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ABSTRACT BOOK

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Blood Transfusion - Bimestrale spedizione in abbonamento postale 70% - Poste Italiane SpA LO/MI

of the PT group. About 6% of the study population had both FVL and the non-O group while only 1.5% had both PTM and the non-O group. The VTE risk was considerably increased in FVL and non-O group (OR 5.74, 95%CI 5.16-6.39; $p < 0.0001$), rather than if just one of the two was present (FV wild type/non-O group: OR 1.78, 95%CI 1.68-1.88; FVL/O group: OR 2.99, 95%CI 2.58-3.47). The corresponding population attributable risk of VTE (PAR) is about 19%. Similarly, risk of VTE was significantly higher in patients with PTM and non-O group (OR 3.27; 95%CI 2.44-4.37; $p = 0.002$), although PAR was considerably lower, about 2%.

Conclusions. The main finding of our metanalysis is that simultaneous presence of FVL and non-O group is a frequent condition and the resulting increased risk of VTE could have clinical impact and prompt therapeutic adjustments. Instead, the association between the prothrombin mutation and the non-o blood group seems to play a less important role in the incidence of VTE.



OC011 - Spontaneous readthrough over recurrent F8 nonsense mutations is associated with residual factor VIII levels: implications for inhibitor risk?

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Background. Nonsense mutations, caused by premature termination codons (PTCs), are frequently associated with “null phenotypes”. However, a process named “ribosome readthrough” may lead to the suppression of the PTC resulting in the synthesis of a full-length protein through incorporation of an amino acid at the aberrant stop position. This might explain why some F8 nonsense mutations are associated with moderate

haemophilia A (HA). The main aim is to evaluate the production of full-length factor VIII (FVIII) arising from the occurrence of readthrough over a wide panel of F8 nonsense mutations.

Methods. Transient expression studies with an optimized chimeric protein consisting of FVIII and a high-sensitivity luciferase in HEK293 cells, and luciferase assays in media and cell lysates. Luciferase activity detected by this sensitive system is directly related to the amount of the full-length luciferase-containing fusion protein as a result of suppression of F8 nonsense mutations. Evaluation of FVIII traces in HA patient plasma through ELISA assays and Western blotting analysis.

Results. F8 nonsense mutations reported in the international database were rationally classified into two groups: a high-frequency group (12 mutations; patient number $n > 10$), resulting from the highly frequent CGA(arginine)>TGA substitution, and a low-frequency group (32 mutations; patient number $n = 1-5$). The latter was further divided into a subset including mutations predicted to be suppressed by reinsertion of the original amino acid, and mutations localized in the B-domain, in which potential amino acid changes inserted during readthrough are predicted to be tolerated. Noticeably, the selected mutations (44 out of 216) have been reported in 384/611 (63%) HA patients.

Strikingly, expression of all F8 nonsense variants led to detectable luciferase activity with a different extent (0.3-7%). These results appeared to be consistent with the impact of the inserted amino acid on FVIII biosynthesis and secretion. Preliminary results revealed trace levels of FVIII in plasma from Italian HA patients affected by 10 of the investigated nonsense mutations.

Conclusions. Data from our expression platform indicate that a relevant number of F8 nonsense mutations, relatively frequent in HA, undergo readthrough and can be associated with residual FVIII protein levels. This might have relevant pathophysiological implications, and might also contribute to interpret the variable susceptibility of HA patients to develop inhibitors upon replacement therapy.