Effects of the factor V G1691A mutation and the factor II G20210A variant on the clinical expression of severe hemophilia A in children – results of a multicenter study

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ABSTRACT

The present multicenter cohort study of 107 pediatric PUPs was performed to determine whether the concomitant inheritance of the factor (F) V G1691A or the F II G20210A mutation influences the clinical expression of severe hemophilia A (HA). Carriers of the FV and FII mutations had a significantly lower annual bleeding frequency (ABF) than non-carriers (p=0.012). Joint damage (Pettersson score) was significantly less severe in patients with thrombophilia (p=0.022). A protective effect of thrombophilic risk factors was shown for ABF (OR [CIs]: 0.7[0.5-0.9]; p=0.02) and the severity of the hemophilic arthropathy (OR [CIs]: 0.06[0.01-0.3]; p=0.0009).

Key words: severe hemophilia A, pediatric PUPs, thrombophilia, Pettersson score, synovitis.

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Hemophilia A (HA) is an X-linked genetic hemorrhagic disorder resulting from a deficiency of blood coagulation factor VIII. The mutation type within the factor VIII gene may influence the clinical severity of hemophilia.[1] It has also recently been suggested that the clinical phenotype of HA is influenced by co-inheritance of the factor V G1691A mutation or the factor II G20210A variant.[2-4] This clinical observation in patients has been supported by findings that the clinical phenotype of severe hemophilia in mice was influenced by the factor V Leiden genotype.[5] Furthermore, data of an in-vitro study have demonstrated a 3-7 fold increase in thrombin generation when factor VIII-depleted plasma was supplemented with different concentrations of factor V Leiden plasma.[6] The aim of the present multicenter cohort study was to investigate the role of the factor V (FV) G1691A mutation and the factor II (FII) G20210A variant, co-inherited with severe HA, with respect to the clinical expression of the disease, i.e. the annual bleeding frequency (ABF), the occurrence and severity of image-proven hemophilic target joints, and the development of synovitis.

Design and Methods

Ethics

The present retrospective review of consecutively recruited pediatric patients with HA was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and was approved by the Medical Ethics Committee of the University of Münster, Germany. With regard to the data presented here, the ethics committee has specifically approved the investigation of established prothrombotic risk factors (PR) possibly co-inherited in pediatric patients with HA.

Inclusion criteria

Previously untreated and undiagnosed Caucasian infants and children (PUP) with severe HA (F VIII activity < 0.01U/mL), aged neonate to 16 years, who had been admitted to the University Children's Hospitals of Frankfurt, Halle, Munich, and Münster, Germany, for the first symptomatic and spontaneous onset of the disease were included in the study.

Exclusion criteria

Pre-treated pediatric patients and children with hemophilia A and inhibitor development (n=23) were not included in the present review.

Outcome measures

The maximum annual bleeding frequency (ABF), the occurrence rate and severity of at least one target joint, and the occurrence of synovitis were defined as outcome measures of interest.

Study population

From October 1985 to December 2001, 147 consecutive Caucasian pediatric PUPs with a first symptomatic onset of HA were recruited from different geographic areas of Germany. Of these, 107 were suffering from severe HA. In 66 patients, treatment, which was at the discretion of the participating centers, was initially *on demand* and was switched to a *prophylactic* treatment regimen when more than two episodes of bleeding into the same joint had occurred within a 6–month period.[7,8] This treatment regimen was maintained as the standard over time in the participating study centers. In the mid 1990s, an intensified treatment protocol was introduced for patients presenting with severe soft tissue bleeding at HA onset. These children received a prophylactic treatment regimen following the first symptomatic hemorrhage. In the children reported here, image-proven hemophilic joint damage (herewith called *hemophilic arthropathy*) was classified according to Pettersson (X-ray score). Joints with a Pettersson score < 1 were classified as normal.[9] In addition, patients with repeated joint bleeding despite adequate prophylaxis, and children with suspected synovitis, were investigated with magnetic resonance imaging (MRI) which is known to be more sensitive to synovitis than X-ray. The MRI classification was made according to Nuss (MRI-score).[10] The maximum ABF of each participating child, based on reviews of patient infusion logs and family reports, was recorded at comprehensive clinical visits. Traumatic or spontaneous joint hemorrhage was defined *a priori* as an acutely painful swollen joint requiring factor replacement therapy. A target joint was defined as more than two bleeding episodes occurring into the same joint within a 3 month period.[11] Synovitis, i.e. the inflammation of the synovium, was diagnosed when synovial hyperplasia and signs of hemosiderin were present in the MRI.[9]

Laboratory analysis

After obtaining informed parental consent, the G1691A polymorphism in the FV gene and the G20210A variant in the factor II gene were evaluated by PCR amplification.[12] The activities of protein C, antithrombin, free protein S antigen and protein C antigen were measured as previously described.[12] The plasma levels of factor VIII were determined by one-stage clotting assays purchased from Behringwerke (Marburg, Germany) using standard laboratory methods. The mutation analysis for HA was carried out as previously described.[13]

Statistics

All statistical analyses were carried out using the StatView 5 software package (SAS Institute Inc., Cary, US). Continuous data were presented as medians and ranges, and evaluated by non-parametric statistics using the Wilcoxon Mann Whitney U test. To compare the frequency distributions of adverse outcome, a univariate analysis was carried out using the χ^2 test or, if necessary, Fisher's exact test. In addition, the effect of the FV G1691A mutation and the factor II G20210A variant on ABF and hemophilic joint damage was assessed by multivariate analysis (logistic regression). Odds ratio (OR) and 95% confidence intervals (CIs) were also shown. p values < 0.05 were considered significant. A correlation analysis was carried out using Spearman's rank correlation test.

Results and Discussion

HA patient population

One hundred and seven children were suffering from severe HA. Median (range) patient follow-up was 14 years (5-24). Fifteen children among the entire study group also carried PR. The prevalence of PR in children with severe HA was in agreement with previously reported data:[4] FV G1691A 7.4% (8/107), once combined with protein C type I deficiency, FV A1691A 0.9% (1/107), and FII G20201A 5.6% (6/107). Deficiency states of antithrombin or protein S were not found in this cohort. The HA mutation spectrum was no different between carriers and non-carriers of prothrombotic risk factors (Table 1). In addition, when comparing patients with inversion 22 with children without this mutation there was no statistically significant association found with respect to the FV or FII carrier status (p=0.6).

No significant differences were found with respect to the treatment methods adopted in the two patient groups (p=0.67: Table 1). At the onset of the disease and without knowledge of the thrombophilia status, treatment was initiated according to the frequency of bleedings. Patients initially received *on demand* therapy, e.g. 50 IU/kg bw, and were switched to thrice weekly prophylactic therapy with substitution of a median (range) dose of 40 IU/kg bw (30-60) factor VIIII concentrate when more than two bleedings (range 3-6) had occurred into the same joint within a 6 month period (Table 2). Furthermore, there was no difference with respect to the source of factor VIII concentrates used.

Maximum annual bleeding frequency and target joints

In patients on prophylactic therapy, ABE remained stable over time. Univariate analysis showed carriers of the FV and FII mutations to have a significantly lower medi-

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

Mutation spectrum	*No thrombophilia*	*With thrombophilia*	*p value**
Inversion 22	42 [45.7]	9 [60.0]	0.6
FV G1691A & AA	–	6	
FII G20210A	–	3	
No Inversion 22	40 [43.4]	6 [40.0]	
FV G1691A & AA	–	3	
FII G20210A	–	3	
Missense**	24 [26.0]	2 [13.0]	0.5
Nonsense	7 [7.6]	–	0.6
stop exon 13			
stop exon 14			
stop exon 18			
stop exon 25			
c.43C⟶T (R-5X)			
codon 1029 TGG⟶TAG			
Large deletion			
exon 14	2 [2.2]	2 [13.0]	0.1
exon 22			
Splice	1 [1.1]	–	1.0
intron 11 IVS11 +5G⟶A			
Frameshift	1 [1.1]	–	1.0
exon 14 2781 del T			
Chromosomal abnormalities	1 [1.1]	–	1.0
translocation (11;X) (q13;q22)			
Not identified so far	4 [4.3]	2 [13.0]	0.2
Total	**82/92 [89.0]**	**15/15 [100]**	

**Univariate analysis; **Exons 4,6,7,8,9,11,12,16,19,20,21,23,25,26.*

Table 2. Patient characteristics.

	No thrombophilia	*With thrombophilia*	*p value**
Year of birth	1990 [1991-1999]	1991 [1982-1999]	0.54
Age at first bleeding: years [median/range]	0.9 [0.1-4.0]	1.5 [0.5-7.1]	0.009
Therapy given: number [%]			
on demand	58 [63.0]	8 [53.3]	0.67
prophylaxis	34 [37.0]	7 [46.7]	
Start of prophylactic regimen: Median/range values (years)	1.3 [0.1-6.7]	1.9 [0.8-7.0]	0.44
Factor concentrates used [%]			
pdFVIII	27.3	33.3	0.33
rFVIII	48.5	55.5	
vWFVIII	24.2	11.2	
Annual bleeding frequency	6 [0-30]	1.8 [0-7]	0.012
Distribution of target joints:			
Total: number [%]	57 [63.3]	8 [57.2]	0.39
ankle	27 [30.0]	5 [35.7]	
knee	19 [21.1]	1 [7.1]	
elbow	6 [6.7]	1 [7.1]	
hip	1 [1.1]	1 [7.1]	
knee and ankle	4 [4.4]	–	
Joint scores: median [range] values; numbers			
Pettersson score	1.3 [0-12]; n=50	1.0 [0-4]; n=6	0.02
Nuss score	6.0 [0-10]; n=36	1.5 [0-8]; n=5	0.06
Median [range] age in years	12 [3-20]	12 [3-19]	0.71
Distribution of synovitis joints:			
Total number [%]	6 of 34 [17.6]	1 of 7 [14.3]	0.9
ankle	3	1	
knee	1	–	
elbow	1	–	
knee and ankle	1	–	

°Univariate analysis.

an ABF than non-carriers (Table 2). Sixty-five out of 107 PUPs (60.7%) developed at least one target joint. Interestingly, the distribution and number of target joints in children with a PR was no different compared with patients without the FV or FII mutation (p=0.39; Table 2). However, the two patient groups differed in the severity of hemophilic arthropathy. The target Pettersson score obtained in 56 of the 65 patients with hemophilic arthropathy was significantly more pronounced in children without PR than in children carrying either the FV mutation or the FII variant. In addition, the more sensitive *Nuss* joint score documented in 41 subjects correlated highly with the Pettersson score (r=0.8; p=0.0004). The multivariate analysis clearly showed a protective effect of PR in children with severe HA for ABF (OR [CIs]: 0.7[0.5-0.9]; p=0.02) and severity of the hemophilic arthropathic joint damage (OR [CIs]: 0.06[0.01-0.3]; p=0.0009). By contrast, no such protective effect was observed for MRI-proven synovitis (OR [CIs]: 0.7[0.07-6.9]; p=0.77). The distribution of affected joints is shown in Table 2. The data obtained in the reported multicenter survey clearly demonstrate that the annual bleeding frequency in children who had remained in the care of one and the same medical team since 1985 was significantly lower in carriers of PR than in non-carriers. However, the present review showed no such protective effect for the development of MRI-proven synovitis, which is mainly attributable to the small number of cases diagnosed. Our results support the clinical and experimental hypothesis that the clinical hemophilic phenotype is influenced by co-inheritance of PR.[2,3,5,6] As Tizzano and co-workers demonstrated we showed that spontaneous bleeding episodes and the number of arthropathies were significantly lower in patients with thrombophilia compared with non-carriers. By contrast, we could found no significant associations between the FV or FII mutation and the intron 22 inversion.[3] In a larger adult HA cohort, Lee and co-workers[14] have also demonstrated a lower annual bleeding frequency along with a significantly reduced use of FVIII concentrate. However, these authors failed to show a protective effect of the FV mutation on the hemophilic arthropathy in their cohort, which contrasts with our observations in the patients reported here. It has been suggested that the frequency of bleeding and the degree of joint damage is not only dependent on the severity of the disease, but also on the

corresponding factor F VIII gene mutation, or the development of inhibitors. In addition, the course of bleeding episodes has been controversially discussed with respect to individualized therapeutic regimens employed by the treating hemophilia center.[15-19] Since 1985, patients enrolled from the different pediatric hemophilia treatment centres in Germany have been treated by the same medical teams using the same treatment protocols. Similar to the Canadian hemophilic cohort recently reported,[11] these patients have been on treatment protocols that have remained unchanged with respect to treatment indications and the criteria chosen to treat a bleeding episode *on demand* or *on prophylaxis*. In both cohorts a similar increasing preference for prophylactic treatment regimens has been observed since the late eighties/mid-nineties.[7,8,11] In addition, in the children reported here, the mutation spectrum in HA subjects did not differ between carriers and non-carriers of PR.[13] Since the treatment regimens were administered without knowledge of the individual thrombophilia status, with no difference between carriers and non-carriers of PR, this observation provides evidence that the thrombophilic gene mutations contribute to the lower bleeding frequency and associated better joint outcome in the children reported. The different results obtained in adults may be mainly attributable to different treatment methods adopted before 1980.[14,19,20] Furthermore, in the elderly hemophilic patient, aging has to be discussed as an additional cause of arthropathic joint damage. It seems likely that the interaction of hemophilic joint damage and age-related arthropathy contributes to a combined effect in the adult patients previously reported.[14,20] The present survey is limited mainly by the small numbers of patients presenting with additional prothrombotic risk factors. Therefore, results of the statistical analyses have to be interpreted with caution. In addition, the long enrolment period with the change of treatment regiments over time, the lack of MRI availability in the entire cohort, and the different sensitivities of imaging scores applied (the MRI score is much more sensitive to synovitis compared with the Pettersson score) are further study limitations. To overcome these shortcomings, prospective large-scale studies in previously untreated hemophilic children are required to obtain further insight into the possible putative effect of thrombophilia on the severity of hemorrhagic disorders, especially of severe HA.

Authors' Contributions

Along with the principal study investigators, e.g. KK, WK, RS, and UN-G, who act as the guarantors, all other investigators (SH, CD, CB, CE, AK, NB) took part in the design, execution and data analysis, and in writing the report.

Conflict of Interest

The author reported no potential conflicts of interest.

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