Absence of Y specific DNA sequences in two siblings with 46XX hermaphroditism

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SUMMARY We report two siblings with 46XX hermaphroditism in whom we were unable to show the presence of Y specific DNA sequences using the DNA probes Y-190, GMGY-7, pHY2.1, pDP34, and 27a. We conclude that an autosomal or X chromosome gene mutation is the most likely mechanism of inheritance in this family with 46XX hermaphroditism.

DNA probes for regions of the Y chromosome have led to identification of Y specific DNA in 46XX and 45XO males, and to the presence of small deletions of the short arm in 46XY females with gonadal dysgenesis.¹ Thus the discordance between karyotype, phenotype, and gonadal histology can be explained by invoking translocation or deletion of a critical segment of the Y short arm, which carries male determining information, the so called testis determining factor. Recently a candidate gene for testis determining factor has been isolated.²

We describe two siblings who are 46XX hermaphrodites and in whom we were unable to identify the presence of Y derived DNA using five different Y specific DNA probes.

Case reports

CASE 1

Case 1 was the first child of unrelated parents. Ambiguous genitalia with hypospadias, a bifid scrotum, and bilateral scrotal gonads were noted at birth. Investigations showed a 46XX karyotype. Three injections of human chorionic gonadotrophin (1000 units) at 3 months of age resulted in a rise in plasma testosterone from 4.1 nmol/l to 6.0 nmol/l. Laparotomy showed no Mullerian structures. A gonadal biopsy specimen showed bilateral ovotestes, and gonadal fibroblast chromosomes were 46XX. Because of the well formed male external genitalia, the absence of female internal genitalia, and the plasma testosterone response to human chorionic gonadotrophin it was decided to rear the child as a male. At 3 years of age the ovarian

component of the ovotestes was excised and the hypospadias repaired.

CASE 2

Case 2 was the second child in the family. His external genitalia were similar to those of his brother, as were the findings at laparotomy. Chromosome analysis showed a 46XX karyotype. Three injections of human chorionic gonadotrophin (1000 units) produced a rise in plasma testosterone from 2.0 nmol/l to 5.2 nmol/l. This child has also been raised as a male and had his hypospadias repaired and the ovarian component of his ovotestes resected at 4 years of age.

The mother of both children is healthy; the father is said to be healthy, but was not available for study.

Methods

DNA was extracted from 10 ml blood from each patient by standard methods. 5 µg DNA was digested with appropriate restriction endonuclease (Northumberland Biologicals) using buffer provided by the manufacturer. DNA was separated by electrophoresis on 0.8% agarose gel and transferred to a nylon filter (Hybond N Amersham) by blotting. The filters were hybridised for 16 hours at 65°C at a final salt concentration of 0.6 M. The probes were labelled by the random priming method and added to the hybridisation solution at a concentration of 10⁶ dpm/ml. After hybridisation, filters were washed with sodium chloride/sodium citrate solution at 65°C to remove unhybridised probe, then exposed to Kodak XAR5 autoradiographic film with intensifying screens at -70°C for one to five days. Before reuse filters were washed with boiling 10 mM TRIS, pH 8.

HYBRIDISATION PROBES

Y-190³ and GMGY-7³ are highly repetitive probes from the short arm of the Y chromosome. pHY2.1 (DYZ2) is a very highly repetitive probe from the long arm of the Y chromosome. pDP34 is a single copy probe on the short arm of the Y with a

homologous sequence in the middle of the long arm of the X chromosome³; 27a is a conserved and Y chromosome unique locus near to testis determining factor.⁴

Results

The figure (a) shows the results obtained with TaqI digest and probe 27a. Only track 2 (from a normal male control) showed any hybridisation. The lack of hybridisation makes it extremely unlikely that testis determining factor was present.

The figure (b) shows the results of hybridisation of Y-190 to a TaqI filter. Again only the track from the normal male control showed hybridisation. This

confirms the lack of Y chromosome material in these children. It also rules out the possibility of mosaicism as the highly repetitive Y-190 sequences would have been detected even if only 1% of 46XY cells had been present.

The other three probes gave the same results; this confirmed the above findings (results not shown).

Discussion

Most cases of true hermaphroditism are sporadic but there have been a few reports of familial hermaphroditism.^{5,6} As in our patients, some of these cases have been characterised by the presence of bilateral ovotestes, absent Mullerian derivatives, and 46XX karyotypes. In theory, sex chromosome mosaics or chimeras, translocations of part of the Y chromosome to the X chromosome or an autosome, or an autosomal gene mutation could all lead to hermaphroditism. Mosaicism and chimerism are unlikely in our cases, however, because of the familial nature of the condition, the absence of cytogenetic evidence of a 46XY cell line in the gonadal fibroblast cultures, and the inability of the DNA probes to detect a 46XY cell line in peripheral lymphocytes. A DNA probe coding for a gonadal determining factor has recently been isolated.² The probe, 27a, used here is extremely close to gonadal determining factor on the Y chromosome, and therefore a translocation of gonadal determining factor is unlikely in view of our inability to detect any 27a fragments.

A gene mutation on an autosome or X chromosome is a much more likely explanation for our patients' familial hermaphroditism. Such a mutation could result in differentiation of a testis or ovotestis in a subject with a 46XX karyotype and in the absence of a Y chromosome. In the female heterozygous state the mutation would be insufficient to induce the indifferent gonad to become a testis but in the homozygous state ovotestes or a testis could differentiate. As there is a sequence closely related to gonadal determining factor on the X chromosome² models have been suggested in which gonadal differentiation is determined by the dosage of factors from the X and Y chromosomes.⁷ Unusual families such as these will provide the material to test such hypotheses.

Figure (a) Autoradiograph of filter of TaqI digest hybridised with probe 27a. (b) Autoradiograph of filter of TaqI digested DNA hybridised with Y-190. Track 1=normal female; track 2=normal male; track 3=mother; track 4 and 5=cases 1 and 2.

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Psychological care of survivors of a fire

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SUMMARY A mother and daughter were the sole survivors of a house fire. Support, maintenance of the parent-child relationship, and monitoring and facilitating the grieving process in a way that was consistent with the developmental stages of the patients were the basis of management.

Case report

Four members of an Asian family of six died in a fire, started by a gas heater while they slept. They were the father, two daughters aged 11 and 2, and a son aged 9. The mother and 7 year old daughter were admitted to the intensive care unit of the district general hospital. They were both suffering from extensive heat and inhalation injuries, and required ventilatory support—the mother for several hours, and the daughter for several days, during which she was sedated and paralysed.

The mother spoke little English, and so most communication was through her local authority social worker who spoke her language. Visitors told the mother that the other members of her family were in another hospital. Three days later she was told of their deaths. She seemed numbed, shed few tears, and asked no questions. Five days after the fire, the daughter had still not been told what had happened. She played quietly with nursing staff and often seemed frightened, but asked no questions about her burns, her presence in hospital, or the whereabouts of her father, brother, and sisters. She avoided contact with her mother. The mother's

father was unable to be with her for another week. Physically, mother and daughter were well enough to be transferred from the intensive care unit.

Psychological aspects of further management

Because we thought that a multidisciplinary team approach would be the best way to help the patients, the paediatrician (GS), child psychiatrist (TJ), social worker, and nursing staff met regularly. These meetings helped us to acknowledge our feelings of hopelessness and our own memories of loss. We were then more able to separate these from our thinking about the needs of this mother and daughter.

We wished to support the relationship between the mother and daughter, so we ensured that when they were transferred they went to adjacent wards so that they could spend as much time as possible together. We also attempted to support their relationship by encouraging the mother in her role as a parent. There were aspects of hospital routine which made this difficult. The authority of a mother and the authority of hospital staff sit together somewhat uneasily, particularly when both the mother and her daughter are inpatients. In this case the mother's authority was undermined when the staff, despite the reluctance of the mother (who was suffering her own psychological injury), told the daughter about the fire and the deaths.

The daughter needed to know about the fire and deaths as soon as possible to end her uncertainty, to allow her to start grieving, and to avoid her hearing inaccurate accounts from other patients. It was