SPECTRUM OF MUTATIONS IN 407 UNRELATED HAEMOPHILIA B PATIENTS

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Introduction

• Haemophilia is an X-linked inherited bleeding disease which is one of the most common coagulation disorders due to impaired Factor FVIII:C for Haemophilia A (1/5000 men) and Factor FIX:C for Haemophilia B (1/25 000 men).

• The severity of the disease is defined by the activity of coagulation factor and it is classified into three clinical phenotypes level: severe with FIX:C <1% of normal, moderate with FIX:C 1-5%, and mild with FIX:C between 5 and 40%.

• FIX:C is encoded by Factor IX gene (*F9*) which maps to the distal end of the long arm of the X chromosome (Xq27). Eight exons encode a single chain polypeptide with six major domains.

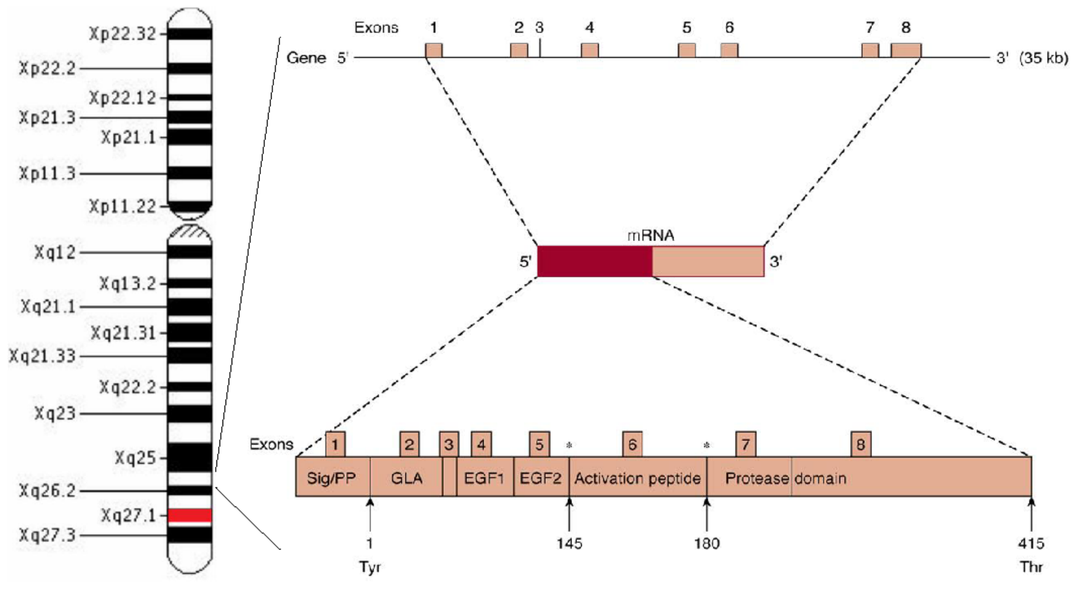


Figure 1: schematic *F9* gene

Aim of the study

➢ **Description and impact of the mutations identified in haemophilia B families**

Population

Diagnosis of Haemophilia B is established on the basis of FIX:C levels

Number of unrelated families: 407

17 (4%) are female presenting normal or low level of FIX:C and no previous family history of the disorder.

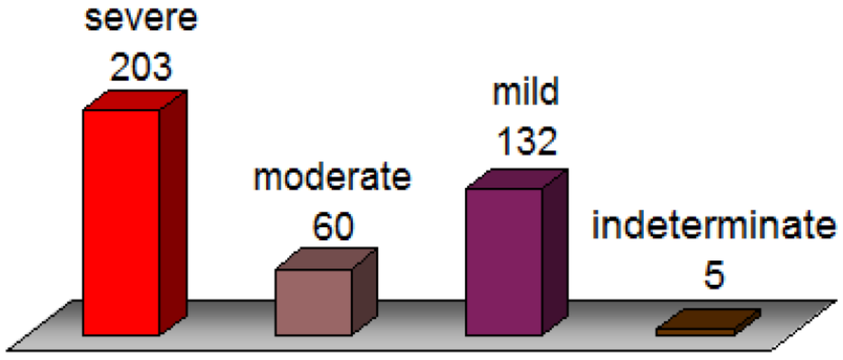


Figure 2: Distribution of severity

Status	n	
family history	228	56%
male without family history	106	26%
female without family history	17	4%
adopted child	2	<1%
unspecified	54	13%

Tableau I: Distribution of status

Methods

❖ DNA analysis

- Systematic analysis of the 8 exons and intron/exon splice junctions, as well as promoter and the 3' untranslated region of the *F9* gene.

- Search for duplication in male patients or heterozygous deletion and duplication in women was performed using a home made method based on quantitative multiplex fluorescent amplification (QFM-PCR) of the promoter, the 8 exons and the polyadenylation signal sequence.

- A specific Long Range PCR for identification of breakpoint deletion for the frequent exon 6 deletion observed in the patients originated from Reunion Island.

- In some families, linkage studies were performed to determine the existence of a common ancestor.

❖ Prediction of the deleterious consequences of mutations

• *In silico* Analysis :

- of missplicing effect : interrogation of bio-informatic tools : splicing-finder, Maxent Scan, HSF.

- for consequences on the structure and function of the protein : POLYPHEN, SIFT, AGVD, sequence alignement

• Segregation studies including healthy men of the concerned family.

• International databases interrogations as well as PubMed and *Haemophilia B Mutation Database*

• French Network of laboratories on genetic of haemophila studies named GENOSTASE

1. Results: Detection rate

Whole population in our cohort	407
Patients with mutation identified	388
Patients without mutation identified	19
Detection rate	**95%**

Tableau II: Detection Rate

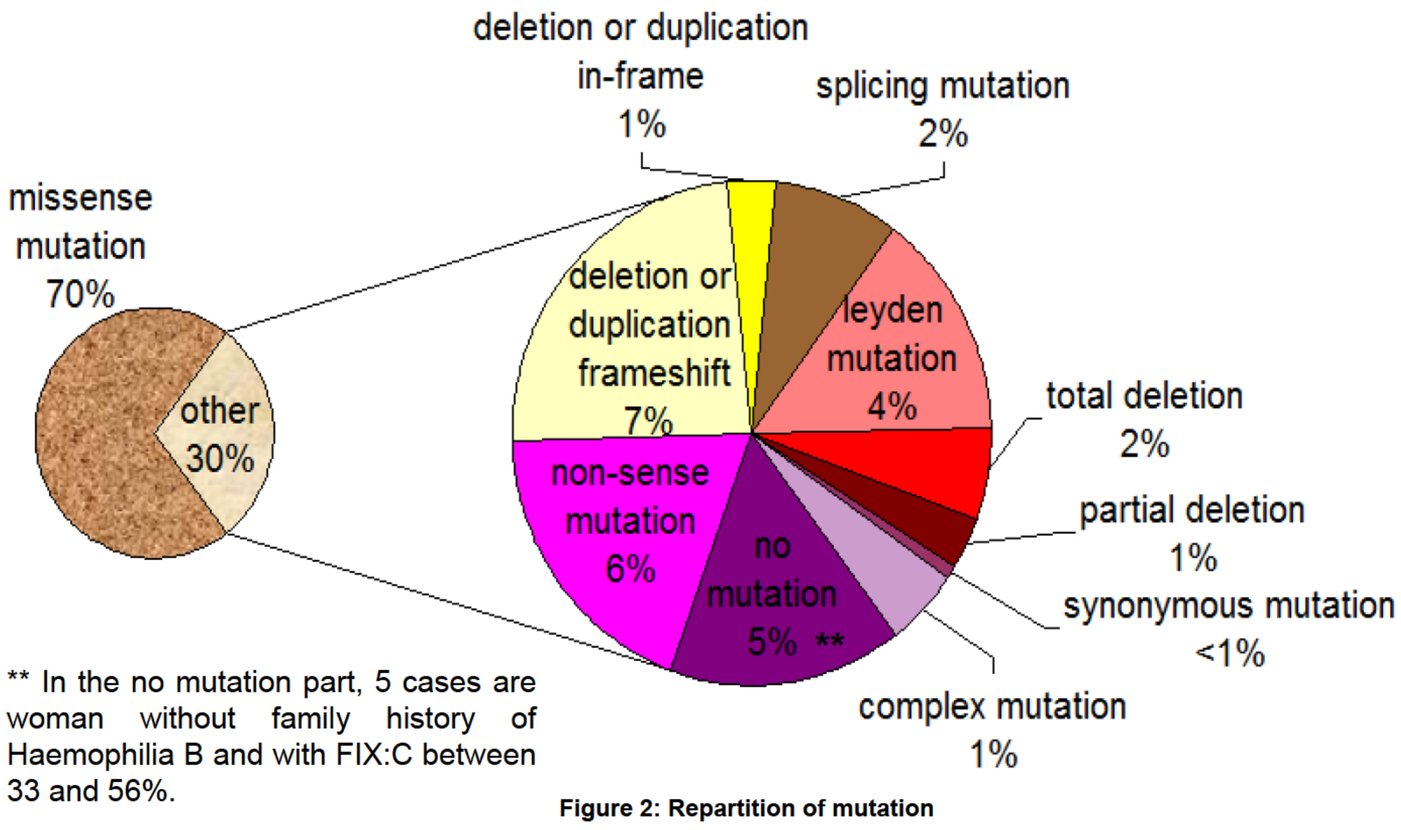
2. Results: Description of mutations

	n	
Total of mutations	410*	
Mutation present in the database by different group	343	83,6 %
Mutation only once previously reported in database by our group	36	**8,8 %**
New mutations only described in this study	31	**7,6 %**

Tableau III: Description of mutations

* We have find two different mutations in three patient.

2. Results: Classification of mutations



** In the no mutation part, 5 cases are woman without family history of Haemophilia B and with FIX:C between 33 and 56%.

Figure 2: Repartition of mutation

Conclusion:

We have identified 31 new mutations whose deleterious character has been succesfuly established.

Synonymous mutation are currently under investigation using minigen studies to study their impact on transcription.

To conclude, 95% of patient are identified which confirm efficiency of our strategy. Only 5% of the patients had no mutation identified after extensive gene analysis whatever the severity of the disease. No duplication has been identified. As, deep intronic mutation suspected of having consequences on splicing cannot be explored by direct RNA studies, exploration of whole gene encompassing intronic region by next generation sequencing will probably help us to solve these cases .

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