# Haemophilia A: the consequences of *de novo* mutations. Two case reports

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## Introduction

Haemophilia A (HA, MIM 306700) is an X-linked recessive disease due to deficiency of coagulation factor VIII caused by mutations in the *factor 8* (*F8*) gene[1]. The disease occurs in males while female carriers are typically asymptomatic[1]. In rare cases HA may occur in females. In the Italian Registry of Congenital Coagulation Disorders (2014 update), 29 female patients are reported out of 3,906 registered cases of HA. Among them, the large majority had mild haemophilia (n=25), whereas two women were diagnosed with severe HA and the other two with moderate HA[2]. The specific conditions underlying HA in women are: (i) a mutant gene in an X-0 female[3,4]; (ii) a mutant gene in an X chromosome plus extreme inactivation of the wild-type X chromosome[5]; and (iii) a female homozygous or compound heterozygous for two mutant *F8* genes[6]. Here we report two cases recently diagnosed at our Centre (in the region of Campania, Southern Italy) with *de novo* mutations that had unusual and relevant clinical implications.

## Cases report and results

The first patient is a 37-year old woman with family history of severe HA. She suffered from easy bruising and menorrhagia although no coagulation tests were ever carried out. At the age of 25, on the occasion of her first pregnancy, she underwent molecular analysis to identify HA carrier status which revealed the large deletion involving exons 1 to 22 of the *F8* gene previously identified in her family members. Thus, after genetic counselling[7], she asked for prenatal diagnosis by chorionic villi sampling[8]. The analysis revealed a male foetus, but the presence of the large gene deletion detected in HA members of the family was excluded. The baby was born free of any complication and he grew normally up to the age of 10 years old, when post-traumatic bleeding symptoms occurred. At laboratory assessment, he was found to have a prolonged activated partial thromboplastin time (aPTT) with a normal prothrombin time (PT) and factor VIII procoagulant activity (FVIII:C) of 6.7%. A normal aPTT mixing test and the absence of factor VIII inhibitors excluded acquired haemophilia, which is very rare in children[9]. Similar results were found in the mother (FVIII:C, 7%). Thus, we sequenced the whole coding region of the *F8* gene in the boy and his mother, revealing the hemizygous c.1569G>T mutation (NM_000132.3) causing p.Leu523Leu in both subjects. This synonymous mutation is known to be associated with a mild phenotype (Factor VIII [F8] Variant Database, www.factorviii-db.org/), consistent with the diagnosis of mild HA in the boy. Similarly, in our affected female who is compound heterozygous for the large gene deletion and the c.1569G>T mild mutation, this latter has a dominant effect, mitigating the clinical and laboratory phenotype. Molecular analysis of *F8* in the patient's father was negative, excluding mutations in the whole coding regions for factor VIII by gene sequencing and confirming paternity[7], while her mother was an asymptomatic carrier of the familial large deletion. This patient adds to the few reported cases of females with HA due to a family mutation and a second *de novo* mutation[6,10]. All such cases have been diagnosed as severe HA in symptomatic females, while the present case is the first, to our knowledge, in a woman with mild haemophilia diagnosed because an affected son was revealed. This case suggests that the number of females affected by mild or moderate HA due to a second *de novo* mutation may be underestimated and that such cases may be diagnosed as HA carriers because only the known familial mutation is tested.

The second case we report is a woman diagnosed as a HA carrier, again due to a mutation of *de novo* origin. This condition is frequently reported in cases of "sporadic" haemophilia but this woman had a family history of severe HA and was referred to our centre while planning her first pregnancy at the age of 32 years. Her aPTT was within the normal range (ratio 1.16) and FVIII:C was low-normal (48%), in the presence of blood group O. Molecular analysis excluded the intron 22 inversion carried by her affected sibling. Two years later, her male son, born after a normal pregnancy, was diagnosed as affected by severe HA due to the

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occurrence of large ecchymoses since the age of 6 months, and laboratory findings of altered aPTT with a normal PT and a FVIII:C <1%. After testing the mother again for the intron 22 inversion (confirmed negative) we extended the molecular analysis by searching for an intron 1 inversion (negative) and sequencing the whole coding region of the *F8* gene, which revealed the c.1753-1G>A mutation (NM_000132.3). In this case the mutation is associated with a severe phenotype because it is predicted to delete the acceptor splicing site of intron 11, likely impairing the splicing pattern of the *F8* gene[11].

## Discussion

Both the cases described in this report highlight the importance of searching for *de novo* mutations when analysis for the family mutation results negative. All current protocols of molecular analysis are based on the search for the family mutation alone and prenatal diagnosis is required only if the familial mutation is present[9,12,13]. *De novo* mutations are frequent in X-linked genetic diseases and are typically point mutations that are spread throughout the gene[14,15]; this means that gene sequencing of the whole coding regions of the *F8* gene would be needed in order to exclude *de novo* mutations. Thanks to the reduction of costs of new, deep-gene sequencing protocols and the availability of scanning procedures, such as denaturing high performance liquid chromatography[16,17], for rapid and inexpensive analysis of the whole coding region of genes, a routine search for *de novo* mutations in cases negative for the family mutation by whole gene scanning could be considered for careful genetic counselling[7,18], in particular when prenatal diagnosis is planned.

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## Web resources

Factor VIII (F8) Variant Database available at: http://www.factorviii-db.org (accessed on 20/07/2016).

## Authorship contributions

FZ and AE performed the molecular analyses; FZ and FA drafted the manuscript; AC and GC critically revised the manuscript; AC and EC followed up the reported patients and collected clinical data; MS and GR identified the cases and were responsible for clinical work; FA supervised the work.

**Keywords:** FVIII, *de novo* mutations, sporadic cases, prenatal diagnosis.

*The Authors declare no conflicts of interest.*

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