published that almost a quarter of patients with moderate and severe Von Willebrand disease (VWD) reported joint bleeds. Those bleeds had a negative impact on health related quality of life and joint integrity, according to patient-reported and retrospective medical file data. However, besides that, little information is available for VWD patients on the prevalence and severity of arthropathy and its influence on joint function and daily life activities.
Aims: To assess the prevalence and severity of arthropathy and its impact on joint function and daily life in moderate and severe VWD patients (trial number NTR 4548).
Methods: Dutch patients with moderate and severe VWD (VWF activity<30 IU/dL and <10 IU/dL, respectively) and documented treatment for at least one joint bleed were invited to participate. The same number of controls (moderate and severe VWD patients without joint bleed treatment) were selected and matched for age (±2 years), gender and FVIII level (±10%). A single experienced physiotherapist conducted the Haemophilia Joint Health Score (HJHS, 0-124). X-rays were made from all joints with prior bleeds, contralateral joints and one control joint. One radiologist scored the X-rays according to Pettersson (PS, 0-13 per joint). Arthropathy was defined as a clinical HJHS score ≥3 or PS>0. All participants completed the Haemophilia Activity List (HAL, 0-100) questionnaire. The Visual Analogue Score (VAS, 0-10 cm) was used to assess joint pain.
Results: 48 patients and 48 controls were included, 60% males, mean age 46 years (range 18-80). Mean FVIII levels were 26 IU/dL in the patients and 31 IU/dL in the controls (p=0.19). More patients had type 3 VWD (19/48 *vs* 3/48 controls). In the control group of patients without documentation on joint bleed treatment, 14/48 patients did report one or more joint bleeds but none of them more than five. In contrast, 56% of the 48 patients had more than five joint bleeds. Arthropathy occurred in 37/48 (77%) patients and 35/48 (73%) controls (p=0.51). Overall, arthropathy occurred in both severe (47/64) and moderate VWD (25/32) and in all three VWD types (22/28 type 1; 30/46 type 2; 20/22 type 3). The median HJHS was significantly higher in the patients compared to the controls (5 *vs* 1.5, p<0.01, maximum score 47 *vs* 29). PS>3 occurred in 2 controls compared to 12 patients (p<0.01) and overall most in type 3 VWD patients (9/22 type 3 *vs* 3/46 type 2 *vs* 2/28 type 1). The total HAL score as well as the scores on the three separate HAL components were significantly lower for the 48 patients compared to the controls (median scores HAL Sum: 88 *vs* 100, p<0.01; Upper Extremity Activities: 93 *vs* 100, p=0.01, Basic Lower Extremity Activities: 87 *vs* 100, p<0.01; Complex Lower Extremity Activities: 80 *vs* 100, p<0.01). Clinically relevant joint pain (mean VAS score >3) was reported by 17 patients and 9 controls (p=0.07).
Summary/Conclusions: Arthropathy, according to our stringent definition, was seen in 77% of patients with moderate and severe VWD treated for joint bleeds. Notably, arthropathy was also found in 73% of matched control VWD patients without joint bleed treatment. Joint function and –integrity in the VWD patients treated for joint bleeds was affected, consistent with higher HJHS and joint X-rays scores. These patients experienced a significant impact on daily life activities (HAL, both upper and lower extremities) and 35% also reported clinically relevant joint pain (VAS).

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A COMPREHENSIVE NEXT GENERATION SEQUENCING TEST FOR THE DIAGNOSIS OF INHERITED BLEEDING, THROMBOTIC AND PLATELET DISORDERS

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Background: Inherited bleeding, thrombotic and platelet disorders (BPDs) are rare diseases affecting approximately 300 individuals per million births. With the exception of haemophilia and von Willebrand disease patients, a molecular analysis for BPD patients is often unavailable. Many specialised tests are usually required to reach a putative diagnosis and they are typically performed in a slow, step-wise manner to control costs. The results of these tests may be used to prioritise genes for Sanger sequencing if a genetic diagnosis is required. This approach causes significant delays and a conclusive molecular diagnosis is often never reached, which can compromise treatment and impede rapid identification of affected relatives.
Aims: Our aim was to design a platform through which accurate and rapid testing of inherited BPDs would be possible.
Methods: We designed a high-throughput sequencing (HTS) platform targeting all 87 known BPD disease genes. The platform can call single nucleotide variants, short insertions/deletions and large copy number variants (though not inversions), which are subjected to automated filtering for diagnostic prioritisation.
Results: We sequenced 159 and 141 samples respectively from individuals with and without previously known causal variants. Among the latter group, 61 cases had phenotypes strongly indicative of a particular molecular aetiology while the remainder had an aetiology that was *a priori* highly uncertain. All the previously detected variants were recapitulated and, when the aetiology was suspected but unknown, a molecular diagnosis was reached in 56 of 61 cases.
Summary/Conclusions: The ThromboGenomics platform provides a comprehensive and affordable DNA-based test to diagnose patients suspected of having a known inherited BPD thereby significantly reducing the current diagnostic delay.

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THE ROLE OF CD72 IN THE REGULATION OF B CELL ACTIVATION THROUGH CD40 IN PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: CD72 is considered to be an important B cell co-receptor for its prominent role in immune regulation. Previous studies show that CD40 signals play a more important role in mechanism of primary immune thrombocytopenia (ITP) by activating B cell and inducing B-cell growth with Ig secretion. NF-κB activation is a pivotal pathway in CD40 signaling. However, the effect of CD72 on B cells activated by CD40 in ITP remains unknown.
Aims: This study aimed to explore the effect of CD72 on B cell activation through CD40 in ITP.
Methods: Activation-associated surface markers (CD80, CD86 and CD40), the proliferation, apoptosis, plasmablasts and platelet-associated IgG were analyzed by flow cytometry in patients with active ITP and health controls. In cultures vitro, peripheral blood mononuclear cells (PBMCs) were stimulated by anti-CD40 in the presence or absence of CD40L, IL-4 and IL-21, autologous platelets. Production of IgG and IgM in the culture supernatants was determined by enzyme-linked immunosorbent assay. The levels of NF-κB P65 and IκBα mRNA were assessed by real-time quantitative polymerase chain reaction.
Results: Our data showed that CD40 expression was significantly decreased on CD19+ B cells after CD72 ligation in ITP patients compared to controls. The higher levels of activation markers CD80, CD86 on CD19+ B cell stimulated by anti-CD40 was reduced by anti-CD72 in ITP patients and controls. CD40 stimulation significantly promoted the survival of CD19+ B cells, and reduced the apoptosis of CD19+ B cells in ITP patients compared to that observed in controls. CD72 ligation corrected the effect of CD40 stimulation on apoptosis and proliferation of B cells. The CD40-mediated the differentiation of B cells into plasmablasts was blocked by CD72 ligation in ITP patients and controls. However, the role of CD72 on B cell differentiation was stronger in ITP patients than in controls. CD72 ligation had only a minor effect on reducing platelet-associated IgG in ITP patients. Following stimulation with anti-CD40 and IL-21, synthesis of IgM by B cell was diminished in the presence of anti-CD72, whereas the IgG levels were barely reduced. The reduced ratio had no difference in ITP patients and controls.CD72 signaling significantly reduces NF-κB P65 and IκBα expression at the mRNA levels in PBMCs activated by anti-CD40 in ITP patients compared to controls.
Summary/Conclusions: These findings indicate that CD72 is a key molecule in regulating B cell activation, proliferation, apoptosis and antibody secretion mediated by CD40 signaling in ITP patients.Thus, CD72 may be involved in the pathogenesis of ITP and antagonizing CD72 could be a novel strategy for the therapy of ITP.

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IMPACT OF BRUTON'S TYROSINE KINASE INHIBITORS ON COLLAGEN-INDUCED PLATELET AGGREGATION: A PHARMACOKINETIC/PHARMACODYNAMIC PERSPECTIVE

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Background: Bruton's tyrosine kinase (BTK) is expressed in platelets (PLTs) and mediates collagen-induced aggregation of PLTs. TEC, a member of the TEC family kinases, is also expressed in human and mouse PLTs. Studies in PLTs from BTK-deficient X-linked agammaglobulinemia patients (Quek 1998; Lipsky 2015), and BTK and/or TEC KO mice suggest that the 2 kinases likely possess redundant functions in collagen-induced aggregation; however, BTK is thought to play a more dominant role than TEC, as BTK deficiency alone reduces robust aggregation at lower collagen concentrations (Atkinson 2003). Ibrutinib (ibr) is a once-daily, first-in-class, covalent inhibitor of BTK approved for various B-cell malignancies. Additional BTK inhibitors (BTKis) have been evaluated in clinical trials, with bleeding as a common adverse event (AE) observed not only for ibr but also for ONO-4059 (Walter 2016), ACP-196 (Byrd 2016), and BGB-3111 (Tam, ASH 2015). Early-phase clinical data reported to date for these follow-on molecules have been limited due to small patient numbers and minimal follow-up, where ACP-196 and ONO-4059 had bleeding events of petechiae and contusion among the most common AEs (hematoma was also frequently reported with ONO), and BGB similarly had a 33% bleeding rate. These compounds are potent BTKis with additional activity against TEC;