Abnormal bleeding phenotype for mild haemophilia B patients with the p.Ile112Thr variation on the gene for factor IX

Céline Row, Pierre Chamouni, Claire Berger, Anne Lienhart, Sandrine Meunier, Mathilde Fretigny, Vincent Dalibard, Marie Viprey, Hervé Chambost, Virginie Barbay, et al.

Received: 28 April 2020 | Revised: 16 July 2020 | Accepted: 16 August 2020

DOI: 10.1111/hae.14146

Haemophilia WILEY

LETTER TO THE EDITOR

Abnormal bleeding phenotype for mild haemophilia B patients with the p.Ile112Thr variation on the gene for factor IX

Haemophilia B is a rare bleeding disorder characterized by low coagulant factor IX (FIX) levels. Mild haemophilia B (mHB) results in a significantly milder bleeding phenotype compared with moderate and severe forms. Muscular haematomas and joint bleeds or haemarthrosis are rare and usually induced by significant trauma. Such patients receive on-demand treatment. Prophylactic treatment is not usually recommended in mHB.[1] Molecular biology is recommended for investigating the aetiology of haemophilia B. Genotype may also be considered as a marker associated with bleeding phenotype. Our initial surprise came from reports of a mHB patient with numerous bleeding episodes, including joint bleeds, despite baseline FIX levels around 40 IU/dL. The c.335T > C variation corresponding to the p.Ile112Thr variation, according to the new Human Genome Variation Society type numbering, was found to be the causal mutation on the FIX gene *F9*. The aim of this study was to identify the genetic variation p.Ile112Thr as a predictive factor for an increased risk of bleeding in mHB patients.

All haemophilia B patients with the p.Ile112Thr variation and follow-up in a French Hemophilia Treatment Center were identified in haemophilia referent genetic laboratories. All *F9* gene exons and flanking introns were sequenced for every first symptomatic patient in each family. If the mutation was reported in the family and X-linked transmission of the disease was respected, a patient was considered as presenting the mutation. Female patients were excluded from the study. Given the retrospective nature of this study, informed consent was not required; however, patient information notes were sent and the study was registered under Clinical Trial Registration number NCT03946384.

In total, 23 males with mHB and the p.Ile112Thr variation were included. Median age at last data collection was 19 years (range 6-67 years), and seven patients (30.4%) were aged under 12. No signs of connective tissue disorder were mentioned in the clinical files.

The median baseline residual plasma FIX activity levels FIX:C, measured by one-stage clotting assay, was 36 IU/dL (range 26-47 IU/dL). The median activated partial thromboplastin time (aPTT) ratio was 1.20s (range 1.07-1.50s), and for 10 patients (43.5%) this ratio was not prolonged. FIX:C was measured by two-stage clotting assay in 3 patients, and the levels seemed similar. Other bleeding disorders, such as fibrinogen anomalies, anomalies in prothrombin time, low factor VIII (FVIII) and XI (FXI), anti-FIX inhibitor, von Willebrand disease, and platelet disorders, were excluded in patients who underwent those tests. Only one thrombin generation assay (TGA) was performed and came back normal.

The median annual bleeding rate (ABR) was 1.0 (range 0.4-9.8), and the median joint-specific ABR was 0.5 (range 0-2.6). More than half of the cohort (56.5%) had an ABR ≥1.0. The number of spontaneous and/or traumatic bleeding events (BE) per year could vary from 0 to 27 in a single patient. The median age at diagnosis was 6 years (range 1-16), and at first BE was 3 years. All patients had at least one BE and their first joint bleed during childhood. A total of 8 patients (34.8%) had joint procedures, approximatively a third of the cohort. Four patients had arthrodesis/plasty, from 1 to 4 joint surgeries/patient, and the median age at first arthrodesis/plasty (total joint replacement, hip, knee, elbow) was 37 years (range 25-52 years). Four patients had other joint procedures (synoviorthesis, meniscectomies, joint infiltrations), from 1 to 4 other joint procedures/patient, and the median age at the first other joint procedure was 31 years (range 14-52 years). The patient who was youngest at the first joint procedure was 14 years old (synoviorthesis). The patients' clinical data are summarized in Tables 1, 2.

Every single patient had received FIX concentrate at least once in their life. A total of 19 patients (82.6%) had on-demand treatment. Four patients (17.4%) had prophylactic treatment with a median ABR of 4.8, a median joint-specific ABR of 1.8, and with a median age at prophylaxis introduction of 31 years.

In this study, we describe patients with mild HB presenting with an abnormal bleeding phenotype. All the patients in our study presented their first joint bleed during childhood, even though spontaneous joint bleeds are unusual in mHB patients.[2] In addition, some significant BE may have been underestimated. Our clinical data were mostly collected from patient treatment diaries, but some BE may not be traced in this type of diary; we therefore described only the minimum number of BE. Moreover, bleeding severity was underestimated because some patients had joint procedures without any joint bleeds recorded. This abnormal bleeding phenotype could justify earlier prophylaxis in these patients. Patients should not be treated on the basis of the initial severity classification based on FIX levels, but rather on the severity of the BE. If the symptoms are similar to those of a severe HB patient, the same therapeutic plan should be chosen because a delay in treatment may be damaging. This genetic variation may thus be deleterious. Out of the 725 HB patients followed in the French national mHB cohort, only 8 have prophylactic treatment, 3 of whom have the genetic variation p.Ile112Thr (FranceCoag, data updated on 05.18.2019).

TABLE 1 Clinical data and treatment

	p.Ile112Thr cohort n = 23
Age (y)	19 (11-32)
Patients under the age of 12 y	7 (30.4)
Joint bleeds	
Age at 1st joint bleed (y)	6 (2-8)
Total N° of joint bleeds	6 (2-14)
Joint-specific ABR[a]	0.5 (0.3-1.1)
Joint-specific ABR ≥1.0	8 (34.8)
Joint procedures[b]	
Patients with joint procedure	8 (34.8)
Patients with arthrodesis/plasty	5 (21.7)
N° of arthrodesis/plasty/patient	1 (1-2)
Age at 1st arthrodesis/plasty (y)	37 (29-52)
Patients with other joint procedures	5 (21.7)
N° of other joint procedures/patient	2 (2-2)
Age at 1st other joint procedure (y)	31 (26-36)
Bleeding events (BE) and diagnosis	
Diagnosed on BE	17 (73.9)
Age at diagnosis (y)	6 (2-7)
Age at 1st BE (y)	3 (1-7)
ABR[c]	1.0 (0.6-1.8)
ABR ≥1.0	13 (56.5)
Treatment	
N° patients requiring substitution at least once in their life	23 (100)
Age at 1st substitution (y)	7 (5-8)
Patients with on-demand treatment	19 (82.6)
Patients with prophylactic treatment	4 (17.4)
Age at prophylaxis (y)	31 (26-33)

Note: Values are median (interquartile range Q1-Q3) or n(%).

[a]Joint-specific ABR, joint-specific annual bleeding ratio or median annual number of joint bleeds.

[b]Joint procedures include arthrodesis and arthroplasties (total joint replacement, hip, knee, elbow) and other joint procedures such as synoviorthesis, meniscectomies and joint infiltrations.

[c]ABR, annual bleeding ratio or median annual number of bleeds.

No discordance between FIX one-stage and two-stage clotting assays was described in our patients in whom those tests were performed. A difference between those assays is well described in FVIII dosage and is associated with certain genetic variations in the *F8* gene.[3] In haemophilia A, clinical symptoms seem best correlated to FVIII levels measured by two-stage clotting assay. Discrepancies between FIX clotting assays have been described in HB patients and could be linked to a genetic mutation, but in those studies two-stage clotting assays gave higher levels than one-stage clotting assays.[4] However, no other articles seem to describe a discrepancy between FIX levels and clinical severity.

This missense variation switches an isoleucine for a threonine amino acid (AA) in the first epidermal growth factor (EGF-1) module, but the implication of such a domain in interaction with factor VIII within the tenase complex has not been fully elucidated.[5] One study built an EGF-1 deletion mutant FIX molecule and showed that the binding affinity of the mutant for interaction with FVIII was impaired.[6]

We tried to understand the impact of p.Ile112Thr variation on the FIX protein. Position p.112 corresponds to the first AA residue after the first beta sheet in FIX EGF-1.[5] In other coagulation factors with two EGF domains, similar to FIX, the AA at this same position is preserved in factor X (isoleucine) and is a leucine residue in factor VII (with physical and chemical features similar to those of isoleucine).[7] However, studying sequence conservation between species, using the Alamut® program (version 2.11.0),[8] shows that Ile112 in FIX is only preserved in 8 species out of 12, which is moderate conservation, indicating little importance

TABLE 2 Characteristics of patients with prophylaxis

	Patient 1	Patient 2	Patient 3	Patient 4
FIX:C baseline one stage, (UI/dL)	32	35	43	45
Age at diagnosis, (y)	3	1	2	8
Joint bleeds				
Age at 1st joint bleed, (y)	8	1	2	8
Total N° of joint bleeds	22	46	64	10
Joint bleeds/year, range	0-4	0-9	0-9	0-3
Joint-specific ABR [a]	1.4	2.6	2.1	0.5
Joint procedures[b]				
N° of joint arthrodesis/plasty	1	0	2	2
Age at 1st arthrodesis/plasty, (y)	25	-	23	26
N° of other joint procedure	0	0	0	7
Age at 1st other joint procedure, (y)	0	-	-	26
Bleeding events (BE)				
Age at 1st BE, (y)	3	1	2	8
Total No of BE	70	91	293	28
BE/year, range	0-14	0-21	1-27	0-6
ABR [c]	4.4	5.1	9.8	1.3
Treatment				
Age at 1st substitution, (y)	8	1	2	8
Age at prophylaxis, (y)	30	14	32	37

[a]Joint-specific ABR, joint-specific annual bleeding ratio or median annual number of joint bleeds.
[b]Joint procedures include arthrodesis and arthroplasties (total joint replacement, hip, knee, elbow) and other joint procedures such as synoviorthesis, meniscectomies, and joint infiltrations.
[c]ABR, annual bleeding ratio or median annual number of bleeds.

of this AA. To predict the impact of point mutation on protein stability, we used CUPSAT software (Cologne University Protein Stability Analysis Tool).[9] The p.Ile112Thr change confers unfavourable torsion, destabilizing the protein. To predict the overall protein impact, we used Poly-Phen-2 (Polymorphism Phenotyping version 2),[10] which considers the variation of interest only as a genetic polymorphism. In all, variation p.Ile112Thr does not seem to have a severe impact on protein activity. However, in these prediction models, FIX was rarely considered as a whole, and intra-molecular interactions and interactions within the tenase complex were often not analysed.

Most of the patients with the p.Ile112Thr variation live in or are from the Normandy region. The patients come from 11 different families and may have a common ancestor. A founder effect should be explored as it could lead to this variation being referred to as haemophilia B Normandy. We should keep in mind that if all the patients are related, an additional possible inherited bleeding disorder could explain the abnormal BE.

We acknowledge the weaknesses of our study. This is a retrospective cohort with patients from different French centre with clinical and biological data relying on patient treatment diaries and medical records. Some clinical data were undocumented, and the data collection periods were different for each patient. Data after the introduction of prophylaxis were not compiled in this study. Biological tests such as two-stage clotting assay, fibrinolysis testing, and TGA were missing for some patients, and we could not test them as it would have made this interventional research.

Our study reveals that the p.Ile112Thr variation on the *F9* gene may be associated with an abnormal bleeding phenotype in mild HB patients. To the best of our knowledge, this is the first study to describe a clinical-biological discrepancy in mild HB. This new information may be beneficial for patients with the p.Ile112Thr genetic variation, as prophylactic treatment could be initiated earlier. It also underlines the importance of molecular biology in finding the causal mutation, even in mild HB patients.

Further studies are needed to complete biological assessments of all patients, and a prospective study, particularly on patient evolution with prophylactic treatment, could be of interest.

ACKNOWLEDGMENTS

The authors would like to thank the panel of specialists in all the HTC involved in this work: Rouen, Lyon, Saint-Etienne, and Marseille. We would also like to thank the team from the FranceCoag coordinating centre for their rapid and efficient work: K. Baumstarck, M.

Boucekine, V. Milien, Y Demay, and P. Auqieur. Finally, we would like to thank all the patients who participated in this study.

DISCLOSURE

C. Berger has received grants/research support from Baxter, Novo Nordisk, and Octapharma and honoraria for participation in symposia by Octapharma, Novonordisk, and Roche-Chugai. The other authors have no conflict of interest.

AUTHOR CONTRIBUTION

C. Row performed the research with considerable help from P. Chamouni, V. Barbay, A. Lienhart, S. Meunier, C. Berger, and H. Chambost who provided patient data and contributed to the concept and design of the study. H. Chambost, M. Viprey, and V. Dalibard supplied data from the national cohort FranceCoag. M. Fretigny contributed to the discussion content of this paper. J. Bovet and C. Row analysed the data, interpreted the results, and drafted the manuscript. All authors revised the manuscript.

Céline Row[1]
Pierre Chamouni[2]
Claire Berger[3]
Anne Lienhart[4]
Sandrine Meunier[4]
Mathilde Fretigny[5]
Vincent Dalibard[6,7]
Marie Viprey[7,8]
Hervé Chambost[9]
Virginie Barbay[2]
Julien Bovet[1]

[1]*Thrombosis and Hemostasis Unit, University Hospital of Dijon, Dijon, France*
[2]*Hemophilia Treatment Center, University Hospital of Rouen, Rouen, France*
[3]*Hemophilia Treatment Center, Pediatric Hematology department, University Hospital of Saint Etienne, Saint Etienne, France*
[4]*Hospices Civils de Lyon, French Reference Centre for Hemophilia, University Hospital of Lyon, France*
[5]*Hemostasis Laboratory, University Hospital of Lyon, Lyon, France*
[6]*Lille CHRU, Hemophilia Treatment Center, Hematology and Transfusion, Lille, France*
[7]*APHM, FranceCoag, Research Department, Marseille, France*
[8]*Aix-Marseille Univ, EA 3279 CEReSS-Health Service Research and Quality of Life Center, Marseille, France*
[9]*APHM, Hemophilia Treatment Center, La Timone Hospital and Aix Marseille Univ, INSERM, INRA, C2VN, Marseille, France*

Correspondence
Céline Row, Thrombosis and Hemostasis Unit, University Hospital of Dijon, Dijon, France. 14, rue Paul Gaffarel. 21000 Dijon, France.
Email: celine.row@chu-dijon.fr

ORCID

Céline Row https://orcid.org/0000-0002-4666-9784

REFERENCES

1. Benson G, Auerswald G, Dolan G, et al. Diagnosis and care of patients with mild haemophilia: practical recommendations for clinical management. *Blood Transfus*. 2018;16(6):535-544.
2. Soucie JM, Monahan PE, Kulkarni R, Konkle BA, Mazepa MA. The frequency of joint hemorrhages and procedures in nonsevere hemophilia A vs B. *Blood Adv*. 2018;2(16):2136-2144.
3. Trossaërt M, Boisseau P, Quemener A, et al. Prevalence, biological phenotype and genotype in moderate/mild hemophilia A with discrepancy between one-stage and chromogenic factor VIII activity. *J Thromb Haemost JTH*. 2011;9(3):524-530.
4. Kihlberg K, Strandberg K, Rosén S, Ljung R, Astermark J. Discrepancies between the one-stage clotting assay and the chromogenic assay in haemophilia B. *Haemoph Off J World Fed Hemoph*. 2017;23(4):620-627.
5. Persson KEM. Role of the N-terminal EGF module of coagulation factor IX in activation of factors IX and X. *Scand J Clin Lab Investig Suppl*. 2002;237:13-18.
6. Qureshi SH, Yang L, Rezaie AR. Contribution of the NH2-terminal EGF-domain of factor IXa to the specificity of intrinsic tenase. *Thromb Haemost*. 2012;108(6):1154-1164.
7. Stenflo J, Stenberg Y, Muranyi A. Calcium-binding EGF-like modules in coagulation proteinases: function of the calcium ion in module interactions. *Biochim Biophys Acta*. 2000;1477(1–2):51-63.
8. Interactive Biosoftware | Creator of the Alamut Software Suite [Internet]. Interactive Biosoftware. [cited 2019 Jul 26]. https://www.interactive-biosoftware.com/. Accessed July 26, 2019.
9. Parthiban V, Gromiha MM, Schomburg D. CUPSAT: prediction of protein stability upon point mutations. *Nucleic Acids Res*. 2006;34(Web Server):W239-W242.
10. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249.