Brief Report

Prophylactic bilateral nephrectomies in two paediatric patients with missense mutations in the WT1 gene

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Abstract

Background. Denys–Drash syndrome (DDS) is associated with mutations of the Wilms’ tumour 1 (WT1) gene, and is characterized by pseudohermaphroditism, a progressive glomerulopathy, and the development of Wilms’ tumour. More than 90% of patients with DDS who carry constitutional intragenic WT1 mutations are at high risk (90%) for the development of Wilms’ tumour. WT1 is a signalling protein with 90% of WT1 mutations occurring in the WT1 zinc finger region as single nucleotide polymorphisms, the majority of which are missense mutations.

Methods. Constitutional DNA was extracted from peripheral blood. Direct sequencing and restriction enzymes were employed to analyse mutations.

Results. Two children, 46XY males who had evidence of pseudohermaphroditism, hypogonadism and renal failure with a glomerulopathy atypical for DDS, but no Wilms’ tumour or nephroblastomatosis, on investigation, prior to transplant, were identified with missense mutations in the WT1 gene, in exons 8 and 9, respectively. The decision to do prophylactic nephrectomies was based on the genetic identification of WT1 mutations supporting a diagnosis of incomplete DDS, with the potential for increased risk of malignancy with the development of Wilms’ tumour. The nephrectomy specimens demonstrated nephrogenic rests (nephroblastomatosis), which have a potential for malignant transformation.

Conclusions. WT1 missense mutations in exons 8 and 9 can be regarded as having the potential for malignant change supporting prophylactic nephrectomy in apparent incomplete DDS patients with end-stage renal disease.

Keywords: Denys–Drash syndrome; missense mutations; nephrectomy; WT1

Introduction

The Wilms’ tumour 1 (WT1) gene, a Wilms’ tumour suppressor gene, is located at chromosome 11p13 and encodes a zinc finger transcription factor involved in the development of kidneys, gonads and other organs [1,2]. The gene has 10 exons, of which exons 1–6 encode domains involved in transcriptional regulation, dimerization and possibly RNA recognition. Exons 7–10 encode the four zinc fingers domains of the DNA-binding domain. The role of WT1 as a tumour suppressor gene has been evidenced by the presence of biallelic inactivating mutations in a subset of Wilms’ tumours [3]. WT1 is expressed during vertebrate development within the kidneys, gonads, spleen and the lining of the abdominal cavity.

Wilms’ tumour arises in 1:10 000 children <15 years of age with median age at presentation of 44 months for patients with unilateral disease and 32 months for patients with synchronous bilateral disease. It is the most common paediatric renal cancer. Mutations in the WT1 gene have been identified in patients with Wilms’ tumour, WAGR syndrome (Wilms’ tumour, aniridia, genitourinary malformation, mental retardation), Denys–Drash syndrome (DDS) and Frasier syndrome (FS).

DDS is a triad of Wilms’ tumour, pseudohermaphroditism and progressive glomerulopathy [4]. There are three clinical categories in DDS: genotypic males with all three abnormalities, genotypic males with nephropathy and ambiguous external and/or internal genital structures, and genotypic females with nephropathy and a WT1 mutation only. All patients show glomerulopathy, usually characterized by the histological finding of diffuse mesangial sclerosis (DMS) and in
rare cases focal segmental glomerulosclerosis (FSGS). Although sporadic Wilms’ tumours carry the WT1 mutation in the germline (5%) or in tumour tissue (6–18%) [3], more than 90% of patients with DDS carry constitutional intragenic WT1 mutations [5].

The aim of this work was to identify mutations of the WT1 gene that would confirm a clinical diagnosis of DDS. The diagnosis of DDS in these children has lead to nephrectomies to protect against the development of Wilms’ tumour as part of their treatment.

Subjects and methods

Constitutional DNA was extracted from the patient’s peripheral blood samples (5 ml) by the SDS–proteinase K method. The polymerase chain reaction (PCR) profile was as follows: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s, 35 cycles. Oligonucleotide primers used for WT1 exon 8 of the human WT1 gene were designed by Dharmidharka et al. [6]. Primers for WT1 exon 9 were designed using the Primer 3 software of BioNavigator. The forward primer is TGTGAAGGCGGAGGCTAGA and the reverse primer is TGTGAAAGAAAGTTTACGCACTTG for exon 9. The BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used for the sequencing reaction. The samples were electrophoresed on an ABI Prism 310 Genetic Analyzer. All steps were performed according to the manufacturer’s instructions. Restriction enzymes MspI and RsaI were employed to analyse mutations according to the manufacturer’s instructions. The DNA fragments were separated on a 10% polyacrylamide gel.

Two paediatric patients were studied. Patient 1 is a 21-month-old XY male who presented with acute on chronic renal failure (creatinine 6.56 mg/dl) at the age of 13 months with anaemia and acidosis. He had a past history of hypoplastic genitalia (hypospadias, undescended testes and a right hydrocele) that were surgically corrected but had no other significant history prior to presentation. There was no significant family history including renal disease and no concurrent illnesses. Initial investigations included abdominal computerized tomography that showed bright kidneys of normal size and a renal ultrasound with increased echogenicity of both kidneys. A moderate sized utricle (vaginal remnant) was seen on a micturating cystourethrogram. Renal biopsy demonstrated glomerulosclerosis and tubular atrophy, a proliferative glomerulonephritis with early crescent formation, double contours, mesangial interposition and proliferation, with ischaemic changes, however, no nephroblastomatosis was seen. Genetic analysis at 15 months of age showed a mutation in the WT1 gene. He remained dialysis dependent switching to peritoneal dialysis prior to transplantation. A living-related transplant with bilateral nephrectomies was performed at 18 months of age. The nephrectomy demonstrated an ‘end-stage’ kidney with changes suggestive of advanced DMS. Nephroblastomatosis was present in both kidneys.

Patient 2 is a 6-year-old XY male, who presented with acute renal failure at 5 years of age with oliguria, hypertension and a creatinine of 5.66 mg/dl. He was commenced on haemodialysis. He had presented with intersex at birth requiring removal of fallopian tube remnants and correction of severe hypospadias. There was evidence of chronic renal insufficiency with echocardiography showing left ventricular hypertrophy and a history of polyuria and polydipsia for 6 months prior to presentation. His renal biopsy showed both acute changes and evidence of more chronic renal damage. Chronic fibrosis, glomerular sclerosis and hypertensive vascular changes with small vessel thrombi were observed. No evidence of nephroblastomatosis was seen on biopsy. Genetic analysis, done on presentation in renal failure, showed a mutation in the WT1 gene. A living-related transplant with bilateral nephrectomies was performed at 6 years of age. Histology of the nephrectomy showed global glomerulosclerosis, FSGS, and small foci of nephroblastomatosis.

Results

WT1 missense mutations in exons 8 and 9 were detected in two patients. In patient 1, a mutation A 1130 G in exon 8 of WT1 was detected, resulting in a wild-type H (histidine) to R (arginine) at codon 377. The original PCR product was 165 bp. The mutation created a novel RsaI site. After digestion with RsaI, the wild-type PCR product was not cut, whilst the mutant allele was cut into 124 and 41 bp bands. For the heterozygote, all three bands (165, 124 and 41 bp) were present on the gel. The results (Figure 1) clearly showed that the first case was a heterozygote carrying the mutation H377R. Parental DNAs examined at the same time confirmed a de novo mutation.

In the second case, a mutation was found in exon 9 of WT1. A single nucleotide C was changed to T at 1180, which substituted an amino acid W (tryptophan) to R (arginine) at codon 394 which is the mutation hot spot of DDS. In contrast to the previous patient, the mutation abolished an existing MspI restriction enzyme site. For the normal allele, the 151 bp PCR product was cut into 81 and 70 bp bands, while the mutant allele could not be cut. The heterozygous genotype, showed all three bands (Figure 2). The second patient was confirmed as a heterozygote of mutation W384R. Parental DNAs examined at the same time confirmed a de novo mutation.

Fig. 1. A mutation (H377R) of exon 8 of WT1 in patient 1. Lane Ma, marker; lane P, patient 1; lane F, father; lane M, mother; lane C, controls (without digestions). For the heterozygote, a 165 bp allele containing exon 8 was cut into 124 and 41 bp fragments due to the novel RsaI site, another allele remains intact in patient 1. The parents only showed an intact 165 bp fragment. The control without digestions shows the same 165 bp fragment.
mutations associated with carcinogenesis have led to prophylactic surgery to prevent malignancy. For example, hereditary non-polyposis colorectal cancer is associated with a variety of gene mutations, and is at high risk for synchronous and metachronous colorectal cancer and endometrial cancer in women. Prophylactic and extended surgery are performed as optional treatment for carriers of this gene mutation [12]. In the area of breast cancer, BRCA1 mutations and a family history of breast carcinoma may lead to mastectomies though this is controversial [13]. In the related syndrome of FS gonadectomy is performed to prevent malignancy [10].

Single nucleotide polymorphisms (SNPs) are increasingly being studied as a determinant of patient outcomes. Missense mutations throughout exons 8 and 9 in a single allele can create a dominant negative gene that guarantees its own failure to survive by leading to renal failure, infertility and cancer. So strong is the association with Wilms’ tumour that SNPs in exons 8 and 9 leading to missense mutations can be regarded as predictive of Wilms’ tumour.

Previously, clinical phenotypes suggestive of DDS have lead to recommendations of nephrectomy at the time of end-stage renal disease (ESRD). Eddy et al. [14] recommended bilateral nephrectomies at the onset of end-stage renal failure in DDS. Schumacher et al. [9] suggested that regular renal imaging may be necessary to evaluate the need for bilateral nephrectomy in patients with WT1 missense mutations who had reached ESRD. It is still debatable whether prophylactic nephrectomies should be carried out in all children with isolated DMS prior to renal transplantation in view of the connection with DDS. Prior to molecular diagnosis Gagnadoux and Habib [15] suggested removal of the ipsilateral kidney at the time of transplantation and monitoring the remnant kidney with periodic ultrasonography in patients with DMS. Our patients would suggest that WT1 missense mutations in exons 8 and 9 can be regarded as risk factors for developing Wilms’ tumour supporting prophylactic early renal transplantation with bilateral nephrectomy in incomplete DDS patients with ESRD.

Discussion

We present two boys with missense mutations in the WT1 gene with renal failure and varying degrees of intersex. Genetic identification of missense mutations in the WT1 gene, in exons 8 and 9 suggested an incomplete DDS, placing them at increased risk of malignancy with the development of Wilms’ tumour. Prophylactic nephrectomies were performed at the time of transplantation. The decision to perform nephrectomies was supported by the pathology of the removed kidneys that demonstrated nephrogenic rests. These are thought to be at risk of malignant transformation and were not demonstrated on the initial biopsy or imaging.

Pseudohermaphroditism and a progressive glomerulopathy are both associated with DDS and FS [4,7]. DDS carries a high risk (90%) of Wilms’ tumour, while FS is associated with the development of gonadoblastoma. WT1 mutations are present in both DDS and FS. The zinc finger region contains 94.5% (70 of 74) of WT1 mutations in DDS [5,6,8,9]. A total of 62.1% of mutations occur in exon 9, with 37.8% of WT1 mutations in DDS occurring at the ‘hot spot’ site R394W in exon 9 as seen in patient 2. The frequency of exon 8 mutations is also high at 24.3%. The majority of mutations in WT1 in DDS are missense mutations (82.4%). FS appears due to donor splice-site mutations in intron 9 in WT1 [10]. When a germline missense mutation is present, the risk of developing a Wilms’ tumour is over 90%.

For patient 1 the identification of missense mutations in the WT1 gene in exon 8 confirmed the clinical diagnosis of DDS. This mutation was previously reported in DDS in 1994. Furthermore, histology of the nephrectomy specimens showed nephroblastomatosis was present in both kidneys. Nephroblastomatosis reflects persistent embryonal remnants in the kidney that are possible precursors of Wilms’ tumour [11]. The identification of missense mutations in the WT1 gene in exon 9 (hot spot mutation R394W) in patient 2 strongly supported the clinical diagnosis of DDS. Furthermore, in the nephrectomy pathology, FSGS and nephroblastomatosis were observed.

In families with a proven cancer pedigree specific mutations associated with carcinogenesis have led to prophylactic surgery to prevent malignancy. For example, hereditary non-polyposis colorectal cancer is associated with a variety of gene mutations, and is at high risk for synchronous and metachronous colorectal cancer and endometrial cancer in women. Prophylactic and extended surgery are performed as optimal treatment for carriers of this gene mutation [12]. In the area of breast cancer, BRCA1 mutations and a family history of breast carcinoma may lead to mastectomies though this is controversial [13]. In the related syndrome of FS gonadectomy is performed to prevent malignancy [10].

References

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