Investigation of a unique male and female sibship with Kallmann’s syndrome and 46,XX gonadal dysgenesis with short stature

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Abnormalities of the craniofacial region and the reproductive tract are a feature of Kallmann’s syndrome (olfactogenital dysplasia) and are associated with anosmia. The syndrome occurs in less than 1 in 10,000 births, with a 5-fold excess of affected males to females. The following modes of inheritance have been observed: autosomal recessive (McKusick, 1994). A gene (KAL1) at Xp22.3 is implicated in the Kallmann’s syndrome manifesting concurrently with ovarian dysgenesis found in this family.

Key words: anosmia/gonadal dysgenesis/hypogonadotrophic hypogonadism/Kallmann’s syndrome/streak ovaries

Introduction

A unique male/female sibship has been identified with both siblings exhibiting hypogonadotrophic hypogonadism with anosmia, which is consistent with the diagnosis of Kallmann’s syndrome. The woman sibling also has ovarian dysgenesis and short stature. A female with both Kallmann’s syndrome and streak ovaries has not previously been reported. Kallmann’s syndrome occurs in less than 1 in 10,000 births, with a 5-fold excess of affected males to females (Jones and Kemmann, 1976). The following modes of inheritance have been observed: X-linked, autosomal dominant with variable penetrance and autosomal recessive (McKusick, 1994). A gene (KAL1) has been isolated from the Xp22.3 region (Franco et al., 1991; Legouis et al., 1991) and mutations in this gene have been found to be responsible for the X-linked form of Kallmann’s syndrome (Meitinger et al., 1990; Bick et al., 1992; Hardelin et al., 1992). The KAL1 locus is believed to undergo X-inactivation (Franco et al., 1991). The extracellular matrix glycoprotein derived from this gene is suggested to be involved in neural cell adhesion and axonal pathfinding and its absence is probably responsible for failure of the neurones secreting gonadotrophin releasing hormone (GnRH) to develop and migrate in embryonic life (Rugarli et al., 1993, 1996). The exclusion of involvement of the KAL1 locus at Xp22.3 in this pedigree is discussed.

Clinicopathological report

The female proband presented at 16 years and 11 months of age, never having menstruated and with no evidence of breast development or any other sign of spontaneous puberty. Birthweight was 3 kg. At presentation her weight was 41.2 kg and her height was 145 cm. She was also completely anosmic. Basal luteinizing hormone (LH) concentrations were less than 1 IU/l and showed no change with i.v. GnRH stimulation (Wyeth-Ayerst HRF®, Ayerst, Rouses Point, USA) and the concentrations of follicle stimulating hormone (FSH) started during the stimulation at 0.8 IU/l and rose to 3.1 IU/l. The anterior pituitary reserve was otherwise normal on dynamic testing. These findings were consistent with a diagnosis of Kallmann’s syndrome (olfactogenital dysplasia). Oestrogen therapy was started, the dose increased after 3 months and cyclical progesterin added. A final height of 156 cm was reached. The patient was advised that gonadotrophin support would be necessary to achieve pregnancy.

At age 30 years the female proband married and desired pregnancy. She was, however, completely refractory to repeated and prolonged attempts at ovulation induction with firstly pulsatile GnRH (Wyeth-Ayerst HRF®) (Jansen, 1993) and later high dose i.m. gonadotrophins (Pergonal®, Laboratories Serono SA, Aubonne Switzerland). During laparoscopic investigation bilateral ‘streak ovaries’ were noted and ovarian biopsy was performed. The uterus and Fallopian tubes appeared normal. Hysteroscopic examination revealed a normal 7 cm long uterine cavity. Ovarian, antinuclear, mitochondrial, parietal cell, islet of Langerhans cell, adrenocortical, rheumatoid factor, acetylcholinesterase, thyroid microsomal and thyroglobulin serum autoantibodies were not detected. Ultrasound examination revealed normal renal tracts.

The proband conceived at age 34 after the transfer of two frozen–thawed embryos, quarantined for the previous 6 months after donor oocyte IVF. Endometrial preparation and support was given with ethinyl oestradiol tablets (Progynon-C; Schering, Berlin, Germany) until 5 weeks after embryo transfer and non-proprietary progesterone pessaries (progesterone powder; Sigma, St Louis, Mo USA) until 7 weeks after the embryo transfer. A healthy female weighing 3395 g was delivered 36 weeks after embryo transfer by elective caesarean section. Vaginal delivery was not attempted due to the clinically
contracted pelvis. Hot flushes were experienced a week into the puerperium and hormone replacement therapy was re instituted.

At presentation it was noted that her brother, age 24 years, had not achieved puberty and had anosmia. The diagnosis of Kallmann’s syndrome in the male sibling was supported by endocrine testing: FSH 0.6 IU/l; LH <1.0 IU/l; prolactin 19 nmol/l; total testosterone 1.2 nmol/l and free testosterone 19 pmol/l. The skeletal phenotype was hypogonadal; height 172 cm, arm span 184 cm, with segmental disproportion (upper segment 73 cm, lower segments 79 cm). The hair distribution was fine, bilateral gynaecomastia was observed and the testicular volumes were 8 ml. Seminal fluid analysis revealed azospermia. Twice weekly injections of human chorionic gonadotrophin (HCG) were then administered for a number of years. Pergonal® treatment initiated spermatogenesis and semen analysis recorded a volume of 4.6 ml, a concentration of $4.6 \times 10^6$ spermatozoa per ml and 50% motility. Sperm morphology was not examined. Androgen replacement therapy was given with s.c. testosterone pellets (Conway et al., 1988).

A further attempt at obtaining spermatogenesis was carried out at age 41 years for the purposes of IVF. Using HCG injections in a dose of 2000 units s.c. thrice weekly (Profasi; Laboratories Serono SA, Aubonne Switzerland) and when a serum testosterone concentration of 10 nmol/l was registered 6 weeks later, FSH was introduced in doses of 150 units thrice weekly (Puregon; Organon, Oss The Netherlands). Four months after the FSH was introduced semen analysis recorded $1.5 \times 10^6$ spermatozoa per ml, 64% of spermatozoa exhibited rapid motility and 6% normal morphology. Further analyses over several months recorded similar semen parameters. Spermatozoa were also frozen for long-term storage. Fertilization was obtained with fresh ejaculated semen and intracytoplasmic sperm injection of all five oocytes retrieved after pituitary down-regulation and ovarian stimulation. Semen analyses were performed in accordance with methodology recommended by the World Health Organization (1992). Pregnancy did not ensue from the transfer of two embryos. The three supernumerary embryos are cryostored.

The siblings have normal intelligence and hearing. Ophthalmic examination of the female sibling offered no evidence of ocular albinism type 1 (OA1). There were no skin efflorescences indicative of ichthyosis. Mirror movements were not evoked in the siblings. The mother did not experience premature menopause. Maternal hyposmia was discerned on anamnesis and clinically. Mirror movements were not elicited in either parent. The parents emigrated to Australia from different districts in Sicily. There was no family history of delayed puberty, infertility, consanguinity or anosmia.

**Magnetic resonance imaging**

Sagittal T1, coronal T1 and FSE images and axial dual echo T2 images were acquired of the pituitary, hypothalamus, anterior commissure, olfactory bulbs and sulci. No abnormality of the pituitary, upper thalamus or olfactory pathway was detected.

**Figure 1.** Overview of the streak ovary. Original magnification ×25.

**Figure 2.** View within the thin ovarian cortex. Original magnification ×200.

**Histopathology of the streak ovary biopsy**

A grey biopsy fragment measuring $13 \times 5 \times 2$ mm obtained from the right streak ovary was embedded whole in paraffin. Sixteen serial sections were prepared and stained with haematoxylin–eosin for microscopic examination. The attenuated streak ovary was characterized by the presence of a thin, densely hypocellular cortex subtending an apparently normal tunica vaginalis and mesothelial surface (Figure 1). Within the thin cortex an occasional primordial follicle (Figure 2) containing a morphologically viable oocyte was noted and observed to be surrounded by cuboidal pregranulosa cells (~1/200 mm²). In the serially sectioned ovarian biopsy there was no other evidence of follicular activity, either present or past. Rare, minute, undistinguished, scars were noted in the cortex, none of which were diagnostic of follicular remnants, and one of which was noted to include a foreign-body giant cell containing foreign material. No serosal inclusions (Mullerian) or inflammatory cell infiltrates were observed in the ovarian cortex. Likewise, the hypotrophic medulla was entirely devoid of follicular structures or remnants (Figure 1). Unlike many streak gonads, no hilus cells (Leydig cells) were observed in the deep medulla or hilar region of the ovary.
Cytogenetic investigations

G-banding with examination of 20 metaphases from peripheral lymphocytes of both siblings confirmed normal karyotypes. High-resolution microscopy was performed for the X-chromosomes, the distal 1q and 10q regions of both siblings, and did not detect abnormalities.

Molecular genetic investigations

Peripheral blood samples from the parents and the two siblings were collected. Genomic DNA was extracted using a modification of the ‘salting out’ method (Miller et al., 1988). The oligonucleotide primers for the KAL1 exons were obtained from Pierre Bouloux (Royal Free Hospital, London, UK). Polymerase chain reaction (PCR) amplification of the KAL1 exon 12 was carried out as previously described (Hardelin et al., 1993; Quinton et al., 1996b). The oligonucleotide primers for the dinucleotide repeat polymorphism at the KAL1 locus (Bouloux et al., 1991) and the oligonucleotide primers for polymorphisms DXS996, DXS7103 were obtained from Research Genetics, Inc. (Huntsville, AL, USA.). PCR reactions for the polymorphic loci DXS996 and DXS7103 were performed according to conditions first described by Jean Weissenbach and obtained from Genome Database (available on the Internet http://gdbwww.gdb.org). PCR products were analysed on 7% (19:1) polyacrylamide gels and visualized using a silver-staining method (Budowle et al., 1991).

PCR using the intragenic KAL1 primers flanking exon 12 resulted in products for both siblings. For the linkage studies the intragenic KAL1 polymorphic locus (Bouloux et al., 1991) was non-informative in the pedigree; however, use of the polymorphic loci, DXS996 and DXS7103, flanking the KAL1 locus, indicated that the siblings have inherited different maternal alleles for these loci (Figure 3). The likelihood of recombination events between these loci, DXS996 and DXS7103, were calculated using centimorgan data, obtained from Cedar Genetics (available on the Internet http://cedar.genetics). DXS996 is stated to be 2.69 centimorgans telomeric to KAL1, while DXS7103 is 3.79 centimorgans centromeric to KAL1. The likelihood of double recombination between these loci was calculated to be 1/981 (or 0.1%).

Discussion

Kallmann’s syndrome shows genetic heterogeneity (McKusick, 1994). Since the isolation of KAL1, the candidate gene for the X-linked form of the disorder, there have been several investigations looking for mutations in this gene. Pedigrees used in these studies have usually experienced at least one affected male either in the maternal family of the proband or among the male siblings. There is an absence of affected females (Hardelin et al., 1993). Carrier females often have anosmia or hyposmia, but there are few, if any, reports of hypogonadotrophic female relatives of a male proband. The central hypotalamic defect in the proband is demonstrated by the dynamic testing results and the findings of the deep medullary/hilar region of the ovarian biopsy. Because gonadotrophin stimulation was absent, unlike many streak gonads, hilus cell development (Leydig cells) was not observed.

Kallmann syndrome-affected women generally occur sporadically in the population with no other affected relatives (P.M.Bouloux, personal communication). Other conditions have been associated with Kallmann’s syndrome, such as mirror movements (bimanual synkinesis), abnormal spatial attention, ocular motor apraxia, unilateral renal dysplasia and more rarely unilateral sensorineural deafness, short clavicles, chorioretinal coloboma, neonatal hydrocephalus, cleft lip/palate, and pes cavus deformity (Hardelin et al., 1993; Quinton et al., 1996a). Renal dysplasia, in particular, often occurs in X-linked Kallmann syndrome patients (Kirk et al., 1994) and there is also a high frequency of single kidneys in these affected individuals (Hardelin et al., 1993). The siblings’ renal ultrasound examinations were normal. This finding cannot be used to discount a KAL1 locus. The finding that the siblings did not elicit mirror movements is reliable neurophysiological evidence that the sibship do not possess a mutation of the KAL1 locus (Quinton et al., 1996a).

It is possible that aberrant cellular interactions might underlie the phenomena of Kallmann’s syndrome and renal agenesis. Renal agenesis does not appear to occur in the non-X-linked Kallmann’s syndrome (Quinton et al., 1996b). Renal anomalies are observed in 45,X ovarian dysgenesis but without olfactory anomalies (Plouffe and McDonough, 1996). The metanephrus and gonadal ridge develop adjacent and a field defect might suggest aberrant cellular interactions. However, the renal development in the sibship appears to be normal. At the time of gastrulation the primordial germ cells begin migration from the caudal primitive streak/epiblast (Lawson and Hage, 1994). The epiblast also differentiates to become the neuroectoderm (Larsen, 1997). Therefore the involvement of germ cells and the olfactory/hypothalamic disorder suggest a possible link at the level of the epiblast development.

There was no evidence in the siblings’ examination to suggest involvement of known contiguous gene syndromes. Contiguous gene syndromes have been described where deletions involving the distal short arm of the X-chromosome have resulted in patients with combinations of short stature, chondrodysplasia punctata, mental retardation, steroid sulphatase deficiency (STS) and Kallmann’s syndrome (Ballabio et al., 1989).
excluded the closest adjacent contiguous gene syndromes, STS (ichthyosis) and OA1, flanking the \textit{KAL1} locus. PCR amplifications of \textit{KAL1} exon 12 and the intragenic \textit{KAL1} polymorphism resulted in appropriate products being found for both siblings, and microdeletions of the entire \textit{KAL1} locus can therefore be excluded.

Although X-linked transmission of the disorder was not indicated in this family, segregation analysis of the disorder with the \textit{KAL1} region of the X chromosome was carried out for confirmation at the molecular level (Figure 3). In view of the allele segregation findings \textit{KAL1} locus involvement would require a double recombination of the maternal allele. The likelihood of a double recombination event was calculated to be 1/981. The denominator, however, is probably underestimated as physical ‘interference’ reduces nearby double recombination frequency (Schmitt \textit{et al}., 1994; Strachen and Read, 1996). The likelihood of \textit{KAL1} locus involvement is therefore probably less than the centimorgan-derived calculation of 0.1 %.

Pedigree information and three translocation reports suggest that there are autosomal forms of Kallmann’s syndrome (Best \textit{et al}., 1990; Casamassima \textit{et al}., 1993; McKusick, 1994; Schinzel \textit{et al}., 1995). The diagnosis of Kallmann’s syndrome in two of the translocation reports is contestable. Best \textit{et al}.
\textit{1990} have possibly erred in contending that a 7q;12q reciprocal translocation case had Kallmann’s syndrome as the gonadotrophin concentrations were actually in the normal range; molecular exclusion of the \textit{KAL1} gene was not performed. The case presented by Casamassima \textit{et al}.
\textit{1993} did not exclude involvement of the \textit{KAL1} gene with molecular investigations; also the FSH concentration of 3 IU/l is not particularly low, and inconsistent with a Kallmann’s syndrome. Schinzel \textit{et al}.
\textit{1995} presented a case in which Southern blot screening did not detect band shifts within the \textit{KAL1} gene. Undetected mutations of the \textit{KAL1} gene could be present. The basal gonadotrophin concentrations were low, which is consistent with Kallmann’s syndrome. In view of the translocation case reported by Schinzel \textit{et al}.
\textit{1995} high-resolution microscopy of the siblings for the distal 1q and 10q regions was performed and an abnormality was not detected. Hypogonadotropic hypogonadism is also seen in the autosomal recessive Boucher–Neuhauser syndrome. However, the sibs do not possess the typical characteristics of choroidal dystrophy and cerebellar ataxia (Rump \textit{et al}., 1997).

The putative \textit{KALP} receptor gene, which has not been cloned, has been proposed as a candidate for the autosomal form of Kallmann’s syndrome (Quinton \textit{et al}., 1996a). A recent study considered idiopathic hypogonadotropic hypogonadism as an alternative manifestation of Kallmann’s syndrome and suggested that the X-linked mode of inheritance accounted for only 36% of 106 cases (Waldstreicher \textit{et al}., 1996). The investigators also found a low prevalence of \textit{KAL1} gene mutation in isolated GnRH deficiency (Georgopoulos \textit{et al}., 1997). It has been suggested, however, that Kallmann’s syndrome should not be considered together with idiopathic hypogonadotropic hypogonadism as the latter usually represents a distinct developmental field defect (Dean \textit{et al}., 1990; Quinton \textit{et al}., 1996b). If so, the proportion of autosomal forms of Kallmann’s syndrome is considerably smaller.

In the syndrome of congenital adrenal hypoplasia and hypogonadotropic hypogonadism the \textit{DAX1} gene, at Xp21.3, is mutated (Zanaria \textit{et al}., 1994). Based upon study of the mouse \textit{DAX1} gene, expression is thought to influence gonadal differentiation (Swain \textit{et al}., 1996). Although the concurrence of gonadal and hypothalamic effects is fascinating, the \textit{DAX1} gene mutation is not, however, known to cause anosmia. Adrenal hypoplasia was not observed in the siblings and an X-linked disorder is unlikely. A \textit{DAX-1} gene mutation in the siblings is therefore unlikely.

It has been observed that X-chromosome deletions associated with complete ovarian failure rather than secondary amenorrhea invariably involve the proximal regions of the X chromosome (Simpson, 1986). These regions are more likely X-inactivated. If one postulates that the causative locus is within this region then one must assume that the female sibling is a manifesting carrier. This assumption requires the occurrence of a further chance event, such as skewed X-inactivation or uniparental isodisomy. Skewed X-inactivation of a normal allele has been documented to occur in Duchenne muscular dystrophy, but infrequently (Azozeifa \textit{et al}., 1995). A homozygous mutated \textit{KAL1} status in the woman is most improbable. Uniparental isodisomy in the woman, with a mutated \textit{KAL1} gene, can be rejected because of the allele heterozygosity found in the three polymorphic sites \textit{KAL1}, DXS996 and DXS7103.

Deletions detected from the long arm of the X-chromosome (Xq) are well recognized as a cause of premature ovarian failure (Krauss \textit{et al}., 1987). The likelihood of contiguous gene deletions in women with ovarian failure accompanying the syndrome of blepharophimosis has been reported (Smith \textit{et al}., 1989). Such deletions appear to be autosomal. Linkage studies suggest that blepharophimosis–ptosis–epicanthus–inversus syndrome type-1 (BPE1) associated with ovarian dysgenesis has been localized to 3q22-q23 (Small \textit{et al}., 1995; Amati \textit{et al}., 1996). The BPE1 syndrome has not been associated with Kallmann’s syndrome.

Seventy-five families with 46,XX ovarian dysgenesis (ODG) have been identified in Finland and some pedigrees suggest the existence of an autosomal recessive gene (Aittomaki, 1994). By systematically searching for linkage in multiplex affected families, a locus for ODG was mapped to chromosome 2p. The follicle-stimulating hormone receptor (\textit{FSHR}) gene was previously assigned to 2p. A search for mutations identified a C566T transition in exon 7 of \textit{FSHR} gene suggested a subset of pathogenetically distinct ovarian dysgenesis patients. Possibly because of residual receptor activity these patients can be identified by demonstrating the presence of ovarian follicles and confirmed by mutation analysis (Aittomaki \textit{et al}., 1996). Hypogonadotropism and the development of spermatogenesis experienced after gonadotrophin therapy both suggest that an FSH receptor deficit is unlikely in the sibship.

Autosomal recessive disorders of single gonadotrophin defi-
cit, of either FSH or LH, can frustrate the normal development of puberty and fertility (Weiss et al., 1992; Layman et al., 1993, 1997). There is evidence that FSH is not required for spermatogenesis (Kumar et al., 1997). In isolated FSH deficiency, however, there is no concurrent deficit of LH release, as was observed in the siblings. Males undergo normal virilization, which was not observed in the male proband (Rabin et al., 1972; Rabin, 1975). Isolated gonadotrophin deficiency would also not account for the observed ovarian dysgenesis. There is a report of 47,XXY gonadal dysgenesis with Kallmann syndrome (Hazard et al., 1986). This would possibly indicate a gonosomal gene effecting Kallmann syndrome. It is difficult to assume an X-linked recessive cause such as a KAL1 locus mutation unless the X-chromosome were duplicated. The duplication error might have led to non-disjunction.

The reasons for failure of germ cell persistence in 46,XX gonadal dysgenesis are unknown. A ‘knock-out’ mouse defective for the DNA mismatch repair gene Mlh1 has been noted to have disturbed meiosis control mechanisms and small ovaries with very few follicles (Baker et al., 1996). No endocrine data were reported and Kallmann’s syndrome has not been associated with DNA mismatch repair gene disorders. These varied reports suggest that more than one gene encodes for factors necessary for the survival of oocytes through reproductive life. There may well be other women who have Kallmann’s syndrome as well as 46,XX gonadal dysgenesis. Such women would be identified by refractory ovulation attempts. There are few reports of ovulation induction and pregnancy results (Sungurtekin et al., 1995).

This is the first report of male and female siblings with Kallmann’s syndrome manifesting concurrently with ovarian dysgenesis. The exclusion of involvement, by linkage analysis, of the KAL1 locus and the presentation of male and female phenotypes suggest that it is highly likely that an autosomal gene is involved. When sufficient pedigrees of females affected with Kallmann’s syndrome are obtained, molecular linkage analyses will become possible. Studies might then reveal a gene involved, albeit continguously, with oocyte survival.

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