European Human Genetics Conference
Virtual Conference
AUGUST 28–31, 2021

P19.029.A

Wide spectrum of *F9* variants in hemophilia B families from the Portuguese population

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INTRODUCTION

Hemophilia B (OMIM#306900) is an X-linked recessive bleeding disorder caused by molecular defects in the Factor IX gene (*F9*), leading to either deficiency or functional abnormality of Factor IX.

Actual data indicate a high heterogeneity of variants in *F9*. Over 1000 different variants have been reported, including pathogenic single nucleotide variants (SNPs), indels and complex variants.

SUBJECTS AND METHODS

86 index patients and 313 relatives were studied. Genomic DNA was extracted from peripheral EDTA blood samples.

F9 variant analysis was performed from total genomic DNA by polymerase chain reaction (PCR) followed either by: i) SSCP (single-stranded conformation polymorphism) and DNA Sanger sequencing (earlier studied families) or ii) direct DNA Sanger sequencing, including the entire coding region, flanking intronic sequences, untranslated leader sequence and a segment of the putative promoter of *F9*.

When no variant was detected by sequencing, *F9* analysis by Multiplex Ligation-dependent Probe Analysis (MLPA) was performed using kit SALSA® MLPA® P207 (MRC Holland), including probes specific for the eight *F9* exons. Comparative analyses was performed using the Coffalyser software.

In samples with no *F9* amplification, multiplex PCR for *F9* flanking regions and array analysis was performed.

Segregation studies were performed in each family.

RESULTS (1)

F9 small substitutions, deletions and insertions identified in the Portuguese population (HGVS nomenclature)

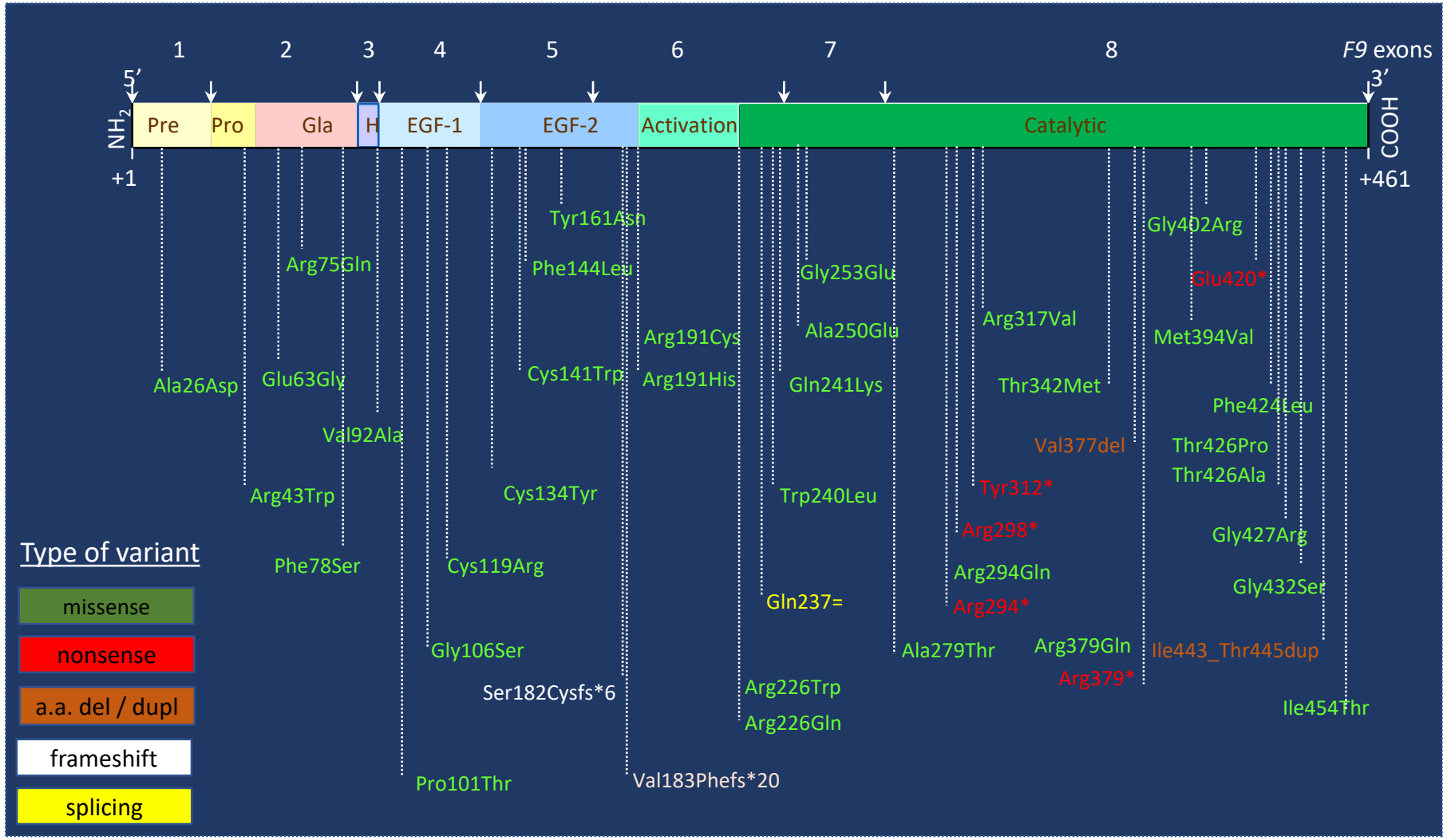
F9 region	cDNA (NM_000133.4)	Protein (NP_007994.1)	HGMD®
Promotor	c.-35G>C	-	CR890138
Exon 1	c.77C>A	p.(Ala26Asp)	CM10302
Intron 1	c.88+5G>T		CS961565
Exon 2	c.127C>T	p.(Arg43Trp)	CM940415
Exon 2	c.188A>G	p.(Glu63Gly)	CM045770
Exon 2	c.224G>A	p.(Arg75Gln)	CM940432
Exon 2	c.233T>C	p.(Phe78Ser)	CM010261
Exon 3	c.275T>C	p.(Val92Ala)	CM001672
Exon 4	c.301C>A	p.(Pro101Thr)	CM010262
Exon 4	c.316G>A	p.(Gly106Ser)	CM940466
Exon 4	c.355T>C	p.(Cys119Arg)	CM940482
Intron 4	c.391+5_391+8delGTAA	(splicing)	CD910509
Intron 4	c.391+5G>T	(splicing)	
Exon 5	c.401G>A	p.(Cys134Tyr)	CM940487
Exon 5	c.423C>G	p.(Cys141Trp)	CM940498
Exon 5	c.432T>G	p.(Phe144Leu)	
Exon 5	c.481T>A	p.(Tyr161Asn)	CM1611745
Intron 5	c.520+1G>T	-	CS910432
Exon 6	c.545_546delCT	p.(Ser182Cysfs*6)	CD930970
Exon 6	c.547delG	p.(Val183Phefs*20)	CD993260
Exon 6	c.571C>T	p.(Arg191Cys)	CM940537
Exon 6	c.572G>A	p.(Arg191His)	CM940534
Exon 6	c.676C>T	p.(Arg226Trp)	CM940545
Exon 6	c.677G>A	p.(Arg226Gln)	CM940541
Exon 6	c.711A>G	p.(Gln237=)	CS920993

F9 region	cDNA (NM_000133.4)	Protein (NP_007994.1)	HGMD®
Exon 6	c.719G>T	p.(Trp240Leu)	CM960587
Exon 6	c.721C>A	p.(Gln241Lys)	CM010269
Exon 7	c.749C>A	p.(Ala250Glu)	
Exon 7	c.758G>A	p.(Gly253Glu)	CM940571
Exon 7	c.835G>A	p.(Ala279Thr)	CM940587
Exon 8	c.880C>T	p.(Arg294*)	CM940593
Exon 8	c.881G>A	p.(Arg294Gln)	CM940591
Exon 8	c.892C>T	p.(Arg298*)	CM940596
Exon 8	c.936C>G	p.(Tyr312*)	CM057691
Exon 8	c.950C>T	p.(Ala317Val)	CM940608
Exon 8	c.1025C>T	p.(Thr342Met)	CM940625
Exon 8	c.1130_1132delTTG	p.(Val377del)	CD910518
Exon 8	c.1135C>T	p.(Arg379*)	CM940663
Exon 8	c.1136G>A	p.(Arg379Gln)	CM940660
Exon 8	c.1180A>G	p.(Met394Val)	CM940678
Exon 8	c.1204G>A	p.(Gly402Arg)	CM000154
Exon 8	c.1258G>T	p.(Glu420*)	CM940708
Exon 8	c.1270T>C	p.(Phe424Leu)	CM940710
Exon 8	c.1276A>C	p.(Thr426Pro)	CM940716
Exon 8	c.1276A>G	p.(Thr426Ala)	CM005426
Exon 8	c.1279G>A	p.(Gly427Arg)	CM010295
Exon 8	c.1294G>A	p.(Gly432Ser)	CM940724
Exon 8	c.1361T>C	p.(Ile454Thr)	CM960610
Exon 8	c.1328_1336dupTATATACCA	p.(Ile443_Thr445dup)	CI931084

(green box indicates novel variant)

RESULTS (2)

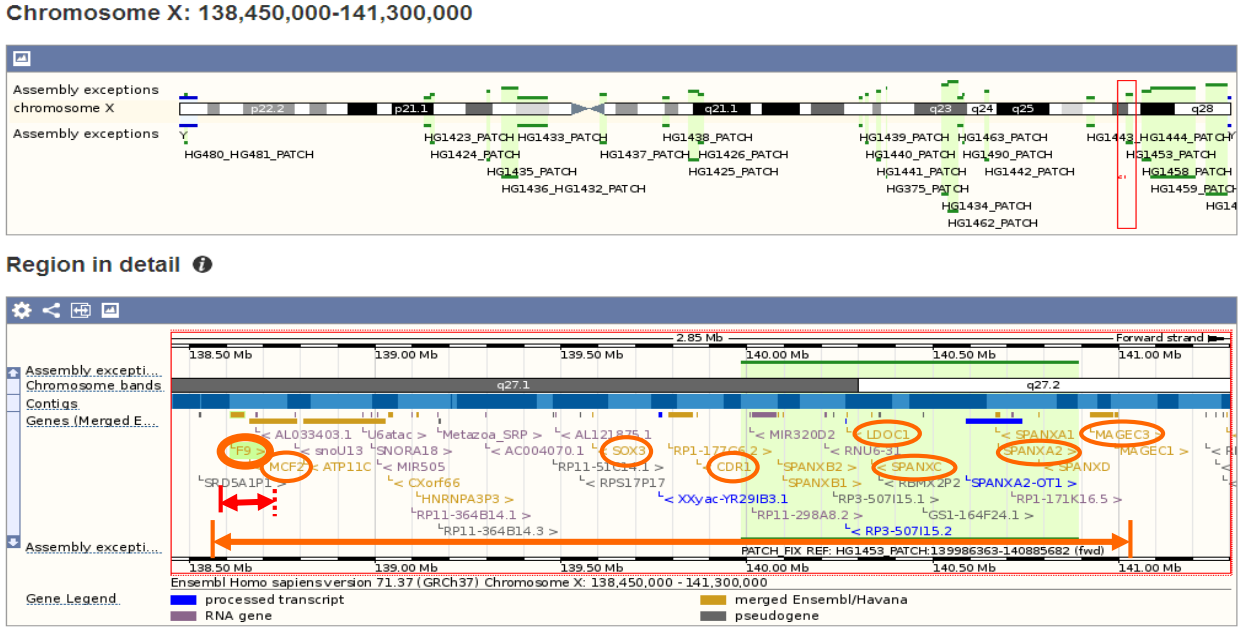
Identified variants in *F9* coding region in the Portuguese population



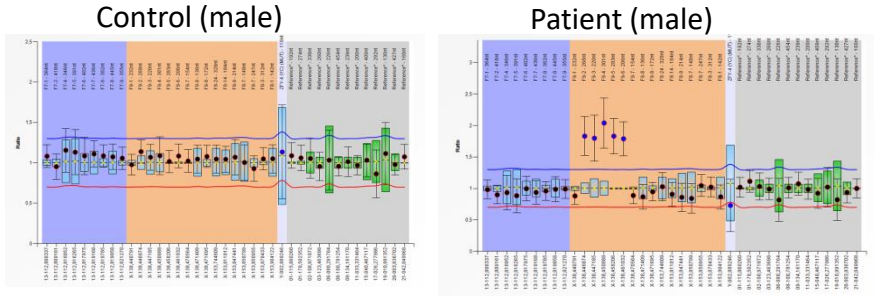
RESULTS (3)

Two gross deletions and a duplication in *F9* coding region in the Portuguese population

Two *F9* gross duplications



F9 exons 2-6 duplication (MLPA results)



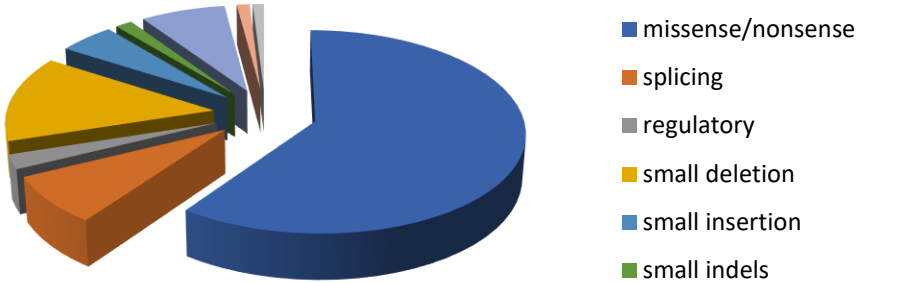
SUMMARY OF RESULTS

- 49 SNPs or small indels (in *F9* promoter, exons or introns)
  - 3 variants not previously described:
    - c.391+5G>T
    - c.432T>G, p.(Phe144Leu)
    - c.749C>A, p.(Ala250Glu)
- 1 *F9* gross duplication (exons 2-6)
- 2 *F9* gross deletions (>59kb and 2,742 Mb extensions)

- Molecular basis of Hemophilia B was identified in all studied families
- Carrier status was established in over 300 women
- 12 prenatal diagnosis were performed

CONCLUSIONS

Spectrum of F9 variants (HGMD data vs Portuguese population)



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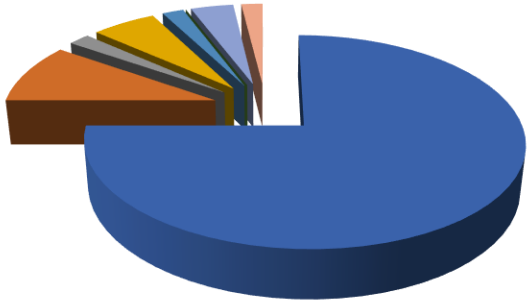

Portuguese population from this study

- The spectrum of *F9* variants identified in the Portuguese population significantly overlaps that observed in other populations.
- Identification of *F9* gene variants in patients allows genotype-phenotype correlations and carrier detection, as well as prenatal diagnosis.
- Sanger sequencing of the coding region and adjacent intronic sequences of *F9* still remains a valid and effective tool for the molecular study of hemophila B, providing information for appropriate genetic counseling and new insights regarding the molecular basis of the pathology.

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Acknowledgements

Authors would like to thank the patients and their relatives, as well as all clinicians that provided samples for this study.
We thank all staff from Unidade de Tecnologia e Inovação – Departamento de Genética Humana – INSA, for Sanger sequencing capillary electrophoresis and data.