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Frasier syndrome: a rare cause of delayed puberty

罕見的青春期延遲原因：Frasier症候群

We report on a post-renal transplant patient who presented with delayed pubertal development at the age of 15 years. She had a normal female phenotype. Blood analysis showed hypergonadotropic hypogonadism. Her karyotype was 46,XY. DNA analysis showed a heterozygous mutation in the *WT1* gene (C to T mutation at position +4 of the splice donor site within intron 9). A diagnosis of Frasier syndrome was made and she underwent laparoscopic gonadectomy. This case illustrates that, while delayed puberty is common in children with chronic illness, clinicians should be particularly aware of the possibility of Frasier syndrome in those with progressive glomerulopathy and delayed puberty. DNA analysis is a useful means of confirming the diagnosis.

本文報告一名15歲女病人，在腎臟移植手術後出現青春期發育延遲。病人女性表徵正常，血液測試顯示高親生殖性腺機能減退。她的染色體組型為46,XY。脱氧核糖核酸分析顯示在威爾姆氏腫瘤一型（*WT1*）基因內出現雜合子突變（C突變為T，出現在間隔子9內的+4剪切提供位），因此診斷她是患上Fraiser症候群。病人接受腹腔鏡性腺切除術。這病例顯示，雖然慢性疾病病童很多會出現青春期延遲，臨床醫護人員仍須加以留意，若持續有絲球體腎病和青春期延遲，便有可能是Frasier症候群。脱氧核糖核酸分析對診斷此症很有效用。

Case report

A girl, born in Mainland China, was well until the age of 6 years when she presented with a recurrent puffy face and generalised oedema in May 1990. Blood results from Mainland China revealed a raised serum creatinine level of 158 μmol/L and blood urea nitrogen of 14.28 mmol/L. Treatment with steroids and Chinese herbal medicine failed to halt her deteriorating renal function. The girl came to Hong Kong in 1993 and came to our care at the age of 11 years. At that time she had developed marked proteinuria of 10 g/d with a serum creatinine level of 376 μmol/L and urea level of 12.6 mmol/L. Her estimated glomerular filtration rate using the Schwartz formula, was 17.3 $mL \cdot min^{-1} \cdot m^{-2}$. Immune markers, including antinuclear antibody, complement C3, C4, anti–glomerular basement membrane, anti-neutrophil cytoplasmic antibody, antistreptolysin-O test, immunoglobulin, and viral titres were negative. Heavy metal screens were negative. A renal biopsy revealed a limited number of viable glomeruli for diagnosis. The patient was diagnosed with steroid-resistant nephrotic syndrome and peritoneal dialysis was commenced at the age of 11 years.

At the age of 13 years she underwent a cadaveric kidney transplant. She made a good recovery: her serum urea level dropped to 6 mmol/L and creatinine to 110 μmol/L. Immunosuppressive therapy consisted of azathioprine, cyclosporin A, and oral steroids. Her steroid dose was tapered to 10 mg daily 1 year after the kidney transplant and she remained well with no rejection episodes. At the age of 15 years, she had not commenced puberty. Physical examination was unremarkable: weight of 52 kg (75th percentile), height of 155 cm (25th percentile), normal pre-pubertal female external genitalia, and no dysmorphic features. Blood analysis revealed primary hypogonadism with a low plasma oestradiol (<37 mIU/L), and raised basal luteinising hormone (54.7 IU/L) and follicular stimulating hormone (181 IU/L). An ultrasound of the pelvis showed an infantile uterus with no gonads detected at either groin. A genitogram demon-

Key words:
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基因，威爾姆氏腫瘤；
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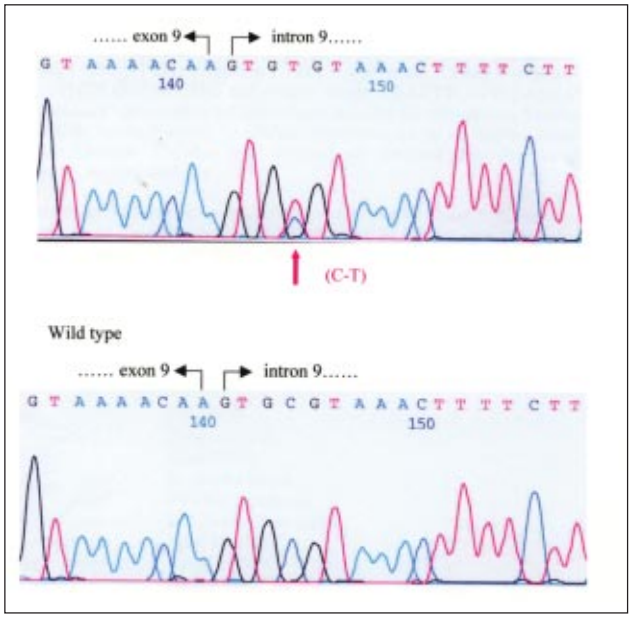

Fig 1. A heterozygous mutation at the splicing donor site of intron 9 (C to T mutation at position +4 of the splice donor site within intron 9) was identified in the patient
The mutation was verified by sequencing in both directions

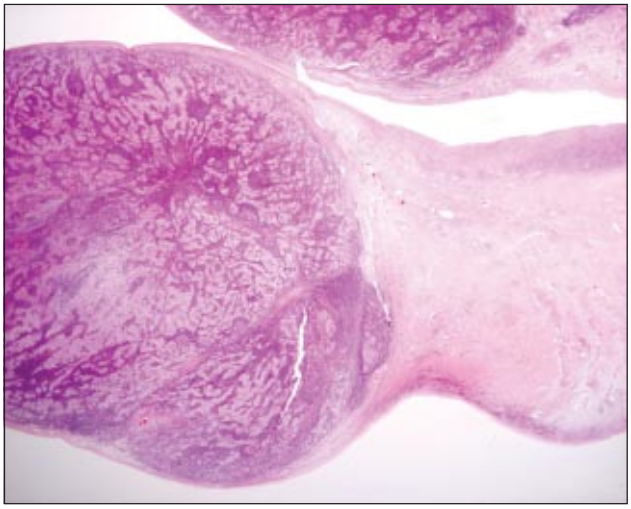

Fig 2. Low-power view (x1) showing germinoma on the left side and gonad with primitive stroma on the right

strated normal female urogenital structures, but her karyotype was 46,XY. The presence of male pseudohermaphroditism together with progressive glomerulopathy and consequent end-stage renal failure were suggestive of Frasier syndrome (FS) or Denys-Drash syndrome (DDS). Further DNA analysis was then performed. Genomic DNA was extracted from the peripheral blood of the patient and a normal control. Polymerase chain reactions (PCR) were performed using the forward primer: CTCACTGTGCCCACATTG and the reverse primer: CAATTTCATTCCACAATAG. The PCR products were purified and then sequenced using the dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer; Applied Biosystems, Foster City, US) and run on an Applied Biosystems 310 Genetic Analyser (Applied Biosystems, Foster City, US). The data were analysed using Applied Biosystems sequencing analysis software. A heterozygous mutation at the splicing donor site of intron 9 of *WT1* gene (C to T mutation at position +4) was identified in the patient (Fig 1), verified by sequencing in both directions. No sequence alteration was found in the DNA samples from peripheral blood of other family members. The mutation analysis supported the clinical diagnosis of FS.

Laparoscopic gonadectomy was performed because of the increased risk of developing germ cell tumours and gonadoblastoma. Intra-operatively, the uterus appeared normal and the fallopian tubes were identified. The gonads were atrophic and were identified near the fallopian tubes. The excised right gonad showed primitive stroma with tubular structures and aggregates of polygonal cells that may represent hilar/Leydig cells. The left gonad consisted of primitive stroma only and a germinoma that measured 1.3 cm (Fig 2). The patient refused further chemotherapy or radiotherapy and puberty was induced with a cyclical oestrogen: progesterone preparation. Four years later no recurrent or secondary tumour was identified.

Discussion

Frasier syndrome is rare and was first described in 46,XY monozygotic twins in 1964.[1] They had pure gonadal dysgenesis, streak gonads with gonadoblastoma and developed end-stage renal disease.[1] More recently a genetic (XY) male with FS was described who developed as a phenotypic female (sex reversal) without ovaries. Mutations in the Wilms' tumour suppressor gene (*WT1*) were identified as the molecular defect at a genetic level.[2]

The Wilms' tumour suppressor gene is located on chromosome 11p13 and comprises 10 exons that include a zinc finger DNA binding domain. The gene is expressed in a wide variety of embryonic tissue including the mesenchymal cells of the foetal kidney, and the stromal cells of the gonads and spleen.[3] Mutations in *WT1* have been identified in patients with FS and DDS. Both diseases result from heterozygous mutation in the *WT1* gene and can present as renal disease, abnormal gonad function, and the development of tumours. In the past, patients with FS would present in the second or third decade of life. Focal segmental glomerulosclerosis (FSGS) with nephrotic syndrome is the most common renal manifestation. Patients with DDS generally present much earlier, under the age of 4 years. Diffuse mesangial sclerosis is the most common histological diagnosis in nephrotic syndrome. Neither FSGS nor mesangial sclerosis respond to immunosuppressive treatment and patients inevitably progress slowly to end-stage renal failure. In our patient, it was unfortunate that an early histological diagnosis was not made and a subsequent

biopsy did not provide useful clues to the underlying aetiology.

Our patient has the classical phenotype described in FS. She is genetically male (XY) but phenotypically female with ovaries absent (sex reversal). Recent reports of patients with FS, diagnosed on the basis of a mutation in *WT1* genes, have included a genetic XX female with normal female external phenotype, and a genetic XY male with normal male phenotype as well as ambiguous external genitalia such as hypospadias and a urogenital sinus described in DDS.[4-7] These phenotypic variations have led to speculation that DDS and FS are variations of the same disease.[6] Sex reversal is often identified when the patient presents for investigation of delayed puberty. The diagnosis may be overlooked in a genetic XX female with normal female phenotype and pubertal changes. In the presence of a family history of FSGS, clinicians should be alert to the possibility of FS with *WT1* mutation.[7]

In FS, point mutation in splice sites within intron 9 of *WT1* has been described.[2] Five different mutations have been reported: +2 T>C, +4 C>T, +5 G>A, +5 G>T, and +6 T>A. The mutation detected in our patient was +4 C>T, the most frequent mutation in FS, accounting for around 52% of reported cases.[8] Familial *WT1* mutations have also been reported in family members with different phenotypes.[7] Nonetheless the mutation seen in our patient was not detected in other family members, thus it was likely to be a de-novo mutation. These mutations cause an inclusion or exclusion of a specific amino acid triplet (lysine, threonine, serine). Normally, KTS-containing (KTS+) isoform doubles KTS-lacking (KTS-) isoform. KTS- isoform has a high affinity with steroidogenic factor 1 and controls normal testicular development. In FS, the splice site mutations abolish the production of KTS+ isoform leading to a reverse KTS+/KTS- ratio.[3,8] Although the discovery of this relationship sheds light on the pathophysiology of FS, the exact mechanisms that impair normal gonadal development remain obscure.

Early diagnosis of FS is important as it enables optimal care and counselling to be provided. The glomerulopathy in FS does not respond to medical treatment and aggressive steroid therapy and other immunosuppressive agents should be avoided to minimise complications. Progression to end-stage renal disease is inevitable and careful monitoring with supportive measures to slow the declining renal function is recommended. Elective gonadectomy is recommended in cases of sex reversal because of the increased risk of gonadoblastoma or germ cell tumours developing in the primitive gonads. A paediatric endocrinologist should determine appropriate hormonal replacement therapy. Family screening for proteinuria is also suggested since *WT1* mutations may be familial.

Conclusion

Genital abnormalities are often associated with renal disease. Although FS is rare and delayed puberty is common in patients with chronic illness, clinicians should be alert to the possibility of this genetic disorder, especially in patients with progressive glomerulopathy and delayed puberty.

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