VWF-related pathologies

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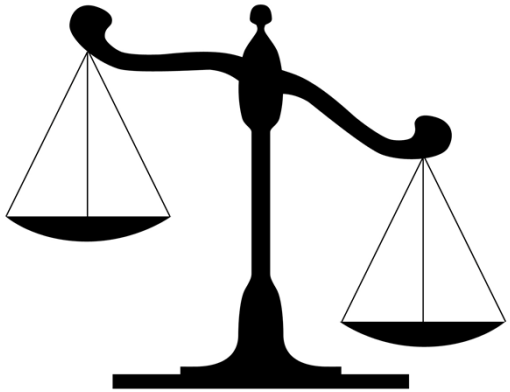
Thrombotic Thrombocytopenic Purpura (TTP)

VWF



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von Willebrand Disease (VWD)

Thrombosis **Haemostasis** **Bleeding**

TTP summary

- Systemic disorder characterized by inappropriate deposition of VWF and platelet rich thrombi throughout the microvasculature, thrombocytopenia, microangiopathic hemolitic anemia, organ failure and death
- Presence of Ultra-large (UL)-VWF-MWMs in plasma
- TTP results from the deficiency of the metalloprotease ADMTS13 that cleaves circulating VWF
- **Rare inherited TTP**
- **More frequent acquired TTP** >
  inhibitory anti-ADMTS13 autoantibodies

Animal models:
Mutant VWF (introduction of a disulfide bond in A2)
Mutant VWF is protected from ADAMTS13-proteolysis
(*in vitro* experiment with transfected cells under flow)
Mouse model by hydrodynamic gene transfer
(recapitulates some of the TTP phenotypes)

VWF-related pathologies
von Willebrand Disease (VWD)

- One of the most frequent inherited bleeding disorder (prevalence of patients with clinically significant bleeding 1:10.000)
- More than 300 mutations
- Extremely heterogeneous > many different **types** have been described based on specific phenotypes
- VWD-type 1 > Quantitative deficiency
- VWD-type 2 > Qualitative deficiency
- VWD-type 3 > Absence of VWF

- Genetic testing is not indicated except for specific cases
  - Because *VWF* is a very large gene 178kb and 52 exons
  - The presence of highly homologous partial pseudogene
  - The gene is highly polymorphic (>300 SNPs reported)

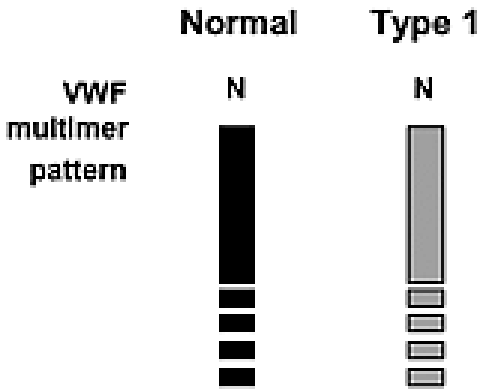
VWF-related pathologies
von Willebrand Disease (VWD)

- Type 1 & 2 generally inherited in an autosomal-dominant pattern
- Type 3 inherited in an autosomal-recessive pattern

- Interestingly not all individuals that have mutations in the *VWF* gene exhibit clinical symptoms (incomplete penetrance)
- Although there is often a significant family history of bleeding associated with the diagnosis of VWD, many family members with low VWF levels may exhibit a range of bleeding symptoms (variable expressivity)

von Willebrand Disease type 1

- VWD type 1 is the most common form of VWD. It affects 80% of all individuals diagnosed with VWD and is traditionally characterized by reduction of functionally normal VWF in the presence of mucocutaneous bleeding.
- Several mechanisms have been shown to cause low VWF levels mostly related to decreased cellular secretion of VWF, including mutations that affect gene expression, protein trafficking, or mild increases in VWF clearance.
- Low amount of circulating VWF but normal multimer distribution



von Willebrand Disease type 1

- The ability to identify a causative mutation in patients with VWD, particularly with type 1, is sometimes difficult:
  - Because mild bleeding is common in the general population
  - And many individuals will have low to borderline levels

- To increase the complexity there are many patients, particularly with VWD type 1, for whom there is no causative mutation identified and **genetic modifiers** outside of the *VWF* gene may play significant roles in the modulation of VWF quantity, function, and multimer status.

  > Therefore, a significant number of patients diagnosed with VWD type 1 may have a complex genetic disorder in which 1 gene coupled with environmental stressors is responsible for the phenotype

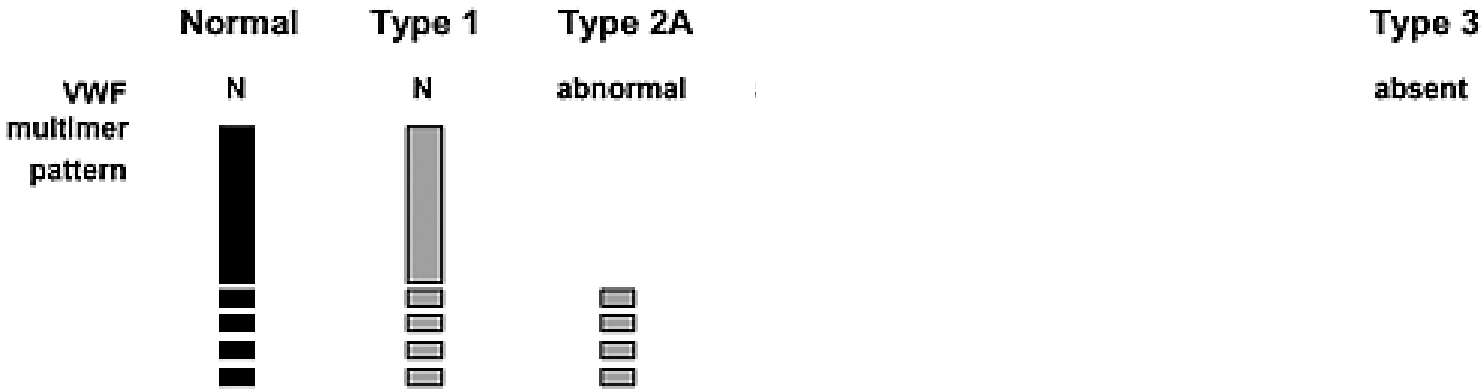
von Willebrand Disease type 3

- VWD type 3 is a severe deficiency of VWF and leads to low FVIII levels.
- The bleeding pattern of these patients is severe and can mimic hemophilia

von Willebrand Disease type 2

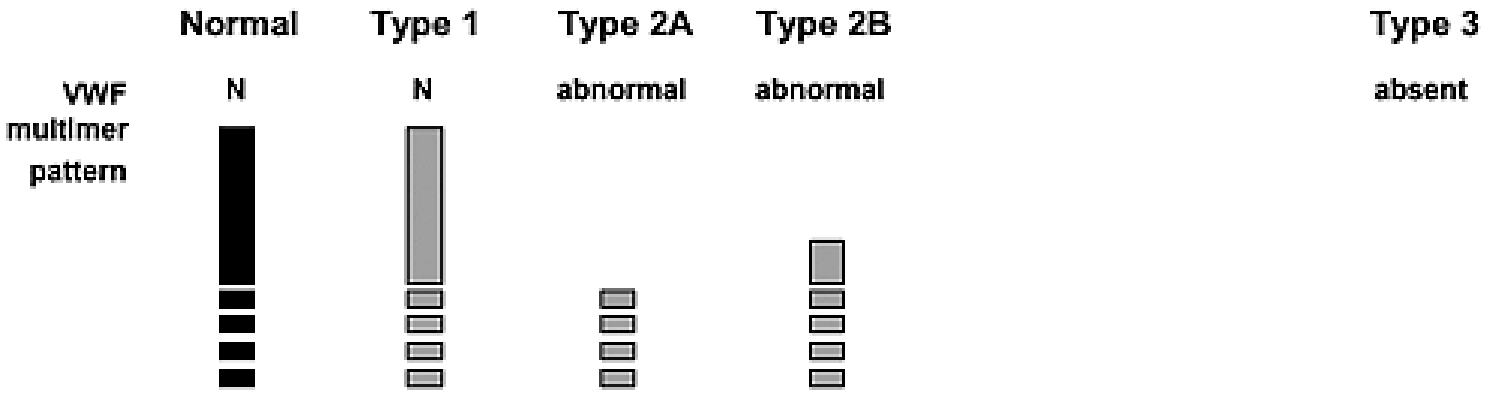
- VWD type 2 is characterized by qualitative defect in VWF
- The specific subtypes are due to mutations in VWF gene that cause abnormalities in the interaction of VWF with its ligands leading to functional defects.

- VWD type 2A is the most common form of type 2. Loss of intermediate and high molecular weight VWF multimers. Due to abnormal synthesis or packaging of VWF or increased susceptibility to ADAMTS13.



von Willebrand Disease type 2

- VWD type 2B is due to increased VWF-platelet GPIb binding because of gain-of-function mutations in *VWF* gene. Platelets aggregates form in circulation and are rapidly cleared. Decreased VWF levels, Loss of HMWM and often but not always thrombocytopenia.



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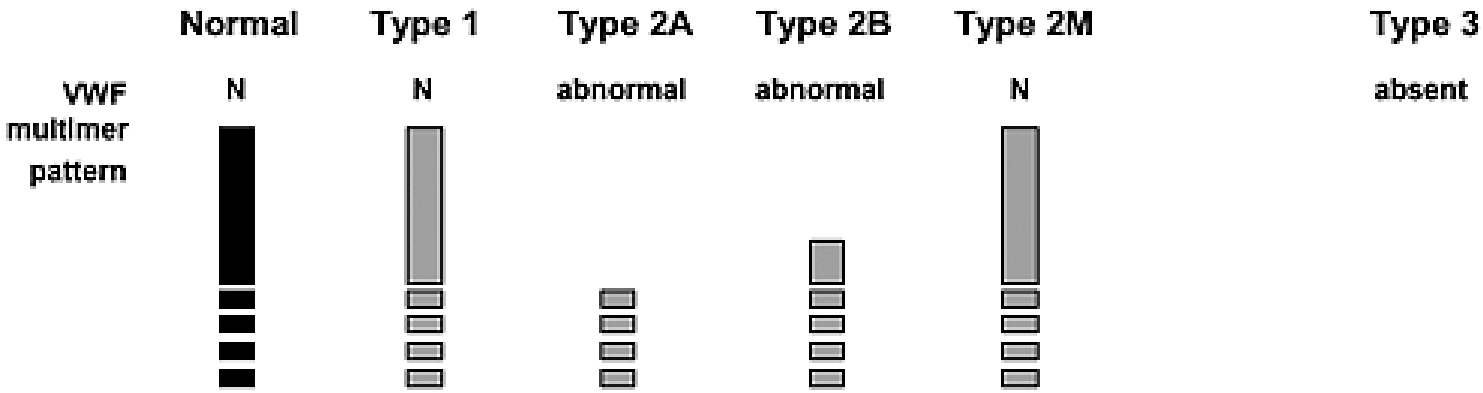
- VWD type 2M is due to mutations that cause decreased interaction between VWF and platelet GPIb. Multimer distribution is normal but they are dysfunctional because they cannot bind platelet properly.

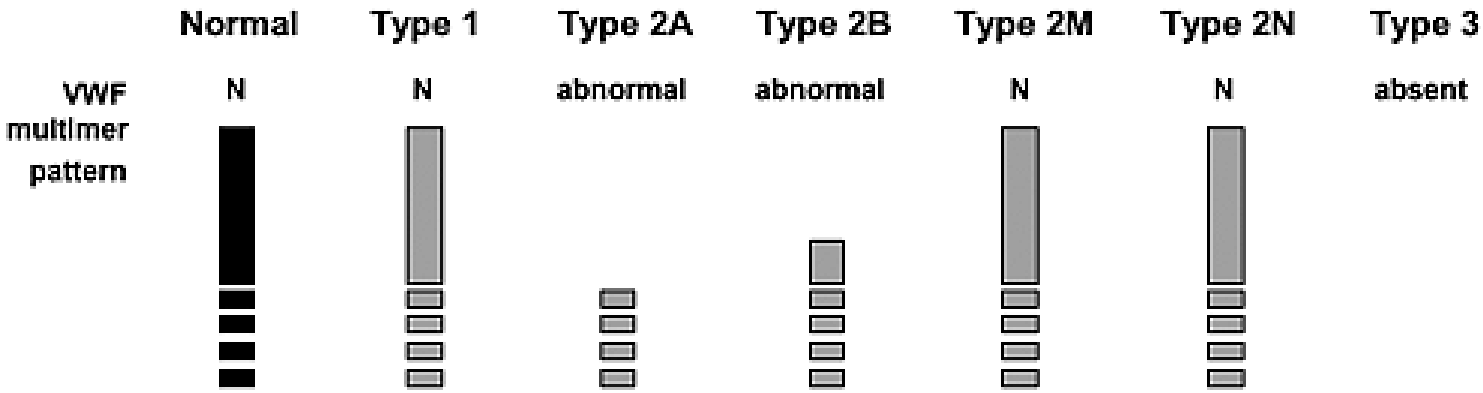


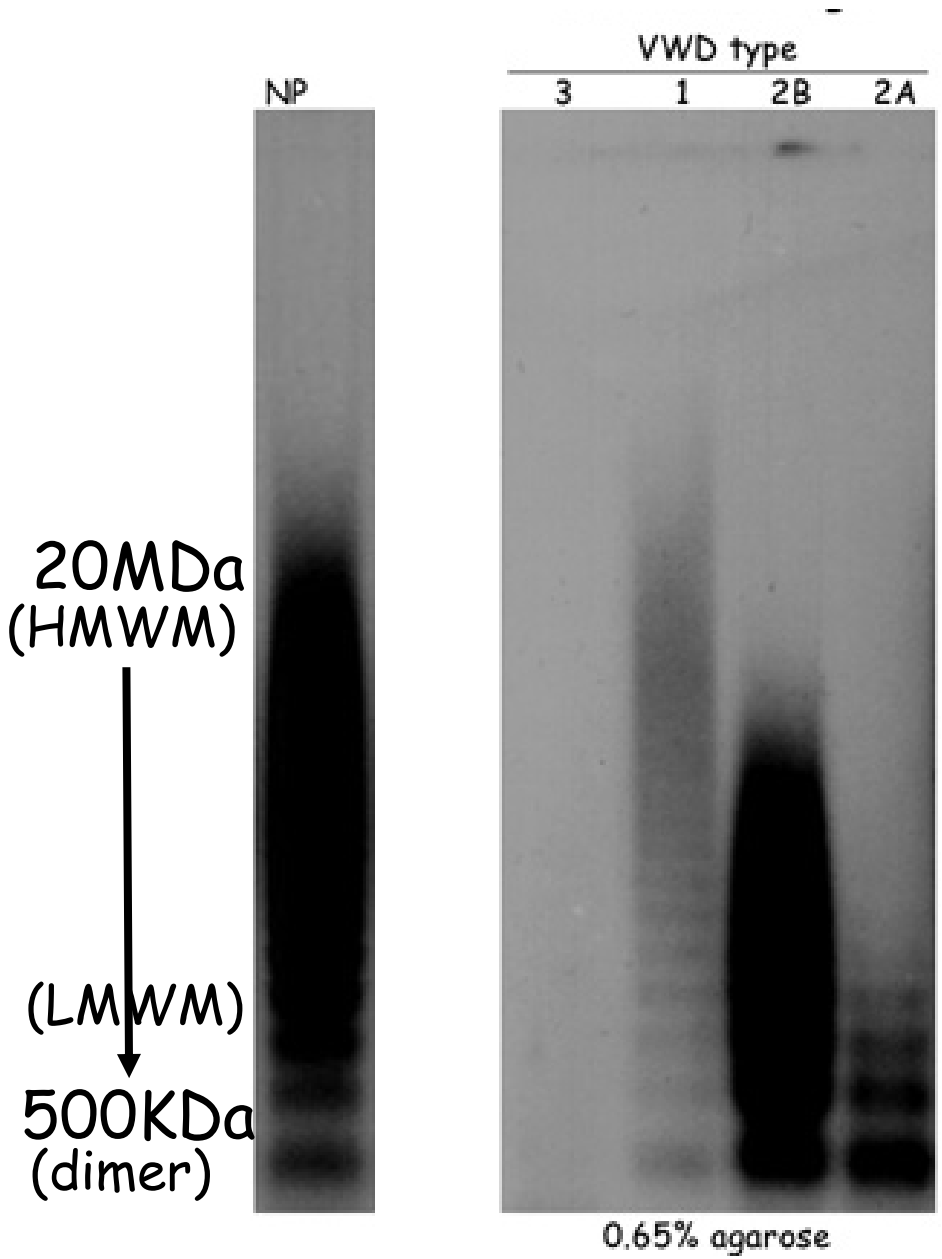
von Willebrand Disease type 2

- VWD type 2B is due to increased VWF-platelet GPIb binding because of gain-of-function mutations in *VWF* gene. Platelets aggregates form in circulation and are rapidly cleared. Decreased VWF levels, Loss of HMWM and often but not always thrombocytopenia.

- VWD type 2M is due to mutations that cause decreased interaction between VWF and platelet GPIb. Multimer distribution is normal but they are dysfunctional because they cannot bind platelet properly.

- VWD type 2N is a rare disorder and result from the inability of VWF to bind FVIII leading to accelerated FVIII clearance. Normally due to mutations in the D'D3 domains (FVIII binding region)




VWD type
NP
3
1
2B
2A
20MDa
(HMWM)
(LMWM)
500KDa
(dimer)
0.65% agarose

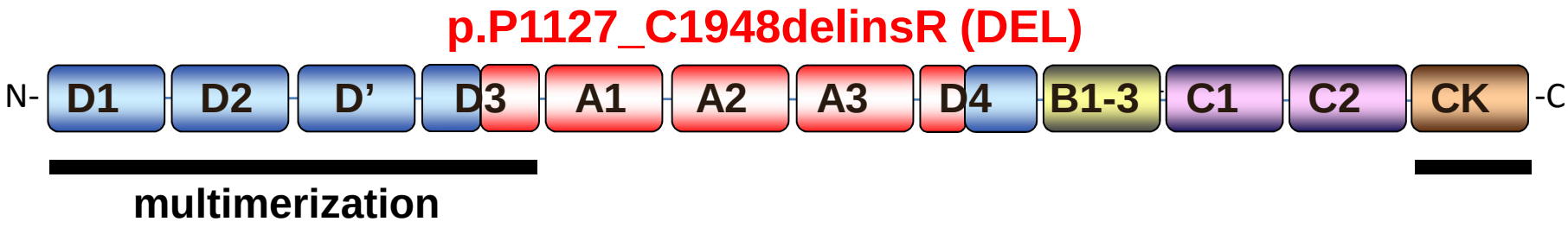
von Willebrand Disease type 2A: a mutation with dominant-negative effect

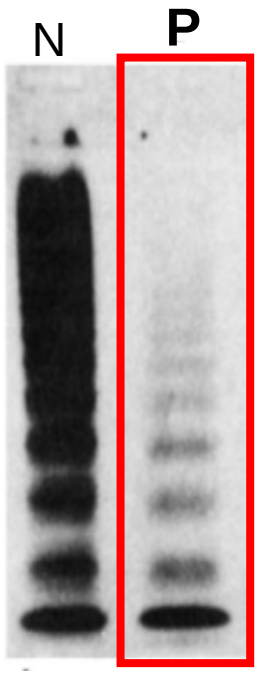
- Dimerization and multimerization make VWF susceptible to mutations with dominant-negative effects.

- Dominant-negative mutations result in non-functional protein that directly interact with and inhibit the activity of the proteins produced by the wild type allele.

- The consequences on the phenotype are more severe compared to a normal heterozygous mutation.

von Willebrand Disease type 2A: a mutation with dominant-negative effect





in-frame connection of exons 25 and 35

- Partial lack of D3 domain leads to a multimerization defect

Bernardi F et al. Blood 1990
Bernardi F et al. Hum Mol Gen 1993
Casari C et al. Blood 2010

Cellular models

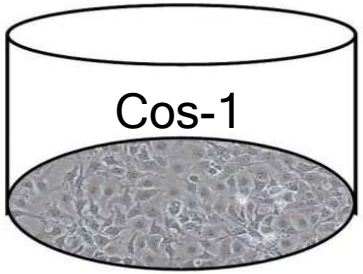
pWT

Wt-VWF expession vector

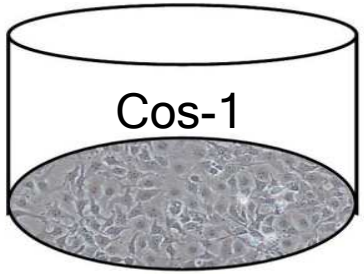
pDEL

Break-point

Del-VWF expression vector
(P1105_C1926delinsR)



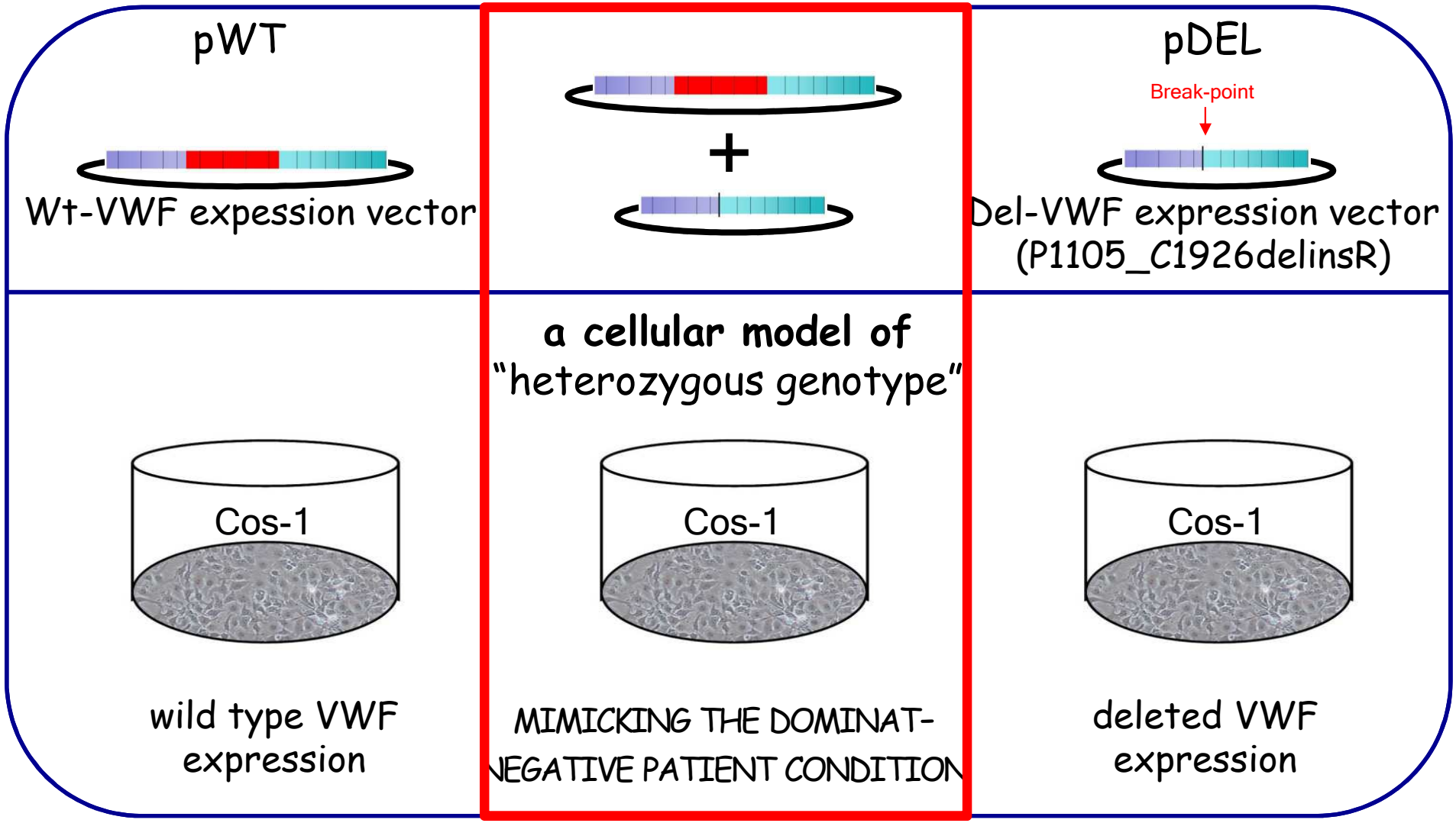
wild type VWF
expression



deleted VWF
expression

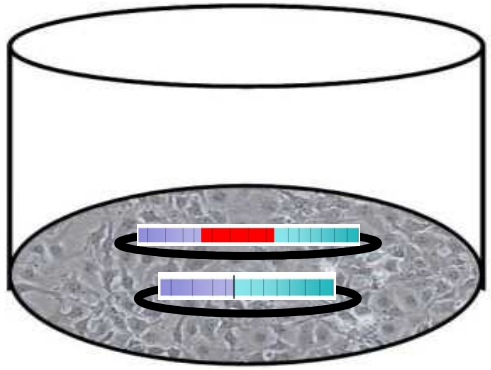
Casari C et al. Blood 2010

Cellular models



Casari C et al. Blood 2010

“heterozygous” cellular model

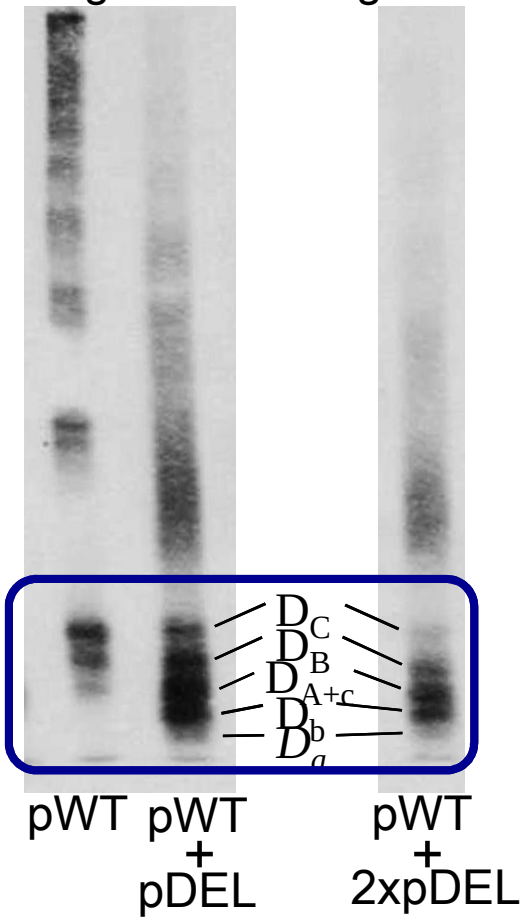


Dominant-negative effect of the VWF deletion

pWT	2x	1x	1x
pDEL		1x	2x

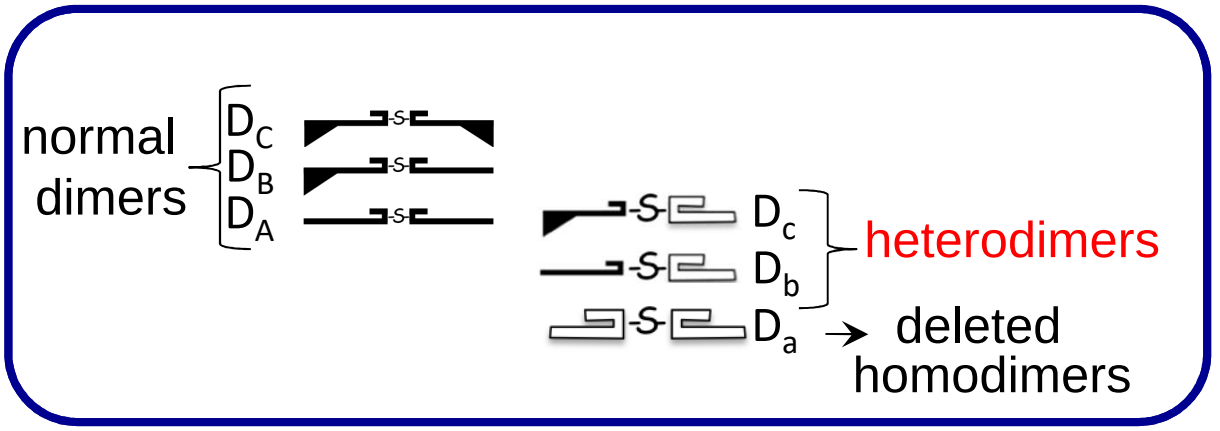
Cellular models

High resolution gels



- Dominant-negative effect of del-VWF
- Formation of heterodimers with wt-VWF

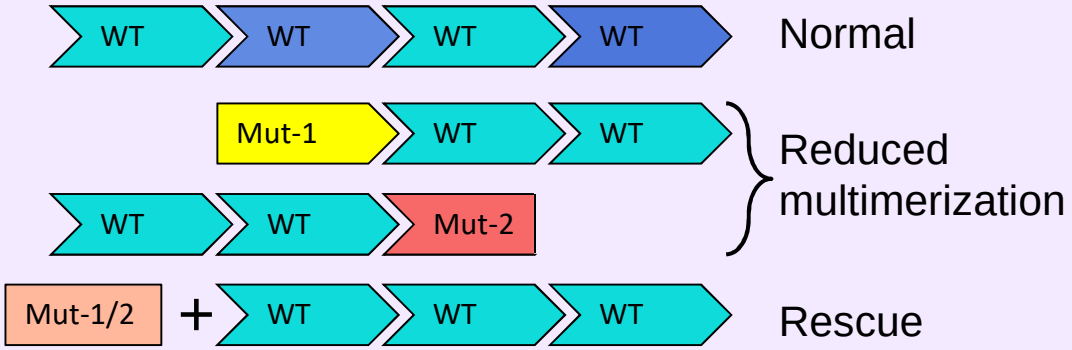
Schematic representation of dimers



Casari C et al. Blood 2010

Strategies to rescue the dominant negative effect of the DELETION mutant

- Combining the multimerization mutation and a dimerization mutation in a single VWF subunit Mut-1/2



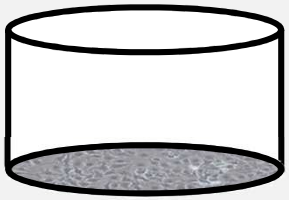
- In the normal situation, multimers are made of subunits originating from the two alleles.
- In both mutants, the multimerization process is interrupted upon the incorporation of the mutant subunit.
- The idea is that by completely preventing incorporation of the double mutant subunit, multimerization will be rescued and multimers of only wild-type subunits will be produced.

Counteracting the dominant-negative effect

in vitro

Plasmids
pSV-hVWF-DEL/C2773R
pSV-hVWF-wt

Co-transfection



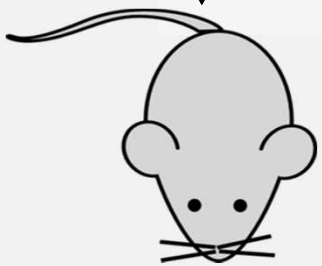
COS-1 cells

in vivo

Plasmids
pLIVE-mVWF-DEL/C2773R
pLIVE-mVWF-wt

Co-injection via hydrodynamic gene transfer

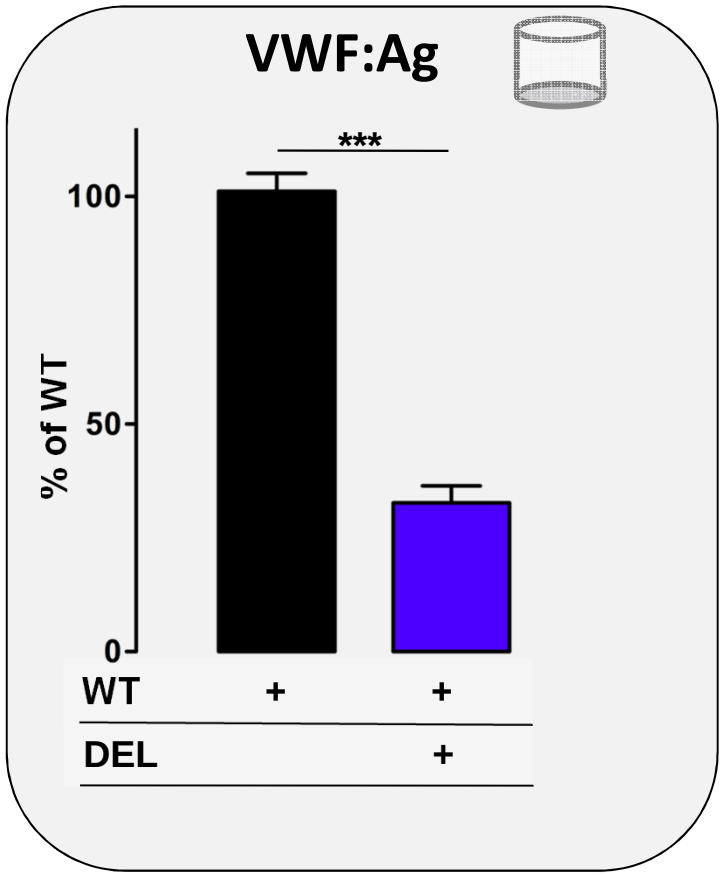
Heterozygous state expression

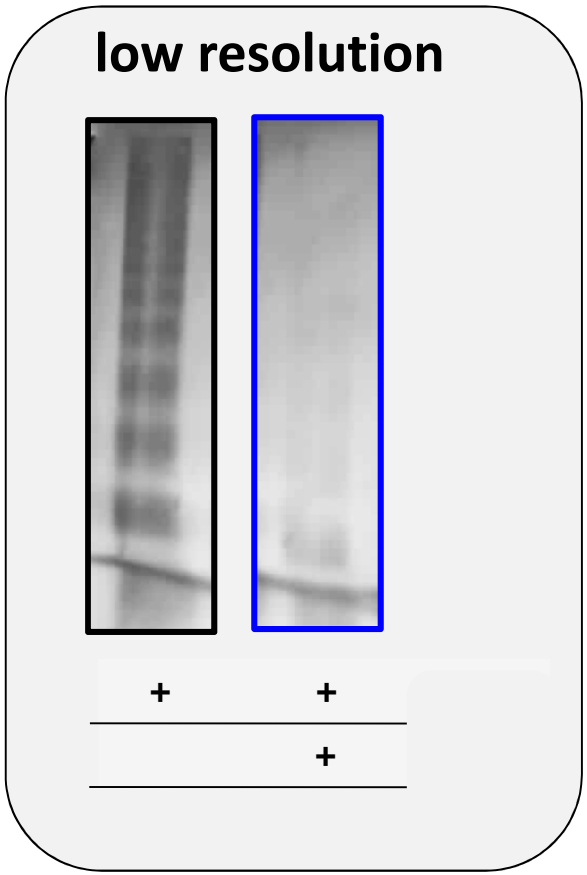


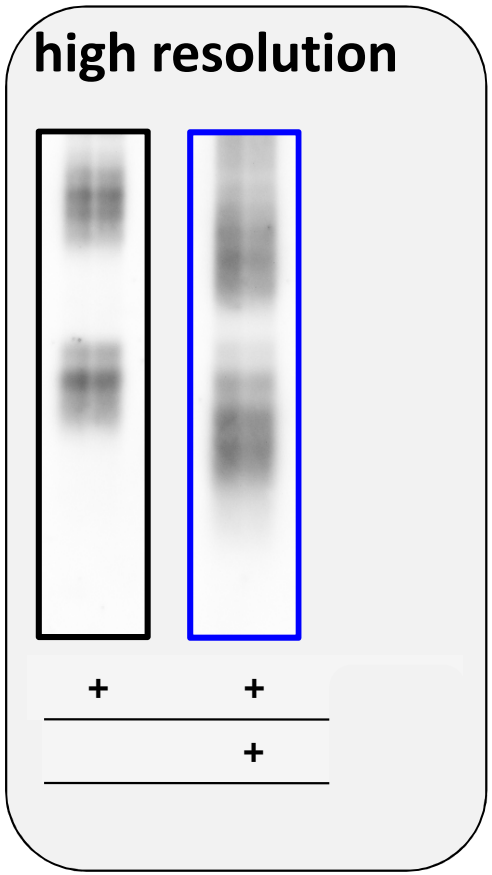
Vwf $^{-/-}$ mice

VWF antigen levels & VWF multimer profile were assessed in conditioned media and in plasma.
Bleeding phenotype was assessed in vivo

Counteracting the dominant-negative effect: DEL/C2773R double mutant -*in vitro*-





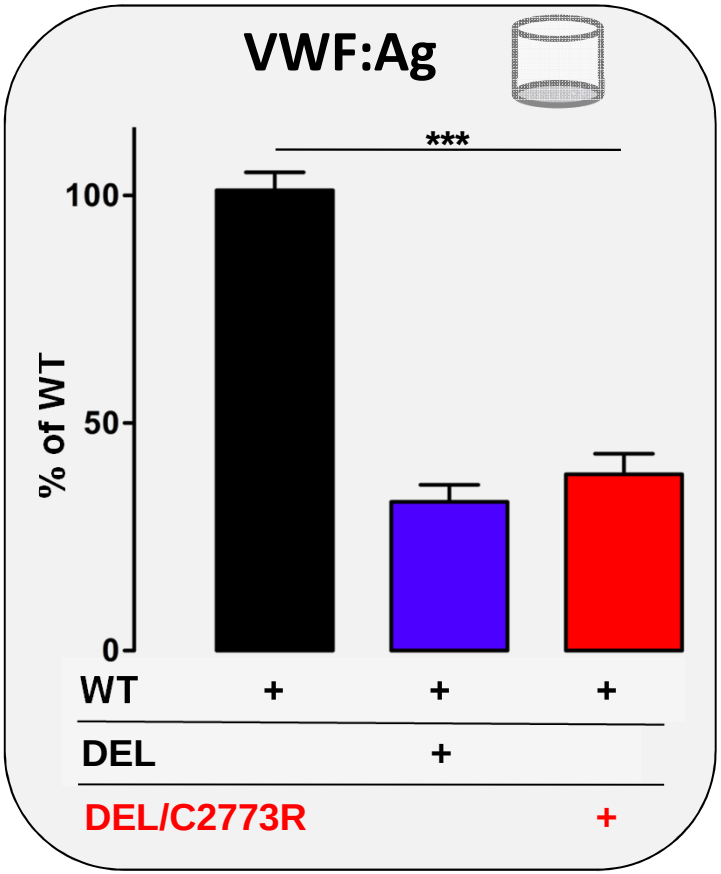


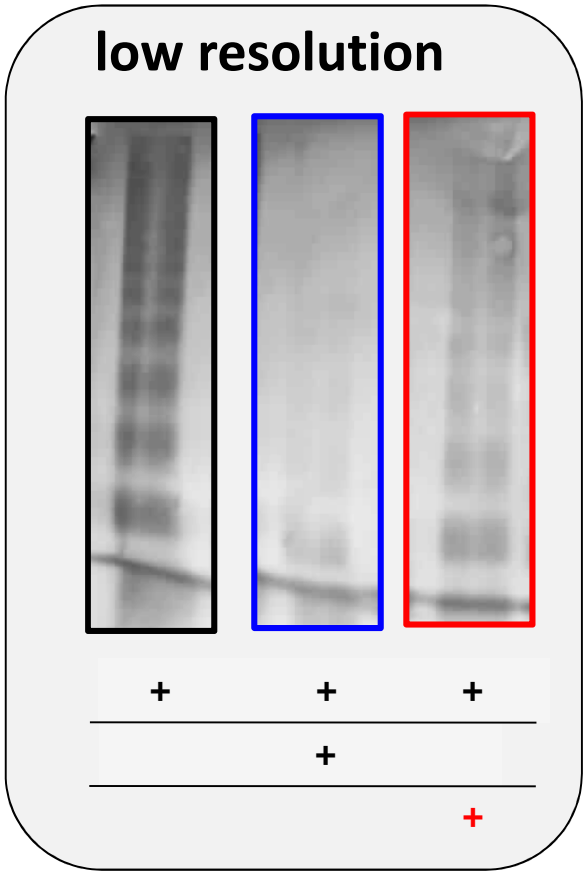
low antigen levels

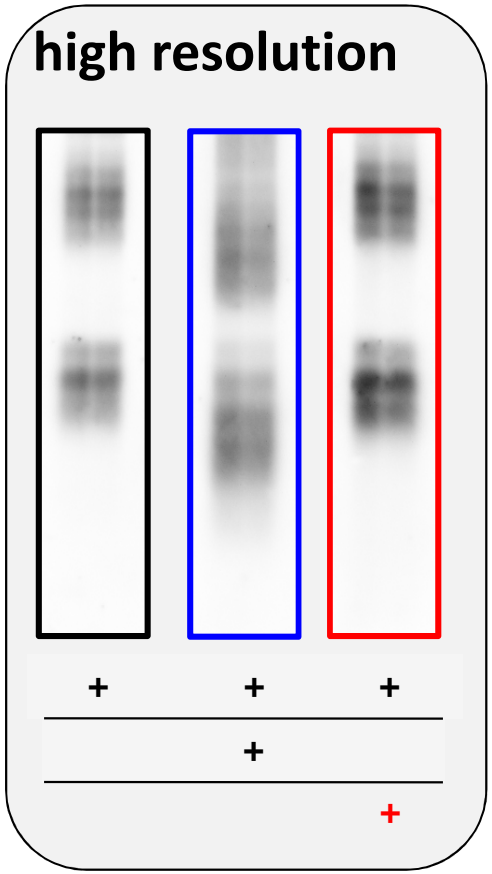
absence of HMWM

smaller bands

Counteracting the dominant-negative effect: DEL/C2773R double mutant -*in vitro*-





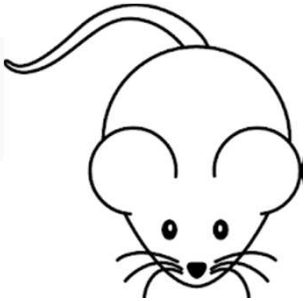


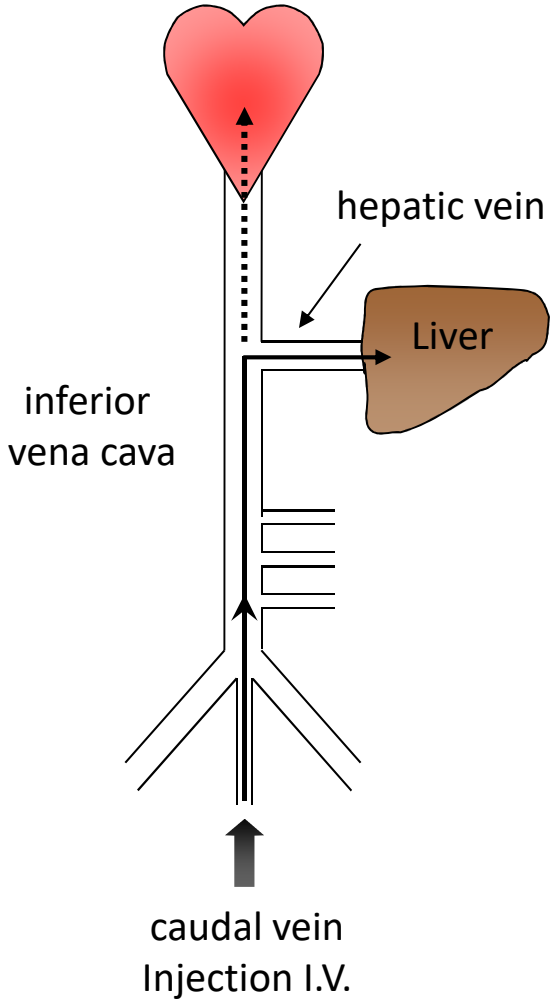
50% of antigen levels

normal multimer profile

normal bands

Hydrodynamic gene transfer





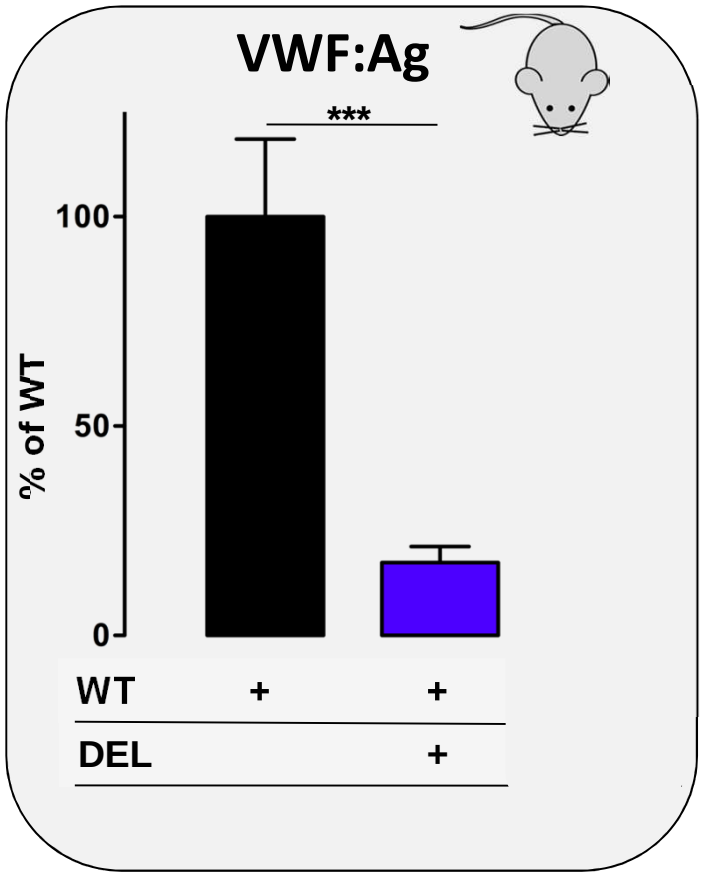
hydrodynamic injection principle

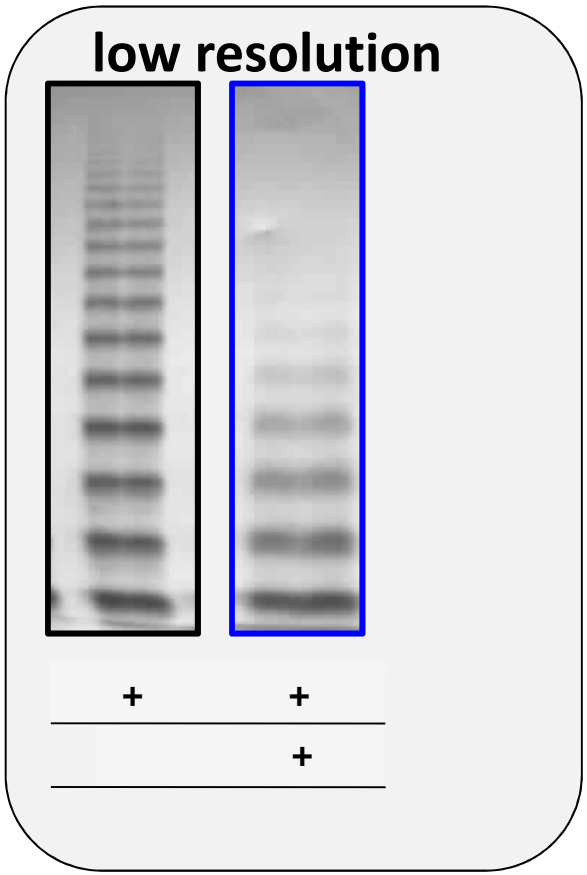
- Rapid injection of large volume of DNA (PLASMID) via the tail vein
- Going beyond cardiac capacity
- Increase of blood pressure
- Reflux to organs connected to the vena cava>

The liver absorbs the majority of the injected volume

- 10 to 40% of transfected hepatocytes

Counteracting the dominant-negative effect: DEL/C2773R double mutant -*in vivo*-





tail bleeding

# of mice that did not occlude the injury

WT 3 out of 7

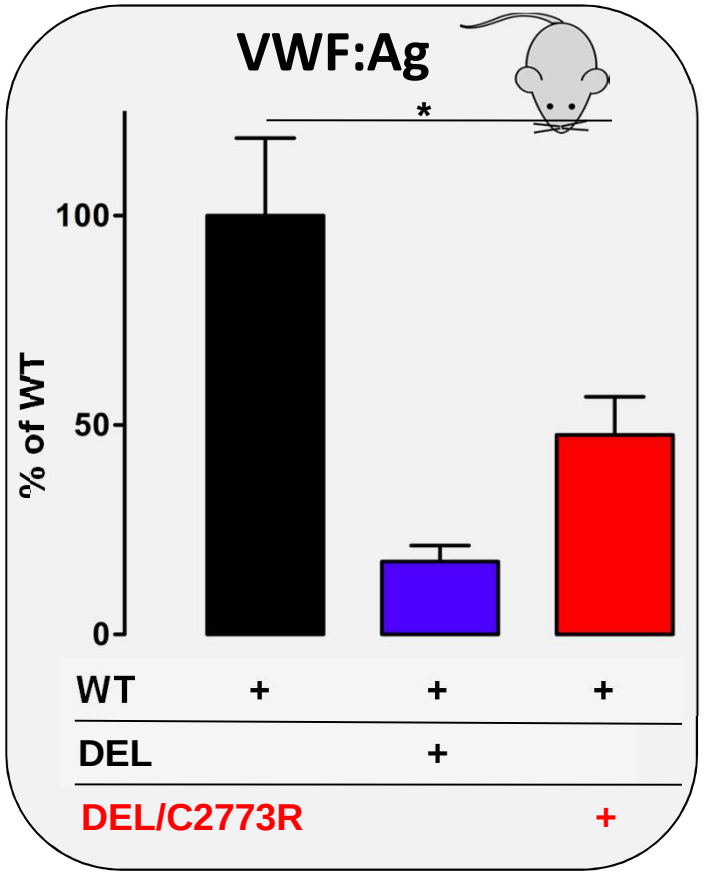
WT+DEL 10 out of 11

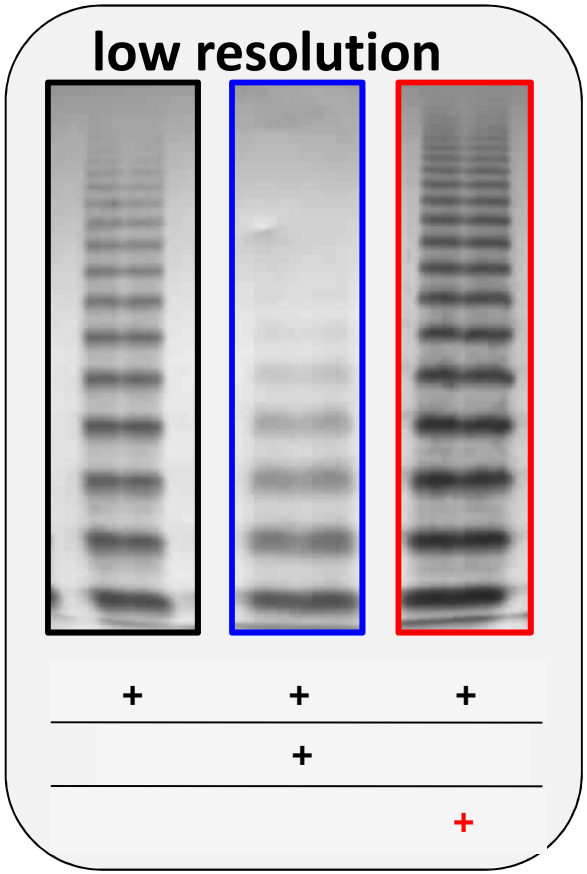
low antigen levels

absence of HMWM

high bleeding risk

Counteracting the dominant-negative effect: DEL/C2773R double mutant -*in vivo*-





tail bleeding

# of mice that did not occlude the injury

WT 3 out of 7

WT+DEL 10 out of 11

WT+DEL/C2773R 5 out of 9

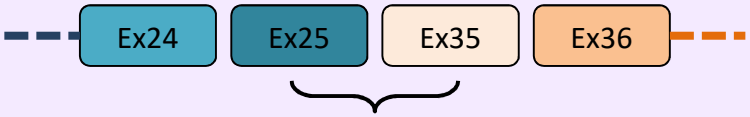
50% of antigen levels

normal multimer profile

bleeding risk similar to WT

Strategies to rescue the dominant negative effect of the DELETION mutant

- Use siRNA to specifically block expression of the dominant negative allele



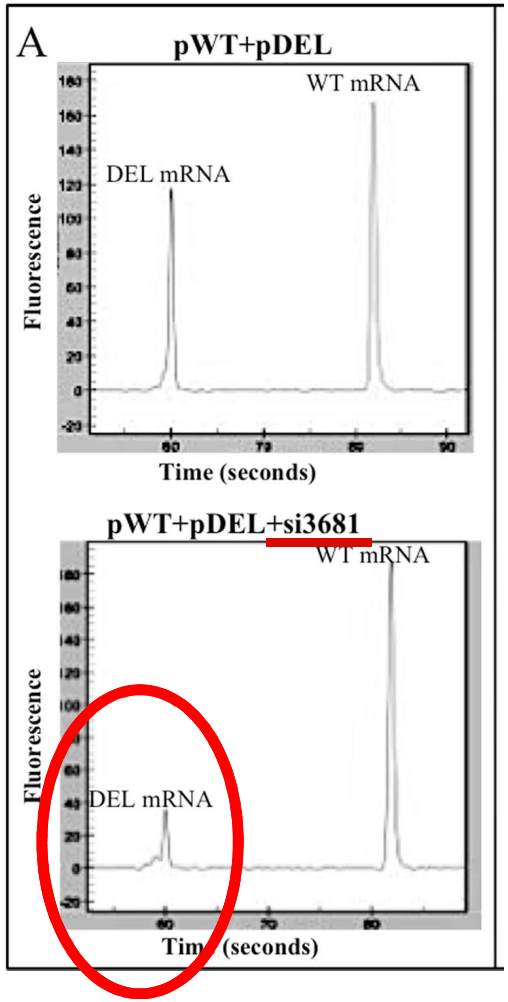
Mutant junction sequence connecting exons 25-35 is target of siRNA
WT-VWF not affected

Casari C et al. Blood 2010

Counteracting the dominant-negative effect: allele-specific RNAi -*in vitro*-

- Capillary electrophoresis of wild-type and deleted mRNA reverse-transcribed PCR products from transfected cells ± siRNA

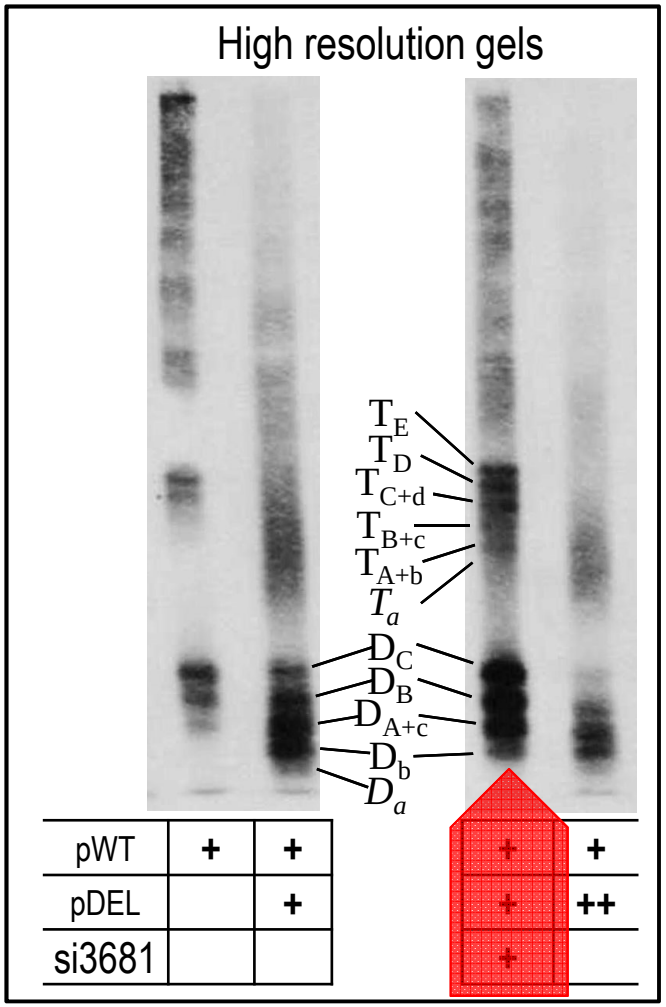
si3681 decreased DEL-VWF mRNA & did not affect WT-VWF mRNA



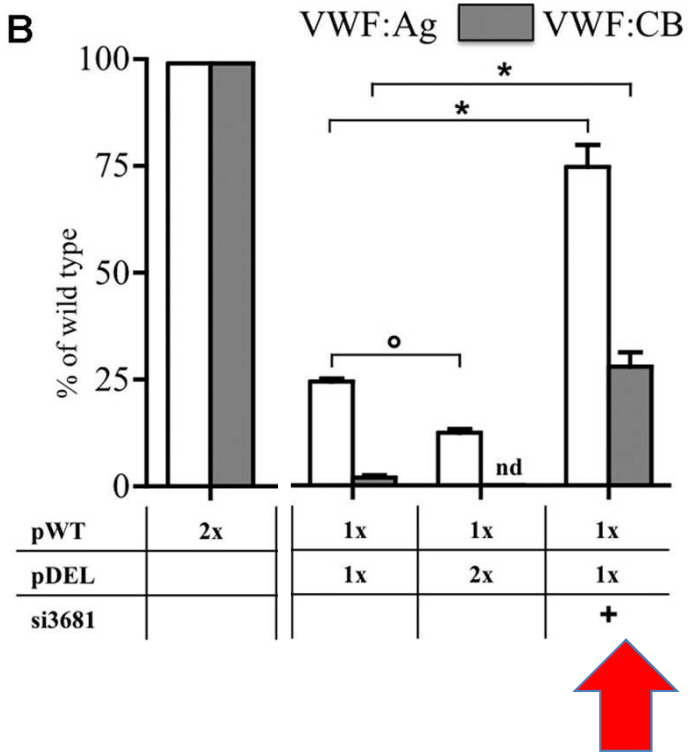
Selective decrease of the deleted mRNA and thus of the deleted VWF

Casari C et al. Blood 2010

Counteracting the dominant-negative effect: allele-specific RNAi -*in vitro*-



- Partial rescue of HMWMs &
- Increased intensity of bands containing wild type VWF



- Partial rescue of the antigen levels of VWF

Casari C et al. Blood 2010