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A Japanese Case with Frasier Syndrome Caused by the Splice Junction Mutation of WT1 Gene

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Abstract. The Wilms' tumor suppressor gene, WT1, plays an important role in the development of the urogenital system and also subsequent normal function of this system. Recently, the splice mutations in intron 9 of WT1 gene have been detected in Frasier syndrome, which is characterized by streak gonads, pseudohermaphroditism, slowly progressive nephropathy and frequent development of gonadoblastoma. Here to elucidate the molecular basis in a Japanese patient of Frasier syndrome, WT1 gene was analyzed by polymerase-chain-reaction (PCR) and direct sequencing. We identified the splice junction mutation in intron 9 of WT1, which is recognized as a mutation hot-spot in intron 9. This finding concludes that 1) the mutation in intron 9 might be the cause of Frasier syndrome, and 2) the mutation hot-spot in Japanese and Caucasian patients is similar.

Key words: WT1 gene, Frasier syndrome, Gonadoblastoma, Splice junction mutation

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THE WT1 gene was originally isolated as a Wilms' tumor suppressor gene and its products are expressed at high levels in the developing urogenital system [1–3]. The mutations of this gene are associated with abnormal urogenital disorder such as Deny-Drash syndrome, Frasier syndrome, WAGR syndrome and Wilms' tumor [1–3]. Among these diseases, Frasier syndrome is characterized by gonadal dysgenesis, progressive glomerulopathy in both genotypic females and males. Furthermore, in this disorder, gonadoblastoma is frequently observed in genetic males [4–7]. Renal manifestations consist of proteinuria in childhood and nephrotic syndrome with the histological changes of nonspecific focal and segmental glomerular sclerosis, progressing to end-stage renal failure in adolescence or early adulthood [4–7]. Recently, the splicing junction mutations of intron 9 of WT1 in Frasier syndrome have been identified as a cause [8–10].

Here, we describe a Japanese patient with Frasier syndrome in whom WT1 gene was analyzed by polymerase-chain-reaction (PCR) and direct sequencing.

Report of a case

The proband was a phenotypic female born to unrelated parents. She had been raised as a female since birth. Her neonatal and childhood development were unremarkable. At the age of 10 years, she was first evaluated for proteinuria. Subsequently, she developed nephrotic syndrome, and an open biopsy of the kidney revealed focal and segmental glomerular sclerosis at age 12. She was noted to have an abdominal mass on routine visit in outpatient clinic at age 14. Computed tomography and ultrasonographic examinations displayed a right pelvic mass and hypotrophic uterus, but bilateral gonads were undetected. By this age, she was devoid of spontaneous secondary sexual development.

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Genital examination revealed normal female external genitalia. Basal plasma levels of follicle-stimulating hormone (46.1 mIU/ml, normal 2–15 mIU/ml) and luteinizing hormone (22.3 mIU/ml, normal< 15 mIU/ml) were elevated. Chromosomes from peripheral lymphocytes demonstrated 46, XY. Her abdominal mass was removed, and gonadectomy was also performed. According to histological findings, her abdominal mass was a right gonadoblastoma, and a left streak gonad was found. Eventually, she progressed to end-stage renal failure and received a cadaver renal transplant at age 17. The patient is now on cyclic estrogen and progesterone therapy and possesses normal renal function. Based on these clinical and laboratory findings, she was diagnosed as a case of Frasier syndrome.

Methods

Informed consent of DNA analysis was obtained from her parents and the patient. DNA was prepared from white blood cells using standard techniques. The primers of PCR were selected in the exon-intron boundaries of WT1 and PCR was performed according to previous methods [8]. Direct sequence of these PCR products was performed using Dye Terminator Amplitaq FS kit (Perkin-Elmer) and analyzed by an ABI 373A automated sequencer (Perkin-Elmer) as described previously [11].

Results

Direct sequencing of all the exons and exon-intron junctions of WT1 gene revealed double bands at the

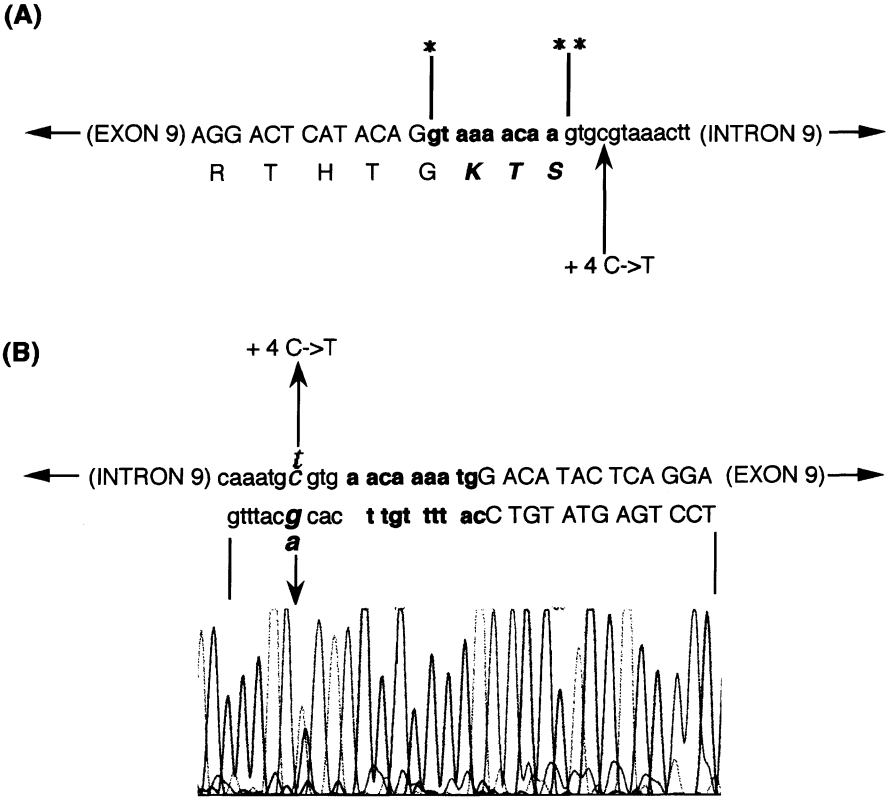

Fig. 1. Sequence of exon 9-intron 9 junction of WT1. (A) Nucleotide and amino acid sequence of exon 9. The K (Lys), T (Thr) and S (Ser) alternative splice isoform is represented in bold. *(first splice site) and **(second splice site) indicate the two alternative splice sites. The mutation in this patient is shown by an arrow. (B) This patient shows heterozygous point mutation in the second splice donor site (+4 C to T). An arrow points to the position of the double bands (G and A) identifying the mutation.

starting of intron 9 at position +4 C and T in this patient (Fig. 1). This nucleotide substitution was not detected in the DNA from either parents, indicating a *de novo* mutation.

Discussion

Recently, the mutations of splice site in intron 9 have been detected in Frasier syndrome. Barbaux *et al.* reported that 5 cases with Frasier syndrome carrying the splicing junction mutations [8]. These splicing junction mutations were also reported in 10 cases of Frasier syndrome by Klamt *et al.* [9]. To date, 15 of 18 cases analyzed show the +4 C to T and +5 G to A mutations. This hotspot in mutation probably is due to the potential to deaminate 5 methylcytosine at +4/+5 CpG dinucleotid. Furthermore, in five Japanese patients with Frasier syndrome, +4 C to T, +5 G to A and a novel +2 T to C mutations in intron 9 were recognized [10]. In our patient, +4 C to T mutation in intron 9 of WT1 was detected, supporting the view that the mutation hot-spot is similar between Japanese and Caucasian patients.

Normally, the WT1 gene is alternatively spliced at the end of exon 9, leading to either the presence or absence of the tripeptide sequence Lysine (K), Tyrosine (T) , and Serine (S) motif between zinc fingers 3 and 4 (+KTS and −KTS form) [8–11]. These two isoforms have different DNA-binding affinities and therefore, possibly, different functions. There is some evidence to suggest that the +KTS form is associated primarily with splice factors and that the −KTS form is associated with transcriptional factor. Disruption of alternative splicing at exon 9 splice donor site in Frasier syndrome might affect the efficiency of alternative splicing, resulting in decrease of the +KTS isoform. Thus, the imbalance of WT1 isoforms might be the cause of this disease [8–10].

In this context, it is of interest to determine whether some isolated cases of steroid-resistant focal glomerular sclerosis may harbor genetic alterations of WT1 gene particularly in 46, XX phenotypically normal females. It is also necessary to evaluate the relationship of these splice mutations and the development of gonadoblastoma.

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