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.

(J Med Genet 1993;30:497-500)

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.¹ 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 lines²; this may reflect the normal mosaic pattern of chromosome inactivation in the heterozygous female.^{3,4} The locus for the

familial form of IP, designated *IP2*, has been assigned to the Xq28 region.⁵ Sporadic cases have been associated with X;autosome translocations with breakpoints at Xp11 and this locus is designated *IP1*.^{6,7}

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 population⁸ and the gene has been mapped to Xq28.⁹ The major site of FVIII synthesis is the liver, with lesser activity in the spleen, kidney, and lymph nodes, but none in leucocytes.¹⁰⁻¹² 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.¹³

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, III-10, III-11, and III-12 were examined personally.

Department of Dermatology, The Hospitals for Sick Children, Great Ormond Street, London WC1N 3JH, UK.

R Coleman
J I Harper

Mothercare Unit of Clinical Genetics and Fetal Medicine, Institute of Child Health, London WC1N 1EH, UK.
S A Genet
A O M Wilkie

Correspondence to Dr Wilkie, Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF4 4XW, UK.

Received 19 November 1992.
Revised version accepted 27 January 1993.

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 haemophiliacs in the 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 antibodies¹⁴ (DAKO Ltd, High Wycombe, Bucks, UK).

GENETIC STUDIES

The X inactivation pattern was analysed using the hypervariable probe M27 β (*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.^{15,16} 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.^{17,18} Segregation of the Xq28 region was studied using the *TaqI* polymorphism of *St14* (*DXS52*).¹⁹

Genomic DNA was extracted from peripheral white blood cells,²⁰ 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 35.6 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 haemophiliac father, and were thus consistent with a diagnosis of mild haemophilia.²¹ By contrast, measurements of FVIII:C and FVIII:C/vWF:Ag in her two older sisters were typical for haemophilia A carriers.²² The mother's coagulation studies were entirely normal.

GENETIC STUDIES

All female family members were heterozygous for the *MspI*/M27 β 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 M27 β allele, indicative of highly skewed X inactivation. By contrast both *HpaII*/M27 β 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 affect-

Coagulation studies of the family.

Family member	FVIII:C 50-150 IU/dl*	vWF:Ag 50-150 IU/dl*	FVIII:C/vWF:Ag 0.8-2.0*
Father (II-5)	12	92	0.13
Mother (II-6)	85	73	1.16
Daughter 1 (III-10)	44	96	0.46
Daughter 2 (III-11)	61	92	0.66
Proband (III-12)	12	135	0.09

FVIII:C = bioassay of FVIII clotting activity. vWF:Ag = von Willebrand factor. FVIII:C/vWF:Ag represents the ratio of the two values. *represents normal range of values.

Figure 2 X inactivation analysis using M27 β . 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 *Bst*XI polymorphism at the *PGK* locus. *Hpa*II digested samples from both subjects showed > 95% loss of one allele, confirming the biased pattern of X inactivation (data not shown).

Analysis of the *St*14/*Taq*I 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 Xq28,⁵ although the lod score was not significant ($\theta_{\max}=0$, $Z=1.2$). 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 M27 β analysis. Skewed X inactivation of peripheral blood cells has previously been observed in rare females manifesting haemophilia.^{13,25} Analysis of methylation at the *DXS*255 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,¹⁰⁻¹² 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,³ red blood cells,⁴ and genomic DNA from peripheral blood and fibroblast cell lines.⁴ Migeon *et al*⁴ 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 M27 β /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 al*²⁶ 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 studies⁵ 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 haemophilia 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.

- 1 Carney RG. Incontinentia pigmenti; a world statistical analysis. *Arch Dermatol* 1976;112:535-42.
- 2 Blaschko A. *Die Nervenverteilung in der Haut in ihrer Beziehung zu den Erkrankungen der Haut*. Wien, Leipzig: Braumüller, 1901.
- 3 Wieacker P, Zimmer J, Ropers HH. X inactivation patterns in two syndromes with probable X-linked dominant, male lethal inheritance. *Clin Genet* 1985;28:238-42.
- 4 Migeon BR, Axelman J, Jan de Beur S, Valle D, Mitchell GA, Rosenbaum KN. Selection against lethal alleles in females heterozygous for incontinentia pigmenti. *Am J Hum Genet* 1989;44:100-6.
- 5 Sefiani A, M'rad R, Simard L, *et al*. Linkage relationship between incontinentia pigmenti (IP2) and nine terminal X long arm markers. *Hum Genet* 1991;86:297-9.
- 6 Hodgson SV, Neville B, Jones RWA, Fear C, Bobrow M. Two cases of X/autosome translocation in females with incontinentia pigmenti. *Hum Genet* 1985;71:231-4.
- 7 Gorski JL, Burright EN, Harnden CE, *et al*. Localisation of DNA sequences to a region within Xp11.21 between incontinentia pigmenti (IP1) X-chromosomal breakpoints. *Am J Hum Genet* 1991;48:53-64.
- 8 Biggs R. Haemophilia treatment in the United Kingdom from 1969 to 1974. *Br J Haematol* 1977;35:481-504.
- 9 Gitschier J, Wood WI, Goralka TM, *et al*. Characterization of the human factor VIII gene. *Nature* 1984;312:326-30.
- 10 Stel HV, van der Kwast TH, Veerman ECI. Detection of factor VIII/coagulant antigen in human liver tissue. *Nature* 1983;303:530-2.
- 11 Wion KL, Kelly D, Summerfield JA, Tuddenham EGD, Lawn RM. Distribution of factor VIII mRNA and antigen in human liver and other tissues. *Nature* 1985;317:726-9.
- 12 Kwast THvd, Stel HV, Cristen E, Bertina R, Veerman ECI. Localisation of factor VIII-procoagulant antigen: an immunohistological survey of the human body using monoclonal antibodies. *Blood* 1986;67:222-7.
- 13 Nisen PD, Waber PG. Nonrandom X chromosome DNA methylation patterns in hemophiliac females. *J Clin Invest* 1989;83:1400-3.
- 14 Short PE, Williams CE, Picken AM, Hill FGH. Factor VIII related antigen: an improved enzyme immunoassay. *Med Lab Sci* 1982;39:351-5.
- 15 Boyd Y, Fraser NJ. Methylation patterns at the hypervariable X chromosome locus DXS255 (M27 β): correlation with inactivation status. *Genomics* 1990;7:182-7.
- 16 Hendriks RW, Kraakman MEM, Mensink RGJ, Schuurman RKB. Differential methylation at the 5' and the 3'CCGG sites flanking the X chromosomal hypervariable DXS255 locus. *Hum Genet* 1991;88:105-11.
- 17 Vogelstein B, Fearon ER, Hamilton SR, *et al*. Clonal analysis using recombinant DNA probes from the X chromosome. *Cancer Res* 1987;47:4806-13.
- 18 van Kamp H, Jansen R, Willemze R, Fibbe WE, Landegent JE. Studies on clonality by PCR analysis of the PGK-1 gene. *Nucleic Acids Res* 1991;19:2794.
- 19 Oberlé I, Camerino G, Heilig R, *et al*. Genetic screening for hemophilia A (classic hemophilia) with a polymorphic DNA probe. *N Engl J Med* 1985;312:682-6.
- 20 Jeanpierre M. A rapid method for the purification of DNA from blood. *Nucleic Acids Res* 1987;15:9611.
- 21 Rizza CR. The clinical features of clotting factor deficiencies. In: Biggs R, ed. *Human blood coagulation, haemostasis and thrombosis*. 2nd ed. Oxford: Blackwell, 1976:231-48.
- 22 Zimmerman TS, Ratnoff OD, Littell AS. Detection of carriers of classic haemophilia using an immunologic assay for antihaemophilic factor (factor VIII). *J Clin Invest* 1971;50:255-8.
- 23 Andrew M, Paes B, Milner R, *et al*. Development of the human coagulation system in the full-term infant. *Blood* 1987;70:165-72.
- 24 Bloom AL. Inherited disorders of blood coagulation. In: Bloom AL, Thomas DP, eds. *Haemostasis and thrombosis*. 2nd ed. Edinburgh: Churchill Livingstone, 1987:393-436.
- 25 Kling S, Coffey AJ, Ljung R, *et al*. Moderate haemophilia B in a female carrier caused by preferential inactivation of the paternal X chromosome. *Eur J Haematol* 1991;47:257-61.
- 26 Harris A, Collins J, Vetrie D, Cole C, Bobrow M. X inactivation as a mechanism of selection against lethal alleles: further investigation of incontinentia pigmenti and X linked lymphoproliferative disease. *J Med Genet* 1992;29:608-14.