Prevalence of discrepancy between one-stage and chromogenic FVIII activity assays and the mutation profile in Polish mild/moderate haemophilia A patients

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Mild hemophilia
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Objectives:

Patients with mild and moderate haemophilia A (MHA) have residual factor VIII activity (FVIII:C) 6-40 IU/dl and 1-5 IU/dl, respectively. There are three main assays of FVIII:C: one-stage (FVIII:C_{1st}) and two-stage (FVIII:C_{2st}) clotting method and chromogenic (FVIII:C_{Chr}) method. Recently significant discrepancy between the three assays has been described. Till now there is no consensus which method should be used to make definite diagnosis of MHA. The goal of our study was to evaluate the prevalence of discrepancy between FVIII:C_{1st} and FVIII:C_{Chr} assays among Polish MHA patients. Additionally, we investigated the correlation between causative mutations in *F8* and FVIII:C_{1st} and FVIII:C_{Chr} results.

Methods:

We examined 51 MHA patients aged 18 – 79 (median 37). In all study subjects coagulation screening tests, FVIII:C_{1st}, FVIII:C_{Chr} and genetic analysis of *F8* gene were carried out. Discrepancy was defined as ratio of FVIII:C_{1st}/FVIII:C_{Chr} ≥2 or ≤0.5.

Results:

Discrepancy was found in 25 (49%) patients with FVIII:C_{1st}/FVIII:C_{Chr} ratio ranging 2–16.7 (median 3.30). In 14 out of 25 discrepant patients (54%) haemophilia severity differed depending on assay used (Table 1). Genotyping analysis revealed 31 causative mutations in *F8* including five novel: c.926C>A (p.Pro309Gln), c.5141C>A (p.Thr1714Lys), c.5153T>C (p.Phe1718Ser), c.5384G>T (p.Arg1795Ile), c.6193T>G (p.Trp2065Glu) [Figure 1]. Fifteen different mutations have been found in discrepant patients and the most prevalent was c.398A>G (p.Tyr133Cys) identified in 5 out of 25 (20%) discrepant patients. In most of these cases (14/15; 93%), the mutated amino acid residues are localized in the A1, A2 and A3 domains at the interface between the subunits (A1-A2, A1-A3, A2-A3).

Conclusions:

Factor VIII assays discrepancy seems to be very common among Polish MHA patients (49% comparing to 30% in German and British studies), therefore haemophilia treatment centres should use both FVIII:C_{1st} and FVIII:C_{Chr} assays in the diagnostic work-out of mild/moderate haemophilia A.

Table 1: Mean values of FVIII activities measured with one-stage and chromogenic assays and the mutation spectrum in patients displaying FVIII:C_{1st} higher than FVIII:C_{Chr}.

n=25	No. Patients	Mutations (HGVS nomenclature)	FVIII domains	FVIII:C_{1st}	FVIII:C_{Chr}	Ratio
1	P-56#	p.Thr294Ile*	A1	25	1,5	16,7
2	P-30#	p.Leu1808Phe	A3	17	1,4	12,1
3	P-4#	p.Met1842Ile	A3	24	4,4	5,5
4	P-27#	p.Tyr655Cys	A2	22	4,2	5,2
5	P-25	p.Arg550His*	A2	44	8,8	5,0
6	P-10#	p.Trp2065Glu[N]	C1	16	3,3	4,8
7	P-12#	p.Phe1804Ile*	A3	19	4	4,8
8	P-1#	p.Tyr133Cys*	A1	6	1,4	4,3
9	P-13#	p.Phe1804Ile*	A3	19	4,5	4,2
10	P-50	p.Gly498Arg	A2	23	5,5	4,2
11	P-15#	p.Arg550Cys*	A2	13	3,2	4,1
12	P-17#	p.Arg550Cys*	A2	5,6	1,5	3,7
13	P-45	p.Arg550His*	A2	35	10,5	3,3
14	P-9	p.Tyr133Cys*	A1	19	6,1	3,1
15	P-26	p.Ala303Pro*	A1	35	11,7	3,0
16	P-49	p.Tyr133Cys*	A1	28	10,8	2,6
17	P-52#	*no mutation detected*		11	5	2,6
18	P-22	p.Tyr133Cys*	A1	17	7,5	2,3
19	P-39	c.5219+3 A>G	-	3	1,3	2,3
20	P-54	p.Tyr133Cys*	A1	12	5,2	2,3
21	P-58	*no mutation detected*		28	13	2,3
22	P-23	p.Ser229Pro	A1	39	19,7	2,0
23	P-37#	p.Gly694Glu	A2	1	0,5	2,0
24	P-47#	p.Gly466Arg	A2	1	0,5	2,0
25	P-24#	p.Arg550His*	A2	74	37	2,0

 # - discrepant patients in whom haemophilia severity differs depending on assay used; * - mutations previously reported to be associated with one-stage and chromogenic FVIII:C assays discrepancy; [N] - novel mutation.

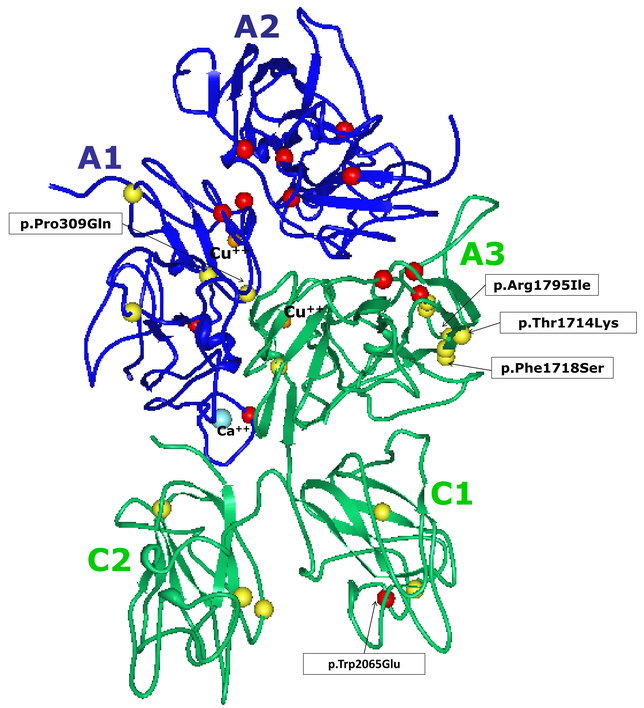

Figure 1: Three-dimensional structure of the B-domain deleted factor VIII protein

FVIII heavy chain is shown in blue, light chain is shown in green. Light blue ball represent calcium ions, the orange one copper ions. Mutations detected in discrepant patients are presented as red balls. Yellow bolls represent mutationts detected in patients with relevant FVIIII:C_{1st} and FVIII:C_{Chr} results.

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