Interaction of incontinentia pigmenti and factor VIII mutations in a female with biased X inactivation, resulting in haemophilia

R Coleman, S A Genet, J I Harper, A O M Wilkie

Abstract
We report a female infant born to a mother with incontinentia pigmenti (IP) and a father with haemophilia A, who manifests both disorders. Analysis of peripheral blood DNA from the infant, her mother, and two female relatives with IP showed a highly skewed pattern of X inactivation. Random patterns were observed in the infant's two sisters, who do not have IP and have normal carrier activity of factor VIII. Preferential inactivation of the X chromosome bearing the IP mutation, probably by negative selection, appears to have unmasked the factor VIII mutation on the infant's other X chromosome. This illustrates an unusual mechanism for the manifestation of an X linked disease in a heterozygous female.

Incontinentia pigmenti (IP) is an X linked dominant genodermatosis. It is seen almost exclusively in females, only 2 to 3% of reported cases having occurred in males. This is attributed to the lethal effect of hemizygosity for the IP mutation in the male. The skin lesions in IP are often linear and may follow Blaschko's lines; this may reflect the normal mosaic pattern of chromosome inactivation in the heterozygous female. The locus for the familial form of IP, designated IP2, has been assigned to the Xq28 region. Sporadic cases have been associated with X-autosome translocations with breakpoints at Xp11 and this locus is designated IP1.

Haemophilia A is an X linked recessive bleeding disorder resulting from deficiency of normal factor VIII activity (FVIII:C). The incidence is 1 per 20 000 of the population and the gene has been mapped to Xq28. The major site of FVIII synthesis is the liver, with lesser activity in the spleen, kidney, and lymph nodes, but none in leucocytes. Female carriers of the FVIII mutation usually have intermediate FVIII:C levels but rarely have clinical haemophilia. In exceptional manifesting cases, highly skewed X inactivation has been observed.

We have recently seen a female infant who carries mutations of both the IP and FVIII genes, inherited from her mother and father respectively. We show that she manifests overt haemophilia A, and that this probably results from selective inactivation of her maternal X chromosome bearing the IP mutation.

Material and methods

SUBJECTS
A 5 week old Caucasian female infant (III-12, fig 1) was referred to the dermatology clinic with the characteristic cutaneous features of

---

Figure 1  Family pedigree showing segregation of the haemophilia A and IP mutations. * represents the miscarriage of twin boys at 5 months and † the miscarriage of a male twin at 3 to 4 months. Subjects I-4, II-5, II-6, II-8, II-10, III-11, and III-12 were examined personally.
IP. She was born at term after a normal pregnancy and delivery to non-consanguineous parents. Vesicular skin lesions, predominantly affecting the lower limbs, were noted at birth. Over the next few weeks these evolved into verrucous eruptions and hyperpigmented streaks characteristic of IP. She was otherwise healthy; in particular she had no bleeding problems. Ophthalmological examination was normal and her development was satisfactory.

There was a family history of IP. Her mother (II-6) had been mildly affected as a child with typical skin lesions and convulsions of unknown cause; examination of her skin is now normal. The mother’s half sister (II-8) was moderately severely affected, with cutaneous manifestations, patchy alopecia, optic atrophy, strabismus, reduced visual acuity, widely spaced, peg shaped deciduous teeth, and subsequent hypodontia. The grandmother (I-4) had no history of the disorder but had miscarried her three male offspring; she has had maturity onset diabetes mellitus, hypertension, and renal failure requiring renal transplantation. Examination showed peg shaped teeth and hypopigmented linear streaks on the back of her legs.

The proband had two older sisters (III-10, III-11) aged 4 and 3 years, neither of whom had any clinical manifestations of IP. The oldest had been investigated for four non-febrile convulsions between the ages of 5 and 18 months, no cause being found.

The proband’s father (II-5) had mild haemophilia A. On average he had two bleeds per year as a consequence of moderately severe trauma and only occasionally required FVIII concentrate. There had been no previously documented haemophilic a family, but assays of factor VIII:C suggested that his mother (I-2), sister (II-2), and niece (III-6) were also carriers.

**COAGULATION STUDIES**

Factor VIII activity was measured by a standard one stage assay using a 10 minute incubation with Kaolin as the activator. Von Willebrand antigen was measured by ELISA using DAKO antibodies (DAKO Ltd, High Wycombe, Bucks, UK).

**GENETIC STUDIES**

The X inactivation pattern was analysed using the hypervariable probe M27β (DXS255), mapping to Xp11.22, in double digests (MspI/BamHI and HpaII/BamHI) (Northumbria Biologicals Ltd) of genomic DNA. At this locus, at least one of the flanking CCGG restriction sites is fully methylated on the active X chromosome, whereas these sites are partially or completely unmethylated on the inactive X chromosome. Methylation differences can be detected by comparing the digest patterns of the isoschizomers MspI and HpaII, as HpaII is unable to cut at methylated CCGG sites. X inactivation patterns were corroborated in subjects informative for the BstXI polymorphism at the PGK locus. BstXI digest patterns of undigested and HpaII digested DNA were compared using a polymerase chain reaction assay. Segregation of the Xq28 region was studied using the TaqI polymorphism of St14 (DXS52).

Genomic DNA was extracted from peripheral white blood cells, digested with appropriate restriction enzymes, size separated by electrophoresis through 0-8% agarose gels, and transferred to nylon membranes (Hybond-N+, Amersham, UK) by Southern blotting. DNA probes were radiolabelled with 32PdCTP and hybridised to the filters overnight. The filters were washed to a stringency of 0.5 x sodium chloride/sodium citrate and 0.1% sodium dodecyl sulphate at 65°C. Autoradiography using intensifying screens and Kodak XARS film was performed for one to 14 days at –70°C.

Cytogenetic studies were performed using standard G banding techniques.

**Results**

**COAGULATION STUDIES**

Initial clotting studies in the proband showed a prolonged Kaolin partial thromboplastin time of 65 seconds (control 35-60 seconds, normal 30 to 45 seconds), with normal prothrombin and thrombin times, a pattern consistent with haemophilia A. The results of FVIII:C and vWF:Ag assays in the family are shown in the table. The proband’s FVIII:C level and FVIII:C/vWF:Ag ratio were reduced to values similar to her haemophilic father, and were thus consistent with a diagnosis of mild haemophilia. By contrast, measurements of FVIII:C and FVIII:C/vWF:Ag in her two older sisters were typical for haemophilia A carriers. The mother’s coagulation studies were entirely normal.

**GENETIC STUDIES**

All female family members were heterozygous for the MspI/M27β polymorphism (fig 2). When genomic DNA from peripheral blood was digested with HpaII, subjects I-4, II-6, II-8, and III-12 (all of whom had IP) showed limit digestion of only one M27β allele, indicative of highly skewed X inactivation. By contrast both HpaII/M27β alleles were visible in III-10 and III-11, who showed no clinical features of IP. The segregation of this polymorphism, which maps close to the IP1 locus, was inconsistent with clinical status, showing three recombinants in four phase known meioses (fig 2). This is not unexpected as IP1 has not been implicated in familial disease. Two family members (I-4 and II-6, both affec-
Interaction of incontinentia pigmenti and factor VIII mutations in a female with biased X inactivation, resulting in haemophilia.

**Figure 2** X inactivation analysis using M278. Southern blot analysis of DNA samples from each family member digested with either BamHI + MspI (track M) or BamHI + HpaII (track H).

ted with IP) were also heterozygous for the BstXI polymorphism at the PGK locus. HpaII digested samples from both subjects showed >95% loss of one allele, confirming the biased pattern of X inactivation (data not shown).

Analysis of the St14/TagI polymorphism in the family was fully informative and showed no recombination with disease status in four phase known meioses (data not shown). This is consistent with the IP mutation in this family equating with IP2, which maps to Xq28, although the lod score was not significant ($\theta_{\text{max}} = 0$, $Z = 1.2$). Chromosomal analysis of the proband was normal (46,XX).

### Discussion

The proband had cutaneous manifestations of IP together with FVIII:C levels, measured at the age of 8 weeks, consistent with mild haemophilia A. Levels of FVIII:C in infants aged 30 and 90 days do not differ statistically from adult values,23 so the early age of measurement is unlikely to have biased the results. Identical FVIII activity (12 IU/dl) was present in the proband and her father; affected males within a single family tend to be of similar severity.24

There are a number of potential explanations for unexpectedly low factor VIII:C in a female. These include von Willebrand's disease, structural or numerical abnormalities of the sex chromosomes, inheritance of FVIII mutations from both parents, uniparental disomy for a familial mutation, and selective inactivation of the normal X chromosome in a heterozygote. Our patient had normal levels of von Willebrand factor and a 46,XX karyotype, eliminating the first two possibilities. Genetic homozygosity is statistically very unlikely, particularly as the mother had normal FVIII:C and FVIII:C/vWF:Ag measurements, and uniparental disomy was excluded by the M278 analysis. Skewed X inactivation of peripheral blood cells has previously been observed in rare females manifesting haemophilia.125 Analysis of methylation at the DXS255 locus (fig 2) suggests that this is the mechanism of haemophilia in our patient, although the tissues where factor VIII is synthesised, primarily the liver,18-42 were not tested directly.

The biochemical basis of the IP mutation(s) is unknown, but the cellular phenotype is presumed severe. In males, the hemizygous state is lethal; in carrier females, those cells in which the IP bearing X chromosome is active may be at a growth disadvantage. An initially random pattern of X inactivation could account for the patchy manifestation of the disease along Blaschko's lines: selection against the active, IP bearing X would lead to progressive healing and an increasingly skewed pattern of X inactivation. Evidence for this selective process in females with IP has been adduced by analysis of X inactivation patterns in hybrid cell lines,2 red blood cells,3 and genomic DNA from peripheral blood and fibroblast cell lines.4 Migeon et al5 reported that skewed X inactivation was frequently, but not always, seen in peripheral blood. Curtis et al (personal communication) found that all 10 affected subjects from two families appearing to segregate for the IP2 mutation showed highly skewed X inactivation patterns.

In agreement with these findings, we observed complete correlation in our family...
between clinical status for IP (affected/unaffected) and X inactivation (non-random/random respectively) in six females. In the affected cases, the preferentially inactivated X chromosome (corresponding to the single fragment seen after M278/HpaII digestion) was always inherited from the mother (fig 2). In the infant III-12, selective activity of the paternal X chromosome (bearing the factor VIII mutation) would give rise to haemophilia; this suggests that non-random X inactivation occurs in the liver in IP. The concurrence of acute cutaneous manifestations (presumably reflecting current or recent expression of the maternal X in skin) with apparently complete inactivation of the maternal X in blood and liver, might reflect a more deleterious effect of the IP2 mutation in the latter tissues.

Recently Harris et al6 studied X inactivation in 23 familial and eight sporadic cases of IP, and failed to observe a consistent correlation between IP status and skewing of X inactivation. The explanation for these different findings is unclear, but may relate to the greater heterogeneity of their clinical material or the existence of additional genetic factors influencing the susceptibility of X chromosomes to inactivation.

Although our family is too small for conclusive localisation of the IP mutation, the concordant segregation of DXS52 is consistent with a location at IP2. Previous studies3 have shown close linkage of IP2 to both DXS52 and the F8C gene in Xq28. It is thus feasible that the proband carries mutant genes in the Xq28 region on both her X chromosomes: IP2 on her maternal X and F8C on her paternal X. Unless recombination between the two loci occurred, all her viable sons would be haemophiliac, and her daughters would either be haemophiliac carriers or have IP.

In summary, this study shows further the value of X inactivation studies in determining carrier status in some families with IP. It provides evidence for X inactivation occurring in tissues other than those known to be a target for the IP gene, and illustrates the potential interaction between two X linked disorders present in a single person.

We would like to thank Janet Cookson for performing the coagulation studies, Les Butler for the cytogenetic analysis, Andrew Curtis for communication of unpublished results, and Richard Gibbons, Susan Lindsay, and Charles Rizza for commenting on the manuscript.