Coexistence of Kallmann syndrome and complete androgen insensitivity in the same patient

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Abstract

Kallmann syndrome (KS) is a developmental disease that combines hypogonadotropic hypogonadism and anosmia/hyposmia. Other congenital abnormalities may also coexist. This present report describes two sisters, aged 13 and 12 years, born from Lebanese consanguineous parents. The two sisters have complete androgen insensitivity (normal female appearance and an XY karyotype) due to a novel mutation, a C-to-G transversion in intron 2 of the androgen receptor gene, resulting in an aberrant splicing leading to an insertion of 66 nucleotides in the mRNA. In addition, the older sister has KS, together with synkinesia and multiple skeletal abnormalities, mainly kyphosis, vertebral abnormalities, and short right hand and feet. Her testosterone, FSH and LH levels were very low compared with her younger sister. No mutation in the \( KAL1 \) and \( FGFR1/KAL2 \) genes were found. This unique report raises the possibility of an autosomal recessive or X-linked form of KS with new phenotypic expression.

Introduction

Kallmann syndrome (KS) is a clinically and genetically heterogeneous disorder defined by the combination of hypogonadotropic hypogonadism and anosmia/hyposmia (1). Other abnormalities such as cleft lip or palate, renal aplasia, deafness, mirror movements of the hands (bimanual synkinesia) or brachymetacarpia may also coexist (2–4). In addition to the chromosome X-linked form of the disease (4–6), autosomal dominant (3, 7) and recessive (8, 9) forms have been reported. To date, two disease genes have been identified, namely \( KAL1 \) located on Xp22.3 for the X-linked forms (4–6) and \( KAL2/FGFR1 \) located on 8 p11.2-12 for an autosomal dominant form (3, 7). Other KAL genes remain to be identified since the vast majority of patients with KS do not carry a mutation in \( KAL1 \) or \( KAL2/FGFR1 \). On the other hand, complete androgen insensitivity (10), is a rare X-linked recessive disorder resulting from mutations in the androgen receptor (\( AR \)) gene, located at Xq11.12 (11). The present report describes two sisters presenting complete androgen insensitivity syndrome (AIS). The older sister, has, in addition, clinical and biological features suggestive of KS. The coexistence of KS and AIS in the same patient has never been reported.

Patients and methods

Case report

Patient 1 and patient 2 are the only siblings born from healthy first cousin Lebanese parents. The family history is negative with regard to any developmental problem, particularly KS or AIS. Patient 1 was first examined when she was 13 years old. She consulted for delayed puberty and bone kyphosis. Her clinical course was unremarkable, except for surgery for bilateral inguinal hernia when she was 1 year old. She had a normal birth, infancy and childhood and attends a normal school. On physical examination, height, weight and arm span were respectively 148 cm (10th centile), 45 kg (50th centile) and 150 cm. There was no breast development and a complete absence of pubic or axillary hairs. The external genitalia showed a normal clitoris with no labial fusion and a rudimentary vagina. Multiple skeletal abnormalities were also noted; more particularly, thoracic kyphosis, a short right hand with swelling and limited flexion of the interphalangeal joints, and short feet more pronounced on the left side (Fig. 1). Neurological examination revealed synkinesia with no other abnormalities.
Smell testing was performed using several common odorants (food, alcohol and perfume) and revealed a complete anosmia. The rest of the physical examination was unremarkable, more particularly dermatoglyphic palm patterns, ophthalmologic evaluation and audiometry. Patient 2, the younger sister, was seen at the same time; she was 12 years of age. Her development was normal. She was also operated for a right inguinal hernia at 10 years old. At physical examination, she had a complete female appearance, with a normal clitoris and a rudimentary vagina. Unlike her sister, breast development was at Tanner stage 2. No pubic or axillary hairs were noted. Her height, weight and arm span were respectively 151 cm (50th centile), 44 kg (60th centile) and 158 cm. None of the physical abnormalities found in her sister was observed.

**Laboratory studies**

In both sisters, complete blood count, blood glucose levels, general urinalysis, plasma and urine amino acid studies, liver and thyroid function studies were normal. Hormonal investigations were performed when the patients were first seen. In the older sister, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol values (by chemiluminescence assays on Immulite DPC, CA, USA) were respectively 0.32 IU/l, <0.1 IU/l and <20 pg/ml (normal prepubertal values <1.6 IU/l, <1.3 IU/l and <20 pg/ml respectively). The FSH and LH peaks after administration of 150 μg gonadotropin-releasing hormone (GnRH) (measuring FSH and LH at baseline and at 30 min, 60 min, 90 min and 120 min) were respectively 1.3 IU/l and 1.9 IU/l. The testosterone level by RIA was 7.4 ng/dl (the normal level for adult females is <86 ng/ml). Pituitary function was assessed by measuring baseline and post-stimulation cortisol, insulin-like growth factor-I, free thyroxine and thyrotropin levels; all the values were normal. Surprisingly, hormonal evaluation of the younger sister was as follow: FSH 4.8 IU/ml, LH 7.8 IU/l, testosterone 163 ng/dl and estradiol <20 pg/ml. In addition, FSH and LH peaks after administration of 150 μg GnRH were respectively 7.5 IU/l and 27.3 IU/l.

**Radiological investigations**

Magnetic resonance imaging (MRI) of the brain was performed for patient 1 and revealed a dysmorphic corpus callosum with agenesis of the rostrum, a small pituitary and an abnormal configuration of the circle of Willis with a right moyamoya-type disease (Fig. 2). Olfactory bulbs were not visualized. Total body X rays showed thoracic kyphosis with a vertebral block on T6 –T7, butterfly T4 vertebrae, and hemi and butterfly T10 –T11 vertebrae. A short right hand and short feet were also observed. Measurements of the lower limbs revealed a 22 mm shorter right limb compared with the left. Echocardiography, abdominal and renal ultrasonography were normal in both sisters. In addition, pelvic ultrasonography detected no uterus, ovaries, or vagina and no seminal or prostate structures in either sister.

**Genetic analysis**

Genetic studies were performed in both sisters after an informed consent form was signed by the parents and the children. Standard chromosomal analysis for both sisters was performed and revealed a normal 46,XY karyotype. In addition, high resolution G- and R-banding karyotype for patient 1 was normal. A search for mutations in the AR gene revealed a C-to-G transversion 3 bp upstream of exon 3 in intron 2 (C/EG-31V52) in the splicing site. The same mutation was found to be heterozygous in
the mother. In order to understand how this novel mutation affects the normal splicing of the mRNA of the AR gene, a region corresponding to exons 1 - 4 was analyzed by RT-PCR for the two affected children and for one control sample. Total RNA was extracted from whole blood using the PAXgene blood RNA kit (PreAnalytiX; Qiagen and BD, Hilden, Germany). Complementary DNA was synthesized by priming with random hexamers in the presence of SuperScript II reverse transcriptase according to the manufacturer’s protocol (Invitrogen Life Technologies, CA, USA). A couple of primers were designed using the program Oligo, version 9.3 (Institute of Biotechnology, University of Helsinki, Finland), to amplify a 493 bp fragment, between exon 1 and exon 4, specific for the AR transcript. Sense (inside exon 1) and antisense (inside exon 4) primers used were as follows: sense, 5’-GGAAAGCGACTTCACCGCAC; antisense, 5’-GGTTGTCTCCTCAGTGGGGCTG. cDNA (0.15 µg) was used as a template for the PCR reaction which was performed in a volume of 25 µl using the PCRx Enhancer System from Invitrogen. The thermocycling conditions for the amplification were initial denaturation at 95°C for 5 min followed by 38 cycles at 95°C for 1 min, 64°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10 min. The total RT-PCR reaction mixtures were electrophoresed on a 2% (w/v) agarose gel incorporating ethidium bromide and visualized under ultraviolet light. The 493 bp expected fragment was obtained for the control sample while an approximately 600 pb fragment was obtained for the two affected children. These two fragments were excised from the agarose gel, recovered with the NucleoSpin Extract II (Macherey-Nagel, Düren, Germany) then sequenced using Big Dye Terminator cycle sequencing procedure (Applied Biosystems, Foster City, USA). Sequencing reactions were run on a ABI automated capillary DNA sequencer 310 (Applied Biosystems). DNA sequences were compared with the GenBank databases utilizing the Blast program available at the web site http://www.ncbi.nlm.nih.gov. Sequence analysis of the normal-sized transcript was normal while in the affected samples an insertion of 66 nucleotides was noted. Indeed, the single base change generated and activated a new 3’ splice site upstream of exon 3, while the normal intron 2 acceptor splice site was disrupted. Thus, the insertion transcript produced a full length AR protein with 22 extra amino acids inserted between codons 589 and 590. Finally, in the older sister, a search for mutations in the coding exons and flanking splice sites of KAL1 and FGFR1/KAL2 as previously described (6, 7) was negative.

**Clinical follow-up**

Eight months later, the testosterone level was still very low in the older sister but markedly increased in the younger sister (23 ng/dl and 280 ng/dl respectively). The older sister was then operated for bilateral orchiectomy and vaginoplasty. Histological findings showed immature seminiferous tubules on the left side and no seminiferous tubules on the right side. Three months after gonadectomy, and before starting hormone replacement therapy, her FSH, LH,
Discussion

The present report describes two sisters with complete androgen insensitivity. Both sisters have a complete female appearance with no pubic or axillary hair, and a 46,XY normal karyotype. In addition, they carry a deleterious point mutation in the AR gene creating a new splicing site that leads to an insertion of 66 nucleotides in the mRNA (insertion of 22 extra amino acids inserted between codons 589 and 590). This insertion probably affects the tertiary structure of the protein, preventing the receptor from having its normal function. Another report found a T→A mutation 11 bp upstream of exon 3 resulting in generation of mRNA with an insert of 69 nucleotides and of a receptor with defective DNA binding (12). Nevertheless, both sisters differed in the clinical expression of their sexual characteristics; in fact, the older sister was completely impuberl, unlike her younger sister who presented signs of breast development (Tanner stage P2). In addition, the younger sister had a high testosterone level, together with inappropriately high levels of FSH and LH, which represents the typical biological profile observed at puberty in AIS. In the older sister, hormonal evaluation showed very low levels of testosterone, FSH and LH, suggesting the coexistence of a hypothalamic–pituitary defect. Moreover, there was no rise in the FSH and LH levels after gonadectomy and the girl presented some typical clinical features of KS, namely anosmia and bimanual synkinesis. Taken together, the clinical and biological features in the older sister confirm the coexistence of KS.

However, our genetic analysis looking for mutations in the KAL1 and FGFR1/KAL2 genes was negative (6, 13). This could be explained by the fact that only the coding exons and adjacent splice sites of KAL1 and FGFR1/KAL2 were sequenced. Therefore, a deleterious mutation in an intron or in the promoter region cannot be excluded. In addition, the molecular analysis of the KAL1 gene has identified an abnormality in the gene in 5–15% of sporadic cases (9, 14, 15) and in only 14% of familial X-linked cases (9). Moreover, FGFR1 mutations account for only 10% of KS cases (7, 16). Thus, it seems more likely that another, as yet undiscovered KAL gene, is responsible for the disease in our patient. The phenotypic appearance of our patient reinforced this idea. Indeed, some of the associated skeletal (such as kyphosis and hemivertebra) and cerebral (such as the dysmorphic corpus callosum and the Moya–Moya syndrome) abnormalities observed have never been described previously in KS. In addition, we did not note the typical findings ascribed to the autosomal dominant form of the disease, such as cleft palate or lip and dental agenesis. Finally, the presence of bimanual synkinesia in our patient may suggest that this finding is not specific to the X-linked form of the disease (3, 7).

In conclusion, our case has reinforced the idea that there is a great clinical and molecular variability in KS. The search for a contiguous gene deletion syndrome using a fine banding karyotype was not relevant. Further research to determine the gene responsible for KS in this patient should be carried out.

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References


