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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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be presumably associated with BAV. In Family 3, of variants (n=4) with minor allele frequency(MAF)<0.02 identified in LTBP4(p.D519_P522del),COL6A3(p.P3082R),ABCC6(p.R724K) and LTBP1(p.S1266F)genes, none has been shown to segregate with BAV.

Conclusions. Segregation analyses on these BAV families unravelled the potential role of some genetic variants as major determinants of the disease while others can be assumed to have at most a modifier role. These data suggest the multifactorial nature of BAV disorder and its complications and highlight the importance of recruiting families with inherited predisposition to BAV in order to better define and demonstrate specific associations between genetic variants and the phenotype.

OC082

SECRETION OF WILD-TYPE FACTOR IX UPON READTHROUGH OVER F9 PRE-PEPTIDE NONSENSE MUTATIONS CAUSING HAEMOPHILIA B

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Background. Nonsense mutations, which introduce premature termination codons (PTCs), are a relatively frequent cause of haemophilia. However, the pharmacological induction of ribosome readthrough over the PTC can lead to the synthesis of a full-length protein, with potential therapeutic implications. A relevant readthrough-mediated rescue of functional protein levels requires a complex interplay between favorable mRNA sequence features and the type of amino acid introduced at the PTC. In this view, nonsense mutations occurring in sequences encoding for protein regions that are poorly conserved, would represent ideal candidates for therapeutic readthrough. In particular, suppression of nonsense mutations affecting the pre-peptide regions of secreted proteins, that are intracellularly removed, would make negligible the impact of missense changes on secretion and function of the mature protein. Therefore in this study we selected three F9 nonsense mutations affecting the pre-peptide and pro-peptide regions of coagulation factor IX (FIX), which cause haemophilia B (HB): the p.G21X (TGA-T) in the variable hydrophobic core, the p.C28X (TGA-A) at the intracellular cleavage site and the p.K45X (TAG-A), affecting the pro-peptide cleavage site.

Methods. Recombinant FIX (rFIX) nonsense and missense variants were transiently expressed in human embryonic kidney 293 cells. Protein levels and isoforms were evaluated by ELISA and Western Blotting, respectively. Pro-coagulant activity of rFIX was evaluated by aPTT-based assays.

Results. Expression of the rFIX-28X and rFIX-45X nonsense variants did not result in appreciable secreted FIX levels. Conversely, the full-length FIX form was clearly detectable from rFIX-21X expressing cells, with estimated levels of 0.4±0.3% of rFIX-wt. The rFIX-21X also significantly responded (secretion, 4.1±0.5% of wild-type; coagulant activity, 4.0±0.3%) to the readthrough-inducer geneticin, while rescue of the p.C28X and p.K45X was prevented by constraints of adjacent cleavage sites. Strikingly, for the p.G21X mutation,

the resulting specific coagulant activity (0.96±0.11) was compatible with normal function, thus suggesting secretion of FIX with wild-type features upon readthrough and removal of pre-peptide. Expression of the predicted readthrough-deriving missense variants (Gly21Trp/Cys/Arg) revealed a preserved specific activity (ranging from 0.84 to 0.98), thus supporting our observation.

Conclusions. Overall, our data indicate that suppression of nonsense mutations in the pre-peptide core preserves mature protein features and specifically identified the p.G21X nonsense mutation as candidate for treatment with readthrough-inducing agents. More generally, this rational approach could be extended to nonsense mutations in protein regions removed during processing and moderately conserved (activation peptides, B-domain), in order to select responsive mutations, and thus patients, to be challenged with readthrough-inducing drugs.

OC083

FACTOR VIII PLAYS A DIRECT ROLE ON BONE CELLS

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Background. Bone disease is a significant complication of haemophilia A (HA). However, the pathophysiology is not exactly known and both processes of bone resorption and bone formation could be hypothesized to be altered. Bone cells express protease-activated receptors (PARs) and FVIII deficiency could play a role also by decreasing thrombin production. The aim of this study concerned the identification of the specific role played by FVIII, von Willebrand Factor (VWF) and thrombin on osteoclasts and osteoblasts.

Methods. *In vitro* assays assessed osteoclastogenesis and osteoclasts proteases activity in the presence of the different coagulation factors (plasma derived VWF/FVIII complex, human recombinant VWF, human recombinant full length FVIII and thrombin). The activity of cathepsin K and matrix metalloproteinase (MMP)-9 was evaluated by real time expression analysis on mature osteoclasts treated for 48h with the aforementioned coagulation factors. Human osteoblasts differentiation was assessed by real time expression analysis of Runx2 and Alkaline Phosphatase (ALP).

Results. The osteoclastogenesis process is strongly inhibited (~45%) by the presence of the VWF and even more if is complexed with FVIII (53% inhibition). Thrombin seems to play an inhibitory, although variable, role (30-50% inhibition). No statistically significant alterations of Cathepsin K and MMP9 expression were revealed in treated mature osteoclasts. Osteoblast differentiation process is affected by coagulation factors. The presence of FVIII, alone or complexed with VWF, leads to an increase expression of ALP (2-fold) and Runx2 (~1.5 fold) compared than controls. In the same way, thrombin