LETTER TO THE EDITOR

A novel p.C1130S mutation in a Finnish family with a complex phenotype of von Willebrand disease

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1. *Supplementary Figures and Tables*

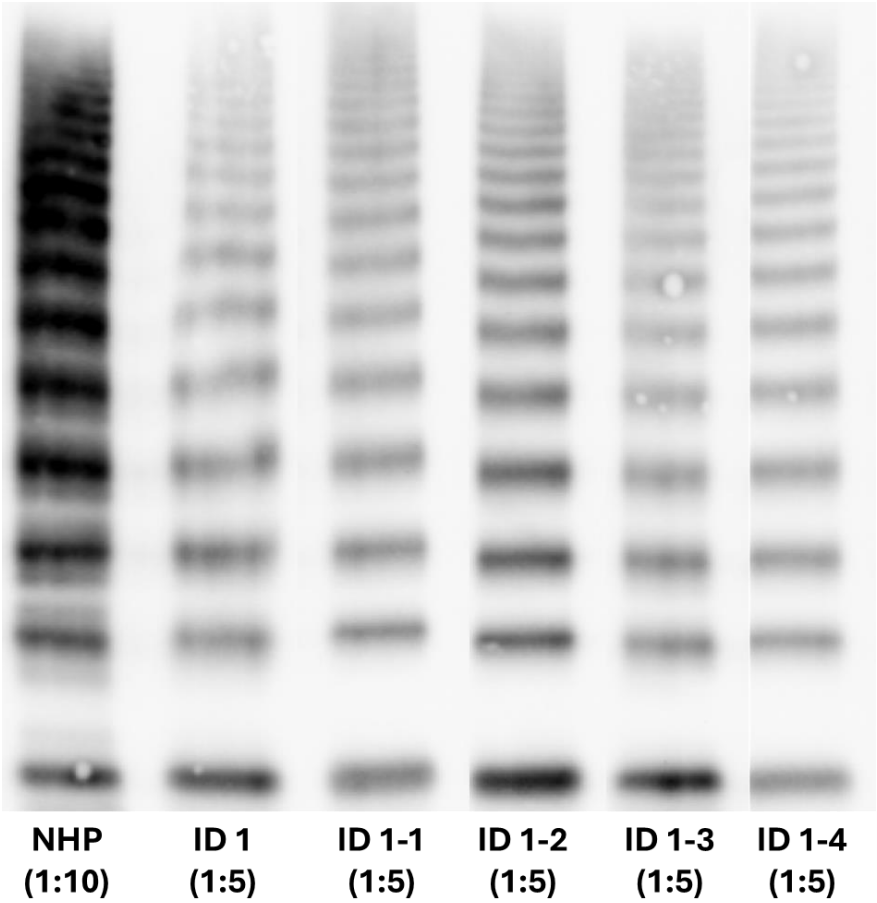


Figure S1. VWF multimer analysis of all family members. VWF multimer analysis was carried out via a 1.5% sodium dodecyl sulphate (SDS) agarose gel electrophoresis, followed by Western blotting and luminescent visualization using polyclonal anti-VWF antibodies labelled with alkaline phosphatase, which was recorded by photo-imaging. For loading the gel, sample dilution of both normal pooled human plasma (i.e;, NHP, normal control), and patient plasmas were performed following the standard procedure described by Budde et al.[1] NHP was loaded onto the gel at a 1:10 dilution, whereas all patient samples were loaded at a 1:5 dilution, as all had VWF:Ag levels below 50 IU/dL. The lanes represent, from left to right, the NHP, the index patient (ID 1), his son (ID 1-3) and his three daughters (ID 1-1, ID 1-2 and ID 1-4). No clearly visible abnormalities in VWF multimers were seen in any of the patients, but a possible subtle loss of the largest VWF multimers might be missed due to limited analytical sensitivity of the assay. Therefore, a densitometric analysis was performed (see Addendum Figure S1 on page 4 of this supplementary file) to assess the number of peaks on the multimer curves. Abbreviations: VWF = von Willebrand factor, SDS = sodium dodecyl sulfate, NHP = normal human plasma pool.

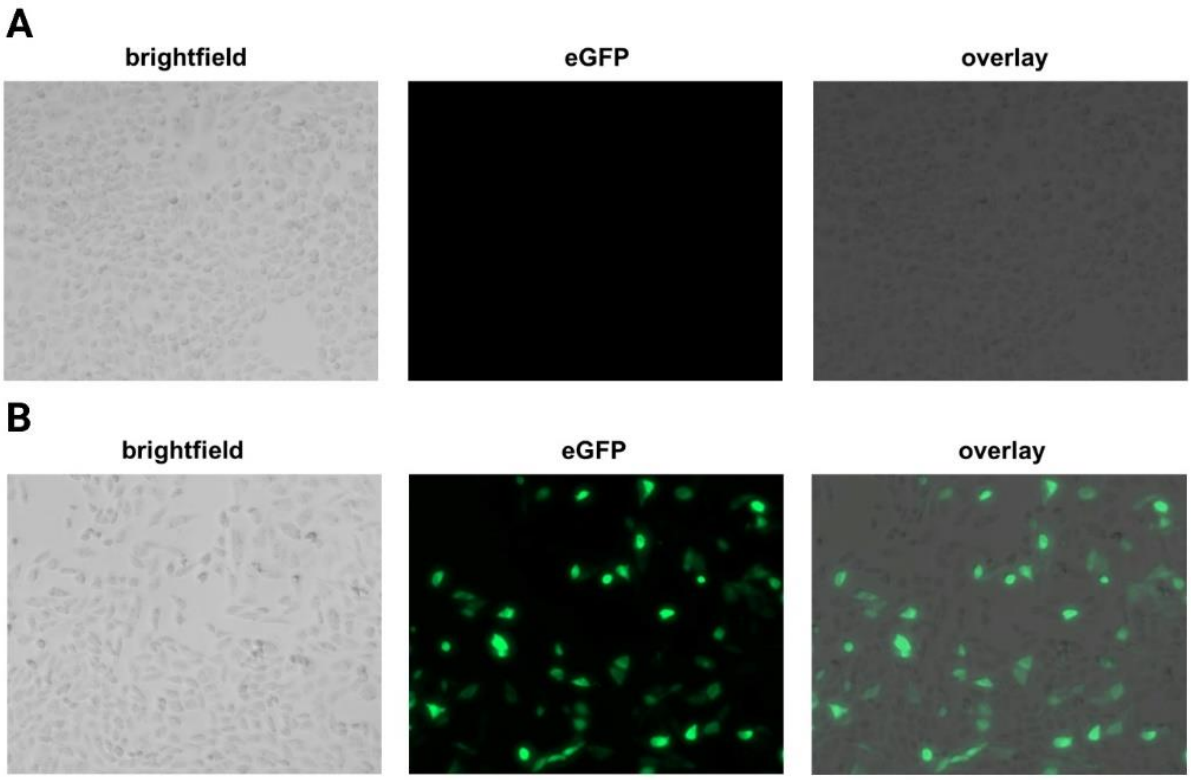
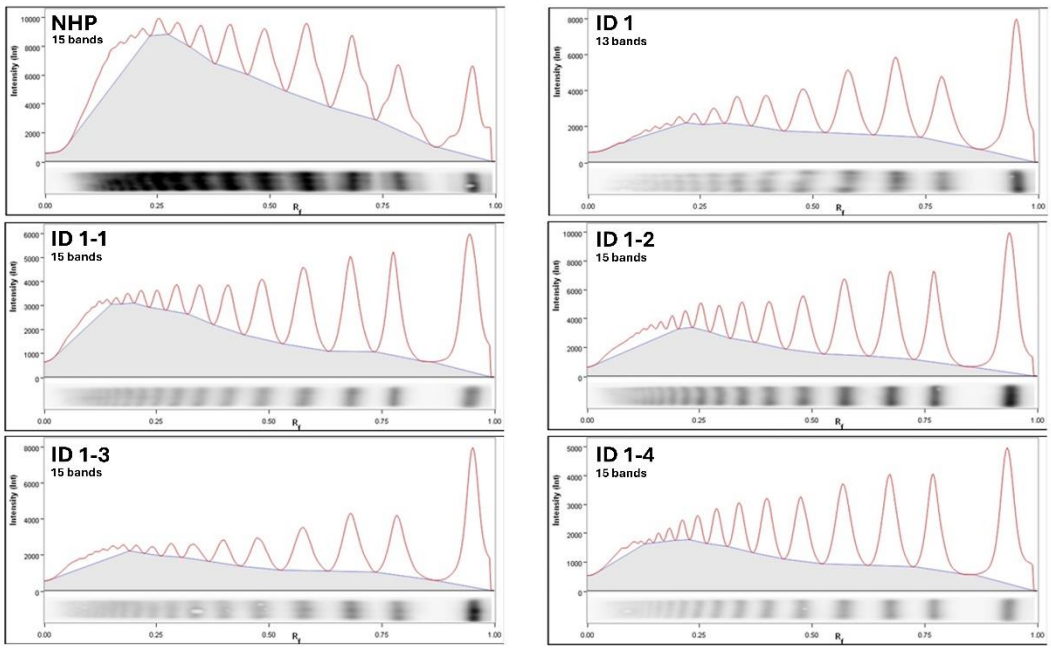


Figure S2. Determination of transfection efficacy in the CHO K1 cells. CHO K1 cells were cultured at 37 °C with 5 % CO_2, in Kaighn's modification of Ham's F12 medium (ref: 21127-022, Invitrogen, Carlsbad, CA) supplemented with 1 % antibiotic-antimycotic (ref: 15240-062, Invitrogen, Carlsbad, CA) and 10 % fetal calf serum (ref: 10500-064, Invitrogen, Carlsbad, CA). Transfection was performed using the jetPRIME® Transfection Reagent (Polyplus transfection, Illkirch, France) according to the manufacturer's instructions. For each transfection, a 3:1 jetPRIME® to DNA ratio was used, with 6 µg of DNA per transfection. When testing heterozygous conditions, 3 µg of both expression plasmids were used. To determine transfection efficiency, 0.6 µg of a Green Fluorescent Protein (GFP)-expressing plasmid (i.e. pMax-GFP; Lonza, Basel, Switzerland) was additionally co-transfected. As a negative control, an Fmock condition containing pure jetPRIME® buffer was studied. Visualization of GFP expression was done via fluorescence microscopy (Zeiss Axio observer fluorescence microscope, Carl Zeiss AG, Oberkochen, Germany). For each flask, both the amount of GFP-expressing CHO K1 cells and total amount of CHO K1 cells were counted on three different fields 48 h after transfection. **A)** Brightfield, eGFP and overlay pictures of the negative Fmock control condition. **B)** Brightfield, eGFP and overlay pictures under an arbitrarily selected test condition to exemplify a condition with a transfection efficacy of 31.02 %. Abbreviations: CHO = Chinese hamster ovary, GFP = green fluorescence protein.



Addendum Figure S1. Densitometry analyses of the VWF multimers of all family members. The results showed that all family members had at least 13 detectable bands, with the father (i.e., ID 1) exhibiting the fewest. In comparison, the normal human plasma pool (NHP) showed 15 bands. After thorough discussion among experts, it was concluded that all family members had a normal VWF multimer pattern, as the presence of at least 13 out of 15 bands was not considered indicative for a pathological multimer distribution. Abbreviations: VWF = von Willebrand factor, NHP = normal human plasma pool.

Table S1. Primers used for site-directed mutagenesis. Site directed mutagenesis was done using the QuikChange® II XL site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA) and was performed according to the manufacturer's instructions.

Primer	Sequence	Plasmid
VWF_mutR854Q_FP	GTGTCTGTCGGGACCAGAAGTGGAACTGCAC	pNUT-VWFR854Q
VWF_mutR854Q_RP	GTGCAGTTCCACTTCTGGTCCCGACAGACAC	pNUT-VWFR854Q
VWF_mutC1130S_FP	GCCCCCAGAGCAGCGAGGAGAGG	pNUT-VWFC1130S
VWF_mutC1130S_RP	CCTCTCCTCGCTGCTCTGGGGGC	pNUT-VWFC1130S

2. *References supplementary data*

1. Budde U, Schneppenheim R, Eikenboom J, et al. Detailed von Willebrand factor multimer analysis in patients with von Willebrand disease in the European study, molecular and clinical markers for the diagnosis and management of type 1 von Willebrand disease (MCMDM-1VWD). *J Thromb Haemost*. 2008;6(5):762-771.