Molecular characterization of Iranian patients with type 3 von Willebrand disease

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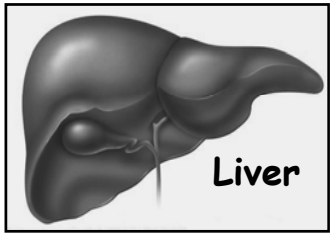
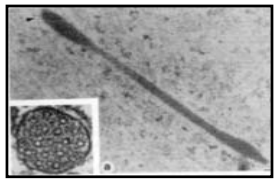
LIFE-CYCLE OF VON WILLEBRAND FACTOR (VWF)

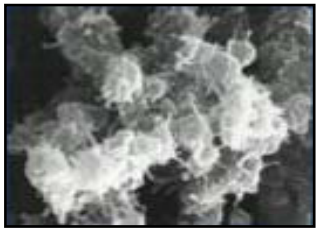


Clearance

Biosynthesis

Storage and secretion in plasma





Formation of the platelet thrombus

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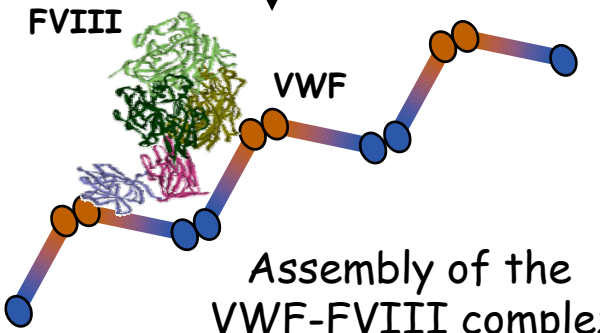

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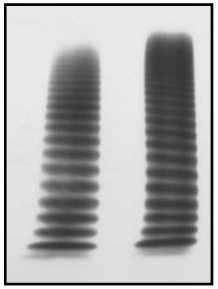

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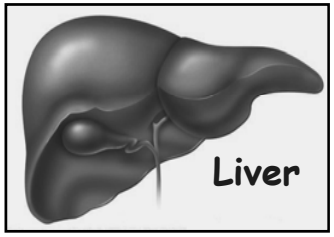

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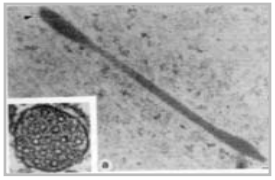

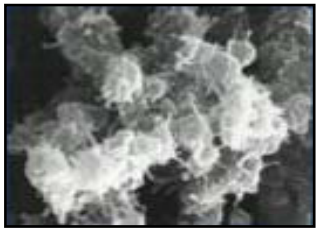

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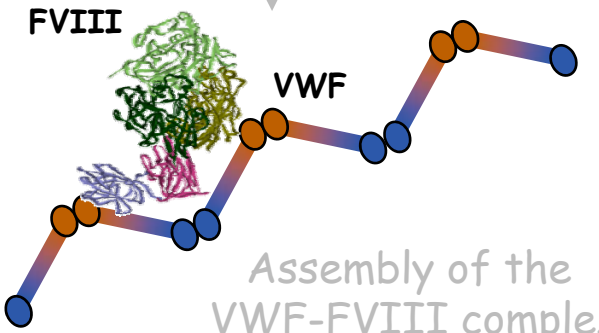

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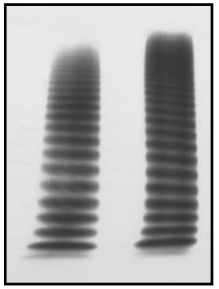
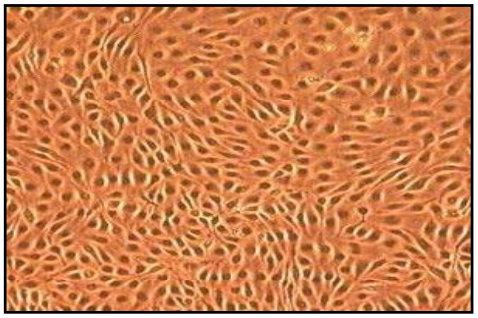
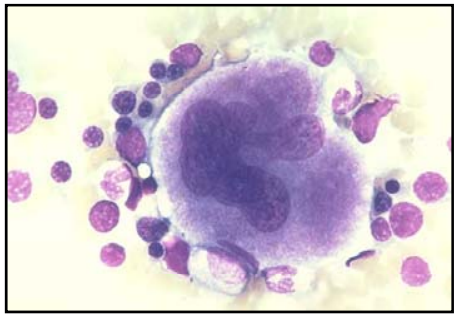


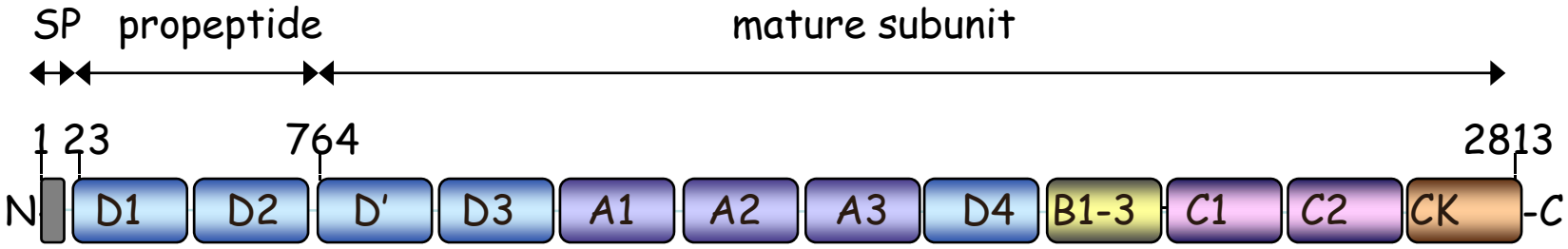
VWF BIOSYNTHESIS



Endothelial cells



megakaryocytes



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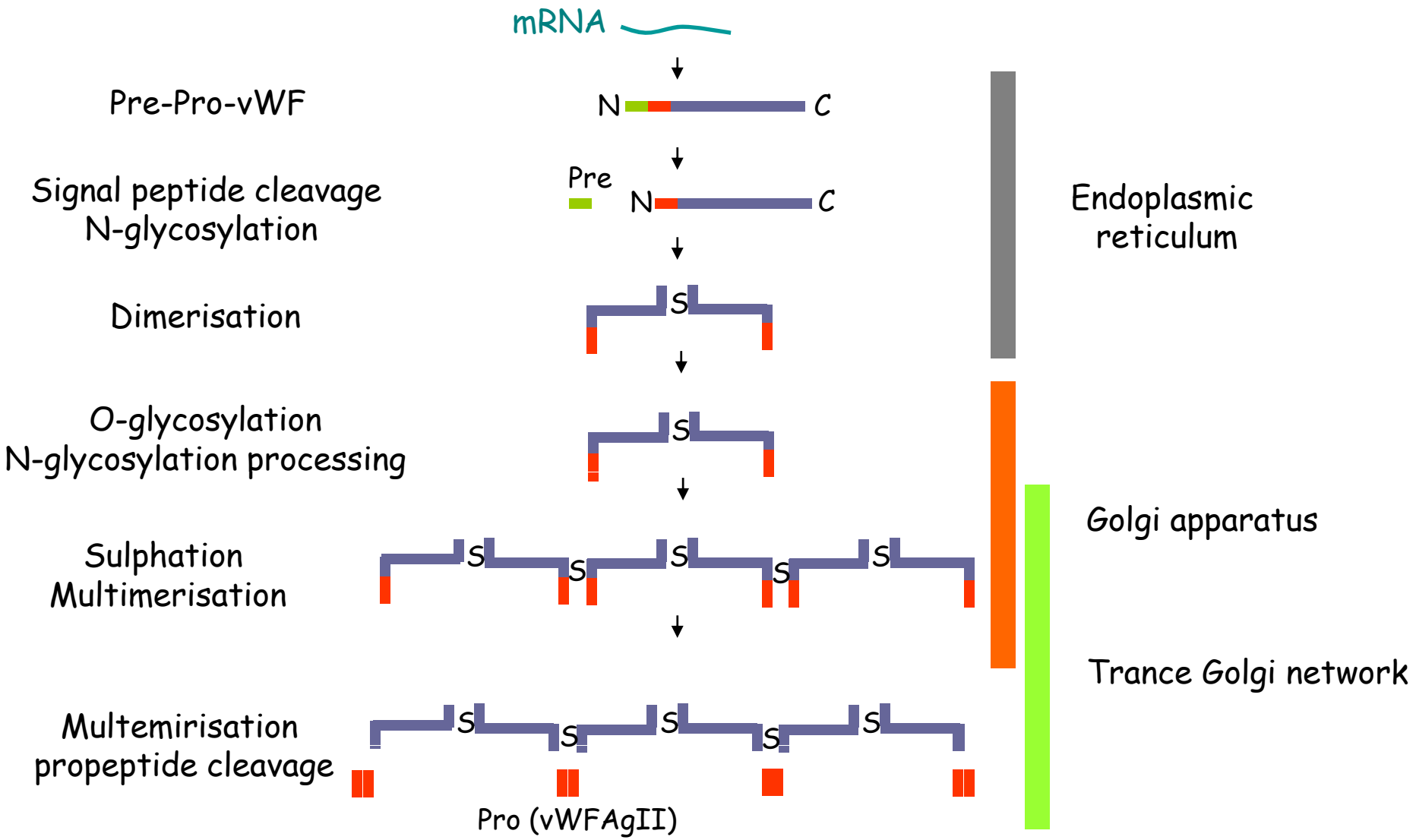

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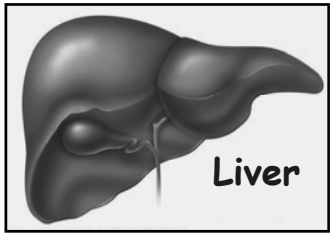

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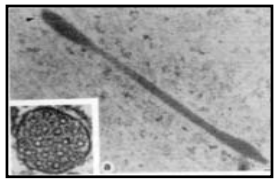

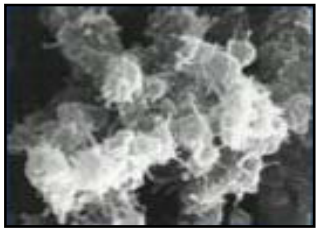

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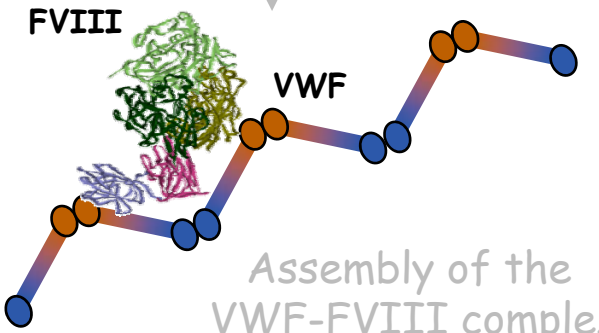

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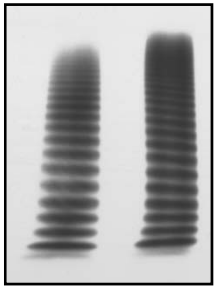

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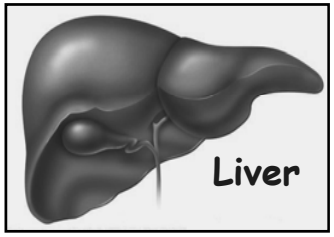

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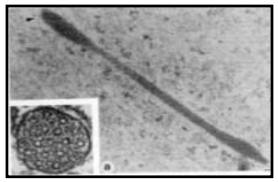

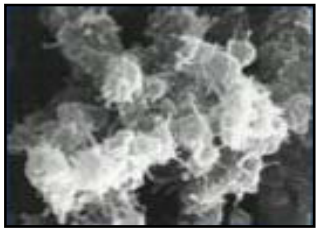

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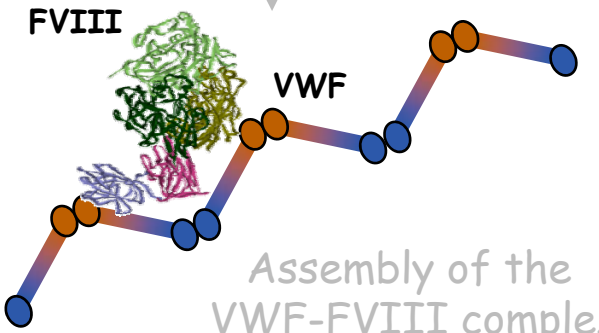

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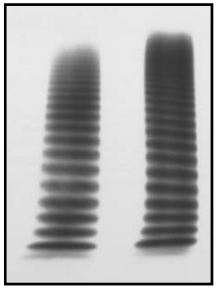

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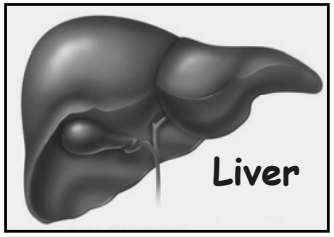

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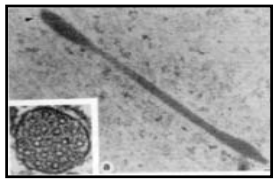

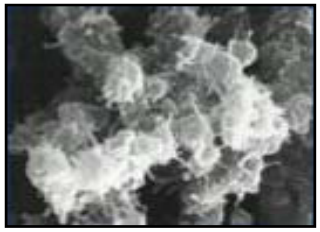

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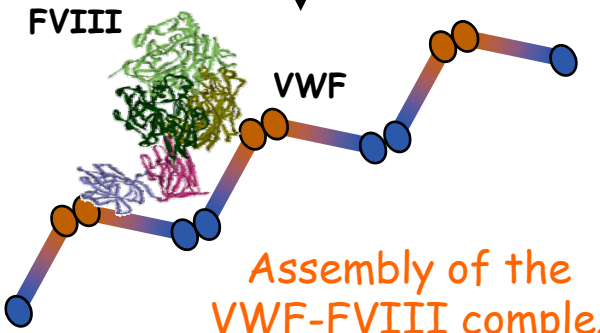

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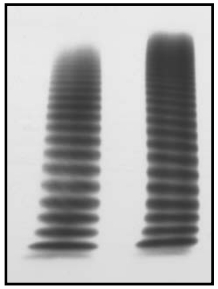

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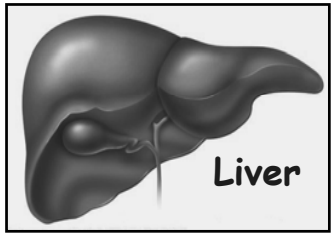

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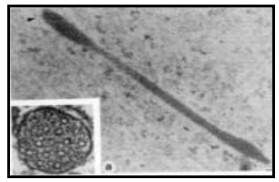

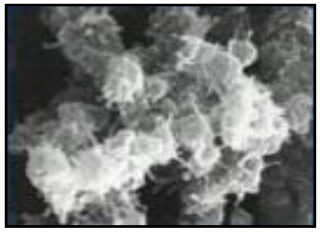

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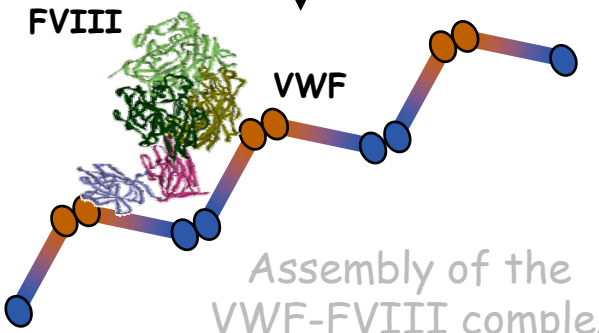

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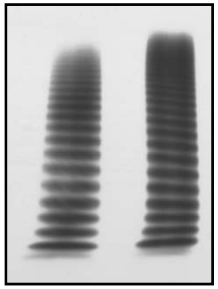

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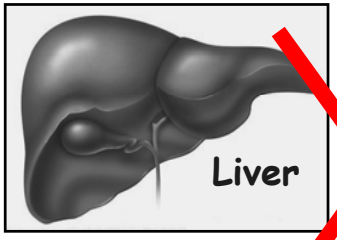

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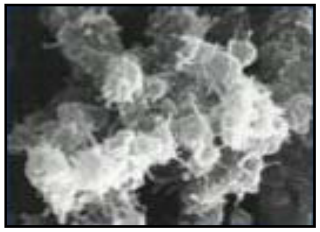
VON WILLEBRAND DISEASE



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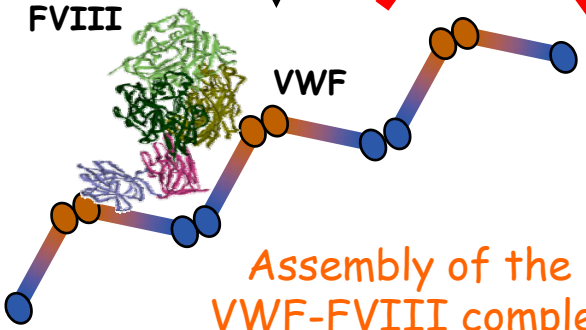

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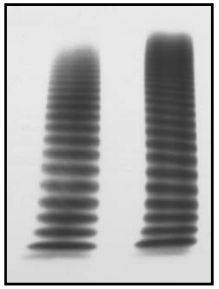


VON WILLEBRAND DISEASE (VWD)

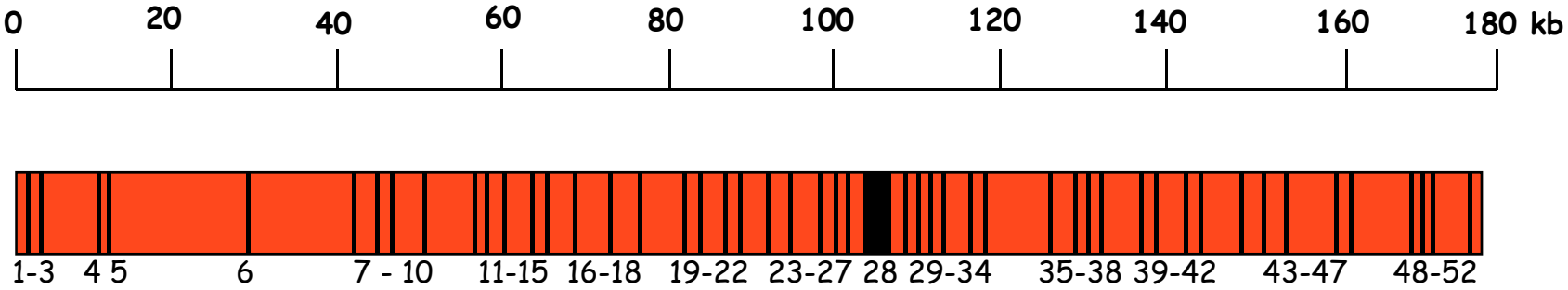
- VWD is the most common inherited bleeding disorder in human with a frequency of about 1:250

- Diagnosed by VWF antigen assay (VWF:Ag), the ristocetin cofactor assay (VWF:RCo) and FVIII pro-coagulant function (FVIII:C)

- Mutation detection helps in accurate diagnosis

VON WILLEBRAND FACTOR GENE

- VWF is encoded by a large gene of ~178kb.
- The gene contains 52 exons.
- It is located at the tip of the short arm of chromosome 12.



VON WILLEBRAND DISEASE (VWD)

VWD has 3 main types

TYPE 1

- ✓ Partial quantitative defects, accounting for ~80% of the cases
- ✓ Limited bleeding symptoms, normal activity of residual VWF

TYPE 2

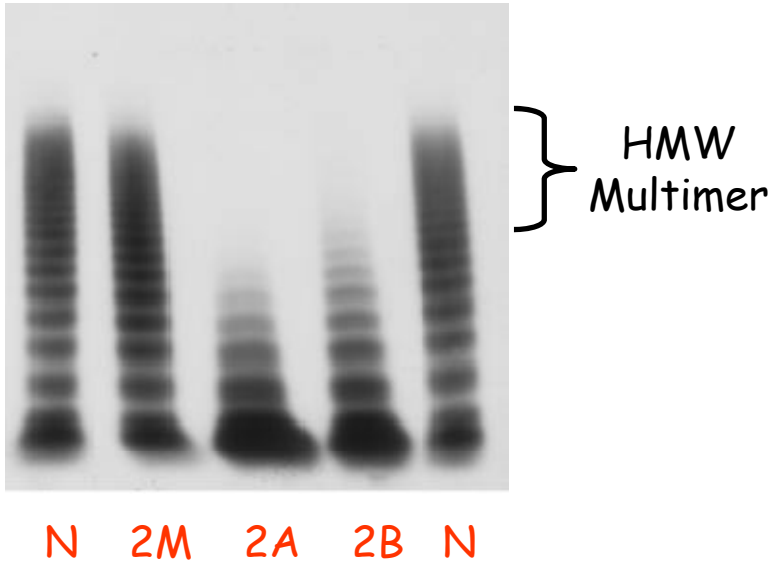
- ✓ Qualitative defects

2A = VWF is more susceptible to cleavage

2B = increased affinity of VWF for GPIb

2M = defective binding of VWF to GPIb

2N = decreased affinity of VWF for FVIII

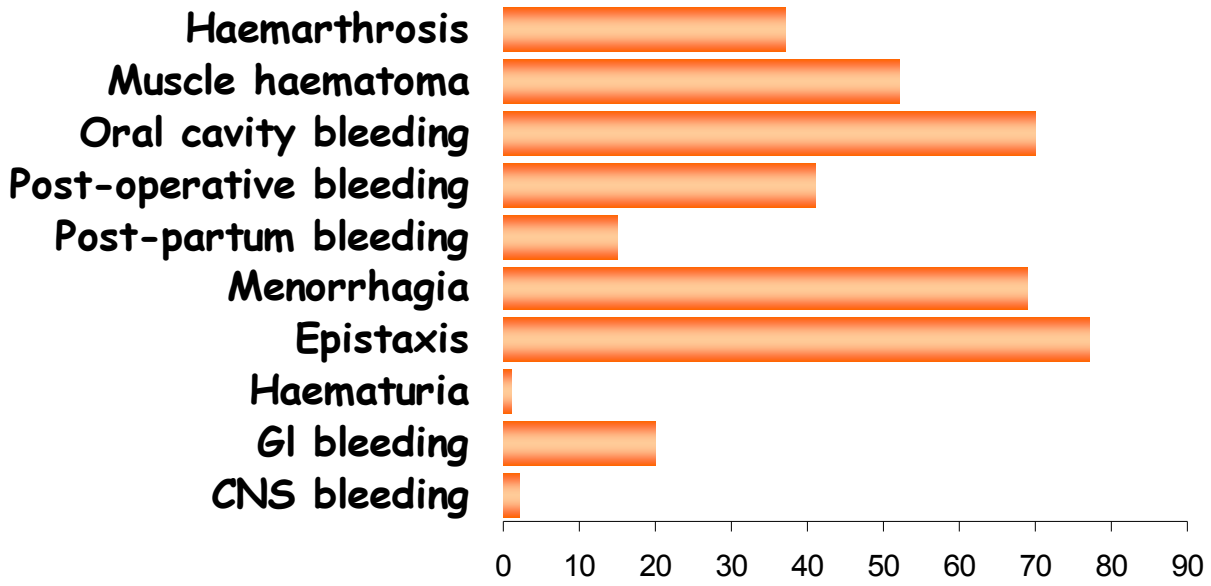


VWD TYPE 3

- Type 3 VWD is a severe autosomal-recessive form of the disease

- VWF levels is extremely low or undetectable and there is a moderate deficiency of FVIII (levels <10%)

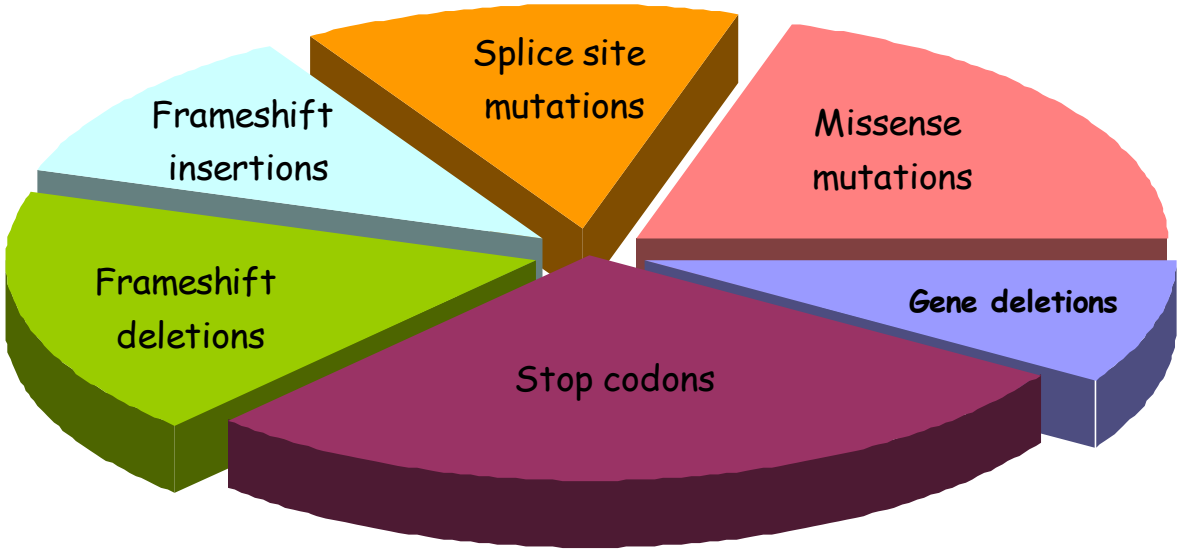
- The symptoms mostly consist of excessive mucocutaneous bleeding such as epistaxis and gingival bleeding



TYPE 3 VWD MUTATIONS

- Mutations may be scattered over the entire gene

- Mutations are mostly two 'null' alleles either in an homozygous or compound heterozygous state



http://www.ragtimedesign.com/vwf/mutation/mutationtableresults.php

VWD TYPE 3

- The prevalence of the disease ranges from 1/million to 2/million individuals globally

- In certain locations where consanguineous marriages are more frequent, the prevalence can be higher

- In 2000, a national registry of inherited coagulation disorders lists 600 Iranian patients, with a prevalence as high as 6/million

  Lak M, Peyvandi F, Mannucci P. M, British Journal of Haematology, 2000, 111, 1236-1239.

TYPE 3 VWD IN IRAN

N°	Sex/age (M/F year)	Nucleotide substitution	Amino acid substitution	Exon	Genotype
1	F/26	652C →T	Q218X	6	Homozygous
2	F/16	1093C →T	R365X	9	Homozygous
3	M/21	1930G →T	E644X	15	Homozygous
4	F/11	2116C →T	Q706X	16	Homozygous
5	F/20	3931C →T	Q1311X	28	Homozygous
6	F/52	4036C →T	Q1346X	28	Homozygous
7	F/36	4975C →T	R1659X	28	Homozygous
8	M/16	5941G →T	E1981X	35	Homozygous
9	M/8	139G→C	D47H	3	Homozygous
10	F/17	6520T →G	C2174G	37	Homozygous
11	M/19	1110-1G →A	FS	10	Homozygous
12	F/18	788del24	263del8	7	Homozygous
13	M/20	7674insC	FS	45	Homozygous
14	M/24	7683delT	FS	45	Homozygous

Baronciani L, Cozzi G, Canciani MT, Peyvandi F, Srivastava A, Federici AB, et al. *Blood Cells Mol Dis* 2003; **30**: 264-70.

ANALYSIS OF TYPE 3 VWD MUTATIONS

- ✓ Blood samples of ten Iranian patients with type 3 VWD were collected
- ✓ VWF gene evaluated by PCR – sequencing method in a 96-well plate format of PCR reaction for each patient to prevent mixing up of the DNA samples
- ✓ All exons and their splice site junctions and flanking regions were amplified
- ✓ At least 30 nucleotides of intronic sequence were included at both sides of each amplicon
- ✓ Primers were designed to ensure no amplification of the Pseudogene

ANALYSIS OF TYPE 3 VWD MUTATIONS

N°	Sex/age (M/F year)	VWF:Ag (U/dl)	Consanguinity	Nucleotide substitution	Amino acid substitution	Exon	Genotype
1	M/24	< 5	YES	5941 G→T	E1981X	35	Homozygous
2	F/25	< 5	NO				
3	M/35	< 5	YES	2443-1 G→C	Splice site	19	Homozygous
4	F/21	< 5	YES	310 C→T	Q104X	4	Homozygous
5	M/48	< 5	YES	2377 C→T	Q793X	18	Homozygous
6	F/29	< 5	YES	3237delA	P1079PfsX39	25	Homozygous
7	F/55	< 5	NO	2377 C→T	Q793X	18	Homozygous
8	F/35	< 5	YES	2377 C→T	Q793X	18	Homozygous
9	M/28	< 5	YES				
10	M/23	< 5	YES	1110-1 G→A	Splice site	10	Homozygous

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2	F/25	< 5	NO				
3	M/35	< 5	YES	2443-1 G→C	Splice site	19	Homozygous
4	F/21	< 5	YES	310 C→T	Q104X	4	Homozygous
5	M/48	< 5	YES	2377 C→T	Q793X	18	Homozygous
6	**F/29**	**< 5**	**YES**	**3237delA**	**P1079PfsX39**	**25**	**Homozygous**
7	F/55	< 5	NO	2377 C→T	Q793X	18	Homozygous
8	F/35	< 5	YES	2377 C→T	Q793X	18	Homozygous
9	M/28	< 5	YES				
10	M/23	< 5	YES	1110-1 G→A	Splice site	10	Homozygous

ANALYSIS OF TYPE 3 VWD MUTATIONS

N°	Sex/age (M/F year)	VWF:Ag (U/dl)	Consanguinity	Nucleotide substitution	Amino acid substitution	Exon	Genotype
1	M/24	< 5	YES	5941 G→T	E1981X	35	Homozygous
2	F/25	< 5	NO				
3	**M/35**	**< 5**	**YES**	**2443-1 G→C**	**Splice site**	**19**	**Homozygous**
4	F/21	< 5	YES	310 C→T	Q104X	4	Homozygous
5	M/48	< 5	YES	2377 C→T	Q793X	18	Homozygous
6	F/29	< 5	YES	3237delA	P1079PfsX39	25	Homozygous
7	F/55	< 5	NO	2377 C→T	Q793X	18	Homozygous
8	F/35	< 5	YES	2377 C→T	Q793X	18	Homozygous
9	M/28	< 5	YES				
10	M/23	< 5	YES	1110-1 G→A	Splice site	10	Homozygous

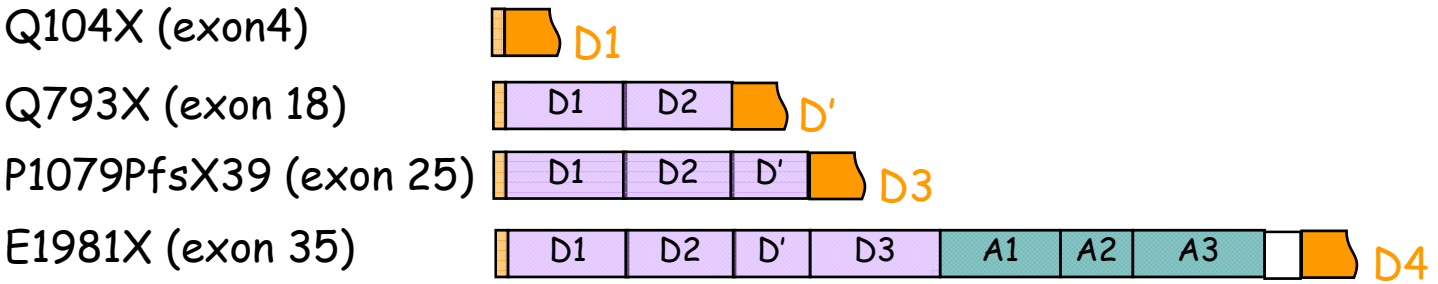
SUMMARY

- ✓ Three entirely new mutations were identified: Q793X, Q104X and 3237delA
- ✓ The Q793X mutation appears relatively common since it was found in three unrelated patients
- ✓ The splice site mutation 24431-1 G➲C had never been identified in an Iranian patient before
- ✓ No inhibitor was detected in these patients

DISCUSSION

The mutations didn't show any correlation with either bleeding severity or developing of inhibitory anti-VWF antibody

Irrespective of the position of the mutations, similar symptoms were observed in type 3 VWD patients:



There are no difference in patients symptoms.

DISCUSSION

There are two hypotheses :

- ✓ Mutations might act by causing intracellular degradation of mutated VWF

- ✓ A mechanism called nonsense-mediated decay pathway degrades mRNA which carries a premature stop codon

DISCUSSION

- In a canine VWD study, following a null allele (255delC) in exon 4, no VWF mRNA nor truncated proteins, were detected in the aortic endothelial cells
(*Haberichter, et al., 2005*)

- A homozygous mutation in exon 45 (7636A➲T) in a type 3 VWD patient strongly reduced the mRNA of mutant allele
(Eikenboom, et al., 1992)

- A homozygous mutation in exon 18 (2430delC) in a type 3 VWD patient caused no decrease in mRNA level
(Mohlke, et al., 1996)

DISCUSSION

The presence of mutant or truncated VWF could be of critical importance

VWF is required for Weibel-Palade body formation

Proteins reported to be present in the **Weibel-Palade bodies**

- VWF and VWF propeptide
- P-selectin
- CD63
- Interleukin-8
- Tissue-type plasminogen activator
- Endothelin
- a1,3-fucosyltransferase VI
- Angiopoietin-2
- Osteoprotegerin

DISCUSSION

Are mutant or truncated VWF capable to potentially form WPBs?

Are type 3 VWD patients faced to more complications ?

- ✓ Inflammation (P-selectin, Ang-2)
- ✓ Angiogenesis (Ang-2)
- ✓ Bone physiology (OPG)

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