Thrombophilia

Genetic heterogeneity of protein C deficiency in Hungary; genotype-phenotype correlations

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Introduction and Aims

In the background of hereditary Protein C (PC) deficiency more than 300 different mutations have been identified, so far. Type I (quantitative) and type II (qualitative) PC deficiencies can be distinguished, the latter can be subdivided according to the result of the chromogenic functional assay (CHR), that gives a normal PC activity in the cases of the rare type IIb variants.

Our aims were to map the mutation spectrum of PC deficiency in the Hungarian population and to predict the potential effect of novel mutations detected in this cohort and to investigate the effect of the different mutations on laboratory results.

Patients and Methods

Non-related individuals having 70% or lower PC activity were selected (n=134). PC activity was determined by clotting *(Siemens, PC Clotting)* and CHR tests *(Siemens, Berichrom PC)* and PC antigen was measured by ELISA *(Diagnostica Stago, Asserachrom PC, Enzyme Immunoassay for PC)*. Direct sequencing of *PROC* gene and MLPA analysis *(MRC-Holland)* were performed to detect potentially causative mutations. The prediction of pathogenicity of novel missense mutations was evaluated with MutPred, PhD-SNP and Poly-Phen-2 in silico tools.

Results

A total of 45 different mutations were identified in 83 index patients. Among them 18 (40%) novel and 27 (60%) known mutations were found (Figure 1.). Of the novel alterations 12 missense, 1 splice site, 4 nonsense and 1 large gene deletion were detected (Table 1.). Most of the mutations (n=29, 64%) resulted in type I, seven (16%) caused type II deficiency from which 5 were type IIb and 9 mutations resulted in uncertain laboratory phenotype (Figure 2.). Among the novel and interesting mutations the p.Ser30Arg mutation, which is located at the signal peptide region is predicted to be a benign mutation by high probability (Table 2.). The p.Arg57Trp, p.Arg57Gln, p.Glu61Lys, p.Cys64Ser, p.Gly109Cys, p.Cys122Phe, p.Arg129His, p.Cys160Arg, p.Ala309Pro, p.Gly392Arg and p.Asp401Asn mutations are predicted to be pathogenic by all the three methods we applied. There is no consensus among the three methods on the pathogenicity of the p.Thr110Arg, p.Arg123Ser, p.Ala333Thr, p.Ala333Asp and p.Ala408Thr mutations.

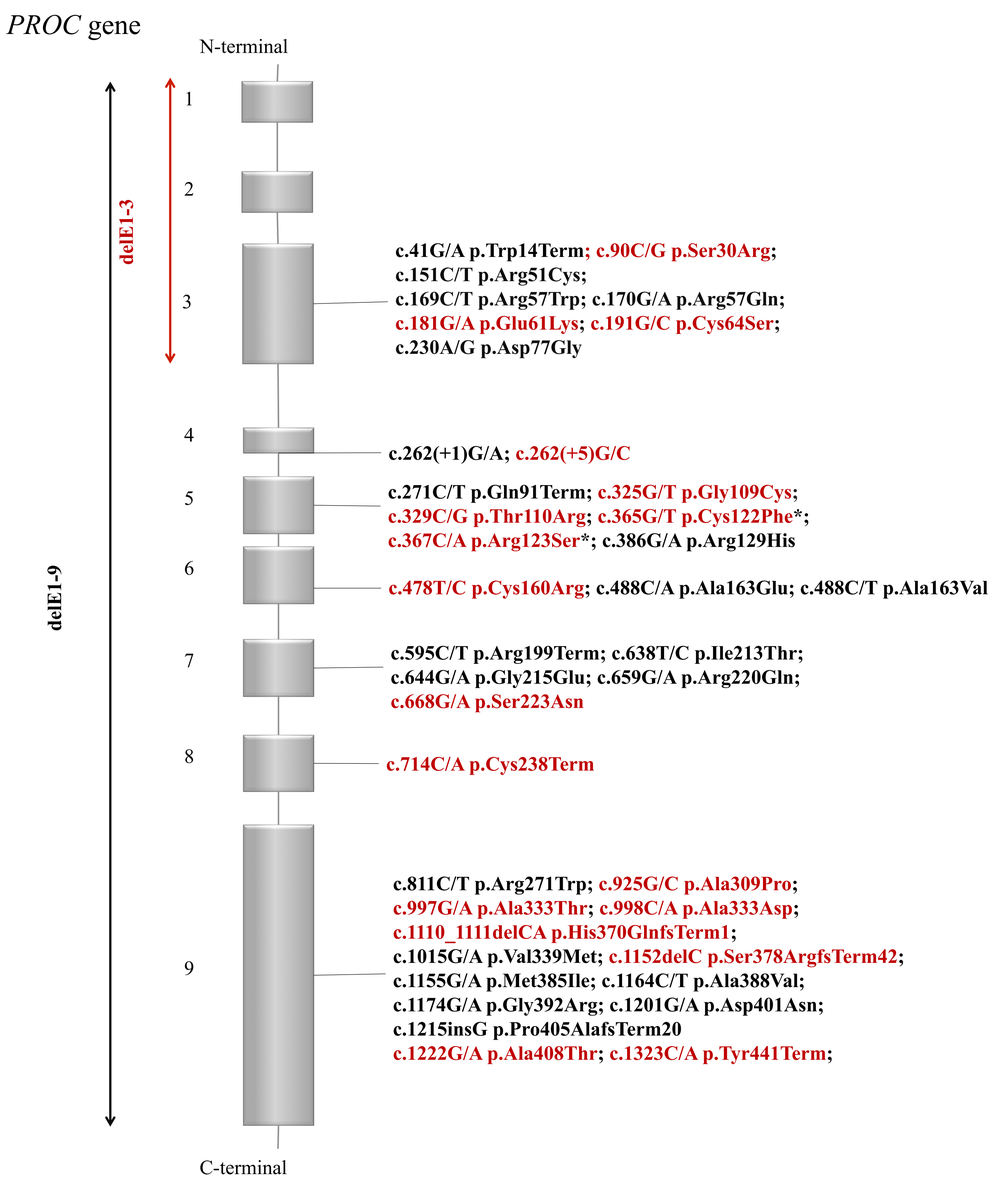

Figure 1. Mutations identified in *PROC* gene.
Mutations indicated in red are novel ones; *, compound mutations

Concerned nucleotide (cDNA numbering)	Concerned aminoacid (mature protein/total polypeptide)	Domain	Type of deficiency	Reference	non-related PCD patients	CL activity % median (min-max)	CHR activity % median (min-max)	Antigen % median (min-max)	VTE	Inherited/ acquired risk factors
c.90C/G	p.Ser30Arg	pre-pro leader	I	novel	1	58	68	68	YES	NO/NO
c.151C/T	p.Arg51Cys	Gla	I/II	[4]	1	53 (46-60)	ND	70 (66-74)	YES	NO/YES
c.169C/T	p.Arg57Trp	Gla	I/IIb	[4]	4	51 (37-68)	83 (63-94)	59 (54-81)	YES (2 patients)	NO/NO
c.170G/A	p.Arg57Gln	Gla	IIb	[2]	2	60 (42-61)	88	87 (73-101)	YES (1 patient)	NO/NO
c.181G/A	p.Glu61Lys	Gla	IIb	novel	1	59	76	78	YES	NO/NO
c.191G/C	p.Cys64Ser	Gla	I	novel	1	18	36	49	YES	YES/NO
c.208A/G	p.Lys70Glu	Gla	IIb?	[5]	1	66	77	ND	NO	NO/NO
c.262+5G/C	-	-	I	novel	1	33 (33-36)	42	41 (35-46)	YES	YES/NO
c.325G/T	p.Gly109Cys	EGF1	I/II	novel	1	55 (51-59)	64 (63-65)	66 (58-74)	YES	NO/YES
c.329C/G	p.Thr110Arg	EGF1	I	novel	1	41.5 (39-44)	46	32.5 (32-33)	YES	NO/YES
c.365G/T	p.Cys122Phe*	EGF1	I	novel	3	39 (19-53)	51.5 (38-65)	39 (32-45)	YES (2 patients)	NO/NO
c.367C/A	p.Arg123Ser*	EGF1	I	novel	3	39 (19-53)	51.5 (38-65)	39 (32-45)	YES (2 patients)	NO/NO
c.386G/A	p.Arg129His	EGF1	I/IIb	[4]	2	57 (53-66)	98 (91-105)	73 (65-81)	NO**	NO/NO
c.478T/C	p.Cys160Arg	EGF2	I	novel	1	41	63	48	YES	NO/NO
c.644G/A	p.Gly215Glu	activation peptide	I/IIa	[1]	7	41 (10-62)	47 (22-67)	53 (22-97)	YES (4 patients)	YES (4 patients)/ YES (2 patients)
c.714C/A	p.Cys238Term	catalytic	I	novel	1	52	49	49	YES	NO/NO
c.814C/T	p.Arg272Cys	catalytic	I	[3]	1	55	70	ND	YES	NO/YES
c.925G/C	p.Ala309Pro	catalytic	I	novel	1	43.5 (35-52)	ND	34 (27-41)	YES	NO/YES
c.997G/A	p.Ala333Thr	catalytic	I	novel	1	64 (59-65)	68	54 (51-55)	YES	NO/NO
c.998C/A	p.Ala333Asp	catalytic	I/IIb	novel	2	54 (44-63)	72	61.5 (49-71)	YES (1 patient)	YES/YES
c.1110_1111delCA	p.His370GlnfsTerm1	catalytic	I	novel	1	54.5 (48-61)	57 (54-60)	46 (41-51)	NO	NO/NO
c.1152delC	p.Ser378ArgfsTerm42	catalytic	I	novel	1	54.5 (54-55)	ND	52.5 (51-54)	YES	NO/NO
c.1174G/A	p.Gly392Arg	catalytic	IIb	[6]	1	57.5 (55-60)	73	89 (79-99)	NO	NO/NO
c.1201G/A	p.Asp401Asn	catalytic	I/IIa/IIb	[6]	5	47 (37-63)	69 (52-78)	69.5 (53-99)	YES (4 patients)	No/YES (1 patient)
c.1222G/A	p.Ala408Thr	catalytic	IIb	novel	1	61 (53-69)	119	92 (88-96)	YES	YES/NO
c.1323C/A	p.Tyr441Term	catalytic	I	novel	3	48 (40-51)	51 (48-53)	41 (33-57)	YES (2 patients) ** (1 patient)	YES (1 patient)/NO
del E1-3	-	-	I	novel	4	57 (39-65)	64.5 (24-70)	55 (48-64)	YES (2 patients)	YES/NO

Table 1. Laboratory results of the novel and interesting mutations.
*CL, clotting test; CHR, chromogenic test; VTE, venous thromboembolism; ND, not determined; *, compound mutations; **, arterial thrombosis; Reference intervals of laboratory tests: CL, Antigen: 70-130%; CHR: 70-140%*

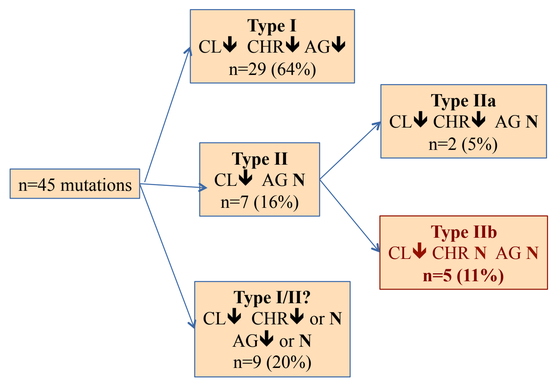

Figure 2. The distribution of laboratory phenotypes.
CL, clotting test; CHR, chromogenic test; AG, antigen; ↓, decreased; N, normal

Mutation	Domain	Type of deficiency	References	MutPred >= 0.5	PolyPhen2 HumDiv	PolyPhen2 HumVar
p.Ser30Arg	pre-pro leader	I	novel	**NO**	**NO**	**NO**
p.Arg57Trp	Gla	I/IIb	[4]	YES	YES	YES
p.Arg57Gln	Gla	IIb	[2]	YES	YES	YES
p.Glu61Lys	Gla	I/IIb	novel	YES	YES	YES
p.Cys64Ser	Gla	I	novel	YES	YES	YES
p.Gly109Cys	EGF1	I/II	novel	YES	YES	YES
p.Thr110Arg	EGF1	I	novel	YES	YES	**NO**
p.Cys122Phe	EGF1	I	novel	YES	YES	YES
p.Arg123Ser	EGF1	I	novel	YES	**NO**	**NO**
p.Arg129His	EGF1	I/IIb	[4]	YES	YES	YES
p.Cys160Arg	EGF2	I	novel	YES	YES	YES
p.Ala309Pro	Catalytic	I	novel	YES	YES	YES
p.Ala333Thr	Catalytic	**I**	novel	**NO**	YES	**NO**
p.Ala333Asp	Catalytic	I/IIb	novel	YES	**NO**	**NO**
p.Gly392Arg	Catalytic	IIb	[6]	YES	YES	YES
p.Asp401Asn	Catalytic	I/IIa/IIb	[6]	YES	YES	YES
p.Ala408Thr	Catalytic	IIb	novel	YES	**NO**	**NO**

Table 2. The results of the prediction of pathogenicity of novel and interesting mutations with in silico tools. These methods use (different) scoring schemes where a mutation can be regarded as probably pathogenic if the score value is exceed (or below) a certain cut-off value.

Conclusions

No founder mutation was identified in the Hungarian PC deficient population and a high number of novel variants were described throughout the whole *PROC* gene. 76% of the mutations associated with thrombotic symptoms and in most of the cases no additional risk factors were demonstrated. Based on the laboratory results type IIb PC deficiency was suspected in 5 cases and uncertain phenotype was demonstrated in 9 cases. In silico tools gave controversial prediction of pathogenicity in 5 cases and predicted a pre-pro leader mutation as benign. In case of type IIb mutations and mutations with uncertain phenotype or controversial in silico prediction biochemical studies are needed to establish their consequences.

References
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ISTH 2017 1538--PB
Thrombophilia
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DOI: 10.3252/pso.eu.ISTH2017.2017
Poster presented at ISTH2017 on:
Tuesday, July 11
ISTH 2017 CONGRESS JULY 8-13 BERLIN GERMANY WWW.ISTH2017.ORG TRANSCENDING SCIENTIFIC BOUNDARIES
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