similar effects. Results from these studies will provide insight into the development of osteoporosis in hemophilia A patients.

PB0828 | Prediction of the Response to FVIII Treatment in Haemophilia A

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Background: Thrombin generation (TG) is frequently measured in haemophilia patients to evaluate their baseline TG level, to monitor their response to therapy and to follow up patients in time. TG is also being used to compare efficacy and potency of potentially new treatment drugs. Many research groups use plasma from healthy controls spiked *in vitro* with an antibody (Ab) in order to mimic haemophilia plasma.
Aims: Our goal was to evaluate whether these *in vitro* mimicked samples have the same TG kinetics as haemophilia A samples and therefor are suitable for research purposes.
Methods: The Calibrated Automated Thrombogram was used to measure TG in plasma samples from healthy controls and haemophilia patients. Samples were spiked *in vitro* with recombinant FVIII (Kogenate, Bayer) and/or FVIII-Ab (PAHFVIII-S, HaemTech). Haemophilia A plasma was spiked with a buffer or with 100% FVIII and 15 μg/ml FVIII-Ab (to mimic haemophilia A again in the presence of the Ab). Plasma from healthy controls was spiked with 6 and 15 μg/ml FVIII-Ab. FVIII was measured using the STA R of Stago (France).
Results: When TG was measured in haemophilia A samples with and without FVIII-Ab, no differences could be observed between both plasma samples. A doses-response of FVIII could be observed for peak height, ETP, time-to-peak and velocity index, but not for lag-time, that seemed to be independent of the FVIII level. The FVIII doses-response in healthy controls with 6 and 15 μg/ml were parallel to each other, indicating that the differences between the two curves was dependent of the remaining FVIII level (94%, 34% and >4% for the samples with 0, 6 and 15 μg/ml FVIII-Ab, respectively).
Conclusions: No substantial differences were observed in TG kinetics, indicating that *in vitro* induced haemophilia samples can be used to determine the response to FVIII treatment in haemophilia A.

PB0829 | Detection of Residual Factor VIII Levels Reveals the Occurrence of Readthrough Over the Majority of F8 Nonsense Mutations

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Background: Nonsense mutations are commonly associated with "null" genetic conditions. However, an event called "ribosome readthrough" may restore the synthesis of full-length proteins through suppression of nonsense mutations and incorporation of an amino acid. This might explain why some *F8* nonsense mutations are associated with moderate haemophilia A (HA).
Aims: To evaluate full-length protein production resulting from occurrence of readthrough over a wide panel of *F8* nonsense mutations.
Methods: Expression of an optimized fusion protein between factor VIII (FVIII) and a high-sensitivity luciferase in HEK293 cells and luciferase assays in media and cell lysates. Evaluation of FVIII in HA patient plasma through Western blotting and ELISA.
Results: *F8* nonsense mutations were subdivided into a high-frequency (12 mutations; patient number, n>10), arising from the highly frequent CGA(arginine)>TGA change, and a low-frequency (44 mutations; patient number n=1-5) group. The latter was rationally classified into two subsets including mutations predicted to be suppressed by reinsertion of the original amino acid, and localized in the B-domain, in which potential amino acid substitutions introduced during readthrough are predicted to be tolerated. Noticeably, the selected mutations (44 out of 216) have been reported in 384/611 (63%) HA patients with nonsense mutations.
Strikingly, expression of all *F8* nonsense variants led to detectable luciferase activity with a different extent (0.3-7%) that appeared to be consistent with the impact of the inserted amino acid on FVIII secretion. The selected panel includes 11 nonsense mutations, found in Italian HA patients, that in preliminary results revealed traces of FVIII protein in plasma.
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 protein levels. This might have relevant pathophysiological implications, and might contribute to interpret the variable susceptibility of HA patients to develop inhibitors upon replacement therapy.

PB0830 | Increased Plasmin Generation in People with Severe Haemophilia

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Background: People with severe haemophilia (PWH) exhibit significant variability in bleeding tendency that is independent of individual factor VIII/IX levels. Despite this, the factor(s) underlying this phenotypic heterogeneity remain largely unknown. In this study, we hypothesised that inter-individual differences in fibrinolytic pathway function may contribute to bleeding heterogeneity in PWH.