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Associazione Italiana dei Centri Emofilia - AICE

Hrvatsko Društvo za Transfuzijsku Medicinu - HDTM

Sociedad Española de Transfusión Sanguínea y Terapia Celular - SETS

Società Italiana per lo Studio dell'Emostasi e della Trombosi - Siset

ABSTRACT BOOK

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was explained by missense changes permitting FVII secretion and function.

Conclusions. Our experimental findings support the notion that the extent of functional readthrough contributes to the variable bleeding phenotype in patients homozygous for F7 nonsense mutations, possibly preventing null conditions even for the most readthrough-unfavorable mutations.

OC078

MUTATION-SPECIFIC CONTRIBUTIONS TO TRACE FACTOR X LEVELS ACCOUNT FOR A LIFE-THREATING PHENOTYPE IN A COMPOUND HETEROZYGOUS FACTOR X DEFICIENT PATIENT

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Background. No factor X (FX)-deficient patients have been reported with complete deletion of F10 genes, or harbouring mutations leading to the total absence of FX activity. Further, F10 gene knockout in mice is lethal. However, a few severe FX deficient infants with life-threatening symptoms have been reported, which might underlie subtle amounts of FX ensuring a minimal haemostasis.

Here, we characterized the novel missense (p.L251P) and nonsense (p.W461X, TAG stop codon) mutations, found in a compound heterozygous proposita, affected at birth by major bleeding symptoms, with radiological evidence of cerebral microbleeds, and currently on prophylaxis with a plasma-derived FX concentrate.

Methods. Expression of recombinant FX (rFX) variants, evaluation of rFX antigen (Ag) secreted in medium and thrombin generation (TG) assays in the presence of specific FX (Fondaparinux) and anti-coagulant (anti-TFPI aptamer) inhibitors.

Results. Residual FX antigen and coagulant activity (PT and aPTT) levels in proposita's plasma were below 1%. Both coagulation parameters in parents were decreased in accordance with their heterozygosity (mother, p.W461X; father, p.L251P). We expressed the corresponding recombinant variants in HEK293 cells. As compared to wild-type rFX (rFX-wt), we detected in conditioned media low Ag levels (rFX-251P, 0.6±0.2%; rFX-461X, 1.3±0.1%) and very low (rFX-251P, 21 min) or not detectable (rFX-461X) ability to shorten TG lag time in FX-deficient plasma (25 min).

Moreover, the missense variants, potentially arising from the spontaneous suppression of the nonsense mutation p.W461X (TAG codon), do not support a functional impact of translational readthrough: the rFX-461Y (TAC) and rFX-461Q (CAG) variants showed very low secretion (Ag: 3.6±1.2% and 1.0±0.3%, respectively) and barely detectable TG activity (19 and 20 min lag times), as compared with 0.5% rFX-wt (14 min) and FX-deficient plasma (25 min).

The residual TG capacity of the rFX-251P variant was FX-dependent, as indicated by Fondaparinux inhibition, which

made TG undetectable, and by inhibition of TFPI (anti-TFPI aptamer), which shortened the TG lag time (from 26 to <24 min).

Conclusions. Our experimental approaches, which contribute to the knowledge of the very severe FX deficiency forms, suggest that i) the p.W461X nonsense mutation, truncating the FX carboxyl-terminal region involved in interactions with prothrombin, produced very low levels of FX variants with negligible activity even after translational readthrough, and ii) the p.L251P missense mutation would contribute, during the foetal and perinatal life, to the trace FX levels and the associated minimal FX-dependent TG capacity. This residual function is candidate to shape the life-threatening, but non-perinatally lethal, phenotype of the proposita.

OC079

RESCUE OF MISSENSE AND SPLICING MUTATIONS IN HAEMOPHILIA A BY A UNIQUE EXON SPECIFIC U1snRNA

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Background. In Haemophilia A (HA) patients, splicing mutations account for about 8-10% of all, a significant proportion if considered that they are largely underestimated. In fact, also the abundant missense mutations can trigger, besides potentially influencing protein biology, aberrant splicing by affecting splicing regulatory elements. The knowledge of molecular mechanisms leading to aberrant splicing led us to the development of new RNA-therapeutics, in particular the engineered spliceosomal U1snRNA targeting specific intronic sequences downstream of donor splice sites (Exon Specific U1, ExSpeU1). This approach enabled us to rescue mutations at donor (5'ss) or acceptor (3'ss) splice sites, as well as within exons. Here, the aim is to characterize the impact on splicing of different HA-causing mutations occurring in F8 exon 19 (at splice sites or within the exon) and develop ExSpeU1 able to correct them for therapeutic purposes.

Methods. Expression of F8 minigenes in human hepatoma cells and dissection of splicing pattern by RT-PCR. Evaluation of ability of ExSpeU1, or the first generation U1, to rescue splicing.

Results. The F8 variants c.6115+1G>A, c.6115+2T>C, c.6115+3G>T, c.6115+4A>G, c.6115+5G>A and c.6115+6T>A, expressed in HepG2 cells, led to complete skipping of exon 19. On the other hand, c.6115+9C>G, c.6113A>G (p.N2038S), c.6037G>A (p.G2013R), c.6053A>G (p.E2018G), c.6108C>T (p.Y2036Y) and c.5999G>C (p.G2000A) impaired to various extent exon 19 inclusion (exon skipping), with variable amounts of correct transcript. The impact of missense changes on protein function showed that the p.N2038S and p.G2013R impair both protein secretion and function (<10% of F8 wt). Notably, the p.E2018G has no effect on protein levels. By screening a panel of ExSpeU1s, we identified a unique ExSpeU1 (U1^{sl}) able to rescue both different splicing-defective mutations, either at splice sites or exonic. In particular, the U1^{sl} promoted (from 0 to ~40%) exon inclusion in presence of c.6115+3G>T, c.6115+4A>G and c.6115+6T>A variants. Notably, the U1^{sl}-mediated rescue, in presence of p.G2013R, p.N2038S and