Key words: plasma cell microaggregates, immunohistology prognosis, MRD, multiple myeloma.

Correspondence: Surender Juneja, The Royal Melbourne Hospital, Grattan St., Parkville, Victoria 3050, Australia. Phone: international +61.3.93428024. Fax: international +61.3.93428022. E-mail: surender.juneja@mh.org.au

References

1. Attal M, Harousseau J, Stoppa A. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. N Engl J Med 1996; 335: 91-7.
2. Blade J, Samson D, Reece D, Apperley J, Bjorkstrand B, Gahrton G, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Br J Haematol 1998;102:1115–23.
3. Wei A, Juneja S. Bone marrow immunohistology of plasma cell neoplasia. J Clin Pathol 2003;56:406–11.
4. Sukpanichnant S, Cousar J, Leelasiri A. Diagnostic criteria and histologic grading in multiple myeloma: histologic and immunohistologic analysis of 176 cases with clinical correlation. Hum Pathol 1994;25:308-18.

Disorders of Hemostasis

Factor XI deficiency: identification of six novel missense mutations (P23L, P69T, C92G, E243D, W497C and E547K)

Factor XI (FXI) deficiency is a rare coagulation disorder associated with bleeding of variable severity but without a clear relationship between bleeding and FXI levels. This study reports the molecular genetic analysis of FXI deficiencies in thirteen patients. Six novel missense mutations were identified: P23L, P69T, C92G, E243D, W497C and E547K.

haematologica 2005; 90:1149-1150
(*http://www.haematologica.org/journal/2005/8/1148.html*)

Factor XI (FXI) is the zymogen of a serine protease that participates in the early phase of blood coagulation.[1] Congenital FXI deficiency is a rare autosomal disorder found predominantly but not exclusively in the Ashkenazi Jewish population and occurs rarely in other populations.[2] So far, around 80 mutations causing FXI deficiency have been reported (available at URL: *http://archive.uwcm.ac.uk/uwcm/mg/search/119891.html*).[3]

Thirteen French patients originating from nine unrelated families and various ethnic groups were studied (Table 1). All 15 exons and exon-intron boundaries of the FXI gene were sequenced. Six novel missense mutations have been identified: P23L, P69T, C92G, E243D, W497C and E547K.

Patient #1 is heterozygous for a novel c8220t substitution in exon 3, leading to the P23L mutation, consistent with his partial FXI deficiency.

In family II (patients #2 and #3), a novel t10663g substitution in exon 5, which predicts a C92G mutation, has been characterized. The C92G mutation involves the disappearance of the C92-C175 disulfide bond and probably impairs the structure of the second apple domain. Therefore, this mutation could alter the assembly or the secretion of the protein. The two affected members presented a different bleeding tendency. This discrepancy could be explained because patient #3 has others hemostatic abnormalities, including an alteration of von Willebrand factor antigen (40%) and of factor VIII (50%). This combined deficiency increases the bleeding tendency.[4] Two novel mutations have been characterized in the catalytic domain in two unrelated patients (patients #4 and #5) who have a partial FXI deficiency. Patient #4 is heterozygous for the W497C mutation which involves the appearance of a new cysteine residue close to a disulfide bond (C496-C563). Patient 5 #bears a heterozygous E547K mutation, affecting the charge of the molecule. These mutations are associated with a reduction of both FXI antigen and activity below the expected values for heterozygous subjects (Table 1). It is possible that these two mutations affect wild-type FXI secretion consistent with a dominant negative effect, as recently demonstrated for two others mutations in the catalytic domain.[5]

In the two patients of family V, a novel g16584c substitution leading to an E243D mutation has been identified. Moreover, the father bears a second mutation, P520L, which has been previously described as a mutation with a modest catalytic defect in functional assays.[6] The contribution of each of these mutations to the deficiency is unclear.

In family VI, the mother was homozygous (patient #8) and her two sons were heterozygous (patients #9 and #10) for the G460R mutation which has been recently described by Mitchell *et al*.[7] This mutation induces a change in the charge and occurs in the vicinity of one of the residues forming the catalytic triad of FXIa (D462), so the activity of the protein could be altered. Moreover, patient 10 had a more severe deficiency than his brother (patient 9). This discrepancy could be explained by the compound heterozygosity of patient #10 who carries, in addition to the G460R mutation, another novel mutation in exon 4, P69T.

Patient #11 is a compound heterozygote for two previously described mutations: the C38R[8] and the P382L[9] mutations. The C38R mutation contributes to FXI deficiency, as this change induces the absence of the disulfide bond between cysteines 32 and 38 in the apple 1 domain.[8] As previously demonstrated,[8,9] these two mutations resulted in the absence of secretion (FXI:C< 1U/dL; FXI:Ag < 1U/dL).

For patient 12 an E297K mutation was predicted from exon 9 sequencing. The patient was heterozygous for this substitution, consistent with his partial FXI deficiency. Two other unrelated cases of E297K mutation have also been found (*Quélin et al., unpublished data*). Patient #13 was found to be homozygous for the T304I mutation previously described by Pugh.[10] This patient has low levels of both FXI:Ag (26 U/dL) and FXI activity (11 U/dL). This is consistent with the findings of Pugh[10] who demonstrated that the T304I substitution causes a reduction of factor XI secretion *in vitro*.[10] Moreover, this patient suffered important bleeding manifestations but other hemostatic abnormalities, such as von Willebrand's disease or platelet dysfunctions, have not been searched for.

Altogether, six novel mutations and five previously described mutations were identified in patients living in the suburbs of Paris. None of these mutations was found in a total of 67 normal chromosomes screened, indicating that they are not common polymorphisms. No recurrent mutation was found, perhaps because there is more intense population mixing in Paris than in other areas of France.[8] Unfortunately, additional family studies are not

Table 1. The patients' origin, laboratory findings and clinical presentation.

Family	Patient	Gender/age	FXI:C (U/dL) NR 70-150 U/dL	FXI:Ag (U/dL) NR 70-150 U/dL	Invasive procedure	Replacement therapy/ Bleeding	Spontaneous bleeding symptoms	Origin	Base Change	Amino acid mutation	Exon
I	1	F/34	30	39	Delivery×1	0/0	0	Black Africa	c 8220 t	**P23L** het	3
II	2 (father)	M/43	47	40	0		0	Vietnam	t 10663 g	**C92G** het	5
	3 (son)	M/14	21	44	0		Epistaxis Ecchymoses	Vietnam	t 10663 g	**C92G** het	5
III	4	F/29	22	25	Delivery×1 Appendicectomy	0/0 0/0	0	Sri Lanka	g 23022 t	**W497**C het	13
IV	5	F/33	23-45	32	0		Epistaxis	NA	g 23444 a	**E547K** het	14
V	6 (father)	M/NA	30-40	NA	0		0	French	g 16584 c	**E243D** het	8
									c 24264 t	P520L het	14
	7 (son)	M/20	30-40	28	0		Easy bruising	French	g 16584 c	**E243D** het	8
VI	8 (mother)	F/40	5	NA	Deliveries×2	0/0	0	Sri Lanka	g 22309 a	G460R hom	12
	9 (son)	M/4	32	NA	NA		0	Sri Lanka	g 22309 a	G460R het	12
	10 (son)	M/2	18	13	NA		0	Sri Lanka	g 22309 a	G460R het	12
									c 9656 a	**P69T** het	4
VII	11	M/58	<1	<1	Dental extraction	0/0	0	Portugal	c 8264 t	C38R het	3
					Meniscusectomy	0/0			c 20699 t	P382L het	11
					Cataract extraction	0/0					
VIII	12	M/31	40-58	52	NA		0	French	g 16845 a	E297K het	9
IX	13	F/36	11	26	Tonsillectomy	Bleeding	Epistaxis	French	c 16867t	T304I hom	9
					Delivery x 1	Bleeding	Menorrhagia				
					Dental extraction	Post-hemorrhage					
					Knee surgery	0/0					

FXI activity (FXI:C) level (normal range 70-150 U dL-1) was measured by an aPTT-based assay with severe FXI-deficient plasma used as substrate (Diagnostica Stago, Asnières, France). FXI antigen level (FXI:Ag, normal range 70-150 U dL-1) was assayed by ELISA based on goat anti-human FXI polyclonal antibodies and a peroxidase-conjugated IgG (Cedarlane Laboratories Ltd, Ontario, Canada). Base numbering refers to the sequence AY191837. NA: not available; Irr: irrelevant. New mutations are in bold type.

possible for the patients described here, hampering demonstration that these mutations are the cause of the FXI defect.

Florence Quélin, Dominique François,‡ Roseline d'Oiron, Benoît Guillet,* Emmanuelle de Raucourt,§ Philippe de Mazancourt*

*Laboratoire de Biochimie et de Biologie Moléculaire, Hôpital Raymond Poincaré, 92380, Garches, France and UVSQ EA2493; ‡Laboratoire d'Hématologie, Hôpital Foch, 92150 Suresnes, France; *Centre de traitement des hémophiles, 94270 Le Kremlin-Bicêtre, France; §Laboratoire d'Hématologie, CHI Poissy-St-Germain en Laye, 78100 St-Germain en Laye, France*

Funding: This work was funded by the Faculté de Médecine Paris-Ile de France Ouest and by Adenosine.

Key words: factor XI, factor XI deficiency, mutation.

Correspondence: Florence Quélin, Laboratoire de Biochimie et de Biologie Moléculaire, Hôpital Raymond Poincaré, 92380 Garches, France. Fax: international +33.1.47107923.
E-mail: Florence.quelin@rpc.ap-hop-paris.fr

References

1. Davie EW, Fujikawa K, Kurachi K, Kisiel W. The role of serine proteases in the blood coagulation cascade. Adv Enzymol Relat Areas Mol Biol 1979;48:277-318.
2. O'Connell NM. Factor XI deficiency. Semin Hematol 2004;41 Suppl 1:76-81.
3. Dossenbach-Glaninger A, Hopmeier P. Coagulation factor XI: a database of mutations and polymorphisms associated with factor XI deficiency. Blood Coagul Fibrinolysis 2005;16:231-8.
4. Bauduer F, Ducout L. Is the assessment of von Willebrand disease prevalence an achievable challenge? The example of the French basque country where blood group O and factor XI deficiency are highly prevalent. J Thromb Haemost 2004;2:1724-6.
5. Kravstsov DV, Wu W, Meijers JCM, Sun MF, Blinder MA, Dang TP, et al. Dominant factor XI deficiency caused by mutations in the factor XI catalytic domain. Blood 2004;104:128-34.
6. Gailani D, Bolton-Maggs PHB, Blinder M, Butler R, Mountford R, Heiny M, et al. Amino acid substitutions in the factor XI catalytic domain associated with factor XI deficiency. J Thromb Haemost 2004; 86 Suppl 1:P1112.
7. Mitchell M, Harrington P, Cutler J, Rangarajan S, Savidge G, Alhaq A. Eighteen unrelated patients with factor XI deficiency, four novel mutations and a 100% detection rate by denaturing high-performance liquid chromatography. Br J Haematol 2003; 121:500-2.
8. Zivelin A, Bauduer F, Ducout L, Peretz H, Rosenberg N, Yatuv R,et al. Factor XI deficiency in French Basques is caused predominantly by an ancestral Cys38Arg mutation in the FXI gene. Blood 2002;99:2448-54.
9. Bolton-Maggs TH, Butler R, Mountford R. Eight novel mutations in factor XI deficient kindreds in Northern Europe. Thromb Haemost 1999;2542:803.
10. Pugh RE, McVey JH, Tuddenham EGD, Hancock JF. Six point mutations that cause factor XI deficiency. Blood 1995;85:1509-16.