adults with Haemophilia, thrombin generation correlates with bleeding risk and severity of Haemophilia. This has not been previously investigated in children.

Aims: To use a paediatric specific thrombin generation assay (ETP), to assess ETP in boys with severe Haemophilia A (< 18 years). To determine correlation of ETP with clinical phenotype, as measured by annual factor usage [units/weight (kg)/annum] and: FVIII level, as well as markers of fibrinolysis (TFPI/TAFI). Venous blood was collected from 32 boys (4/12–17 years) with severe Haemophilia A. ETP was performed on PPP using the subsampling method which directly measures the Alpha2-macroglobulin-bound component (physiologically inactive). Factor VIII, TAFI, free and total TFPI assays were performed. Factor VIII results varied from < 1% to 55% depending on recent therapy. No patient had evidence of a significant inhibitor. Poor correlation was observed between FVIII and Log ETP/Log Peak TG. In addition no significant correlation was noted between ETP characteristics and clinical phenotype (FVIII usage). Fibrinolytic markers (TFPI/TAFI) did not correlate with ETP parameters or FVIII usage. A modest correlation was observed between FVIII and free TFPI ($r = 0.44$, $P < 0.02$). Analysis of the subset of patients ($n = 14$) with FVIII < 1% (no recent factor usage) revealed no significant correlation between TG parameters (ETP and Peak TG) and annual FVIII usage. A modest correlation was observed between free TFPI and Log ETP ($r = -0.6$, $P < 0.02$) in this group. The subsampling Thrombin Generation assay used in this study appears insensitive to FVIII%. This may indicate that the concentration of tissue factor within the thromboplastin is sufficiently high such that FVIII is not a rate limiting component. Our results suggest ETP does not differentiate phenotype within our cohort of severe Haemophiliacs. Variation within the study population, which included both boys on prophylaxis (high FVIII%) and on demand (low FVIII%) treatment may also have impacted on the findings.

Disclosure of interest: V. Ignjatovic, Sanofi-Aventis, Clinical Research Fellowship.

PP-WE-532

Detection of large rearrangements by MLPA in severe hemophilia A patients

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Detection of large rearrangements by MLPA in severe hemophilia A patients. N. Lannoy1, I. Abinet1, C. Lambert3, Ch. Vermylen2, K. Dahan1 C. Hermans3. (i) Center of Human Genetics, (2) Departement of Paediatrics, (iii) Departement of Haematology, Université Catholique de Louvain, Belgium. Determination of large genomic rearrangement (deletion and duplication) responsible of disease was a challenge in the past with time consuming and critical standardization techniques: Southern blotting, multiplex PCR-LC, Real-time PCR. The recent arrival of MLPA (Multiplex Ligation-dependant Probe Amplification) gene dosage kit made it possible to detect these rearrangements quickly and easily in a single reaction. Large deletions from one exon to the complete Factor 8 (F8) gene account for about 5% of the causal mutations of severe X-linked hemophilia A leading to an absence of factor VIII. MLPA analysis was performed in 38 DNA unrelated Belgian patients with severe hemophilia A after a negative genetic testing for intron 22 and 1 inversion. DNA of three unrelated carriers presenting a factor VIII deficiency in the absence of a family history or in absence of paternal investigation were also included for identification of the causal mutation. MLPA assay identified three large deletions (exon 5 for 1st, exon 15 for the second and exons 23–26 for the third) and two large duplications (exon 1 and exons 1–22). For carriers without familial contribution, a decreased copy number compared to control DNAs of exon 1–12 in patient 1, of exon 2 in patient two and of exon 26 extending to the 3'UTR region in the third patient was identified. In conclusion, MLPA technology is efficient to search large genomic rearrangement in patients with severe hemophilia A. If deletions are common, a very few cases with duplication was reported. This technique is also warranted in female patients presenting with 'de novo' factor VIII deficiency or without familial investigation predicting the severity of the disease.

Disclosure of interest: none declared.

PP-WE-533

Unusual intron 1 inversion with concomitant large duplication within the F8 gene in a severe hemophilia A patient

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Hemophilia A is a common inherited bleeding disorder, caused by factor VIII deficiency as a result of mutations in the factor VIII gene. Intron 1 and 22 inversions, small insertions/deletions and point mutations are the most common genetic defects responsible for severe hemophilia A. However, in 2%–5% of patients with severe hemophilia A (FVIII: C < 1IU/dL), no F8 gene mutation is found. An exon 13 duplication was described in a patient with mild hemophilia and more recently, seven other duplications were published. Here, we describe a severe hemophilia A patient with a large duplication within the F8 gene associated with an abnormal pattern of intron 1 inversion.

Material and Methods: The patient exhibited a severe phenotype (FVIII:C < 1 UI/dL, FVIII:Ag < 1%) and did not develop an inhibitor. The intron 1 inversion was detected by long fragment PCR as described by Bagnall et al (2002). A DNA sample was also analyzed by an MLPA kit P178 (MRC Holland, Amsterdam, the Netherlands) which is a quantitative method able to detect large rearrangements. Duplicated exons were also analyzed by Q-PCR (Quantitative PCR), as well as the promoter region and the FUNDC2 gene (located 4 kb upstream from the F8 gene).

Results: An unusual pattern of intron 1 inversion was detected in this patient: normal pattern for Int1h2 amplification and presence of three bands in Int1h1 PCR: 1.5 kb (normal band), 1 kb (inverted band) and an additional intermediate band (about 1.3 kb). The MLPA analysis showed the highest peaks for exons 1–14. The Q-PCR analysis confirmed the duplication of these exons. We also detected a duplication of the F8 gene promoter region and the FUNDC2 gene.

Conclusion: Recently, several complex rearrangements have been described: combination of deletion/insertion or deletion/insertion/intron 22 inversion. To our knowledge, this is the first description of complex rearrangement associating unusual intron 1 inversion and large duplication in a severe hemophilia A patient.

Disclosure of interest: none declared.

PP-WE-534

Evaluating therapy in hemophilia: development of an MRI protocol

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Magnetic Resonance Imaging (MRI) is currently the most sensitive imaging modality for detection of joint lesions in hemophilia. If early, subtle changes could be detected by MRI in young adults,