119.5 kb insertion at intron 1. The 119.5 kb insertion appeared to be a duplicated-sequence ranging from truncated *FUNDC2* to *int1h*-2 in the 3' flanking region of *BRCC3*.

Conclusions: We identified a complex *F8* abnormality with a large deletion and a large insertion in a patient with severe HA. It seemed that the complex rearrangements were caused by template switching and *int1h* homologous recombination in spermatogenetic genomic replication.

PB0213 | Favourable Recombinant Factor IX Pharmacokinetics Outcomes in Severe Hemophilia B Patients with FIX Activation Site Mutations

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Background: In Hemophilia B (HB), low levels of wild-type/dysfunctional endogenous Factor IX (FIX) molecules are candidate to influence the pharmacokinetics (PK) of infused FIX. *F9* genotypes, responsible for variant FIX biosynthesis, could help interpreting the PK outcomes, but this relationship has not been yet demonstrated in HB patients.

Aims: To investigate *F9* genotype-associated molecular mechanisms underlying recombinant FIX (rFIX) PK in the frame of the *"F9 Genotype and PK Hemophilia B Italian Study" (GePKHIS).*

Methods: PK evaluation of full-length rFIX by coagulant assay (FIX:C). Characterization (ELISA, chromogenic assay) of FIX variants transiently-expressed in HEK293 cells.

Results: Evaluation of PK profiles in severe (FIX:C < 1%) Italian HB patients with missense changes at position 226 showed noticeable FIX:C levels (≥3%) still detectable at 72-96 hours post-infusion (p.Arg226Trp), and long half-life (HL) parameters (Beta HL=42.4 hours) in 2-compartment model analysis (p.Arg226Gln).

Substitutions at Arg226, located in the FIX activation site (P1) resulting in the FIXaβ, are recurrent in HB (p.Arg226Gln, n=7/52; p.Arg226Trp, n=9/44; Italian/International Hemophilia B mutation database).

We expressed all FIX variants with naturally occurring (Gln/Trp/Gly/Pro/Leu) missense changes, and in addition designed substitutions abolishing (Ala/Asp/Glu) or mimicking (Lys) biochemical features of wild-type Arg226. All substitutions were responsible for secreted antigen levels comparable (50-80%) to those of wild-type FIX. Antigen levels, measured after variant expression, were slightly lower than those observed in the corresponding HB patients. All missense changes produced very low chromogenic activity, except the Arg226Lys variant (60-80%).

Conclusions: Combination of data in patients and recombinant variants suggests that well-secreted but inactive endogenous FIX molecules favorably influence the rFIX PK. We hypothesize that endogenous 226-mutated FIX molecules, partially cleaved (FIXα), might occupy activation/receptor complexes involved in removal of FIX from plasma, thus lowering infused rFIX clearance. Particularly, these mechanisms could favor the PK final phase, characterized by higher endogenous FIX/rFIX ratio.

PB0214 | Evaluation of Single-site and Multisite Precision of Factor IX Measurement

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Background: Precision and accuracy of factor IX determination is of highest importance to properly screen, diagnose and manage haemophilia B patients. Accuracy can be ensured through proper activity assignment and link to international standards. Precision rely mostly on the properties of the assay methodology used (combination of reagents, instrument and data acquisition).

Aims: We evaluated single-site and multisite precision of FIX measurement with STA®-ImmunoDef IX and STA®-C.K. Prest® reagents, following EP05-A3 CLSI guidelines recommendations.

Methods: Samples used were 5 native individual samples spanning the assay measuring range.

They were tested with 3 lots of STA®-ImmunoDef IX and 1 unique lot for all other reagents.

For single-site precision, a total of 20 runs were measured in duplicates with 2 runs per day, on both STA R® and STA Compact®.

Multisite precision was determined through experiments on 3 sites, 5 runs per site (on 5 different, non-consecutive days), 5 replicates per run, on STA R® only.

All reagents and instruments were from Stago, Asnières-sur-Seine, France.

Results: Checking results with Grubbs' test, less than 1% of them were outliers and all sites kept the calibration curve of day 1 to determine FIX levels along the whole experiment.

For sample 1, an additional point was included in the calibration curve and used only if needed (some very low results were otherwise out of range).

Results were calculated utilizing ANOVA tests.

Conclusions: FIX measurement with STA®-ImmunoDef IX and STA®-C.K. Prest® is very precise and reproducible both single-site and multisite, even at low FIX levels, without the need for any recalibration. When considering less variables than this full precision study, serial dilutions of FIX showed that down to 0.7% FIX, CVs observed on 8