Bleeding manifestations in heterozygotes with congenital FVII deficiency: a comparison with unaffected family members during a long observation period*

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ABSTRACT

Objectives: To determine whether heterozygotes with FVII deficiency have a bleeding tendency or not.

Patients and methods: Eighty-four patients (OK) heterozygous for FVII deficiency, at the onset of the study, were paired with unaffected family members and followed for a long period of time (mean 22.6 years) for the occurrence of bleeding. Diagnosis of heterozygosis had to be based on family studies, clotting, immunological assays and genetic analysis.

Results: The mean FVII activity level was 0.51 IU/dl (range 35–65) and 94 IU/dl (range 88–118) in the heterozygotes and in the normal counterparts, respectively. Documented bleeding manifestations occurred in eight heterozygotes and in seven normal subjects. Statistical analysis of the difference was not significant. Bleeding manifestations were easy bruising, bleeding after tooth extractions, menorrhagia, epistaxis with no difference among the two groups. There was no strict correlation between bleeding and FVII activity levels.

Conclusions: The study indicates that heterozygotes for FVII deficiency show rare bleeding manifestations which are also present in the unaffected family members with normal FVII levels. This indicates that Factor VII activity levels played no role in the occurrence of the bleeding symptoms. Furthermore, FVII levels of around 0.40 IU/dl are capable of assuring a normal hemostasis.

KEYWORDS
Bleeding; heterozygotes; factor VII deficiency

Congenital FVII deficiency is the most frequent defect among the rare bleeding disorders (RBD) [1–4]. First described in 1951 by Alexander et al. in an American family [5], it was subsequently reported in several countries, all over the world.

The existence of the defect had been suspected before 1951 on the basis of multiple cross-correction studies among normal, coumarin and FV-deficient plasmas [6,7]. Factor VII deficiency is, together with FXIII deficiency, the only clotting defect whose existence was postulated before the discovery of an actual patient [4].

The defect is usually subdivided into two types, namely type I and type II. The Type I deficiency is characterized by a concomitant decrease of FVII activity and antigen. On the contrary, in the Type II defect there is a discrepancy between FVII activity which is always low and FVII antigen which is normal or borderline, in any case, always much higher than FVII activity [8,9].

Homoyzgotes or compound heterozygotes with a FVII deficiency of less than 10% of normal present a sure but variable bleeding tendency in the sense that there is no strict correlation between FVII levels and severity of bleeding [1,10–14].

Heterozygotes, with FVII levels around 0.40–0.60 IU/dl of normal, are variably reported as asymptomatic or mildly symptomatic. Altogether, available data are scanty and inconclusive. Even good reviews or series studies on FVII deficiency fail to supply sure information in this regard [1–3,9,11].

The purpose of the present study is to report an investigation on the occurrence of bleeding in 84 patients with heterozygous FVII deficiency, followed for a long period of time in comparison with a control group made up by sex- and age-matched unaffected family members.

Patients and methods

Eight patients with known heterozygos for FVII deficiency were taken into consideration. All these patients were studied in Padua during the years 1968–2011. Some were the object of previous papers [15–22], some are unreported. At diagnosis, each patient was matched with an unaffected family member (same gender, age ± 5 years). In eight instances, such match could not be achieved for the lack of suitable subjects. In this case, the match was found among distant relatives, cousins, uncles or aunts once removed. Age varied from 8 to 61 (mean age 29.3); 45 patients were females and 39 were males. In the control group, age varied between 10...
and 63 (mean age 31.1); the same number of female and male were present.

Both the heterozygotes and the normal subjects were followed every 1–2 years. In case of occurrence of bleeding, the patient or the normal counterpart was requested to report to Padua where they were evaluated by one of us (A.G.) or by the physicians of a local hospital. In the latter case, the physicians of the local hospital were then contacted by us and the bleeding event was evaluated. The duration of the observation period varied between 3 and 36 years, and the mean observation period was 22.6 years. At the onset of the study, the following score was set to classify the bleeding manifestations: easy bruising, epistaxis, bleeding after tooth extraction, surgery or delivery 1 point; spontaneous hematomas 2 points; spontaneous hematuria or gastrointestinal bleeding 3 points; spontaneous hemarthrosis or replacement therapy 4 points; intracranial bleeding 5 points. Bleeding due to evident or overt external traumas was excluded. The bleeding manifestations had to be spontaneous or not related to known traumas, but in the case of delivery, surgery and tooth extraction. Easy bruising and epistaxis had to occur at least twice during the observation period in order to be accepted as a bleeding manifestation. At the time of the bleeding episode, a platelet count was always carried out, besides a PT and an aPTT, to rule out thrombocytopenia or other defects. If a patient or a control failed to appear at the scheduled control for the consecutive times, he or she was eliminated from the study together with his or her paired normal subject or patient.

FVII assays were carried out at onset and then every 1–2 years or according to the need (preparation for surgical procedures, deliveries, tooth extractions).

At least three tissue thromboplastins were used at the onset for the FVII assay (rabbit brain, human placenta or human recombinant and ox-brain thromboplastin). Afterwards, a rabbit brain thromboplastin was always used since it was shown to yield, in all patients, the lowest FVII activity level. The persons who carried out the FVII activity assays were not aware of the type of patients or controls (symptomatic or asymptomatic) being investigated.

FVII antigen evaluation was carried out by electro immunoassay using an antiserum supplied by Behring Laboratories (Marburg, Germany) or by an ELISA method (Asserachrom FVII, Stago Laboratories, Asnières, France).

The large majority of the heterozygous patients were members of families which contained also heterozygotes. In 12 cases, no homozygotes or compound heterozygotes were present in the family. However, diagnosis was firmly established even in these cases by means of clotting and immunological tests and by family study. Molecular biology techniques were also available for most families. The mutations found were Arg304Gln; splicing mutation IVS2-3C>A; Phe328Ser; Cys70stop; Ala294Val. Two families could not be studied by molecular biology techniques; however, diagnosis was equally firmly established on the basis of clotting, including cross-correction studies, immunological investigation and hereditary pattern. Statistical analysis of results was carried out by means of the Chi square and odds ratio (OR) tests. The patients and the normal controls were informed of the scope of the study which was carried out according to the Helsinki convention.

Results

The mean FVII activity level obtained by rabbit brain thromboplastin in all 84 patients was 0.51 IU/dl (range 35–65 IU/dl). The level obtained in the control group was 0.94 IU/dl (range 88–118). Mean FVII antigen was 51% of normal (range 42–62) in the patients with ‘true’ or Type 1 deficiency. It was normal in the FVII Padua patients and in the control group: 96.2%, range 90–125%, and 94.5%, range 88–115%, respectively (Figure 1).

There were eight bleeding episodes in the heterozygous group and seven in the control group. Such episodes were: easy bruising (two cases), bleeding after tooth extraction (2), epistaxis (2), menorrhagia (2) in the affected group. In the control group, we observed the same bleeding manifestations but for only one case of easy bruising. The main features presented by patients or controls who had bleeding manifestations are gathered in Table 1. There were no other bleeding manifestations. In particular, no undue bleeding was noted at delivery. The bleeding scores were 8 and 7, respectively, for the heterozygotes and the normal controls. The difference in the prevalence of bleeding was not statistically significant. Deliveries and surgical procedures and tooth extractions were never accompanied by excessive bleeding in either group. There were 16 deliveries among the heterozygous group and 14 in the control group. Tooth extractions were 11 and 12 for the heterozygotes and the normal counterparts, respectively. Finally, there were eight surgical procedures among the heterozygotes: appendectomy (2), inguinal hernia repair (2), tonsillectomy (2), repair of superficial cuts (2) and seven in the control group: appendectomy (2), inguinal hernia repair (2), tonsillectomy (1), sutures of accidental cuts (2). No prophylaxis with FFP or FVII concentrates was ever practiced in the affected group on the basis of the positive family history. One unit of whole blood (350 ml) was given to a heterozygous woman because of a pre-delivery anemia. No blood transfusions were given to the control group. There was no strict correlation between bleeding episodes and FVII levels (Figure 1). At the time of bleeding, platelet...
count and PTT were normal and the clotting pattern was similar to what observed at onset. There were six nonhemorrhage-related deaths in the affected group and five in the normal counterparts. Four patients (one male and three females) dropped out of the study. Their unaffected counterparts were also eliminated at the same time. No peculiar or preponderant disease was noted during the observation period in any of the two groups. In the case of death or dropping out of the study of patients, the normal counterparts were also eliminated. At the end of the study, the total number of patients was 74.

Discussion

The accurate knowledge of a hemostatically safe level of a given clotting factor is an important fact in clinical practice. The study of heterozygotes with congenital bleeding disorders may give an important contribution to the solution of the problem. Unfortunately, there is a lack of adequate studies in the literature (1). Most of the information are obtained from the studies of families which contain both homozygotes and heterozygotes [23–25]. The ideal study would be represented by a comparison of the prevalence of bleeding among heterozygotes in comparison with that observed in a group of sex- and age-matched unaffected family members over a long period of observation.

This type of study is difficult to perform for the following reasons: (1) limited number of patients available in a single center, (2) limited availability of controls (unaffected family members), (3) long period of observation. These facts explain the lack or the scarcity of this type of studies. As far as we know, only one study concerning FX is available [24]. Multicenter studies could be a solution of the problem, but they would implicate other limitations, for example lack or reduced homogeneity in the evaluation of patients and controls.

As far as heterozygotes with FVII deficiency are concerned, available data are equivocal, to say the least, some indicating lack of bleeding, others indicating the opposite. No matched study is available. The matter is complicated by the observation that even for homozygotes or compound heterozygotes with this deficiency, there is no strict correlation between Factor VII level and bleeding symptoms [1,10]. Factor VII deficiency is surely the clotting defect most frustrating in this respect.

Owing to what seen for patients with clearly decreased FVII levels, often less than 10% of normal, it is not surprising that an accurate evaluation might become very difficult or even impossible for heterozygotes who have a FVII level of 40–60% of normal.

However, the results of the study leave no doubts. No difference in the prevalence of bleeding has occurred between the heterozygotes and the unaffected family members. Of particular significance is the observation that no undue bleeding was noted during delivery, a known important risk factor. This indicates that FVII levels around 0.40–0.50 IU/dl are hemostatically adequate.

There is a limitation to the study which should be underscored.

Twenty one of these heterozygotes (29%) belonged to the FVII Padua (Arg304Gln) variant which is characterized by only a mild bleeding tendency even among the homozygotes, whereas heterozygotes are
almost always asymptomatic [6]. However, this bias is not significant since even in case of true deficiency, the incidence of bleeding is very low and not significantly different from that of the normal counterparts. Furthermore, the selection of the family members was carried out always taking into account the type of defect. In other words, the normal counterparts of the heterozygotes for FVII Padua were always selected among their family members and the same occurred for the cases of true deficiency.

There seems to be a clear difference between FX and FVII deficiency. A recent paired and sequential study has shown, as suspected, that heterozygotes for this deficiency may have a mild bleeding tendency [26].

Claims that heterozygotes for FVII deficiency could have a mild bleeding tendency are anecdotal, often based on questionnaire studies and lack both an adequate sequential follow-up and an adequate normal counterpart [1,23–25].

It has to be remembered, in fact, that occasional bruising, epistaxis, menorrhagia or bleeding after tooth extractions may occur even in normal subjects. This is particularly true for bleeding after tooth extractions, where local factors, such as techniques, local fibrinolysis, vascularization of mucosa and dental condition, play an important role [27,28].

Furthermore, there is another element which may influence the incidence of bleeding in heterozygotes, namely the attribution of a bleeding manifestation to an already diagnosed defect using inadequate controls. Unaffected family members represent the ideal controls because they often live in the same environment, have common nutritional habits, frequently are involved in the same social and working activities.

The blind attribution of the level of the clotting defect to the patients being examined gives a powerful support to the plausibility of the results here presented.

Comparison with other vitamin K-dependent clotting factors deficiencies and with FV defects can be made only with FX deficiency since a similarly structured study has been recently published [26]. There is no possible comparison with FII or FV deficiency since no adequate study has been published so far. Sporadic data concerning FII or FV deficiency are nonconclusive [2,29–31].

The comparison with FIX deficiency (Hemophilia B) is impossible because of the different hereditary pattern, no heterozygotes exist.

The comparison with FX deficiency is striking. In this case, about 29% of patient presented a mild bleeding tendency characterized by easy bruising, epistaxis and bleeding after tooth extraction or surgery [26].

It is hopeful that further studies on the heterozygous population with these RBD will be able to supply adequate information on the hemostatically safe level of these factors. As far as FVII deficiency is concerned, the present study clearly indicates that levels of 0.40 IU/dl appear to be hemostatically safe.

Disclosure statement
No potential conflict of interest was reported by the authors.

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Table 1. Main features of symptomatic heterozygotes or symptomatic unaffected family members. n.a.: not available; E.B.: easy bruising; O.C.: oral contraceptives; T.E.: tooth extraction.

| Subjects | Age, Gender | FVII activity | FVII antigen | Type of bleeding | Mutation | Therapy | Comments |
|----------|-------------|---------------|--------------|------------------|----------|---------|----------|
| Het.1    | M 50        | 50            | 55           | T.E.             | n.a.     | Local   |          |
| Het.2    | M 55        | 90            | 55           | Epistaxis        | Arg304Gly| Local   | FVII Padua |
| Het.3    | F 45        | 50            | 100          | Menorrhagia      | Ala294Val| Local   |          |
| Het.4    | F 62        | 100           | 90           | Menorrhagia      | Arg304Gly| O.C.    | FVII Padua |
| Het.5    | M 45        | 50            | 100          | T.E.             | Arg304Gly| Local   |          |
| Het.6    | F 65        | 60            | 100          | E.B.             | Phe328Ser| Local   |          |
| Het.7    | M 36        | 40            | 100          | E.B.             | Cys70stop| None    |          |
| Het.8    | F 60        | 55            | 100          | Epistaxis        | IVS2–3C>A| Local   |          |
| Nor. 1   | M 90        | 100           | 100          | T.E.             | Local    |         |          |
| Nor. 2   | F 110       | 95            | 100          | E.B.             | Local    |         |          |
| Nor. 3   | M 88        | 90            | 100          | Epistaxis        | Local    |         |          |
| Nor. 4   | M 90        | 105           | 100          | Epistaxis        | Local    |         |          |
| Nor. 5   | F 88        | 86            | 100          | Menorrhagia      | O.C.     |         |          |
| Nor. 6   | F 115       | 100           | 100          | Menorrhagia      | O.C.     |         |          |
| Nor. 7   | M 90        | 95            | 100          | T.E.             | Local    |         |          |
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