A total management system of carrier diagnosis for hemophilia using gene analysis : Results from 24 women in 15 Japanese families with hemophilia

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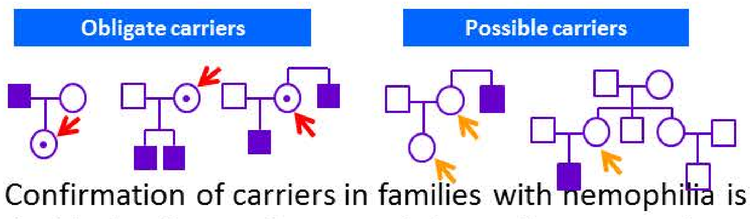
INTRODUCTION

Hemophilia A and B are hereditary bleeding disorders with an X-linked recessive inheritance pattern, caused by gene mutations in coagulation factor VIII (FVIII) or IX (FIX) that result in the absence or reduced activity of FVIII or FIX.

Women who have the genetic mutation for hemophilia on one of their X chromosomes are carriers. There are two definitions for carriers due to their genetic situations: obligate carriers and possible carriers.



Confirmation of carriers in families with hemophilia is valuable for these subjects and their relatives. Until recent years, carrier diagnosis was performed by standard pedigree analysis and conventional coagulation factor assays. However, because factor activity varies by such factors as X-lyonization, pregnancy, hormone level, aerobic exercise, chronic inflammation, individual difference, and daily variance, the subjects who underwent carrier diagnosis using blood coagulation tests have not been precisely identified.

OBJECTIVES

We aimed to apply gene analysis to carrier diagnosis of hemophilia and to establish a precise carrier diagnosis system for use in hemophilia patients widely.

METHODS

Informed consent was obtained from each patient and their family members who participated in the study. The study was approved by the Ethics Committee of Tokyo Medical University, Tokyo, Japan.

Table 1 Carrier diagnosis for hemophilia

Pedigree analysis (Family tree)
Blood coagulation test (*Clotting Factor assay*)
Factor VIII activity・vWF antigen・Factor IX activity
Gene analysis (*Genetic testing*)
PCR-direct sequencing, Long-PCR, MLPA

1. Factor VIII and IX assays

FVIII and FIX activity was measured with a functional assay based on APTT with Platerin-AAUTO (KYOWA KIRIN) and FVIII- and FIX- deficient plasma (IL).

von Willebrand factor (vWF) antigen level was determined with a latex coagulation method.

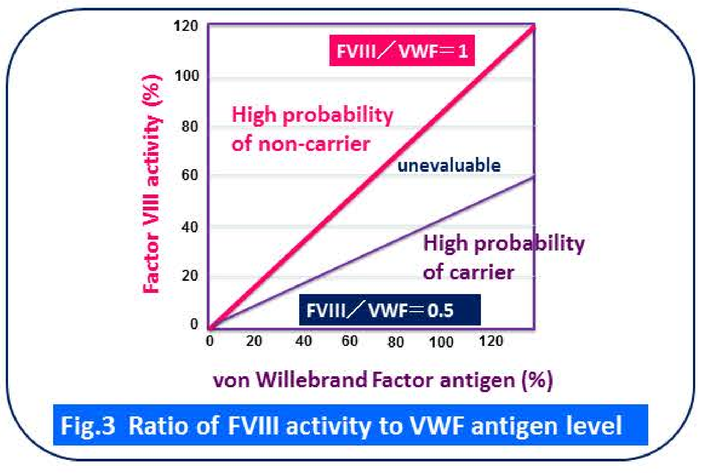


Fig.3 Ratio of FVIII activity to VWF antigen level

2. Gene analysis

Genomic DNA was isolated from leukocytes.

PCR-direct sequencing Purified PCR products with *TaKaRa LA Taq*™ (Takara BIO) were sequenced with a 3730 DNA Analyzer (Applied Biosystems).

Long-PCR For the detection of inversion (*F8*, intron 22), we performed long-PCR with KOD FX Neo (Toyobo).

MLPA For the identification of large deletions and duplications, we performed an MLPA assay using SALSA® P178 F8 and P207 F9 probemix (MRC-Holland).

RESULTS

1. Development of a total management system

A total management system was constructed (Table 1,2), and the *F8* gene analysis system flow-chart was generated (Fig.1).

Table 2 Flowchart of a carrier diagnosis for hemophilia

1. Application to our system (by attending physician)	
2. Submit an application form	
3. Review of the application（ethical and social issues）	
4. Informed consent (on site)	Patients (hemophilia)
5. Specimen collection	
6. Gene analysis and blood coagulation tests	
7. Explain the results of gene analysis to patient	
8. Informed consent (on site)	Subjects (possible carriers)
9. Specimen collection	
10. Gene analysis and blood coagulation tests	
11. Explain the results of diagnosis for carrier status (gene analysis and blood coagulation tests)	
12. Counseling based on the results of carrier diagnosis	
13. Counseling , following-up ,and carrier care	

Fig.1 *F8* gene analysis system flow-chart

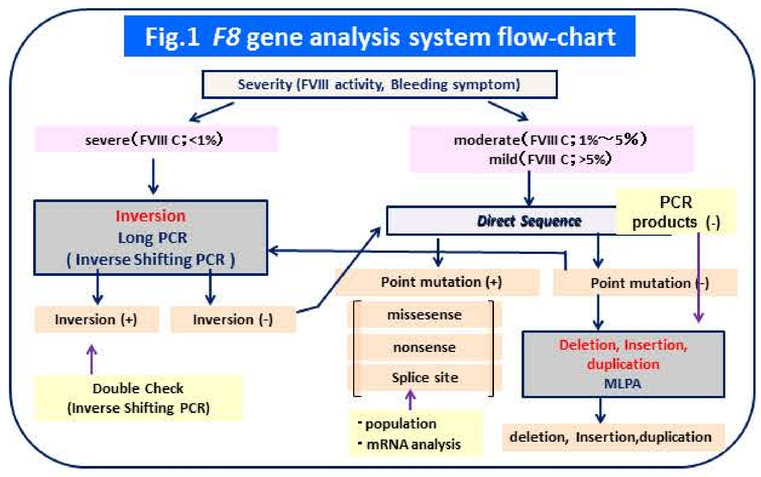


Table 3 Subjects

	Women	Families
Hemophilia A	20*	13
Hemophilia B	4	2

* including 1 obligate carrier

2. Subjects

Twenty women who belonged to 13 families with hemophilia A and 4 women who belonged to 2 families with hemophilia B were analyzed for carrier detection by gene analysis (Table 3).

Reasons for application to the carrier diagnosis system by the 23 women were presented as pie graph as in Fig. 2.

Fig.2 Reason for the application to the carrier diagnosis

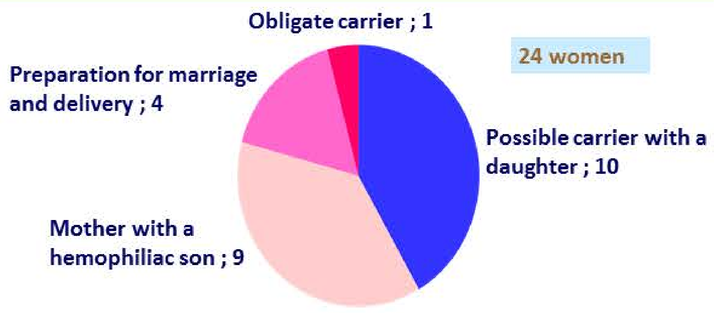


Table 4 Diagnostic testing for carrier status

	Hemophilia A	Hemophilia B
Carrier	17*	4
non-carrier	3	0

* including 1 obligate carrier

Table 5 Results of gene assay for carrier

Hemophilia A 17 women	Inversion	7
	Missense mutation	6
	Deletion	3
	Nonsense mutation	1
Hemophilia B 4 women	Missense mutation	4

Table 6 Comparison of clotting factor assay with genetic assay

Blood Coagulation Test	Genetic testing: Mutation (+) (Carrier)	Genetic testing: Mutation(-) (non-carrier)
FVIII/vWF : 0.6↓ (Carrier)	1	0
FVIII/vWF : 0.6↑ (non-carrier)	9	1

* Blood coagulation test perform more than three times on a different days.

3. Diagnostic testing for carrier status using gene analysis

Seventeen women were diagnosed as carriers and 3 women were diagnosed as non-carriers in the families with hemophilia A (Table 4). Inversion of intron 22 in the heterozygous state was identified in 7 women. Six missense mutations, 3 deletions, and 1 nonsense mutation were identified in the heterozygous state. Four women were diagnosed as carriers in the 2 families with hemophilia B and missense mutations were identified in the heterozygous state (Table 5).

Blood collections of 11 subjects were performed more than three times on different days, and the ratio of FVIII activity to vWF antigen level was calculated (Fig.3). Concordance between blood coagulation assay and genetic assay was only observed in one carrier and in one non-carrier (Table 6).

CONCLUSION

1. We constructed a total management system of carrier diagnosis as a central laboratory for hemophilia in Japan using gene analysis and conducted carrier diagnosis of 24 women based on this system.
2. We identified causative mutation in 20 of 23 women who were possible carriers of hemophilia A and B and they were diagnosed as carriers.
3. The genetic test can determine the causative mutation directly and possesses high reliability.
4. The rate of accurate diagnosis of carriers by blood coagulation tests is remarkably low.
5. Our carrier diagnosis system is expected to play an important role in enhancing the quality of life of carriers as a widely available method in Japan.

DISCUSSION

The genetic tests which can determine the exact genetic mutation, is very useful in carrier diagnosis. In contrast to genetic tests, the rate of accurate diagnosis of a carrier by blood coagulation tests is remarkably low. Blood coagulation tests are useful for the judgment of coagulation function of carriers, it is a medical checkup for carriers, however, we cannot recommend that subjects undergo only a blood coagulation test for carrier diagnosis.

In the present situation in Japan, the genetic tests are performed as a part of a special study only in institutions that specialize in hemophilia. Also, the cost of gene analysis is expensive, and the genetic test may take many months until getting the results. DNA of at least one of patients in family members is necessary for genetic test. Thus, there are complex issues, ethical and cultural concerns, in genetic testing for carrier diagnosis. Therefore, we tried to construct a new total management system of carrier diagnosis for hemophilia as a central laboratory in university hospital setting.

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