SELEZIONE PUBBLICA N. 2025N59, PER ESAMI, PER L'ASSUNZIONE A TEMPO INDETERMINATO E PIENO DI N. 1 PERSONA NELL'AREA DEI FUNZIONARI, SETTORE SCIENTIFICO-TECNOLOGICO, PRESSO L'UNIVERSITÀ DEGLI STUDI DI PADOVA. TECNICO DI LABORATORIO SPECIALISTA IN MALATTIE TROMBOTICHE-EMORRAGICHE.

QUESITI COLLOQUIO

ELENCO A

1. TEST DI LABORATORIO PER LA DIAGNOSI DEL LUPUS ANTICOAGULANT
2. ESPRESSIONE IN VITRO SU COLTURE CELLULARI DEI MUTANTI DEL FATTORE VIII

3. Dall'articolo "**Partial F8 gene duplication (factor VIII Padua) associated with high factor VIII levels and familial thrombophilia"** Paolo Simioni, Stefano Cagnin, Francesca Sartorello Gabriele Sales, Luca Pagani, Cristiana Bulato, Sabrina Gavasso, Francesca Nuzzo Francesco Chemello, Claudia M. Radu, Daniela Tormene, Luca Spiezia, Tilman M. Hackeng, Elena Campello, and Elisabetta Castoldi

THROMBOSIS AND HEMOSTASIS

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KEY POINTS

- We report a partial *F8* duplication associated with markedly elevated FVIII levels and venous thrombosis in 2 Italian families.
- This duplication contains transcriptional activators that may be involved in the upregulation of *F8* messenger RNA and protein expression.

High coagulation factor VIII (FVIII) levels comprise a common risk factor for venous thromboembolism (VTE), but the underlying genetic determinants are largely unknown. We investigated the molecular bases of high FVIII levels in 2 Italian families with severe thrombophilia. The proband of the first family had a history of recurrent VTE before age 50 years, with extremely and persistently elevated FVIII antigen and activity levels (>400%) as the only thrombophilic defects. Genetic analysis revealed a 23.4-kb tandem duplication of the proximal portion of the *F8* gene (promoter, exon 1, and a large part of intron 1), which cosegregated with high FVIII levels in the family and was absent in 103 normal controls. Targeted screening of 50 unrelated VTE patients with FVIII levels ≥250% identified a second thrombophilic family with the same *F8* rearrangement on the same genetic background, suggesting a founder effect. Carriers of the duplication from both families showed a twofold or greater upregulation of *F8* messenger RNA, consistent with the presence of open chromatin signatures and enhancer elements within the duplicated region. Testing of these sequences in a luciferase reporter assay pinpointed a 927-bp region of *F8* intron 1 associated with >45-fold increased reporter activity in endothelial cells, potentially mediating the *F8* transcriptional enhancement observed in carriers of the duplication. In summary, we report the first thrombophilic defect in the *F8* gene (designated FVIII Padua) associated with markedly elevated FVIII levels and severe thrombophilia in 2 Italian families. (*Blood.* 2021;137(17):2383-2393)

Leggere e tradurre il seguente paragrafo

FVIII, which was long believed to be produced by hepatocytes, is actually mainly synthesized in (liver sinusoid) endothelial cells and secreted in plasma as an inactive precursor. Von Willebrand factor (VWF) serves as a carrier of FVIII and prolongs its half-life in the circulation. After proteolytic activation, FVIIIa acts as an essential cofactor of FIXa in the activation of FX, playing a pivotal role in the amplification of blood coagulation. FVIII is encoded by the F8 gene (186 kb; 26 exons), which is located on the long arm of chromosome X (Xq28).

4. IN CHE MODO GARANTIREBBE LA CORRETTA GESTIONE, CONSERVAZIONE E SICUREZZA DEI DATI GENETICI E BIOINFORMATICI PRODOTTI DURANTE LE ANALISI, NEL RISPETTO DELLE BUONE PRATICHE DI LABORATORIO E DELLA NORMATIVA VIGENTE?

ELENCO B

1. TEST DI LABORATORIO PER LA DIAGNOSI DI EMOFILIA ACQUISITA
2. ESPRESSIONE E PURIFICAZIONE DI PROTEINE RICOMBINANTI MUTANTI DELLA PROTROMBINA

3. Dall'articolo "**Partial F8 gene duplication (factor VIII Padua) associated with high factor VIII levels and familial thrombophilia"** Paolo Simioni, Stefano Cagnin, Francesca Sartorello Gabriele Sales, Luca Pagani, Cristiana Bulato, Sabrina Gavasso, Francesca Nuzzo Francesco Chemello, Claudia M. Radu, Daniela Tormene, Luca Spiezia, Tilman M. Hackeng, Elena Campello, and Elisabetta Castoldi

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FVIII is the missing factor in hemophilia A, a recessive X-linked bleeding disorder affecting ;1 in 5000 males. More than 2000 different F8 gene mutations responsible for hemophilia A have been reported, including several F8 gene rearrangements.

4. COME DOCUMENTEREBBE E RENDEREBBE RIPRODUCIBILE L'ANALISI BIOINFORMATICA SVOLTA, IN MODO CHE I RISULTATI POSSANO ESSERE VERIFICATI O RIUTILIZZATI DA ALTRI OPERATORI DEL LABORATORIO?

ELENCO C

1. I TEST PER LO STUDIO DELLA AGGREGAZIONE PIASTRINICA NEI PAZIENTI EMORRAGICI: METODICHE ED INTERPRETAZIONE
2. ESPRESSIONE IN VITRO SU COLTURE CELLULARI DEI MUTANTI DEL FATTORE IX

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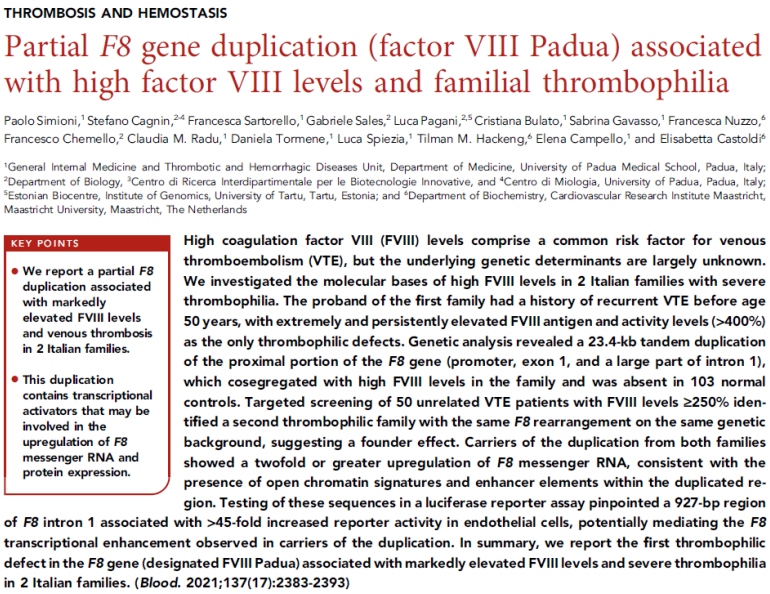

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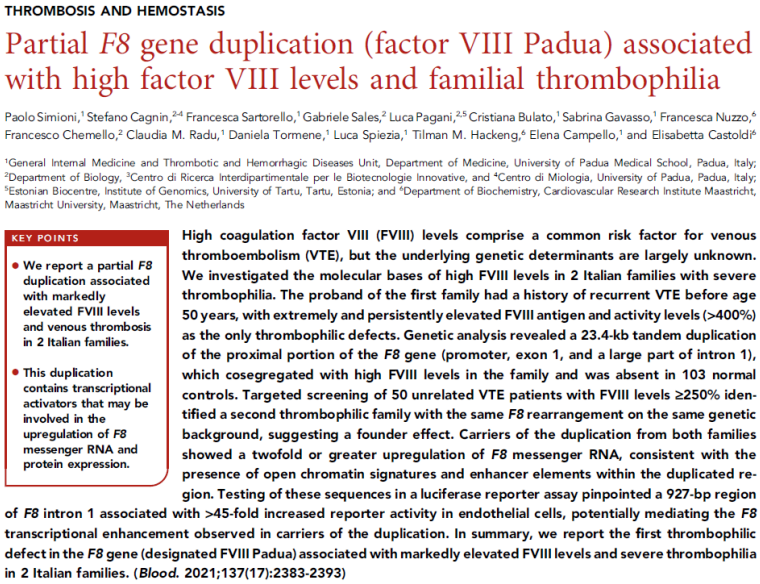
Whereas FVIII deficiency leads to bleeding, high FVIII levels have been consistently and dose-dependently associated with an increased risk of VTE. In the Leiden Thrombophilia Study, it was estimated that participants with FVIII clotting (FVIII:C) $150%, representing ;11% of the healthy population, had a fivefold increased risk of a first episode of VTE, and similar findings have been reported for recurrent VTE. Therefore, high FVIII levels represent a prevalent and relatively strong predisposing factor for VTE.

4. QUAL È, SECONDO LEI, IL RUOLO DEI SOFTWARE BIOLOGICI E DEI DATABASE GENETICI NELLO STUDIO DEL RAPPORTO GENOTIPO-FENOTIPO E NEL SUPPORTO ALLA RICERCA TRASLAZIONALE O CLINICA?

ELENCO D

1. I TEST COAGULOMETRICI E CROMOGENICI NELLA DIAGNOSI DI EMOFILIA A E B
2. DIFETTI GENETICI PROTROMBOTICI DEL FATTORE VIII ED ESPRESSIONE IN VITRO

3. Dall'articolo "**Partial F8 gene duplication (factor VIII Padua) associated with high factor VIII levels and familial thrombophilia"** Paolo Simioni, Stefano Cagnin, Francesca Sartorello Gabriele Sales, Luca Pagani, Cristiana Bulato, Sabrina Gavasso, Francesca Nuzzo Francesco Chemello, Claudia M. Radu, Daniela Tormene, Luca Spiezia, Tilman M. Hackeng, Elena Campello, and Elisabetta Castoldi



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Elevated FVIII levels tend to cluster in families and have an estimated heritability of 30% to 60%. The best-known determinants of FVIII levels are the ABO blood group and VWF levels,29 but additional genes affecting FVIII levels have recently been identified via genome-wide association studies. In contrast, there is limited evidence for an effect of common genetic variation in the F8 gene on FVIII levels. However, it has been reported that an increased copy number of the F8 gene is associated with higher FVIII levels and VTE occurrence.

4. COME VERIFICA L'AGGIORNAMENTO, L'AFFIDABILITÀ E LA CORRETTA INTERPRETAZIONE DEI DATI OTTENUTI DA DATABASE GENETICI PUBBLICI?