F8 GENOTYPE SPECIFIC INHIBITOR RISKS IN ARGENTINE PATIENTS WITH SEVERE HA AND PARTICULAR RISK ESTIMATION OF DIFFERENT MUTATION TYPES

Liliana Rossetti[1‡], Claudia Radic[1], Martín Abelleyro[1], Vanina Marchione[1], Irupé Szurkalo[1], Laura Primiani[2], Daniela Neme[2], Miguel Candela[2,3], Miguel de Tezanos Pinto[2,3], Carlos De Brasi[1,3].

[1] Laboratorio de Genética Molecular de la Hemofilia, IMEX, CONICET-Academia Nacional de Medicina (ANM); [2] Fundación de la Hemofilia *Alfredo Pavlovsky*; [3] Instituto de Investigaciones Hematológicas, ANM. Buenos Aires, Argentina.

[‡]Email: rossetti@hematologia.anm.edu.ar

BACKGROUND

Haemophilia A (HA) is an X-chromosome inherited disorder associated with deleterious mutations in the coagulation factor VIII gene (*F8*). The development of inhibitory antibodies is a serious complication that occurs in 15-30% of patients with severe HA in response to replacement therapy with FVIII. Version 2012 of the WFH Global Annual Survey (WFH, Annual Survey, 2013) informed that 286 out of 2075 people with HA presented with clinically identified inhibitors in Argentina, indicating the significance of their comprehensive characterization in our patients. Both genetics and non-genetics factors have been implicated to influence inhibitor formation (Astermark, 2006). Among patient's genetics, several studies have shown that the type and location of the haemophilia causative mutation is the most decisive risk factor for inhibitor formation (Oldenburg et al, 2002).
This study describes a comprehensive and multicentre countrywide *F8* genotype characterization study of Argentine patients with HA and inhibitors using a cost-effective laboratory scheme, particularly useful for developing countries. The analysis between the type and location of *F8* mutation allows us to associate specific HA patients with locally estimated inhibitor risks.

OBJECTIVES

1. To characterise the phenotype causative mutations in Argentine patients with severe haemophilia A with and without inhibitors countrywide.

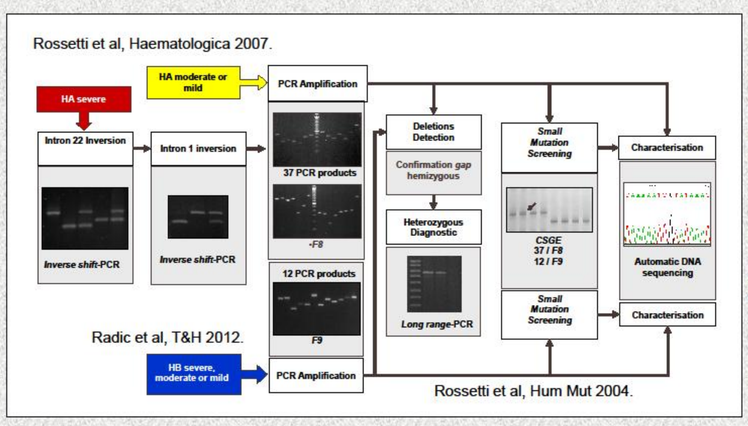
2. To estimate locally-specific risks for developing inhibitors associated with each specific *F8* mutation type/location and mutational risk groups.

METHODS

Studied populations: We studied DNA samples from 278 severe HA patients, classified by inhibitor status (INH): N (negative), T (transient, <6 month), LR (low responders, 1-5 UB/dl) and HR (high responders, >5 UB/dl). These populations represent the entire series of severe HA patients. To estimate the risks for developing FVIII INH associated with each *F8* mutation type/location, we considered an Argentine unbiased group of sHA patients (n=107) showing an Inhibitor Prevalence (IP=(LR+HR)/(N+T+LR+HR) of 17.6%. The comprehensive population of Argentine patients with sHA (n=278, 97 cases and 181 controls) was considered to estimate relative inhibitor risks (OR) associated with each mutation type/*F8*-location.

Molecular algorithm analysis for *F8* and *F9* genes: *F8* intron 22 and intron 1 inversions were analysed by inverse shifting-PCR (Rossetti-Radic et al, 2008). All the relevant sequences of *F8* and *F9* were represented in 37 and 12 PCR-amplifications, respectively that were designed for mutation screening by CSGE (conformation sensitive gel electrophoresis). Large deletions were defined as a consistent absence of PCR-product amplification of a contiguous group of exonic sequences. Small mutation screening was performed using CSGE and Sanger DNA-sequencing (Rossetti et al, 2007).

Statistical Analysis: Chi-square test was applied to analyse contingency table of Inhibitor status (i.e., HR, LR, T and N) *vs F8* mutation inhibitor risk groups (i.e., High, Medium and Low risks). These analysis was achieved by use of GraphPad Prism 4.0 software.



Updated molecular algorithm for a comprehensive mutational analysis of the entire *F8* and *F9* in Argentina.

The three points to enter the scheme are indicated. Severe HA is indicated as S-HA, moderate or mild HA, M-HA, and severe, moderate or mild HB is indicated as S/M-HB.

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RESULTS

We studied the haemophilia causative mutation in 278 severe HA cases: 97 showed positive INH, including 79 with HR and 18 with LR, and 181 were INH negative (N).
We characterized the causative mutation by application of a laboratory algorithm including inverse shifting-PCR for *F8* inversions, 37 PCR-amplifications for gross deletion detection, and for small-mutation screening by CSGE, and DNA-sequencing.
The case/control study (97/181) permitted estimation of *F8* genotype–specific inhibitor risks [OR; IP (CI95%)] in sHA patients classifying a high-risk group including multi-exon deletions [3.98; 59% (20–100)], Inv22 [1.72; 23% (18–28)] and nonsense in FVIII-LCh [1.35; 23% (7,6–63)]; an intermediate risk group including single-exon deletions, indel frameshifts and nonsense-HCh; and a low-risk group represented by missense defects [0.15; 2,9% (0.9–9,4)] (Table 1).
In addition, this approach allowed performing multiple mutation associations of more than one molecular defect, e.g.: small mutations *vs* INV22/INV1 & deletions resulted in [0.46; 10,6% (7.1–15,2)] predicting more than twice times less risk of INH developing for sHA patients without large rearrangements. This analysis may be associated side-by-side with the techniques forming the traditional algorithm used in most laboratories for sHA molecular diagnosis worldwide (1st INV22/INV1 analysis and 2nd large deletion detection) that normally requires less time than small mutation screening allowing a rapid estimation of patient-specific INH risks.

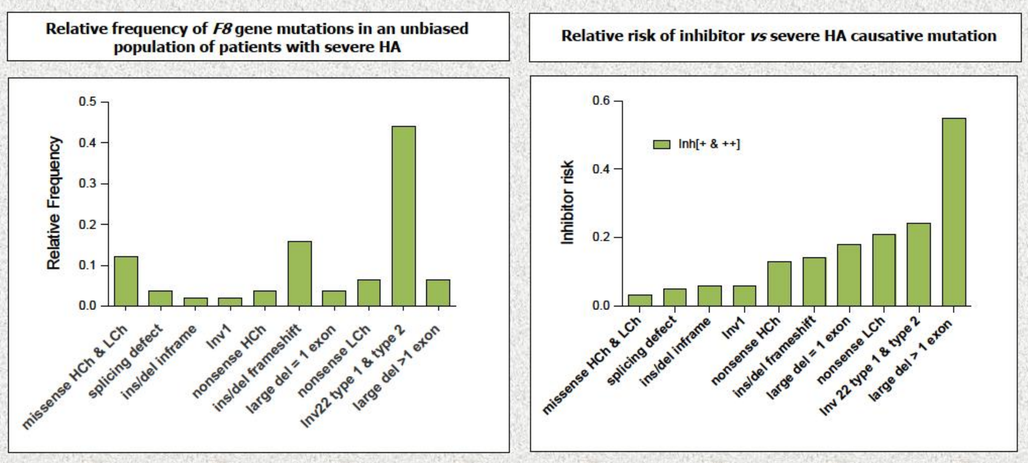

Figure 1. Left panel: *F8* mutation frequencies of an unbiased Argentinean population of severe HA patients. Right panel: Inhibitor relative risks of *F8* mutations in the same population.

Table 1. FVIII inhibitor development *vs F8* mutation type/location in Argentine patients with severe HA by single mutation type and multiple mutation associations

Mutation Type	SHA[1] [%]	Cases[2] N= 97	Controls[3] N= 181	OR[4](CI 95)	IP[5](CI 95) [%]	P value[6]
INV22	44.0	56	80	1,72 (1,05-2,84)	23 (18-28)	0,0333*
INV1	1.9	0	3	0,26 (0,01-5,12)	4,7 (0,2-83)	0,5539
MED	6.5	8	4	3,98 (1,17-13,57)	59 (20-100)	0,0276*
SED	3.7	3	5	1,12 (0,26-4,81)	20 (4,8-74)	1
NS.LCH	6.5	5	7	1,35 (0,42-4,38)	23 (7,6-63)	0,758
NS.HCH	3.7	5	11	0,84 (0,28-2,49)	15 (5,1-42)	1
FS.I/D	15.9	15	29	0,96 (0,49-1,89)	17 (9,3-29)	1
IF.I/D	1.9	0	2	0,37 (0,02-7,75)	6,6 (0,3-100)	0,544
MS	12.2	3	32	0,15 (0,04-0,50)	2,9 (0,9-9,4)	0.0002**
SPD	3.7	2	8	0,46 (0,09-2,19)	8,2 (1,7-37)	0,5021
Mutation Group[7]						
INV22/1	0.459	56	83	1,61 (0,98-2,65)	22,2 (17,4-26,5)	0,0779
INV/MED	0.524	64	87	2,10 (1,25-3,49)	23,4 (19,5-26,6)	0,0054**
INV/M/SED	0.561	67	92	2,16 (1,28-3,63)	23,0 (19,5-25,8)	0,0035**
F8 Small Mutations[8]	0.439	30	89	0,46 (0,27-0,78)	10,6 (7,1-15,2)	0,0035**

[1] SHA [%]: Natural (unbiased) Mutational Prevalence in Severe HA showing an absolute inhibitor prevalence estimation of 17.6% (N=107), similar to those previously reported in the same population [11].
[2] Cases: Permanent Inhibitor Positive cases (high and low responders).
[3] Controls: Inhibitor Negative or Transient.
[4] OR: Inhibitor Likelihood Odds Ratio (Mutation positive/Mutation negative); (CI95): Confidence interval of 95%.
[5] IP [%]: Absolute Inhibitor Prevalence; (CI95).
[6] P value: Fisher Exact Test P value; *P<0.05 significant; **P<0.01 highly significant.
MED: Multi Exon Deletion; SED: Single Exon Deletion; NS.LCH: Nonsense Light Chain; NS.HCH: Nonsense Heavy Chain; FS.I/D: Frameshift Ins/Del; IF.I/D: In-Frame Ins/Del; MS: Missense; SPD: Splicing Defect.
[7] Mutation Group (different mutation type association): i.e., INV22 and INV 1 vs small mutations.
[8] *F8* Small Mutations vs *F8* large rearrangements (includes inversions and multi-exon and single exon deletions).

CONCLUSIONS

In conclusion, the Argentine series of sHA patients presents similar global and mutation-specific inhibitor risks than the HA database and published series.

This case-specific information that can be obtained in approximately 15-20 days may be valuable for designing fitted therapies and follow-up protocols in patients with high risk for inhibitor development.

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