Original Article

Multicystic dysplastic kidney and Kallmann’s syndrome: a new association?

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Abstract

Background. Kallmann’s syndrome is characterized by anosmia and hypogonadotrophic hypogonadism. Radiographic studies of teenagers and older subjects with the X-linked form of the syndrome have shown that up to 40% have an absent kidney unilaterally. Although this has been attributed to renal ‘agenesis’, a condition in which the kidney fails to form, little is known about the appearance of the developing urinary tract either pre- or post-natally in individuals with Kallmann’s syndrome.

Methods. We describe two brothers who had features of Kallmann’s syndrome, most probably of the X-linked variety, who both had a major urinary-tract malformation detected before birth.

Results. The brothers were found to have unilateral multicystic dysplastic kidneys on routine antenatal ultrasound scanning and both underwent surgical nephrectomy of these organs post-natally. Immunohistochemical studies on the younger sibling revealed hyperproliferative dysplastic kidney tubules which overexpressed PAX2, a potentially oncogenic transcription factor, and BCL2, a cell-survival factor, surrounded by metaplastic, α smooth-muscle actin-positive stroma: similar patterns have been observed in patients with non-syndromic multicystic dysplastic kidneys.

Conclusions. Our results describe a new type of urinary-tract malformation associated with Kallmann’s syndrome. However, since multicystic kidneys tend to involute, only when more Kallmann’s syndrome patients are screened in utero or in early childhood using structural renal scans, will it be possible to establish whether multicystic kidney disease is a bona-fide part of the syndrome.

Keywords: anosmia; gonadotrophin-releasing hormone; hypogonadism; Kallmann’s syndrome; multicystic dysplastic kidney; renal agenesis

Introduction

In 1944 Kallmann et al. [1] described the familial nature of a syndrome comprising anosmia and hypogonadotrophic hypogonadism. These clinical features result from defective migration of gonadotrophin-releasing hormone neurones from the medial olfactory placode epithelium into the anterior hypothalamus and failure of olfactory bulb genesis [2]. Mutations of KAL-1, a gene encoding a putative cell-signalling molecule, anosmin 1, have now been described and validate the disruption of this gene as the cause of the X-linked form of the syndrome [3,4]. The KAL gene is expressed in the embryonic forebrain in the first trimester of human development [5]. The incidence of Kallmann’s syndrome is 1/10000 in males and 1/50000 in females. It is usually inherited in an X-linked recessive manner but autosomal dominant and autosomal recessive forms also exist. Patients with Kallmann’s syndrome commonly present with delayed puberty but the diagnosis may also be suspected in early life because of micropenis and cryptorchidism. A range of abnormalities has been described in the X-linked form of the disorder, including mirror movements of the hands (synkinesis) [6], and unilateral renal agenesis [7]. In this report we describe a possible association between Kallmann’s syndrome and multicystic dysplastic kidney.

Subjects and methods

Immunohistochemistry of surgically removed kidney tissue was performed as previously described [8–10]. Briefly,
paraffin-embedded tissue was sectioned at 5 μm, dewaxed, microwaved for 10 min, and incubated with the following antibodies: mouse monoclonal anti-human BCL2 (clone124, Dako), an anti-apoptotic protein expressed in non-syndromic dysplastic kidney epithelia [8]; rabbit anti-PAX2 (71–6000, Zymed), a potentially oncogenic transcription factor protein expressed in non-syndromic dysplastic kidney epithelia [8, 9]; mouse monoclonal anti-proliferating cell nuclear antigen (PCNA; Ab1, Oncogene Science), a surrogate marker of mitotic cells, also expressed by non-syndromic dysplastic kidney epithelia [8, 9]; mouse anti-α smooth muscle actin (αSMA; A2547, Sigma), a cytoskeletal protein expressed by metaplastic tissues around dysplastic tubules in non-syndromic dysplastic kidneys [10]. Primary antibodies were detected with appropriate second antibodies and colour generated by the ABC peroxidase kit (Dako). Finally, nuclei were counterstained with haematoxylin.

DNA was extracted from peripheral blood, taken from the mother and both brothers, using QIAamp columns and protocol (Qiagen, U.K. Cat# 51304), and subjected to genetic analysis. For the brothers, all 14 KAL-1 exons were individually amplified by PCR, using intronically located primers [11], and the products were analysed by automated sequencing (carried out by the Microchemical Unit, Babraham Institute, Cambridge, UK). Analysis was also carried out on a CA repeat polymorphism located within an intron of the KAL genomic sequence for all three individuals.

Results

The structure of the family is shown in Figure 1.

Patient 1 was found to have multiple cysts in the left kidney on routine antenatal ultrasound at 15 weeks gestation. A left-sided multicystic kidney and normal right kidney was confirmed by ultrasound scan postnatally. Intravenous urogram showed a normal hyper trophyed right kidney but no function on the left. The patient subsequently underwent left nephrectomy at 2 years of age. Histology confirmed the presence of dysplastic tubules surrounded by metaplastic stroma (not shown), characteristic appearances of renal dysplasia [8, 12]. At 6 weeks he was noted to have micro penis and cryptorchidism and he required bilateral orchidopexies when 7 years of age. At 9 years of age, his basal gonadotrophin levels were below the threshold of the assay (<1 U/l). At 30 and 60 min after administration of 100 μg luteinizing hormone releasing hormone (LHRH), his luteinizing hormone (LH) levels were 1.1 U/l and 1.4 U/l, and follicle-stimulating hormone (FSH) levels were <1.0 and 1.0 U/l respectively. Although this response was compatible with normal prepuberty, it was also considered to be consistent with hypogonadotrophic hypogonadism. A prolonged human chorionic gonadotrophin (HCG) test, using 2000 U HCG twice weekly for 3 weeks, showed a suboptimal increase of serum testosterone from 1.0 to 3.3 nmol/l. At 12 years of age the patient was still clinically prepubertal with each testis less than 1 ml volume. He was noted to have mirror movement of the hands (synkinesis) and test of his sense of smell confirmed anosmia. His blood pressure and kidney function, as assessed by plasma creatinine, were consistently normal and he had a normal male karyotype. Baseline LH and FSH levels have remained less than 1.0 U/l at 16 years of age in keeping with the diagnosis of Kallmann’s syndrome with unilateral multicystic dysplastic kidney. He has received oral and intramuscular testosterone preparations and is now being treated with pulsatile subcutaneous gonadotrophin-releasing hormone to assess testosterone production in the longer term and to induce an increase in testicular volume. Both the mother and sister of patient 1 have two normal kidneys. His sister has progressed through puberty normally and has a regular menstrual cycle at 14 years of age.

Patient 2 is the half-brother of patient 1 and they share the same mother. He is 2½ years of age. Antenatally, at 20 weeks gestation, he presented with an ultrasonographic appearance compatible with a right-sided multicystic dysplastic kidney. Postnatal ultrasonography at 4 weeks of age confirmed a multicystic dysplastic kidney on the right with a normal left kidney. There were two cysts in the region of the right kidney measuring 2 and 3 cm in diameter, but with no normal renal tissue identified. [99mTc]dimercaptosuccinic acid isotope renogram showed no uptake on the right side but normal left renal function (Figure 2). The

![Fig. 1. Family pedigree showing the two affected siblings.](image)

![Fig. 2. 99mTc dimercaptosuccinic acid (DMSA) scan of patient 2. The right kidney is not visualized; consistent with a multicystic right kidney. The left kidney appears normal.](image)
patient was also noted to have a micropenis with normally descended testes. At 2 months of age, his
stretched penile length was abnormally short at 2.1 cm
[13]. LH and FSH concentrations were less than 1 U/l
and testosterone levels were also below the assay
threshold of 1 nmol/l at 4 months of age. Assessment
by a neurodevelopmental paediatrician (MG) at 2.5
years included video-analysis of grasp and release
activities with bricks and pegboard. This showed sym-
metrical onset of finger flexion to grasp, and exten-
sion to release on the opposite side to the voluntary
movement of either right or left hand. Whilst this
can be a normal finding in early life, the precision
of these mirror movements when compared to chil-
dren of a similar age was striking [14]. The clinical
picture and biochemistry were thought to be con-
sistent with a diagnosis of hypogonadotropic hypo-

gonadism in an infant with a multicystic dysplastic
kidney. At 6 months of age his plasma creatinine
was 51 μmol/l—normal range 25–55—and his blood
pressure was normal at 85 mmHg systolic. He under-
grew right nephrectomy at 6 months of age and histo-
logy confirmed the characteristic appearances of
dysplastic tubules and cysts (Figure 3A,B). No normal
glomeruli were seen and dysplastic tubules were
surrounded by poorly differentiated stromal tissues,
indicating a lack of normal mesenchymal to epithelial
conversion in this tissue compartment. These features
are characteristic of the histological lesion known as
‘renal dysplasia’ [12]. Immunohistochemistry (Figure
3C–G) revealed metaplastic αSMA-positive ‘collarettes’
around dysplastic epithelia, which expressed PAX2,
PCNA, and BCL2. Cells in the poorly differentiated
tissues around dysplastic tubules did not express
significant levels of either PAX2 or BCL2.

Analysis of the KAL gene (Xp22.3) did not reveal
any mutations after direct sequencing of all 14 exons
and splice site regions: similar negative results have
been described in a significant number of X-linked
Kallmann’s syndrome patients [15] and would also be
expected in the autosomal phenocopies of the syn-
drome. An analysis of a CA repeat polymorphism
located in the KAL-1 locus showed the same pattern
for both maternal X chromosomes and so it has not
been possible to definitively prove that the same X
chromosome was inherited by her two sons using this
methodology.

Discussion

Despite the lack of direct genetic evidence, we suggest
that this kindred has X-linked Kallmann’s syndrome
based on the family structure and the occurrence of
synkinesis, a feature characteristic of X-linked disease
[6]. Furthermore, given that the incidence of unilateral
 multicystic dysplastic kidney is 1 : 5000 births [16],
and that of Kallmann’s syndrome in males is 1 : 10000,
we suggest that the occurrence of the two diagnoses
in these two individuals is significant. It is not possible
to confirm with absolute certainty a diagnosis of
hypogonadotropic hypogonadism in the prepubertal
child (patient 2) but in this family the occurrence of
micropenis with low gonadotrophin and androgen
levels at 4 months of age, when the hypothalamo–

teritary gonadal axis is normally active [17], is highly
suggestive of the diagnosis. The extreme nature of the
synkinesis adds further support to this conclusion.

Hydropsia of the rhinencephalon may be observed on

MR imaging in subjects with Kallmann’s syndrome
[18] but we could not justify administering a general
anaesthetic to a small child when the results would not
affect subsequent management.

Based on radiological studies of teenage and older
individuals with Kallmann’s syndrome, kidney agen-
esis appears to be the commonest urinary-tract mal-
formation association with the syndrome [7], although
urinary-tract duplication and hydrenephrosis are also
reported [19,20]. Unilateral absence of functional
kidney tissue occurs in up to 40% of X-linked patients
[7], and these individuals are predisposed to develop
hypertension, proteinuria and even chronic renal
failure from the second decade [21]. While it has
been assumed that such patients have renal ‘agenesis’
resulting from a failure of kidney formation, there
is little information regarding urinary tracts in fetuses
or children with the syndrome. This is relevant to our
study because an identical radiological appearance to
renal agenesis, i.e. a lack of functional and structural
kidney tissue as assessed by radioisotope and ultra-

sound imaging, can result from the pre- or post-natal
regression of a multicystic dysplastic kidney [22,23].
We speculate that some of the cases of renal ‘agenesis’
detected in older patients with X-linked Kallmann’s
syndrome may originate as multicystic dysplastic
kidney which subsequently involute.

Non-syndromic multicystic dysplastic kidneys have
a remarkable life cycle, which can be correlated with
aberrations of cell turnover and gene expression [10].
First, there is a period of overgrowth, with expression
in hyperproliferative dysplastic epithelia [8–10] of
PAX2, a transcription factor causing renal cyst for-

mation in transgenic mice [24], and BCL2, a survival
factor expressed in normal nephrogenesis [25]. Since
PAX2 and BCL2 expression is a feature of both
non-syndromic cases, including those associated with
in utero urinary obstruction [8,9], and also Kallmann’s
syndrome, we speculate that this lesion may be a ‘final
common pathway’ resulting from diverse teratogenic
and primary genetic events. Overgrowth of the multi-
cystic dysplastic kidneys is generally followed by
involution [22,23], at least partly driven by apoptosis
[26]. A minority of multicystic dysplastic kidneys fail
to regress and, as in our two patients, such organs are
often surgically removed because of a perceived low
risk of development of hypertension, driven by renin
secretion, and renal-tumour formation [27–29]. How-
ever, whether the incidence of these ‘complications’
significantly exceeds those in the general population,
is debatable.

The metanephros, or human kidney precursor,
appears at 5 weeks gestation and comprises the ureteric


Fig. 3. Histology of the multicystic dysplastic kidney of patient 2. In all sections, nuclei are counterstained with haematoxylin. (A) Low-power view shows cyst (c) and an area of dysplasia (d). (B) High-power view shows a typical dysplastic tubule (t) surrounded by a ‘collarette’ in stromal (s) tissue. (C) Immunostaining for αSMA reveals expression (brown) of this protein in the stromal tissue proximate to the dysplastic tubule. (D) Immunostaining for PAX2 reveals expression of this protein (brown) in the dysplastic tubule epithelium. (E) Immunostaining for PCNA localizes this protein (brown) to nuclei of the dysplastic tubule epithelium. (F) Adjacent section to E, with primary antibody omitted, to show the absence of unspecific immunostaining. (G) Immunostaining for BCL2 localizes this protein (brown) to the cytoplasm of dysplastic tubule epithelia. Bar in A is 60 μm and in all other frames is 10 μm.
bud epithelium, a Wolffian (mesonephric) duct outgrowth, which branches to form collecting ducts, and renal mesenchyme, which undergoes an epithelial transformation to form nephrons [10]. The bud also forms the renal pelvis and ureter epithelium. Part of the urinary bladder trigone is derived from the Wolffian duct, as it is absorbed into the posterior wall of the cloaca. KAL-1 transcripts are expressed in the embryonic human central nervous system and urinary tract [5], and anosmin-1, the protein encoded by this gene, immunolocalizes to interstitial matrix and basement membranes of the Wolffian duct and early generations of ureteric bud branches [30]. Furthermore, cell culture experiments suggest that protein encoded by KAL-1, anosmin-1, has cell-adhesion properties [31]. These data are consistent with a role for anosmin-1 in mediating cell adhesion during growth of the mesonephric duct/ureteric bud lineage. KAL-1 mutations may therefore cause a primary failure of growth of these tissues, leading to renal agenesis. Classical microdissection studies and recent gene expression studies [10] indicate that dysplastic renal tubules are poorly branched derivatives of the ureteric bud that terminate in cystic dilatations. It is therefore possible that KAL-1 mutations can generate renal dysplasia as well as agenesis, since the same lineage is implicated in both lesions. On the other hand, it is currently unclear why cystic overgrowth or a failure of growth occurs in any particular instance.

An alternative explanation for the occurrence of Kallmann’s syndrome and multicystic kidney in more than one individual from the same family is that each condition is aetologically separate and caused by mutations of different genes. In fact, non-syndromic multicystic kidney can be inherited [32], and while some evidence points towards a locus on chromosome 6 [33], recent data suggest that an intronic polymorphism of the angiotensin II receptor type 2, a gene on Xq that modulates apoptosis in development, predisposes males to a variety of urinary-tract malformations, including renal dysplasia [34]. Here, a report from Colquhoun-Kerr et al. [35] may be relevant. They studied a family with individuals affected by mutation-proven X-linked Kallmann’s syndrome, some of whom also had renal agenesis; however, other members of the kindred did not have Kallmann’s syndrome (and had an apparently normal KAL-1 gene) but did have renal malformations. A final theoretical possibility is that the generation of urinary-tract malformations in X-linked Kallmann’s syndrome requires the summation of effects between a KAL-1 mutation and other, yet to be identified, genetic changes.

In conclusion, our results provide preliminary evidence that multicystic dysplastic kidneys may be associated with Kallmann’s syndrome. However, because these organs tend to involute prenatally or in early childhood, it is only when more Kallmann’s syndrome patients are screened in utero or in early childhood using structural renal scans that it will be possible to establish whether multicystic kidney disease is a bona-fide part of the syndrome or a chance association.

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