Gonadal mosaicism for incontinentia pigmenti in a healthy male

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Abstract

Incontinentia pigmenti (IP) is a genodermatosis that segregates as an X linked dominant trait with male lethality. The disease has been linked to Xq28 in a number of studies. A few affected males have been documented, most of whom have a 47,XXY karyotype. We report a family with two paternally related half sisters, each affected with IP. The father is healthy, clinically normal, and has a 46,XY normal male karyotype. Linkage analysis of 12 polymorphic markers (two X linked and 10 autosomal) confirms paternity. X inactivation studies with the human androgen receptor (HUMARA) indicate that the paternal X chromosome is inactivated preferentially in each girl, implying that this chromosome carries the IP mutation, and that the father is a gonadal mosaic for the IP mutation.

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Incontinentia pigmenti (IP) was recognised as a discrete clinical entity as early as 1906.¹ The most prominent phenotypic features involve the skin and its derivatives, the eye, and the central nervous system. The penetrance of the disease appears near 100%, but its expression is highly variable, even within families. Familial cases of IP fail to show male to male inheritance and include a high rate of spontaneous abortion of male fetuses, or an abundance of female children, suggesting that IP is an embryonic

lethal in males. The most recent substantive case review included 653 patients, of whom only 16 were phenotypic males.² Among males apparently affected with IP who have had chromosome analysis, most have been shown to be 47,XXY.^{3,4} Other male survivors may have had undiscovered chromosomal aneuploidies or, if accurately diagnosed, the disease may have resulted in postzygotic "half-chromatid" mutation.^{5,6} There is sufficient overlap of IP with other dermatological conditions to raise concern that some males were diagnosed inappropriately.⁷ Convincing evidence for gonadal mosaicism has not been presented previously.

IP has been mapped to Xq28 by a number of studies. Historically, the gene has been localised to that area of Xq28 surrounding the factor VIII gene.⁸ Currently, the closest linkage has been shown with marker DXYS154.⁹

Three families in which the IP mutation appears to have originated in a male progenitor before segregating through the subsequent generations have been recently identified.¹⁰ Since IP is lethal in males, a mutation on such a paternal X chromosome must have arisen de novo. In each of those families, only one woman was affected with IP in the first generation, implying that the mutation was unique to her. Further study of two of the three families identified the paternal X as preferentially inactivated in the first affected woman (data not shown). Highly skewed X inactivation has been observed in peripheral blood lymphocytes in 92% of females with IP (A Scheuerle, in preparation), and appears to be a hallmark of this mutant X chromosome. Small family sizes complicate this observation by limiting the number of potentially affected pregnancies.

We report a family (fig 1) in which there are two half sisters affected with IP who share a common father (fig 2). The father has a normal male karyotype (fig 3). The father and the second mother have been examined and are healthy. The mother of the first child is not available for detailed examination, but is reported normal by reliable observers. The mothers are not known to be related. None of the three has skin pigmentation changes or a history of IP. This inheritance pattern has not been reported previously and suggests gonadal mosaicism for the mutation in the father.

Methods

CASE REPORTS

Patient 1 (I-2). The father was born at term after an uncomplicated pregnancy. He denied

Figure 1 Pedigree and haplotypes of the family. Subjects for whom a sample is available are marked with an asterisk. SAb = spontaneous abortion.

Figure 2 (Top) Patient 2 at 8 years of age. There is a reticular pattern of pigmentation change on the right flank and abdomen. (Bottom) Patient 3 at 8 weeks. Note the linear vesiculopustular lesions on the left thigh and groin.

personal or family history of skin, hair, eye, or tooth abnormalities. There is no consanguinity reported in either of his marriages, nor other known family history of birth defects, mental retardation, spontaneous abortion, or early infant death. At the time of examination, he was a healthy 36 year old. He has a normal male 46,XY karyotype (fig 3).

Patient 2 (II-1). The older daughter was born at term after the second pregnancy of a healthy 30 year old Hispanic woman. The mother's one previous pregnancy resulted in a spontaneous abortion at 9 weeks. The gender of that fetus was not identified. There is no known family history of incontinentia pigmenti. IP was diagnosed after birth when linear vesicles were present on the trunk and extremities. These were later replaced by tan-brown pigmentation in a swirled pattern (fig 2, top). A biopsy of the skin was reportedly performed in Brazil and the histology confirmed the diagnosis of IP. At the age of 9 years, she is in good health and has no hair, tooth, nail, ophthalmological, or neurological abnormalities.

Patient 3 (II-2). The younger daughter was born at 27 weeks' gestation because of preterm labour (aetiology unknown) to a then healthy 41 year old G3P0121 Hispanic woman. The mother received prophylactic penicillin during the pregnancy because of a positive RPR (she

Figure 3 Peripheral blood lymphocyte karyotype of patient 1, courtesy of Stanford University Medical Center, Department of Cytogenetics.

Figure 4 Results of HUMARA methylation analysis of family XL241. H=sample digested with *HpaII* before amplification (a completely digested sample will not amplify). R=sample digested with control enzyme *RsaI* before amplification. Each subject is represented in two adjacent lanes. C=control. The first two lanes are a normal control. The second two lanes are a sample known to have skewed X inactivation. The father, designated a carrier, completely loses the band in the H lane as expected. His single X chromosome should be unmethylated, digest completely with *HpaII*, and fail to amplify. The available normal mother shows no skewing. Both affected daughters show loss of one band indicating that they have preferential inactivation of one chromosome. In this case, the remaining band is that which is methylated (inactivated) and is the paternal allele for both girls.

had been treated for syphilis two years earlier). There were no other known exposures to illnesses or chemicals. She stopped smoking cigarettes early in this pregnancy and denied alcohol intake. This mother's previous two pregnancies had resulted in spontaneous abortions at 4 and 10 weeks' gestation. The gender of these fetuses is unknown. The birth weight of II-2 was 1295 g, birth length was 40 cm, and birth head circumference was 26 cm. Her Apgar scores were 5, 6, and 8 at one, five and 10 minutes, respectively. There is no known family history of unusual rashes or pigmentary changes. Neonatal complications included bilateral grade I intraventricular haemorrhage and stage I "retinopathy of prematurity", each of which had resolved on subsequent examinations. Within the first week of life, several vesicles and vesiculopustules were noted on her trunk and extremities. A scraping of one of the vesicles showed numerous eosinophils. Bacterial, fungal, herpes simplex, and varicella cultures were negative. Over the next few weeks, these lesions increased in number and coalesced into linear configurations on the extremities, trunk, axillae, and groin (fig 2, bottom). Her peripheral blood eosinophil count was normal at birth, began to rise after 3 weeks of age, reached a peak of 31% at 4 weeks of age, and returned to normal by 14 weeks. A skin biopsy from the right leg vesicle at 8 weeks of age showed eosinophilic spongiosis with intraepidermal blisters containing eosinophils and monocytic cells. The vesicular lesion of the trunk and extremities resolved after 4 months. At age 11 months, she had not manifested any pigmentary changes, her hair and nails were normal, and she was diagnosed as having mild gross motor delay.

DNA PREPARATION

DNA prepared by standard phenol/chloroform extraction from peripheral blood lymphocytes was used in the analysis.

LINKAGE ANALYSIS

Linkage analysis was performed with four distal Xq markers covering the region of the chromosome in which the IP gene is thought to reside: DXS998,¹¹ DXS52(VNTR),¹² F8C,¹³ and DXYS154.¹⁴ Analysis of the human androgen receptor gene (HUMARA) for X inactivation was performed as described previously.¹⁵

PATERNITY ANALYSIS

Paternity analysis was done by methods previously published,^{16,17} with STR polymorphisms HUMHPRTB, HUMFABP, HUMCD4, HUMCSF1PO, HUMTHO1, HUMPLA2A1, HUMF13A01, HUMCYARO4, HUMLIPOL, D6S366, HUMFESFPS, and HUMARA.

Results

Haplotype analysis of the family shows, as expected, that each girl received the same paternal X chromosome, as indicated by Xq27-tel markers (fig 1). Analysis of X inactivation patterns using the HUMARA(CAG)_n shows that each of the two girls has non-random X inactivation with preferential inactivation of the paternal X chromosome (fig 4). These data support the clinical diagnosis of IP in both girls, and suggest that each inherited the disease gene from her father. The father shows no symptoms or signs of IP, so he must carry the mutation as a gonadal mosaic.

Analysis of the HUMHPRTB STR polymorphism (AGAT)_n showed a mutation in the number of tetrad repeats between the father (I-1) and his younger daughter (II-1). This phenomenon has been reported previously with an average mutation rate for STR tetrads of 2.1×10^{-3} .¹⁸ Paternity testing with this marker showed an exclusionary event in this meiosis, even when the published mutation rates for locus HUMHPRTB are incorporated. Addition of three markers, including one on the X chromosome, allowed appropriate calculation of a paternity index. There were no other exclusionary events, indicating that the result at HUMHPRTB is most probably a result of mutation or recombination within this DNA locus.

The probability for paternity was calculated for II-1 to be 99.73%, with nine STR polymorphisms. For II-2, the probability for paternity was 99.99%, with 12 STR polymorphisms. A prior probability of 0.5 was used for each calculation.

Discussion

Preferential inactivation of the paternal X chromosome in the affected daughters presented both previously and here is consistent with

published observations, and may be considered confirmatory of the clinical diagnosis in the girls. Migeon *et al*¹⁹ reported five affected, presumably heterozygous, women who showed X inactivation patterns with selection against the IP X chromosome in both skin fibroblasts and white blood cells. Support for preferential inactivation was also found in a couple affected by both IP (the mother) and haemophilia A (the father). Non-random inactivation of the IP chromosome apparently unmasked the abnormal factor VIII gene on the alternative chromosome in one daughter who manifests both conditions.²⁰ Most recently, Curtis *et al*²¹ have reported skewed X inactivation in two families with IP.

The analysis of observations of the family presented is consistent with gonadal mosaicism in the father. This phenomenon is similar to that documented, for example, in osteogenesis imperfecta.²² The multiple spontaneous abortions are, however, inconsistent with this hypothesis. If the father is carrying a mutation on one of his X chromosomes, his offspring are expected to be 50% affected daughters and 50% normal sons. He should not produce affected sons or normal daughters without a recombination between his sex chromosomes. Tissue from the abortuses is not available for analysis.

Neither of the biological mothers in this family has any medical history consistent with IP. It is possible that either or both of these mothers carries the IP mutation as the variable expressivity of the IP may lead to underascertainment of mildly affected women. However, the disease is uncommon enough that we consider it unlikely for the father to have associated with two affected, but undiagnosed, women from a random population.

One previous report described an apparent father-daughter pair with a classical IP phenotype.²³ An analysis of this family has been interpreted to support the half chromatid mutation model.⁶ It is possible that our family also represents such a model. In such an event, the father would be expected to have 25% normal daughters, 25% affected daughters, and 50% normal sons. Again, however, if the spontaneous abortions are related to IP, they would not be expected under the half chromatid model.

The embryonic lethality of IP may not be limited to the affected males. It is possible that some increased wastage of female fetuses may occur also. Our hypothesis is that the normal form of the "IP" gene is required for placental development, and is maternally imprinted (inactive when inherited from the mother). In the family presented above, this would leave female fetuses without an active, normal gene, leading to placental maldevelopment and miscarriage. Survival of two affected daughters may have resulted from "fortunate Lyonisation" towards or reactivation of the normal maternal gene. In either hypothetical event, the placenta would

be comprised of a majority of cells in which the normal gene was active, allowing for survival of the fetus. This phenomenon would also account for the embryonic/fetal lethality in males and the lack of findings in those miscarriages of significant malformations other than hydrops fetalis.²⁴

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