Effects of the Factor V G1691A Mutation and the Factor II G20210A Variant on the Clinical Expression of Severe Hemophilia A (< 2%) in Children – Results of a Multicenter Study

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Summary

It has been recently shown that the first bleeding onset in children with severe HA carrying prothrombotic risk factors is significantly later in life than in non-carriers. The present multicenter study was performed to determine whether the factor (F) V G1691A or the F II G20210A are associated with decreased annual bleeding episodes (ABE) in 104 pediatric PUP patients with severe HA consecutively admitted to German pediatric hemophilia treatment centers. Treatment was initiated according to the frequency of bleedings, and most patients received on demand therapy with a switch over to prophylactic therapy 3x/week when more than two bleedings (range 2–6) had occurred into the same joint. Prospective median (range) patient follow-up was 14 (5–24) years. Heterozygosity of the FV mutation was found in 8 subjects, homozygosity in one, and 5 children carried the FII mutation once combined with protein C-deficiency. Carriers of the FV and FII mutations had significantly fewer ABE than non-carriers ($p = 0.004$). 65 of 104 PUP patients had developed at least one target joint. The Pettersson score (median/range: 1/0–12) available in 56 patients is clearly dependent on age ($p = 0.039$), and on ABE ($r = 0.42$; $p = 0.007$). The »Nuss« joint score available in 32 subjects highly correlated with the Pettersson score ($r = 0.8$; $p = 0.0004$). Data presented here give evidence that the clinical expression of severe HA in children is influenced by the co-expression of the FV and FII mutation.

Introduction

Hemophilia A and B are X-linked genetic hemorrhagic disorders resulting from deficiencies of blood coagulation factor VIII or IX. Subjects suffering from plasma levels of factor VIII coagulant activity or factor IX below 1% of normal are classified as severe hemophiliacs [1]. While mild or moderate hemophilia is not always diagnosed during childhood, severe hemophilia is generally diagnosed at an early age [2–6]. Although bleeding symptoms correlate with the levels of the remaining factor activity, it is reported that some hemophilic subjects with factor VIII levels < 1% do not all bleed with the same severity [7, 8].

Besides the possibility that the mutation type within the factor VIII gene may influence the clinical severity of hemophilia [9, 10], it has been recently suggested that the clinical phenotype of severe hemophilia A is influenced by co-inheritance

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with the factor V G1691A mutation [11]. In addition, we have recently demonstrated that the first symptomatic bleeding onset in children with severe HA carrying prothrombotic risk factors is significantly later in life than in non-carriers [12].

The present study was therefore conducted to unravel the role of the factor V (FV) G1691A mutation or the factor II (FII) G20210A variant, coinherited with severe hemophilia A with respect to the clinical expression of the disease, e.g. the bleeding frequency.

Methods

Ethics

The present survey on consecutively recruited pediatric patients with hemophilia was performed in accordance with the ethical standards laid down in a relevant version of the 1964 Declaration of Helsinki and approved by the medical ethics committee at the Westfälische Wilhelms-University, Münster, Germany. With special regards to the data presented here, the ethical committee has approved the investigation of established prothrombotic risk factors possibly coinherited in pediatric patients with hemophilia A.

Inclusion Criteria

Untreated Caucasian infants and children (PUP) with previously undiagnosed severe hemophilia A (FVIII activity < 1%) aged neonate to 16 years admitted to the university pediatric hospitals in Frankfurt, Halle, Munich and Münster, Germany, on the first symptomatic and spontaneous onset of the disease. In the patients enrolled the classification of HA based on the FVIII activity was confirmed by using the same aPTT reagents and factor VIII-deficient plasma in the patients investigated.

Exclusion Criteria

Pretreated pediatric patients, subjects in whom surgery- or major (birth-) trauma-induced bleeding had occurred. In addition, patients with prenatal diagnosis of HA were excluded, as well as children with diagnosis of hemophilia before the first bleeding episode. Children with hemophilia A and inhibitor development were not included in the present survey.

Study Period

From October 1985 to December 2001, 144 consecutive Caucasian pediatric PUP patients with a first symptomatic onset of hemophilia A were recruited from different geographic areas of Germany.

Study Population

Of the 144 consecutively recruited children, 104 were suffering from severe HA. Due to individual decisions of the participating centers the majority of patients were initially treated »on-demand« and were switched over to a »prophylactic« treatment regimen when more than two bleeding episodes had occurred into the same joint within a 12-month period [13, 14]. In children with joint bleedings imaging of the affected joints was performed according to Pettersson [15], and in addition, in patients with a Pettersson score of 0, as well as in cases with suspected synovitis a MRI was additionally requested. The MRI classification was performed according to Nuss [16, 17]. Due to ethical reasons in children without clinical hints of joint bleedings no roentgenograms of the joints were performed. In each participating child the annual frequency of bleeding events (ABE) was recorded at comprehensive clinical visits based on review of patient infusion logs or the family report. Joint hemorrhage was defined as an acutely painful swollen joint necessitating factor replacement therapy. A target joint was defined when more than two bleeding episodes had occurred into the same joint within a three-month period [18, 19].

Laboratory Analysis

With informed parental consent the G1691A polymorphism in the FV gene and the G20210A variant in the factor II gene were detected in patients with severe hemophilia A by PCR amplification [20, 21]. Activities of protein C, antithrombin, free protein S antigen and protein C antigen were measured as previously described [22]. The plasma levels of factor VIII were measured by one-stage clotting assays purchased from Behringwerke/Marburg, Germany using standard laboratory methods. Mutation analysis for hemophilia A was performed as described earlier [23].

Statistics

Calculations of medians, ranges and nonparametric statistics (Mann-Whitney, Spearman rank correlation) were performed with the Stat view 5.0 programs. The significance level was set at 0.05.

Results

HA Patient Population

Of the 144 consecutively recruited children, 104 were suffering from severe HA. Median (range) patient follow-up was 14 years (5–24). The HA mutation spectrum was no different between carriers and non-carriers of prothrombotic risk factors ($p = 0.3$: Table 1). In addition, no significant differences were found with respect to

Table 1. Spectrum of mutations in children with HA with respect to coinherited prothrombotic risk factors

	– Thrombophilia number [%]	+ Thrombophilia number [%]	
Inversion 22	35 [53.8]	6 [60.0]	
Missense	17 [26.2]	1 [10.0]	
Nonsense	6 [9.2]	0	
Large deletion	2 [3.1]	1 [10.0]	
Splice	1 [1.5]	0	
Frameshift	1 [1.5]	0	
Chromosomal abnormalities	1 [1.5]	0	
not identified so far	2 [3.1]	2 [20.0]	
Total	65 [72.2]	10 [71.4]	P=0.3

treatment modalities performed in both patient groups (p = 0.24): without knowledge of the thrombophilia status treatment was initiated according to the frequency of bleedings, and most patients received initially »on-demand« therapy, e.g. 50 IU/kg bw, and were switched over to prophylactic therapy 3x/week with the substitution of a median(range) dose of 40 IU/kg bw (30–60) factor VIIII concentrate, when more than two bleedings (range 2–6) had occurred into the same joint within a 12-month period. »On-demand« therapy was applied to 58 children without prothrombotic risk factors (64.4%) compared to 7 patients in the thrombophilia group (50%), and prophylactic factor VIII administration was administered in 32 subjects without additional thrombophilias compared with 7 children in carriers of the FV or FII mutation (p = 0.24).

Prevalence of Prothrombotic risk Factors in German Children with Severe HA

From the entire study group 14 children carried additionally prothrombotic risk factors. The prevalence of prothrombotic risk factors in children with severe HA was no different from previously reported data [12]: FV G1691A 7.7% (8/105), FV A1691A 1.0% (1/104), FII G20201A 4.8% (5/104), once combined with protein C type I deficiency. No deficiency states of antithrombin or protein S were found in this cohort of hemophilic patients.

Annual Bleeding Episodes

Carriers of the FV and FII mutations had significantly fewer median (range) ABE than non-carriers (p = 0.004): 1.3 (0–7) versus 5.5 (0–36) respectively. 65 of 104 PUP patients developed at least one target joint: the distribution within the two patients groups, however, was no different (no thrombophilia: ankle n=27, knee n=19, elbow n = 6, hip n = 1, knee & ankle n = 4; with thrombophilia: ankle n = 5, knee, elbow or hip n = 1 each [p = 0.76]). The median (range) Pettersson score performed in 56

patients at a median (range) age of 12 (4–24) years was 1(0–12) in children without thrombophilic risk factors compared with 1(0–4) in children carrying either the FV mutation or the FII variant. The Pettersson score was clearly dependent on age ($p = 0.039$) and on ABE ($r = 0.42$; $p = 0.007$). The »Nuss« joint score documented in 32 subjects highly correlated with the Pettersson score ($r = 0.8$; $p = 0.0004$).

Discussion

The purpose of the present study was to investigate whether the presence of prothrombotic risk factors influences the bleeding frequency in children with severe hemophilia A. In addition, in HA patients suffering clinically from joint bleedings the association between the bleeding frequency and the joint status were investigated. In addition to our previously findings, that the coinheritance of a thrombophilic gene mutation is associated with a later onset of a first symptomatic bleeding in HA patients, data of this multicenter survey give evidence that the clinical phenotype of severe HA in children is influenced by the presence of the FV and FII mutation: our results clearly demonstrate that the annual bleeding frequency in children treated since 1980 by the same treating physicians over a median time of 14 years was significantly lower in carriers of prothrombotic risk factors compared to non-carriers, thereby supporting the hypothesis of Nichols et al. [11], that the hemophilia phenotype is influenced by coinheritance with prothrombotic risk factors. The latter is also supported also by the findings of Lindvist et al. showing that women carrying the FV G1691A mutation experienced less intrapartum blood loss [24]. Our data, however, are in contrast to data reported by Arbini et al. and data shown by Lee et al. in adult patients [25, 26], showing that the proportion of severe hemophiliacs whose mild clinical course could be attributed to coinheritance with the FV G1691A mutation tended to be small.

Pediatric patients with severe HA typically experience frequent bleeding episodes into joints or soft tissues [2, 3, 5], necessitating on-demand or prophylactic treatment twice or thrice weekly with individual amounts of factor VIII concentrates. The frequency of bleeding and the outcome with respect to joint damage investigated with the Pettersson score [27] has been discussed to be not only dependent on the severity of the disease, but also on the corresponding factor F VIII gene mutation, or the development of inhibitors [8]. In addition, the course of bleeding episodes is also controversially discussed with respect to individual therapeutic regimen performed by each hemophilia center [28–33]. Patients enrolled from the different pediatric hemophilia treatment centers in Germany, and due to the higher bleeding tendency not including children with inhibitor development, were treated since 1980 by the same medical teams. In similarity to the Canadian hemophilic cohort recently reported [19], treatment protocols used in the German pediatric PUP patients enrolled here have not changed with respect to treatment indications, and the criteria chosen to treat a bleeding episode »on-demand« or »on-prophylaxis« with an uniform increase of prophylactic treatment regiments since the late eighties [13, 14]. In addition, in the children reported here the mutation spectrum in HA subjects were no different in carriers and non-carriers of prothrombotic risk factors. Thus,

since the treatment regiments were administered in the PUP patients presented here without knowledge of the individual thrombophilia status, and since the treatment regimens were no different between carriers and non-carriers of prothrombotic risk factors, evidence is given, that the thrombophilic gene mutation itself is responsible for the lower bleeding frequency associated with the better joint outcome in the children reported. The different results obtained from adults [25, 26], however, may be mainly due to different treatment modalities used before 1980 [32], e.g. the non-availability of purified factor VIII concentrates leading to a higher rate of untreated bleeding episodes in older hemophiliacs, as well as a lower rate of prophylactic administration of non-purified factor VIII preparations. In the elderly hemophilic patient, however, aging has to be discussed as an additional cause of arthropathic joint damage, which is normally not present in the young: therefore it seems to be likely, that the interaction hemophilic joint damage and age-related arthropathy will be combined in the adult patients previously reported [25, 26]. The latter, however, makes a clear differentiation difficult, if the origin of a joint arthropathy diagnosed in adult hemophiliacs is mainly based on the number of bleeding episodes, the process of aging, the interaction with prothrombotic risk factors, or a combination of multiple contributing factors respectively.

In conclusion, data of the cohort study presented here suggest that the hemophilic phenotype is influenced by the presence or absence of prothrombotic risk factors, e.g. the factor V or factor II variant. However, to obtain further insight into the possible putative effect of these prothrombotic risk factors on the severity of hemorrhagic disorders, especially of severe hemophilia A, prospective large-scale studies in previously untreated hemophilic children are required.

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