A Skewed Lyonization Phenomenon as Cause of Hemophilia A in a Female Patient

To the Editor:

X-chromosome inactivation occurs early in female mammalian development to achieve dosage compensation with males. This event is random for each cell and is passed on to the progeny of the cell in a stable manner.[1] Females heterozygous for an X-linked disease are mosaics with respect to X-chromosome expression; therefore, phenotypic evidence of rare recessive disorders in women is an unusual phenomenon. It can result from (1) extreme lyonization in a heterozygote, (2) X-chromosome abnormalities, or (3) homozygosity at the disease locus.

Performing a clonal X-activation analysis at the DNA level with Southern blot technique could be a useful approach to the correct diagnosis and genetic counselling in symptomatic carriers of X-linked disease such as hemophilia A (HA). For this purpose, M27β probe (locus DXS255),[2,3] with a heterozygosity rate of greater than 90%, seems particularly useful in assessing clonality in cell populations. This probe, identified on X-cen p11.22, shows a multiallelic polymorphism due to a VNTR of 26 nucleotides detectable by digestion of genomic DNA with the restriction enzyme *Msp* I and its methylation-sensitive isoschizomer *Hpa* II. This method can demonstrate the activation (methylation) or inactivation (not methylation) of X-chromosomes.[4] We report two females with a family history for HA and low level of clotting factor VIII showing a mild HA phenotype.

GV is a 33-year-old woman (FVIII:C 5%; FVIII:vWAg 80%) born from an HA carrier and a healthy father. Her karyotype was 46, XX; no acquired inhibitor against factor VIII was detected; and restriction fragment length polymorphism (RFLP) analysis with an intragenic probe to FVIII confirmed an HA carrier status. To explain this particular phenotype, 20 μg of genomic DNA of the proband and her parents was digested with *Pst* I to show the polymorphic allele at DXS255. An aliquot of *Pst* I digestion of only the women was cleaved with *Hpa* II enzyme that recognizes the sequence C CGG flanking the DXS255 locus only if the internal C residue is unmethylated. The analysis with this probe showed that the paternal X-chromosome is unmethylated and, in this context, inactive (Fig 1). The densitometric measurement on autoradiography scanned with

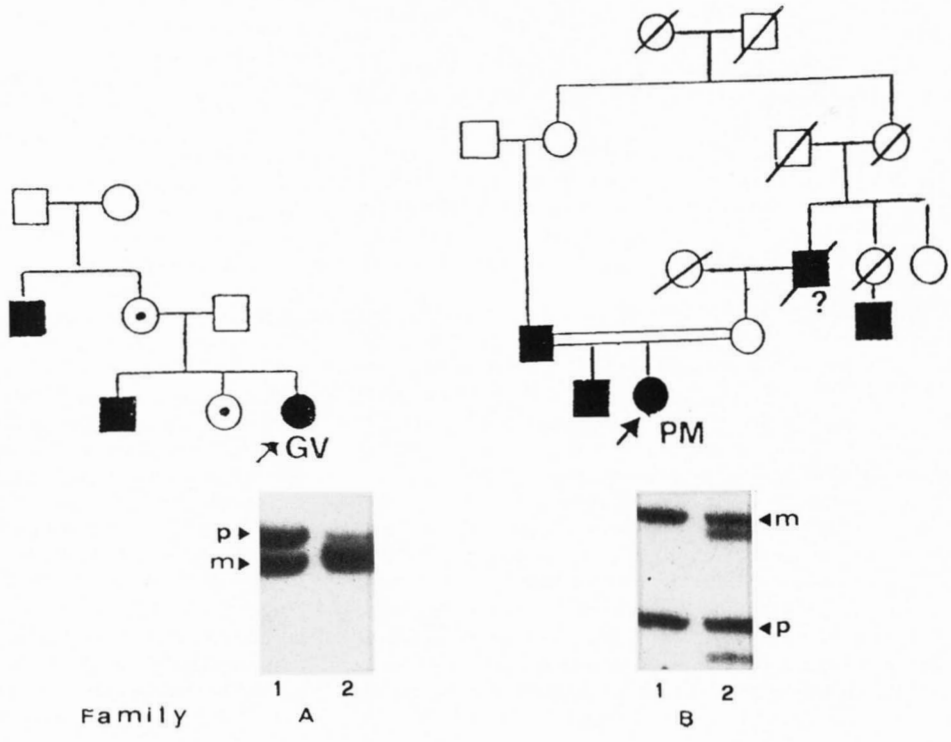

Fig 1. Southern blot of probands' DNA cleaved with *Pst* I (lanes 1) and *Pst* I + *Hpa* II (lanes 2), hybridized with M27 probe, allows us to distinguish the paternal (p) and maternal (m) allele, respectively. Family A: after *Hpa* II digestion (lane 2), only the paternal allele of GV is cleaved, whereas the maternal allele is resistant to cleavage, confirming a nonrandom X-inactivation pattern. Family B: PM maternal and paternal alleles are both cleaved by *Hpa* II, demonstrating a balanced X-inactivation (lane 2).

a laser densitometer showed an allelic cleavage ratio (ACR), calculated from *Pst* I/*Hpa* II bands, of almost 100. These data showed that the maternal *Pst* I allele is fully resistant to *Hpa* II cleavage, which is so fully methylated and, in this case, active. Because the maternal chromosome bears a factor VIII gene with a mutation, the proband's phenotype can be explained by these findings.

Using this probe, we also studied another female patient with a low FVIII:C level whose defect was not caused by an unbalanced lyonization.

PM is a 48-year-old woman (FVIII:C 12%; FVIII:vWAg 100%). Her parents were relatives. Her father and her brother had the same FVIII:C level (11%), whereas her mother was the daughter of a possible hemophilic patient (insufficient anamnestic data). Karyotype analysis of the proband showed no abnormalities and no acquired inhibitor against FVIII was detected. M27β probe showed a balanced lyonization, as also attested by an ACR value of 1.6. Therefore, the heterozygosity for this probe allowed us to exclude the possibility of uniparental disomy and to hypothesize the homozygosity for HA-defective gene through the haplotype analysis with RFLPs.

The characterization of the molecular defect causing the disease should confirm this supposition.

In conclusion, the ability to measure the degree of X-chromosome inactivation is very useful in explaining the mechanism of the symptomatic phenotype in women heterozygous for HA gene alteration.

Maura Acquila
Daniela Caprino
Patrizia Bicocchi
Pier Giorgio Mori
Department of Pediatric Hematology/Oncology
"G. Gaslini" Children's Research Hospital
Genova, Italy
Anna Rita Tagliaferri
Hospital of Parma
V General Medicine Division
Parma, Italy

REFERENCES

1. Lyon MF: X-chromosome inactivation and developmental patterns in mammals. Biol Rev 47:1, 1972

2. Fraser NJ, Boyd Y, Brownlee G, Craig IW: Multi-allelic RFLP for M27β, an anonymous single copy genomic clone at Xp11.3-X cen. Nucleic Acids Res 15:9616, 1987

3. Fraser NJ, Boyd Y, Craig I: Isolation and characterization of a human variable copy number tandem repeat at X cen-p 11.22. Genomics 5:144, 1989

4. Boyd Y, Fraser NJ: Methylation patterns at the hypervariable X chromosome locus DXS255 (M27β): Correlation with X inactivation status. Genomics 7:182, 1990