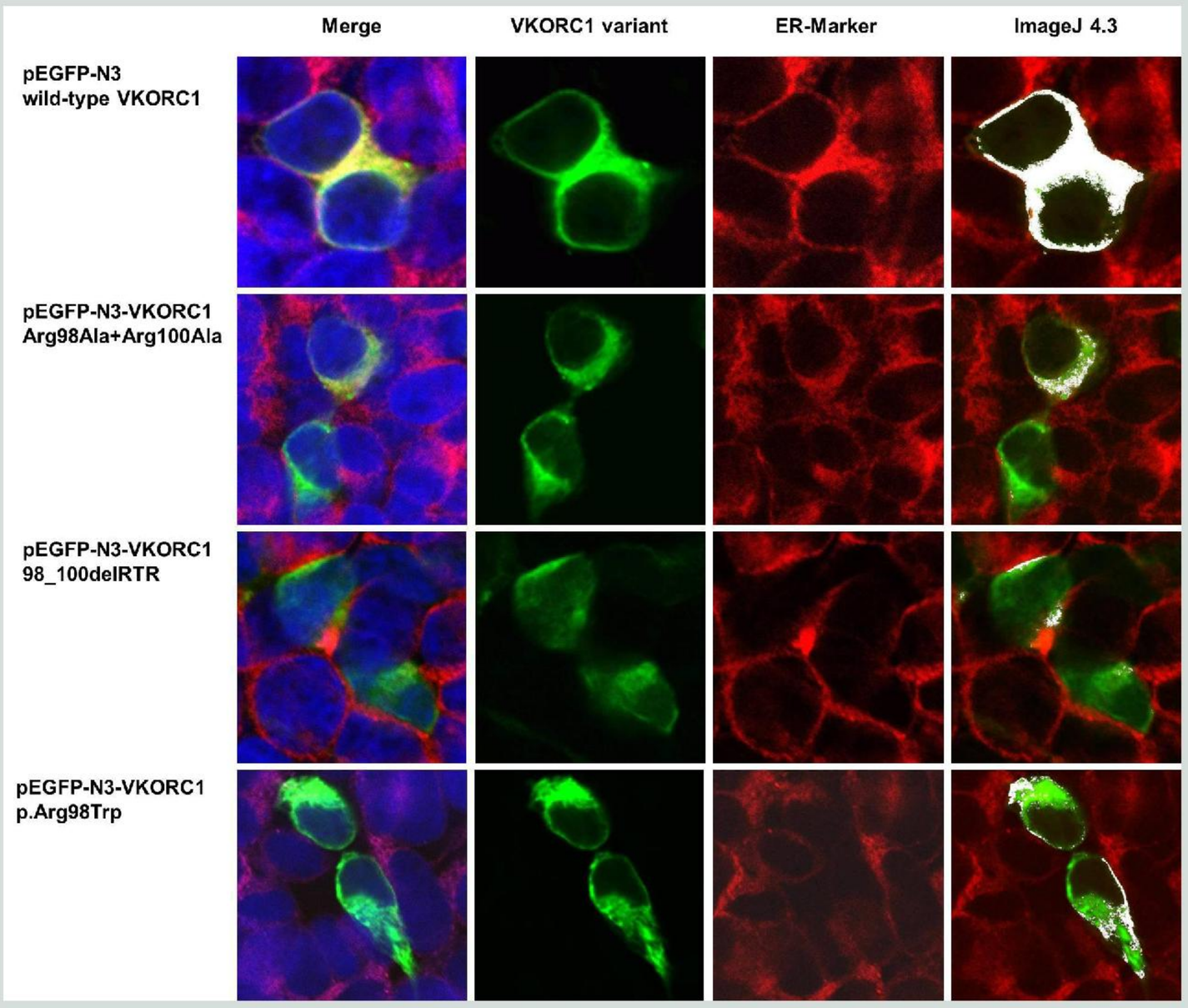

Fig.1: Co-localization images for wild-type hVKORC1 and the mutated di-Arginine motif variants in HEK293T cells.

This figure shows representative co-localization images of immunostained *HEK293T* cells for wild-type hVKORC1, Arg98Ala+Arg100Ala, the 98_100delRTR variant and the human p.Arg98Trp mutant variant, respectively. Each panel is composed of four images; the first one is a merged picture of the green coloured GPF tagged hVKORC1 protein, the red stained ER by PDI antibody and AlexaFluor 594 as secondary antibody and the blue counterstained nucleus (ToPro3). The second image shows cells expressing the GFP tagged hVKORC1 protein in green only, followed by an image in which the ER is stained in red only. The last image shows co-localized regions as white dots using the co-localization highlighter plug-in embedded in ImageJ version4.3. The images clearly show a decrease in ER co-localization from the wild-type to the deletion variant.

Tab.1: Degree of co-localization of VKORC1 variants with the ER.

This bar graph represents the comparative mean Pearson´s coefficient and Mander´s R coefficient for wild-type and the mutated variants affecting the di-Arginine motif. Error bars represent the standard deviation. The mean Pearson´s coefficient (grey bars) and Mander´s R (black bars) has been calculated from n=10 to 24 ROI´s (regions of interest). All calculations for Pearson´s and Mander´s R coefficient have been performed on the Image J version 4.3 visualization and analysis software.