A structure–function analysis in patients with prekallikrein deficiency

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ABSTRACT

Objective: To investigate the structure–function relation in prekallikrein (PK) deficiency. PK is one of the proteins of the contact phase of blood coagulation which at the present time is the object of a revival of interest.

Methods: All patients with PK deficiency who had been investigated by molecular biology techniques are the object of the present investigation. Details of patients were obtained from personal files and a time-unlimited PubMed search. Only cases with a molecular-biology-based diagnosis were included.

Results: Twelve families were included. The total number of missense mutation was 10, together with 3 stop codons and 2 insertions. These mutations involved mainly exons 11 and 14. There were eight proved homozygotes and three compound heterozygotes. In one instance, homozygosity was probable but not proved. In nine cases, the defect was Type I, whereas it was Type II in the remaining three. No bleeding manifestations were present in 11 of the 12 probands. One proband had epistaxis, but she had hypertension. Altogether, four patients had hypertension and one of them had also two myocardial infarctions.

Conclusions: Despite the paucity of cases, it was established that the majority of mutations involved the catalytic domain. It is auspicious that future reports of patients with this disorder should include molecular studies. This would certainly contribute to the understanding of the contact phase of blood coagulation.

KEYWORDS
Prekallikrein; deficiency; hypertension; bleeding; molecular studies

The contact phase of blood coagulation is composed of three main factors, namely FXII, prekallikrein (PK) and Factor XI. High-molecular-weight kininogen (HMWK) also participates in the process as a co-factor. A central role is played by FXII, which, once activated, activates both PK and FXI. PK, once activated to kallikrein by aFXII, plays a role in controlling blood pressure by cleaving HMWK and releasing bradykinin. PK will also activate tissue plasminogen activator and therefore stimulate fibrinolysis [1]. These are the two main functions of PK, namely vasodilatation with consequent hypotension and increased fibrinolysis. Both functions may have a great potential role in vascular physiology and pathology. FXI, once activated, initiates, instead, the intrinsic clotting system by activating FIX. PK structure is similar to that of FXI and this explains why they are both activated by aFXII. There are also differences since PK is not activated by thrombin. Furthermore, PK, once activated, has the capability of enhancing FXII activation. FXI, on the contrary, lacks these two properties [1]. Therefore, it appears that PK plays an important role in the contact system. It has no clotting capability but it may cause vasodilatation, decrease blood pressure, stimulate fibrinolysis and serve as a potentiating effect in the activation of FXII [1–3].

Despite this important role played, PK has been poorly investigated when compared with other components of the contact phase of blood coagulation [4]. FXII and its activation by glass and other foreign surfaces have surely received more attention. Whereas it seems firmly established that patients with PK deficiency do not bleed, it is not clear yet if the defect might play a role in the pathogenesis of hypertension and other cardiovascular conditions [4]. Occasional reports of patients with PK deficiency who presented myocardial infarctions (MI) or other vascular diseases had suggested its role in this type of disorders, but they have been underestimated [5–7]. The picture is clouded by the fact that molecular biology studies on the subject have been limited [4]. No structure–function analysis on PK deficiency has ever been carried out. The purpose of the present study is to analyze all reported cases of PK deficiency that have been investigated by molecular biology techniques in an attempt to find clues that could suggest a direct pathogenetic role.

Patients, material and methods

All patients with PK deficiency who have been studied by molecular biology techniques are the object of the present study. Two extensive PubMed searches were carried out in January 2014 and again in February 2017. The search was carried out using pertinent
Results

A total of 12 families with a total of 20 patients were gathered [8–18]. The main features of these families are reported in Table 1. PK activity levels varied between less than 1% and 13% of normal. PK antigen level was evaluated only in 10 cases. It was found low in seven cases, and normal or reduced in three cases. The total number of mutations was 10. One mutation (Cys529Tyr in exon 14) was seen in four families. In three of these families, the mutation was at the homozygous level. In the fourth family, it was at the heterozygous level in combination with Trp383Stop codon forming a compound heterozygosis. Mutation Gly401Glu was seen in two families. All other mutations, Arg94Stop codon, Gly104Asp, Cys548Tyr, Asp558Glu, Trp499Stop codon and Arg541Gln, were present only in one family. There were also two cases of T insertion in intron 7.

There were nine homozygotes among the probands and three compound heterozygotes. One homozygous patient was double homozygous for mutations Gly104Arg + Asn124Ser. In one instance [15], homozygosity was likely but not proved since no family study was carried out. Furthermore, this case had a PK level of 13% [15]. The exons involved were mainly exon 11 and exon 14. There were 10 missense mutations, 3 stop codons and 2 insertions (Table 1). Only one patient showed a bleeding tendency [15]. Five patients (four propositi plus a family member) had instead hypertension and one of them had also two MI (Table 2).

Discussion

PK is a γ globulin with a molecular weight of about 86 kDa and an approximate plasma concentration of about 40 µg/ml. About 75% of plasma PK circulates bound to HMWK while only 25% circulates free [1]. The human PK gene contains 15 exons and 14 introns and is located on chromosome 4 (4q, 34–35) close to that of FXI. The structure of the protein is also similar to that of FXI [1]. Once activated by FXIIa, it is transformed in a heavy chain and in a light chain held together by a disulfide band. The heavy chain is involved in complexing with HMWK, whereas the light chain contains the catalytic domain [1].

PK deficiency is a rare disease since only about 150 cases have been reported [4]. Unfortunately the number of families investigated by means of molecular studies is very scanty. The paucity of data is a limitation of the study. Another limitation refers to the fact that we directly investigated only three of these families [8,17,18]. For the other families, we had studies to conform with the information supplied by the authors who published the respective papers.

The new mutations seen in these 12 families were 10 since 1 mutation (Cys529Tyr) of exon 14 was seen in 4 families and another (Gly401Glu) in 2. Finally, T insertion in intron 7 was present in two families. Since PK deficiency is undergoing a revival because of the potential association with hypertension and other vascular disorders, it is ausplicable that more cases should undergo a molecular genetic analysis [4,19]. This will allow the ascertainment of the possibility that some mutations might be more strictly correlated than others with vascular diseases. Mutations in exon 11 and exon 14 which control the catalytic domain of the light chain are more frequently involved in PK deficiency (Figure 1).

In only three cases, the mutations (Gly104Arg, Arg94Stop and Gly104Arg + Asn124Ser) involved the second Apple-shaped structure of the heavy chain [11,12]. This study of molecular-biology-proven cases confirms, in general, the common impression that the defect is not accompanied by bleeding. The only exception to this rule is represented by the patient described by Dasanu et al. [15]. However, it is not sure that the bleeding manifestations reported in this patient were due to PK deficiency. The bleeding manifestations were mainly mucosal, and concomitant defects have not been excluded (association with Rendu-Osler disease, thrombocytopathy and hyperfibrinolysis). Since the bleeding manifestations were epistaxis and the patient had hypertension, it could be surmised that the latter was the cause of the former. In this regard, it has to be noted that the patient underwent seven deliveries and no bleeding was noted [15].

The lack of bleeding in patients with PK deficiency is based also on the observation that even among the patients who have not been studied by molecular techniques (about 140 cases) no sure hemorrhagic diathesis has ever been reported but in one other instance [20]. In this latter case, the diagnosis of PK was doubtful even on the basis of the clotting methods, since no PK deficient substrate was used for the PK assays [20].
| Author (year)          | Age and sex | Consanguinity | Eponymous | PK activity % | PK antigen % | Background | Genotype | Mutation (Exon) | Comments                                                |
|------------------------|-------------|---------------|-----------|---------------|--------------|------------|----------|----------------|---------------------------------------------------------|
| Lombardi et al (2003)  | 14, M       | No            | Padua     | <1            | Traces       | Italian    | Comp. Het. | Trp383Stop + Cys529Tyr (11 and 14) | A sister similarly affected                             |
| Shigekiyto et al (2003)| 47, M       | n.r.          | Tokushima | <1            | 25           | Japanese   | Hom.     | Gly401Glu (3)    | Parents were first cousins                               |
| Jones et al (2004)     | 79, M       | Yes           | None      | <1            | 4            | Caucasian  | Hom.     | Arg94Stop (3)     |                                                                 |
| Katsuda et al (2007)   | 53, M       | Yes           | Seki      | 1–3           | Near Normal  | Japanese   | Double Hom. | Gly104Arg + Asn124Ser (5) | An124Ser polymorphism present. A brother and sister similarly affected |
| Francois A et al (2007)|             |               |           |               |              |            |          |                |                                                         |
| Case 1                 | 63, M       | no            | None      | <1            | 7            | Portuguese | Hom.     | Cys529Tyr (14) | Unrelated to previous case                              |
| Case 2                 | 38, M       | n.r.          | None      | <1            | 7.5          | French     | Hom.     | Cys529Tyr (14) | Unrelated to family presented by Shigekiyto et al. [9]   |
| Nagaya et al. (2009)   | 69, F       | Yes           | None      | <1            | <10          | Japanese   | Hom.     | Gly401Glu (11) | No family study. Proposita had bleeding tendency         |
| Dasanu and Alexandrescu (2009) | 75, F | n.r. | None | 13 | N.r. | Jamaican | Probably homo. | Cys529Tyr (14) | Unrelated to family presented by Girolami et al. [10] |
| Maak et al (2009)      | 14, M       | n.r.          | None      | 1             | N.r.         | German     | Comp. het. | T insertion + Cys548Tyr (intron 7) (15) | One brother also affected                               |
| Girolami et al (2010)  | 40, M       | No            | Cordoba   | 4             | 90           | Italian-Argentinian | T insertion + Asp558Glu (intron 7) (15) | One brother and two sisters similarly affected          |
| Nakao et al (2011)     | 40, M       | No            | None      | <10           | N.r.         | Japanese   | Hom.     | Trp495Stop (13) | Idiopathic thrombocytopenia also present                |
| Girolami et al (2014)  | 31, M       | No            | Cordoba 2 | <1           | <1           | Spanish-Argentinian | Hom. | Arg541Gln (15) | An aunt similarly affected                               |

Note: N.r. = not reported; Hom. = homozygote; Comp. Het. = Compound Heterozygote.
However, a word of caution in this regard is indicated. Recent studies have in fact demonstrated that defects or abnormalities of FXII or of FIX may be associated with both bleeding and thrombosis, depending on the site of the mutation [21,22]. Therefore, at this stage, it cannot be completely excluded that a similar pattern may occur also in PK deficiency. Unfortunately, there is only one example of a molecular characterization of a PK-deficient patient with bleeding [15]. The mutation is Cys529Tyr; however, the same mutation is reported in three other patients without any bleeding. Therefore, the possibility of a dual behavior for PK defects (asymptomatic or bleeders) remains unlikely and remote.

The finding that 3 of the 12 probands had cross-reacting material in their plasma is not surprising. Similar cases have been reported [23]. It is clear, therefore, that PK defects have to be distinguished, as other clotting proteins, in Type I (concomitant decrease of activity and antigen) and Type II (low activity with normal or slightly reduced PK antigen). We do not know yet if certain mutations are associated with the Type II defect. In the present study, the patients with Type II defect had the following mutations: Gly401Gln, Gly104Asp and T insertion + Asp558Glu.

Despite the paucity of data, it is possible to indicate that most of the mutations so far described involve the
catalytic domain (Figure 1). Due to the recent revival interest in the contact phase of blood coagulation, it is surprising that so few families have been studied by means of molecular biology techniques. It is more surprising that new case reports are being accepted without such studies. This is useless for the simple reason that it is well established that patients with PK deficiency have no bleeding tendency. It would be more important to know if the defect plays a role in hypertension and other cardiovascular disorders [4,19].

A clarification of the relation existing between PK deficiency and vascular diseases would come from a better understanding of the structure–function link in the PK protein. It could be, for example, that only certain mutations are more frequently associated with hypertension than others. For these considerations, only adequately investigated families should be published in the future.

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