Ann Hematol (2012) 91:633–636
DOI 10.1007/s00277-011-1295-z

LETTER TO THE EDITOR

Are *int22h*-mediated deletions a common cause of hemophilia?

Miguel Martín Abelleyro · Liliana Carmen Rossetti · Claudia Pamela Radic · Miguel Candela · Irene Beatriz Larripa · Carlos Daniel De Brasi

Received: 19 April 2011 / Accepted: 7 July 2011 / Published online: 20 July 2011

Dear Editor,

Hemophilia A (HA) (OMIM 306700) is an X-linked inherited bleeding disorder caused by deleterious mutations in the coagulation factor VIII gene (*F8*). Even though there is a broad diversity of HA-causative mutations, an uncommon type of rearrangement—a large DNA inversion involving *F8* intron 22 (Inv22)—accounts for approximately one half of severely affected patients. Inv22 was formerly described by Lakich et al. [1] and Naylor et al. [2]. A collaborative international effort estimated that Inv22 is the cause of 43% (35%, 7%, and 1% for Inv22 type I, type II, and rare types, respectively) of severe HA cases worldwide with minor geographical or ethnical differences [3], in close agreement with our corresponding Argentinean series (42% of Inv22, and 34% and 7% for type I and type II, respectively) [4]. Naylor et al. [5] indicated that Inv22 originates by homologous recombination between well-defined duplicons (*int22h*) of 9.5 kb located one copy within *F8* intron 22 (*int22h*-1, *h*1) and the other, inversely oriented, from a group of two extragenic copies (*int22h*-3, *h3* for Inv22 type I and *int22h*-2, *h2* for type II). It was formerly believed that *h2* and *h3* were equally oriented (i.e., head to tail). However, Ross et al. [6] determined that *h2* and *h3* are inversely oriented (i.e., head to head), both embedded in the arms of a large imperfect palindrome (Fig. 1). This finding prompted Bagnall et al. [7] to hypothesize recombination between these arms interchanging the location of the extragenic *int22h* copies and generating a non-deleterious inversion polymorphism in Xq28, i.e., *h123* and *h132*. In this scenario, Inv22 type I may be generated from intrachromosomal recombination between *h1* and *h3* on the most frequent variant *h123* whereas Inv22 type II may be generated between *h1* and *h2* on the least frequent *h132* (Fig. 1). Moreover, on each of these normal structural variants of the X chromosome, recombination between *h1* with either equally oriented copies (*h2* or *h3*) may generate deletions (Del22) or duplications (Dup22) but not inversions [7]. More precisely, Del22 type I would be generated by recombination between *h1* and *h3* on variant *h132* whereas Del22 type II by recombination between *h1* and *h2* on variant *h123* [8] (Fig. 1).

Notwithstanding these theoretical speculations, until recently, no such Del22 mutation has been unequivocally reported in the literature, and Del22 has been suspected to be extremely deleterious, even to compromise the viability of hemizygous males [8]. Unexpectedly, a recent paper by Abou-Elew et al. [9] detected three cases of Del22 (two Del22 type II and one type I) by the use of inverse-shifting PCR (IS-PCR) [10] in a group of 13 Egyptian patients with severe HA. In this scenario and previous beliefs on the phenotype of *int22h*-mediated deletions (Del22), the aims of this scientific letter are, first, to report the lack of Del22 in a series of *int22h*-mediated rearrangements from Argentina and, second, to provide a simple practical approach to

Electronic supplementary material The online version of this article (doi:10.1007/s00277-011-1295-z) contains supplementary material, which is available to authorized users.

M. M. Abelleyro · L. C. Rossetti · C. P. Radic · I. B. Larripa · C. D. De Brasi (✉)
Departamento de Genética,
Instituto de Investigaciones Hematológicas,
Academia Nacional de Medicina de Buenos Aires,
Pacheco de Melo 3081,
Buenos Aires 1425, Argentina
e-mail: cdebrasi@hematologia.anm.edu.ar

M. Candela
Departamento de Hemoterapia y Hemofilia,
Instituto de Investigaciones Hematológicas,
Academia Nacional de Medicina de Buenos Aires,
Buenos Aires, Argentina

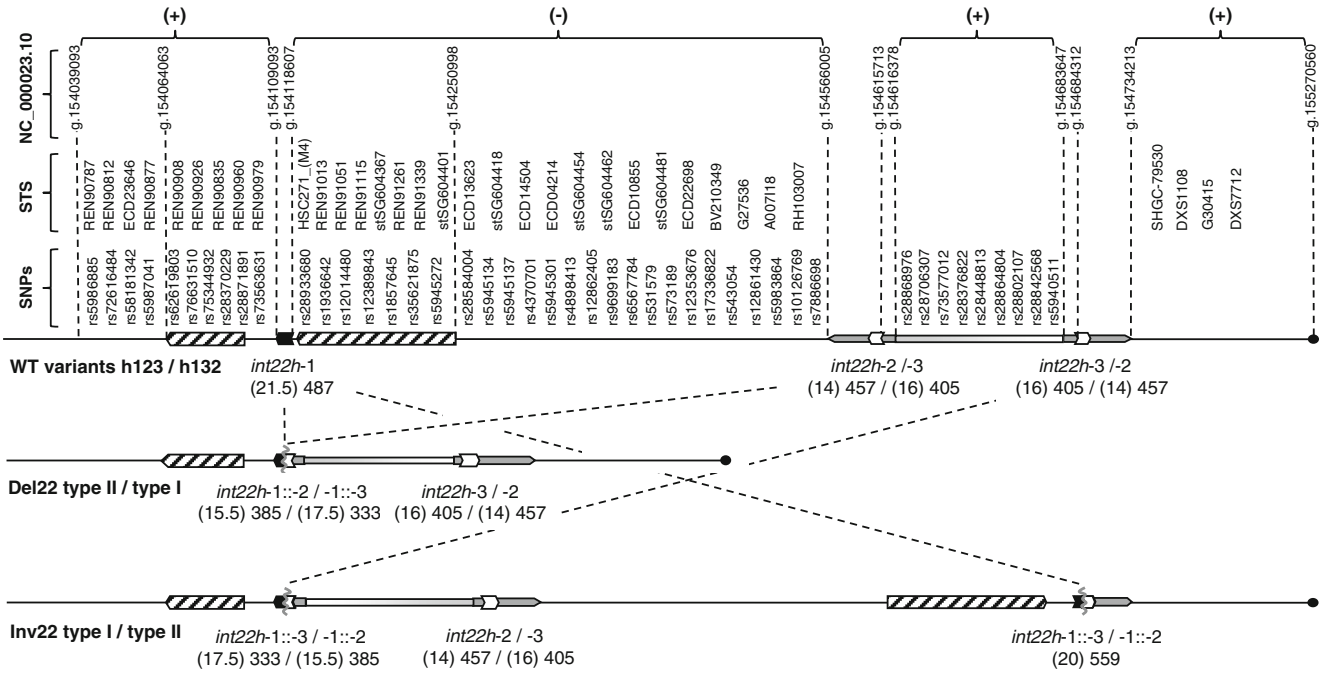

Fig. 1 Schematic view of HA-causative *int22h*-mediated rearrangements and genetic markers involved. Normal wild type variants (*WT*), alleles *h123* and *h132* (denoted as *h123/h132*), from a non-deleterious inversion polymorphism are depicted in the *middle*. *F8* gene sequences are shown as *hatched boxes*; *int22h*-1 is shown as a *closed chevron*; and *int22h*-2 or -3, as *open chevrons* embedded in the centromeric and telomeric arms of a large imperfect palindrome. Each *int22h* sequence is associated with two numbers: the *number between parenthesis* indicates the Southern blot signal in kilobase (e.g., (21.5) for *int22h*-1-associated *Bcl*I restriction fragment) [1] and the *free number* indicates the IS-PCR specific signal in base pair (e.g., 487 for the same *int22h*-1-associated fragment) [10]. The second linear scheme represents Del22, NC_000023.10:g.154118607_154615713del. Allele Del22 type II would be originated from recombination between *h1* and *h2* on WT variant *h123*, whereas Del22 type I, from *h1* and *h3* on WT variant *h132*. Chimeric *int22h* sequences are denoted as *double colons* (e.g., *int22h*-1::-2 represents the chimera between *h1* and *h2*). *Below*, the third linear scheme represents the classical Inv22 type I with recombination between *h1* and *h3* on the WT variant *h123* and type II with recombination between *h1* and *h2* on the WT variant *h132*. On *top*, approximate location of two types of genetic markers (*STSs* sequence tagged sites and *SNPs* single nucleotide polymorphisms) that should be involved being present (*plus sign*) or absent (*minus sign*) in hemizygous patients with HA caused by Del22 either type I or II, indistinctly. Some important nucleotide coordinates on the NCBI build 37.1 of the X chromosome (NC_000023.10) are shown. A list with additional information of genetic markers, such as its exact coordinates on the X chromosome, specific primer pairs for STS amplification, and genes involved in the Del22, is supplied in the Supplementary Table S1

identify the gap extent to further support the molecular diagnosis of Del22 until it is fully characterized both at genotypic and phenotypic levels. This latter information will be important for Abou-Elew and colleagues to provide further support to Del22 diagnosis of their three Egyptian patients [9] in addition to IS-PCR.

Genomic DNA samples from 79 patients with severe HA and 16 controls (8 males and 8 females) from the general Argentinean population were screened for *int22h*-related rearrangements by the use of the approach of IS-PCR [10]. Our institutional ethics committee approved the study and a written informed consent was obtained in all cases. Briefly, 2 μg of peripheral blood leukocyte-extracted DNA samples from patients and controls was subjected to protocols for *Bcl*I restriction, fragment end ligation (ligation in a total volume of 400 μl), and two separated multiplex PCR analysis, i.e., diagnostic (dt) and complementary (ct) tests using published primers and conditions [10]. PCR products were analyzed by 1.5% agarose gel electrophoresis and documented.

Out of 79 patients, 42 with severe HA (and all 16 cases from the control healthy population) showed a signal of 487 bp in the dt and signals of 457 and 405 bp in the ct, typical pattern of normal variants; 32 patients (41%) showed a pattern indicative of the Inv22 type I with a signal of 333 bp in the dt and signals of 457 and 559 bp in the ct; and 5 (6%) showed a pattern of the Inv22 type II with a signal of 385 bp and 405 and 559 bp in the dt and ct, respectively. Notably, in contrast with the recently reported Egyptian patients with HA, none of our cases showed IS-PCR patterns associated with the Del22 type I or type II (i.e., dt/ct, 333/457 and 385/405 bp, respectively) (Fig. 1).

Because of Del22 hemizygous mutation, there will be a lack of PCR templates within the gap interval, which spans *F8* exons 1–22, and at least six entire genes, along with a number of sequence tagged sites (STSs) and single

nucleotide polymorphisms (SNPs) (a list of some evenly spaced markers is provided in Fig. 1 and some amplification details in Supplementary Table S1). Although full characterization of Del22 gap range is desirable, a pilot experiment based on multiplex PCR amplification of four or five selected STS markers within the gap would provide a rapid answer on whether or not the observed Egyptian Del22-affected cases are actually the predicted 0.5-Mb deletions.

Figure 1 indicates the diagnostic relationship between *int22h* rearrangements and each specific signal of IS-PCR and classical Southern blot analysis [1]. Rossetti et al. [10] coined the name Del22 type I and II to denote *int22h*-mediated deletions that resemble the patterns of Inv22 types I and II, respectively, but with the loss of the 20-kb signal.

There are several reports of noncanonical Southern blot patterns. Andrikoviks et al. [11] presented a series of severe HA-affected patients with rearrangements involving *F8* intron 22 and compiled previous findings of aberrant Southern blot patterns. Among this rearrangement compilation, Windsor et al. [12] and Poon et al. [13] showed Southern blot analysis evidence compatible with Del22 type II and Del22 type I, respectively, in patients with severe HA and no additional unrelated phenotype features. Interestingly, Windsor et al. [12] documented that a patient with this aberrant Southern blot pattern also showed a gross deletion that removes *F8* exons 1–22 as well as sequence 5′ to the gene, and high titer FVIII inhibitor, typically associated with large multidomain deletions [14].

However, on a theoretical basis, hemizygous Del22 associates with the loss of a number of genes in addition to *F8* (exons 1–22). Five of these are well characterized on Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim/): *MTCP1* (*300116), *BRCC3* (*300617), *VBP1* (*300133), *RAB39B* (*300774), and *CLIC2* (*300138). Supplementary Table S1 provides additional information of these Del22-involved genes, their locations, potential functions, mutational effects, and references. Although highly speculative, the potential consequences of this additional function loss include benign clonal or leukemic T cell proliferation (*MTCP1*); increased cell sensitivity to ionizing radiation and a defect in G2/M checkpoint arrest (*BRCC3*); hemangiomas in the retina, brain, spinal cord, adrenal gland, etc. (i.e., von Hippel–Lindau-like syndrome (VHL), as *VPB1* is a chaperon of VHL protein, a tumor suppressor associated with von Hippel–Lindau disease) (*VPB1*); X-linked mental retardation associated with autism, epilepsy, macrocephaly, and obesity (*RAB39B*); and increased Ca^{2+} release from internal stores in cells from cardiac and skeletal muscle and fetal liver (*CLIC2*) (Supplementary Table S1).

Taking into account all these considerations regarding the actual involvement of Del22 in severe HA, it would be essential to provide a full characterization of all patients with Del22 compatible patterns both at genotypic and phenotypic levels. Accordingly, it would be important to address a systematic Del22 mutation analysis worldwide under a collaborative multicenter international effort.

Acknowledgments We thank the hematologists Raúl Pérez Bianco and Miguel de Tezanos Pinto and the scientists Christiane Dosne Pasqualini, Derrick Bowen, Anne Goodeve, Amy Curto, and Eduardo Tizzano for their help in the different phases of the work.

Fundings This study was supported by grants from René Barón Foundation, Alberto J. Roemmers Foundation, Florencio Fiorini Foundation, the National Research Council, Agencia Nacional de Promoción de la Científica y Tecnológica, and the World Federation of Hemophilia.

References

1. Lakich D, Kazazian HH, Antonarakis SE, Gitschier J (1993) Inversions disrupting the factor VIII gene are common cause of severe haemophilia A. Nat Genet 5(3):236–241. doi:10.1038/ng1193-236
2. Naylor J, Brinke A, Hassock S, Green PM, Giannelli F (1993) Characteristic mRNA abnormality found in half the patients with severe haemophilia A is due to large inversions. Hum Mol Genet 2(11):1773–1778. doi:10.1093/hmg/2.11.1773
3. Antonarakis SE, Rossiter JP, Young M, Horst J, de Moerloose P, Sommer SS, Ketterling RP, Kazazian HH Jr, Negrier C, Vinciguerra C, Gitschier J, Goossens M, Girodon E, Ghanem N, Plassa F, Lavergne JM, Vidaud M, Costa JM, Laurian Y, Lin S-W, Lin S-R, Shen M-C, Lillicrap D, Taylor SAM, Windsor S, Valleix SV, Nafa K, Sultan Y, Delpech M, Vnencak-Jones CL, Phillips JA, Ljung RCR, Koumbarelis E, Gialeraki A, Mandalaki T, Jenkins PV, Collins PW, Pasi KJ, Goodeve A, Peake I, Preston FE, Schwartz M, Scheibel E, Ingerslev J, Cooper DN, Millar DS, Kakkar VV, Giannelli F, Naylor JA, Tizzano EF, Baiget M, Domenech M, Altisent C, Tusell J, Beneyto M, Lorenzo JI, Gaucher C, Mazurier C, Peerlinck K, Matthijs G, Cassiman JJ, Vermylen J, Mori PG, Acquila M, Caprino D and lnaba H (1995) Factor VIII gene inversions in severe haemophilia A: results of an international consortium study. Blood 86:2206–2212
4. De Brasi CD, Candela M, Cermelj M, Slavutsky I, Larripa I, Bianco RP, De Tezanos Pinto M (2000) Intron 22 factor VIII gene inversions in Argentine families with severe haemophilia A. Haemophilia 6(1):21–22. doi:10.3324/haematol.11112
5. Naylor J, Buck D, Green PM, Williamson H, Bentley D, Giannelli F (1995) Investigation of the factor VIII intron 22 repeated region (int22h) and the associated inversion junctions. Hum Mol Genet 4 (7):1217–1224. doi:10.1093/hmg/4.7.1217
6. Ross MT et al (2005) The DNA sequence of the human X chromosome. Nature 434(7031):325–337. doi:10.1038/nature03440
7. Bagnall RD, Giannelli F, Green PM (2005) Polymorphism and hemophilia A causing inversions in distal Xq28: a complex picture. J Thromb Haemost 3(11):2598–2599. doi:10.1111/j.1538-7836.2005.01566.x
8. Bagnall RD, Giannelli F, Green PM (2006) Int22h-related inversions causing hemophilia A: a novel insight into their origin and a new more discriminant PCR test for their detection. J Thromb Haemost 4(3):591–598. doi:10.1111/j.1538-7836.2006.01840.x

9. Abou-Elew H, Ahemed H, Raslan H, Abdelwahab M, Hammoud MD, Arnaout H (2011) Genotyping of intron 22-related rearrangements of F8 by inverse-shifting PCR in Egyptian hemophilia A patients. Ann Hematol 90(5):579–584. doi:10.1007/s00277-010-1115-x
10. Rossetti LC, Radic CP, Larripa IB, De Brasi CD (2008) Developing a new generation of tests for genotyping hemophilia-causative rearrangements involving *int22h* and *int1h* hotspots in the factor VIII gene. J Thromb Haemost 6(5):830–836. doi:10.1111/j.1538-7836.2008.02926.x
11. Andrikovics H, Klein I, Bors A, Nemes L, Marosi A, Varadi A, Tordai Attila (2003) Analysis of large structural changes of the factor VIII gene, involving intron 1 and 22, in severe hemophilia A. Haematologica 88(7):778–784. doi:10.1182/blood.V99.1.168
12. Windsor S, Taylor SAM, Lillicrap D (1994) Direct detection of a common inversion mutation in the genetic diagnosis of severe hemophilia A. Blood 84(7):2202–2205
13. Poon MC, Low S, Sinclair GD (1995) Factor VIII gene rearrangement analysis and carrier determination in hemophilia A. J Lab Clin Med 125(3):402–406
14. Oldenburg J, Pavlova A (2006) Genetic risk factors for inhibitor to factors VIII and IX. Haemophilia 12(Suppl 6):15–22. doi:10.1111/j.1365-2516.2006.01361.x