Familial Elevated Factor VIII in Children With Symptomatic Venous Thrombosis and Post-Thrombotic Syndrome

Results of a Multicenter Study

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Objective—To evaluate the role of factor (F) VIII in children with non-cancer related venous thrombosis (DVT), post-thrombotic syndrome (PTS) or recurrent DVT.

Methods and Results—FVIII levels were measured in White patients and age- and gender-matched healthy controls. Heritability of factor VIII was estimated in 99 pedigrees by the variance component method implemented in SOLAR. The group of 103 patients showed higher median values of FVIII than 206 controls [FVIII:Ag, 115 versus 96 IU/dL, $P<0.0001$; FVIII:C, 119 versus 106 IU/dL, $P=0.0009$], and had a significantly increased odds ratio (OR) for fibrinogen-adjusted elevated FVIII levels [FVIII >90th percentile versus values below the cut-off: FVIII:Ag, OR 4.3, 95% confidence interval (CI) 1.5 to 12.1; FVIII:C, OR 5.5, CI 2.03 to 15.06]. PTS occurred in 19 of 59 children and persisted in 5 individuals. Recurrent DVT was seen in 8 patients. The heritable(h2)/household(c2) components were calculated for FVIII:Ag levels (h2, 0.48 ± 0.15, $P=0.0008$; c2, 0.21), and FVIII:C (h2, 0.61 ± 0.15, $P<0.0001$; c2, 0.41). When incorporating h2 and c2 in the estimate, the phenotypic variance for FVIII:Ag levels is predominantly explained by h2, whereas c2 stayed significant in the model for FVIII:C ($P=0.00002$).

Conclusions—Elevated FVIII levels increase the DVT-risk in children. **(*Arterioscler Thromb Vasc Biol*. 2006;26: 1901-1906.)**

Key Words: factor VIII and children ■ body mass index ■ venous thrombosis ■ post-thrombotic syndrome ■ recurrent thrombosis

Venous and arterial thromboses, although still rare in childhood, have been increasingly diagnosed and recognized in pediatric populations: symptoms of thrombotic manifestation have been reported in 0.07/10 000 children, in 5.3/10 000 admissions of children, and in 2.4/1000 admissions of newborns to intensive care units.[1–4] Among the entire childhood population, neonates are at a greater risk of thromboembolic complications than older children. The incidence of vascular accidents decreases significantly after the first year of life, with a second peak during puberty and adolescence.[1,2] Numerous clinical and environmental conditions result in elevated thrombin generation with subsequent thrombus formation in infancy and childhood.[2–7] In addition, various genetic prothrombotic defects have been discussed as risk factors for thrombotic events in adult and pediatric patients.[8–15] The association of multiple hemostatic prothrombotic defects or a combination of prothrombotic risk factors with acquired environmental or clinical conditions greatly increase the risk of thrombosis not only in adults but also in infants and children.[12–14]

Several studies have demonstrated that elevated factor (F) VIII concentrations are associated with an increased risk of a first episode of venous thrombosis (DVT) to occur in young and elderly adults, whereas the role of elevated FVIII in recurrent DVT is still enigmatic.[16–24] Thus, the present study was performed to evaluate the role of familial elevated FVIII in children with DVT.

Materials and Methods

Ethics

The present study was performed in accordance with the ethical standards laid down in the updated relevant version of the Declaration of Helsinki and was approved by the medical ethics committee

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of the Westfälische Wilhelms University, Münster, Germany. Signed informed parental consent was obtained for each study participant.

Study Design and Study End Points

The present study is a case–control study (match: 1 case versus 2 controls) to assess the association between elevated FVIII levels and the onset of first DVT. In addition, on an explorative basis, the patients enrolled were prospectively followed with respect to the study end points "post-thrombotic syndrome (PTS)" and "recurrent DVT" for a minimum of 24 months (December 2004).

Patients

The analysis included 103 unselected pediatric patients presenting with their first symptomatic DVT who were consecutively enrolled between January 2000 and December 2002 as previously described.[9,11,12] Based on the German population denominator the patient enrollment was 2.0/1 000 000 living children <18 years. Five of 103 children (4.8%) enrolled had a positive family history of DVT (defined as DVT before the age of 50 in affected family members).

Inclusion Criteria

A thrombotic event confirmed by standard imaging methods, ie, compression sonography, venography, computed tomography (CT), spiral CT, or magnet resonance imaging (MRI) and perfusion lung scans, qualified for the diagnosis of DVT. Patients older than 18 years at onset of their first DVT, children of non-White origin, patients with incomplete clinical or laboratory work-up (established prothrombotic risk factors in White German children, FVIII), and subjects lost to follow-up were not enrolled (n=7). Patients were also excluded during the acute thromboembolic phase and if they had experienced arterial thrombosis or stroke, and recurrent DVT before the study was initiated. Further exclusion criteria were premature birth (≤36 gestational weeks), ongoing liver, renal, or inflammatory diseases, malignancies, and concurrent treatment regimens known to influence FVIII levels (oral contraceptives, steroids).

The definition of PTS, adapted from adults, was based on objective signs, eg, an increase in calf or ankle circumference by 2 to 4 cm, dark pigmentation of the skin, venous telangectasia, varicose veins, or open ulcer.[25,26] PTS was defined as "transient" when it was diagnosed once only and did not recur during the predefined follow-up period. DVT/recurrent DVT in the deep veins of the leg was diagnosed when venography performed in the acute phase of a new vascular accident showed fresh thrombotic material within a lumen of the vein, ie, a new intraluminal filling defect compared with the previous tests. Thrombus extension in children with leg thrombosis was diagnosed when initial thrombotic material was distributed within the affected vessels: distal DVT<proximal DVT<pelvic DVT<proximal and pelvic DVT. Body mass index (BMI) calculated as kg/m^2 was recorded in patients and controls.

Anticoagulation After Symptomatic DVT

At the discretion of the participating study centers, childhood patients with a first DVT received thrombolytic therapy (n=7), unfractionated heparin (n=42), and twice daily low molecular weight heparin (LMWH; n=54) during the acute thrombotic onset. In addition, as standard care, either vitamin K antagonists (INR 2.0 to 3.0) or LMWH (once daily: 4-hour anti–factor Xa activity 0.3 to 0.6 IU/mL) were administered for another 6 to 9 months. Children with distal or proximal DVT of the legs were instructed to wear compression stockings during the day.

Clinical Routine Procedures Performed by the Participating Centers

Routine diagnostics included physical examination by an experienced pediatrician, serial oscillometric blood pressure recordings, and standard imaging methods at first thrombotic onset as well as 6 to 8 weeks and 6 months later (before withdrawal of anticoagulation). After discontinuation of antithrombotic therapy, asymptomatic pediatric patients were revisited every 3 months and at prolonged intervals after the second year (yearly).

Control Group

The control group included 255 healthy White children. These controls, who had no history of chronic disease or of thromboembolic events and were not on medication at the time of recruitment, presented as outpatients for evaluation before minor surgery (planned circumcisions and hernias) or bone marrow donation.[9,11,12] Healthy children with a positive family history of early thromboembolism/myocardial infarction or stroke were not accepted as controls.

Heritability Study

Ninety-nine pedigrees including biological parents and siblings (376 subjects, females n=184) were available for a study on the possible heritability of FVIII levels versus household and environmental effects.

Blood Sample Collection

Blood sample collection from patients, relatives, and controls was done at the study center in Münster in the morning after a 12-hour fasting period (infants 4 to 6 hours); samples were drawn by peripheral venipuncture into plastic tubes containing 1/10 by volume of 3.8% trisodium citrate (Sarstedt) and were immediately placed on melting ice. The blood samples from patients were collected 6 to 12 months after the acute thrombotic event and at least 6 weeks apart from anticoagulation. Platelet-poor plasma was prepared by centrifugation at 3000*g* and 4°C for 2×20 minutes, aliquoted in polystyrene tubes, stored at −70°C, and thawed immediately before assay. DNA extraction was performed by a spin column procedure (Qiagen) as previously described.[9,11,12,15] For the present FVIII study blood samples were drawn from otherwise healthy patients, relatives, and controls with normal hemograms and no evidence of further diseases.

Laboratory Tests

Standard laboratory techniques[9,11,12,15] were used to investigate the factor V G1691A (FV) and factor II G20210A (FII) mutations, concentration of Lp (a) (intrassay-coefficient of variation [ICV], 4.5%; run-to-run coefficient of variation [RCV], 4.2%), PC (ICV, 1.6%; RCV, 5.0%), PS (ICV, 2.2%; RCV, 4.2%), AT (ICV, 3.1%; RCV, 6.5%), and tissue factor pathology inhibitor (TFPI) levels (ICV, 5.2%; RCV, 4.0%) in patients and controls. Samples from 69 patients and 181 controls were available for TFPI measurements. Whereas FVIII:Ag levels were investigated in frozen plasma samples with a commercially available ELISA (Asserachrom VIIIC:Ag, Diagnostica Stago; ICV, 4.5%; RCV, 4.0%), FVIII:C activity (FVIII:C; ICV, 2.6%; RCV, 3.3%) was measured with a one-stage clotting assay with FVIII-deficient plasma (Dade Behring) on a Dade Behring BCS analyser. VWF:Ag (ICV, 1.3%; RCV, 3.9%) was measured using Hemosil (Instrumentation Laboratory) on an ACL 9000 (Instrumentation Laboratory). Because data on normal values for FVIII in children are lacking, FVIII:Ag concentrations, FVIII:C activity levels, and von Willebrand factor (vWF):Ag levels >age-dependent 90th percentiles derived from healthy controls were used as cut-off values. These values were also differentiated for the influence of blood group 0 versus non-0 (Table 1). As an acute phase reactant, fibrinogen was measured according to Clauss (Dade Behring BCS analyser). Quantitative D-dimer levels were measured by a latex-enhanced turbimetric test (D-dimer plus, Dade-Behring; ICV, 2.0%; RCV, 2.9%), and f1.2 was investigated using a commercially available ELISA technique (f1.2 micro, Dade-Behring; ICV, 6.2%; RCV, 9.5%). Cut-off values were derived from healthy pediatric controls (>90th percentile). For all plasma-based assays a clotting abnormality was regarded as a defect only if the level was outside the normal range/percentile.[27] Apart from the classification based on age-dependent normal reference ranges and confirmation of a suspected protein-based prothrombotic defect in a second plasma sample (3 to 6 months later), the presence of a hemostatic defect in at least one first degree relative or the identification of a causative gene mutation were considered confirming criteria for its hereditary nature.

TABLE 1. Cut-Off Values by Age (90th Percentile, Derived From 255 Healthy Controls). FVIII and vWF Cut-Off Values Are Also Differentiated for Blood Groups 0 Versus Non-0

Age	FVIII:Ag (IU/dl)	FVIII:C (IU/dl)	Von Willebrand Factor Ag (IU/dl)	D-dimer (mg/l)	F1.2 (nmol/l)	BMI (kg/m^2)
3 to 6 months	135	134	138	0.34	2.5	18.2
	118 [150]†	130 [137]†	136 [141]†			
6.1 to 12 months*	129	139	129	0.34	1.6	18.3
	101 [130]†	128 [136]†	127 [135]†			
1 to 9 years*	127	141	112	0.34	1.3	20.9
	106 [168]†	122 [150]†	99 [118]†			
9.1 to 18 years*	162	143	133	0.34	1.2	25.0
	126 [173]†	131 [167]†	111 [142]†			

*Reference values used for paediatric patients: blood sampling 6 to 12 months after acute thrombotic onset.
†Adjusted for blood groups 0 vs non-0 [brackets].

Statistical Analysis

Statistical analyses were performed with the StatView 5 software package (SAS Institute). Heritability (h2) for factor VIII levels was estimated using the variance component method implemented in SOLAR.[28] This method allows the total phenotypic variance to be partitioned into a proportion caused by polygenic effects (h2) and a proportion caused by random environmental effects (e2), including household effects (c2). The calculated heritability thus estimates the total variance of a trait explained by genetic effects. Prevalence rates of prothrombotic risk factors in patients and control subjects were calculated by descriptive statistics and compared by χ^2-analysis or by Fisher exact test, if appropriate. To compare the rate of prothrombotic risk factors between patients and controls and to evaluate the interaction between elevated FVIII levels and other thrombophilic risk factors, odds ratios (ORs) together with 95% confidence intervals (CIs) were estimated from multivariate analysis using a logistic regression model: risk factors significantly associated with DVT in children in the univariate model were included in the multivariate analysis. To further investigate whether an acute phase may confound the thrombotic risk associated with high FVIII levels, fibrinogen levels were incorporated in the multivariate logistic regression model and FVIII values were adjusted for fibrinogen levels. For variables with a non-Gaussian frequency distribution, data were presented as medians and ranges. All evaluations and comparisons within patients and between patients and controls were done using the Mann–Whitney test. The significance level was set at 0.05. Correlations were determined by Spearman rank correlation test.

Results

Patients

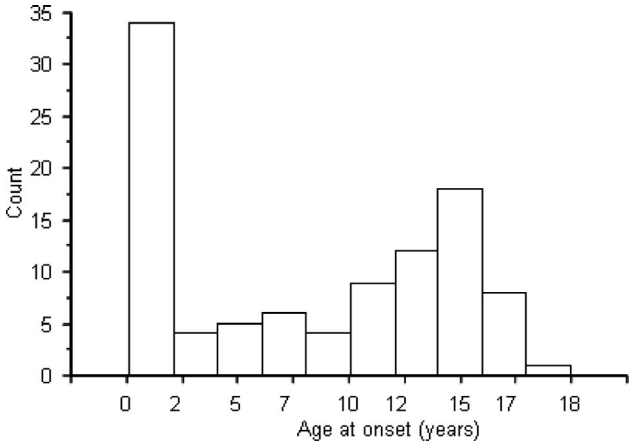
Patients (46 female and 57 male) consecutively recruited in- and outpatients with a median (range) age of 8.2 (neonate to 18) years were investigated: three children who fulfilled all inclusion criteria and were initially enrolled for the study missed their follow-up visit in Münster. The age distribution is shown in the Figure. Thrombosis site and underlying medical conditions are summarized in Table 2.

Case–Control Analysis

The risk of DVT in this pediatric cohort increased with age per year (OR 1.2, 95% CIs 1.1 to 1.2). Blood group 0 versus non-0 did not play a significant role in pediatric patients compared with control children (OR 1.05, 95% CIs 0.5 to 2.2). Further, there was no statistically significant difference between patients and controls with respect to BMI >95th age-dependent percentile (OR 0.94, 95% CIs 0.3 to 3.05). No significant associations were found between different types/sites of thrombosis and elevated factor VIII levels ($P=0.88$) or other thrombophilic risk factors ($P=0.3$).

The median level (range) of FVIII:Ag was 115 IU/dL (51 to 375) in children with DVT compared with 96 IU/dL (50 to 192: $P<0.0001$) in the control group. FVIII:C activity levels showed a median (range) value of 119 IU/dL (60 to 215) in patients compared with 106 IU/dL (50 to 176) in healthy controls ($P=0.0009$).

Median values (range) of vWF:Ag did not differ between patients and controls (110 IU/dL [38 to 165] versus 102 IU/dL [61 to 175], $P=0.15$). FVIII:Ag and FVIII:C levels were significantly correlated ($P<0.0001$; $r=0.522$), and vWF:Ag showed a weak correlation with FVIII:C ($P=0.0001$; $r=0.357$) and FVIII:Ag ($P=0.002$; $r=0.274$). FVIII:Ag was elevated above the 90th age-specific percentiles in 24 of 103 patients (23.3%) compared with 15 individuals in the control group (7.3%, $P<0.0001$). When using the activity method, elevated FVIII:C above the 90th age-specific percentiles was found in 20 children with DVT (19.5%) compared with 9 controls (4.4%, $P<0.0001$). The corresponding ORs (CIs) in the univariate logistic regression analysis (Table 3) with respect to the FVIII increase per IU/dL (gradual response) adjusted for age and fibrinogen levels were 1.02 (1.007 to 1.029) for FVIII:Ag and 1.02 (1.01 to 1.034) for FVIII:C. In the multiple regression



Age distribution of 103 children with venous thrombosis.

TABLE 2. Characteristics of Patients and Controls

	Study Group n=103	Control Group n=206
Age (median range), years	8.2 (0.6 to 18)	7.8 (0.6–17.5)
BMI, kg/m^2	16.7 (7.3–38.6)	15.8 (7.9–25)
Gender, female	n=46 (44.7%)	n=95 (45%)
Thrombus Location		
Cerebral veins	33 (9)*	...
Proximal DVT (leg)	22	...
Proximal pelvic DVT	15 (5)*	...
Renal veins and pelvic DVT (n=7)	14 (4)*	...
Portal veins	3 (2)*	...
Distal DVT (leg)	9	...
Subclavian veins	2 (2)*	
Pulmonary embolism and leg DVT (n=3)	5 (2)*	
Underlying Diseases		
Trauma/immobilisation‡	25 (9)*	...
Infection§	26 (10)*	...
Overweight‖	9	...
Oral contraceptives	8	...
Vitium cordis	3 (2)*	...
Dehydration	2	...
Steroid administration	4 (2)*	...
Thrombocytosis	1	...
Lupus	1	...
Diabetes type 1	2	...
Nephrotic syndrome	1 (1)*	...
Fetopathia diabetica	1	...
Miscellaneous<n=3	1	...
Otherwise healthy/idiopathic	19	206

*Central venous line-associated; ‡bed rest >4 days.

§Appendicitis, encephalitis, gastrointestinal infections, mastoiditis, meningitis, osteomyelitis, otitis, sepsis, sinusitis.

‖Overweight defined as BMI kg/m^2 >90th age-dependent percentile.

analysis (Table 3), levels of FVIII above the age-specific 90th percentiles retained their statistically significant association with DVT in children. For children experiencing DVT during infancy the fibrinogen-adjusted FVIII:Ag-OR was 5.5 (1.04 to 29.4) versus 4.8 (1.4 to 16.3) in older children, and 6.6 (1.3 to 35.7) versus 4.2 (1.2 to 14.3) for FVIII:C.

Heritability Study

The heritability estimate was found highly significant for elevated FVIII:C (h2, 0.61±0.15; $P<0.0001$) as well as for FVIII:Ag levels (h2, 0.48±0.15; $P=0.0008$). The estimates for the component of household effects were c2, 0.21 for FVIII:Ag levels and c2, 0.41 for FVIII:C levels. When modeling the household polygenic model to incorporate both h2 and c2 in the estimate, it became evident that for FVIII:Ag levels the phenotypic variance is predominantly explained by h2, whereas c2 was removed from the final model as non-significant. Interestingly, the opposite result was observed for FVIII:C, where the h2 component was removed from the final model and c2 stayed significant ($P=0.00002$).

TABLE 3. Factor VIII and Other Prothrombotic Risk Factors in Children With a First Onset of Venous Thrombosis (univariate multivariate analysis). In Addition, the Influence of Blood Group Non-0 Is Shown

Factor of Interest	Odds Ratio 95% CIs	*Adjusted Odds Ratio 95% CIs
FVIII:Ag >90th percentiles	6.7	4.3
	2.7–16.7	1.5–12.1
FVIII:C >90th percentiles	5.02	5.5
	1.9–12.8	2.03–15.06
vWF:Ag	2.10	...
	0.45–9.80	
FV G1691A	2.6	2.8
	1.32–5.21	1.14–7.05
FII G20210A	4.3	9.09
	1.08–17.06	1.40–58.84
Lipoprotein(a) >30 mg/dl	3.8	4.6
	2.04–6.97	2.05–10.27
TFPI <10th percentiles	4.36	5.2
	2.17–8.77	2.3–11.79
Antithrombin-, protein C-, or protein S-deficiency	6.4	12.57
	1.70–23.8	2.78–56.82
Blood group non-0	1.0	...
	0.47–2.3	

*Risk factors significantly associated with DVT in univariate analysis were included in the multivariate model.

The median (range) D-dimer concentration was 0.16 mg/L (0.1 to 1.9) for children with DVT compared with 0.18 mg/L (0.1 to 0.9) in controls ($P=0.7$). In addition, levels of f1.2 were no different between patients and controls (DVT, 0.8 nmol/L [0.2 to 4.6] versus 0.9 nmol/L [0.5 to 3.4]; $P=0.4$).

Cohort Analyses (Explorative)

Patients were prospectively followed for a median (range) period of 48 months (24 to 120). Transient PTS defined as one of the study end points occurred within 12 months of thrombotic onset in 19 of 59 children (32.2%) with DVT of the legs and pelvis. Seven male and 12 female patients were affected. Interestingly, 10 of 15 children in this group developed leg and/or pelvic DVT during puberty at a median age of 13.5 years (range 0.2 to 18 years). The transient PTS was associated with the initial thrombus extension (OR 3.9, CI 1.13 to 13.45), increasing age (OR 1.2, CI 1.07 to 1.32), and BMI (OR 1.2, CI 1.1 to 1.4). The increase of FVIII:Ag per IU/dL (OR 1.0, CI 0.99 to 1.01) or FVIII:C (OR 1.0, CI 0.97 to 1.02) did not play a significant role for acute PTS in the cohort investigated. During the regular follow-up visits at 24 months, however, persistence of PTS was finally diagnosed in 5 of 59 children (8.5%; male n=1). Increased leg circumference, pigmentations of the skin, or venous telangectasiae were no longer seen, neither at home, as reported and documented by the patient/parents, nor at the clinical follow-up visits. Recurrent thrombosis occurred in 8 of 103 patients (7.8%, cerebral venous

thrombosis n=3, leg DVT n=4, pulmonary embolism n=1). Because of the small number of children affected with persistent PTS or recurrent DVT, no further statistical calculations were performed.

Discussion

In the present study in children with non-cancer related DVT the rate of elevated FVIII:Ag levels was 23.3% (FVIII:C, 19.5%) in patients compared with 7.3% (FVIII:C, 4.4%) in the controls. Confirming the findings in adult patients with DVT and small scale case series from Turkish children,[16–24,29] the data presented here give evidence that elevated FVIII levels >age dependent 90th percentiles contribute to the odds of DVT in White children.

To date, the proportion of White children with DVT in whom one or more prothrombotic risk factors have been identified is ≈50%, whereas no thrombophilia has been found in the remaining subjects.[9–15] In similarity to the Dutch prospective 2-year thrombosis registry,[7] children consecutively recruited with a first DVT presented their first event in >80% of cases associated with underlying medical conditions, including central venous lines.[13,14] In addition, the typical distribution of thrombosis onset has been shown to have a first peak during the neonatal period and a second peak during puberty (Figure, 2 and 7). Thus, in contrast to our recently published cohort with idiopathic thrombosis only,[12] the present population has general applicability for White children. However, the rate of prothrombotic risk factors associated with DVT in this study, eg, the FV mutation, the FII variant, deficiency states of AT, PC, PS, or TFPI, and elevated Lp(a), is no different from controlled data in neonates, infants, and children previously published by the authors.[12,30,31]

Possible mechanisms thought to be associated with elevated FVIII are the enhancement of thrombin formation or the induction of acquired activated protein C (APC) resistance, but the molecular mechanisms that underlie elevated FVIII are still not clear.[32,33] vWF and blood group are important determinants of the FVIII level in plasma.[34–37] In the present study, neither blood group nor vWF levels showed significant associations with a first thrombotic onset in children. Family studies pointed toward a genetic influence on FVIII levels which persist over time and are not attributable to an acutephase reaction.[20–22,35,37–40] Our study is in agreement with these reports and presents further confirmation; household components were modeled in the equation and the heritability scores for FVIII levels were thus further refined. Interestingly, FVIII:Ag levels in our cohort are predominantly explained by heritable components plus environmental effects, whereas FVIII:C levels are predominantly explained by household and environmental effects. The true underlying mechanism for this observation has not yet been defined and requires further exploration. However, it is conceivable that basal FVIII:AG levels are more subject to genetic control than FVIII:C activity, which is influenced by a variety of environmental factors. Thus, the phenotypic variance explained through environmental and household effects is likely to override the phenotypic variance explained through genetic factors.

Similar to recently reported pediatric data the recurrence rate observed in our study was 7.8% in children with DVT who had been followed for a median of 48 months. In contrast to Goldenberg et al,[41] elevated FVIII levels did not contribute to the risk of PTS in the cohort presented here.

The limitation of the present study and of other pediatric cohorts reported[3–7,13,29,41] is mainly attributable to the rarity of the disease. To obtain more reliable data in children with DVT, PTS, or recurrent DVT in comparative as well as prospective follow-up studies, larger cooperative international surveys are necessary to better address this topic. In such studies standardization issues and differences in ethnical background have to be taken into consideration.

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Disclosure(s)

None.

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