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Case Report

A novel *WT1* missense mutation presenting with Denys–Drash syndrome and cortical atrophy

Valérie Schumacher[1], Julia Thumfart[2], Matthias Drechsler[1], Maximillian Essayie[3], Brigitte Royer-Pokora[1], Uwe Querfeld[2] and Dominik Müller[2]

[1]Institute of Human Genetics, Heinrich-Heine University Düsseldorf and [2]Department of Pediatric Nephrology and [3]Department of Pediatric Endocrinology, Charité Children's Hospital Berlin, Germany

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Introduction

Germline heterozygous missense mutations in the Wilms' tumour suppressor gene 1 (*WT1*) cause Denys–Drash Syndrome (DDS), which is characterized by a progressive glomerulopathy (mainly diffuse mesangial sclerosis), genital abnormalities in genotypic males and a predisposition to Wilms' tumour [1]. The protein exerts its function as a tissue-specific zinc finger transcription factor by binding to the promoter of its target genes and, thus, regulating their transcription. Loss of WT1 function through mutations results in the misregulation of these target genes and, subsequently, in developmental defects. The expression pattern of *WT1* mRNA together with data obtained from *WT1* deficient mice indicate that the function of the gene is not restricted to the urogenital system, but involves the development of other organs, such as spleen, adrenal glands, heart, mesothelium, spinal cord and brain [2–5]. We report a novel germline *WT1* missense mutation in a genotypic male patient with DDS and cerebral atrophy.

Case

The child was the second offspring of healthy, unrelated Caucasian parents. Pregnancy was complicated by fetal cardiac arrhythmias and myocardial hypertrophy. Both were not confirmed after birth. Abnormalities of amniotic fluid or placenta size were not noted. The child was born at term with a birth weight of 3100 g. A horseshoe kidney was already diagnosed prenatally. Although external genitalia had male appearance at birth, the child displayed anorchia and bilateral inguinal hernias. On surgical exploration during hernia repair, only blind ending testicular cords without any signs of testicular or epididymic tissue were found; they were not removed.

At the age of 6, months a routine cerebral ultrasound showed hydrocephalus *e vacuo*. The child developed poorly during the following months. Besides a convergent strabism, the patient showed progressive psychomotor delay. Computed tomography and magnetic resonance imaging scans during follow-up revealed cerebral cortical atrophy and thinning of corpus callosum in the first year of life (Figure 1). Funduscopy at the age of 12 months remained without pathological findings. At the age of 1 year, overt proteinuria (4.0 g/24 h/1.73 m^2) was discovered accompanied with a rapid decline of renal function. At the age of 3 years, the patient was started on haemodialysis and then switched to peritoneal dialysis. At the age of 4.5 years, one half of the horseshoe kidney was removed to reduce proteinuria. Histologically, the kidney showed diffuse mesangial sclerosis. The child was successfully transplanted at the age of 5 years with a kidney graft from his father. At the time of surgery, the remnant kidney was also removed. There were no signs of malignancy (Wilms' tumour or gonadoblastoma). DDS was therefore considered as incomplete.

To confirm DDS on the molecular level, *WT1* mutational analysis was performed. Using the polymerase chain reaction (PCR)/single-strand conformation polymorphism analysis as described previously [6], an altered banding pattern was found for the exon 8 PCR product. Direct sequencing revealed a novel germline heterozygous nucleotide exchange from G → T at position 1107 (Figure 2A), resulting in an amino-acid substitution from Gln → His at codon 369. As seen in Figure 2B, glutamine is highly conserved between different species and its exchange to

Correspondence and offprint requests to: Dominik Müller, MD, Department of Pediatric Nephrology, Charité Children's Hospital, Augustenburger Platz 1, 13353 Berlin, Germany.
Email: dominik.mueller@charite.de

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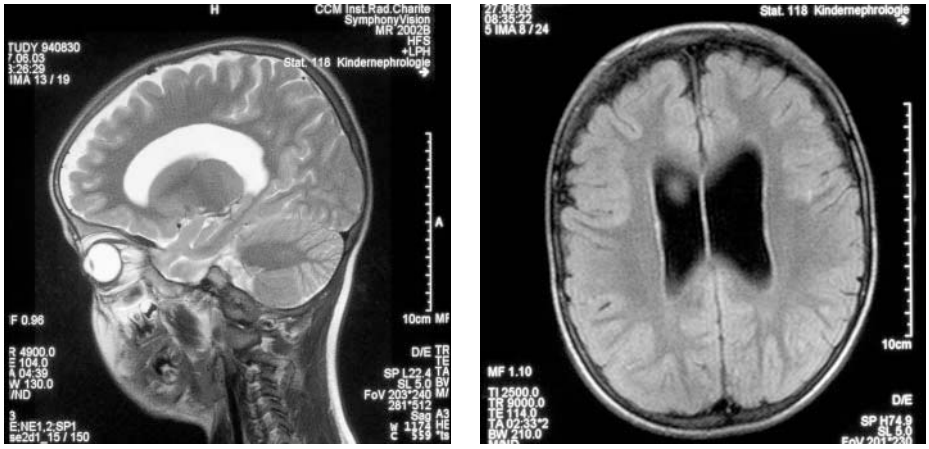
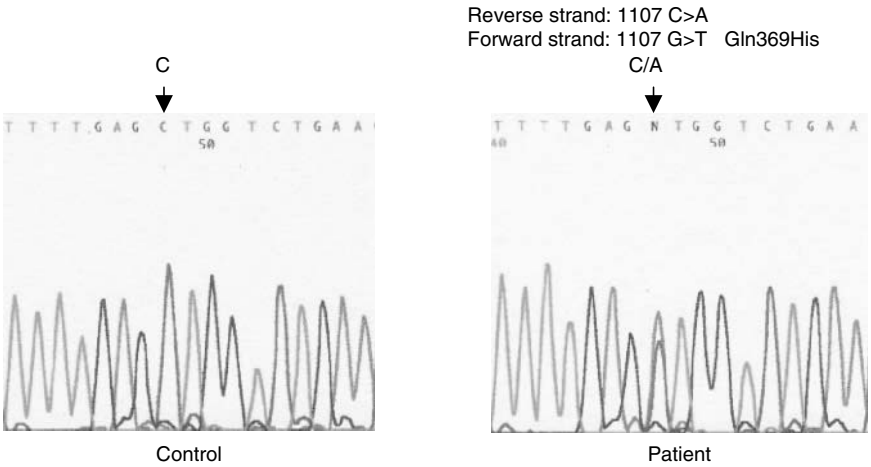


Fig. 1. Magnetic resonance image of the brain at the age of 5 years. It demonstrates cerebral atrophy and thinning of corpus callosum.

(A)



(B)

```
sp|P19544|WT1_HUMAN       YQCDFKDCERRFSRSD Q LKRHQRRHTGVKPFQCKTCQRKFSR
sp|P49952|WT1_RAT         YQCDFKDCERRFSRSD Q LKRHQRRHTGVKPFQCKTCQRKFSR
sp|P22561|WT1_MOUSE       YQCDFKDCERRFSRSD Q LKRHQRRHTGVKPFQCKTCQRKFSR
tr|Q9I8A0|Q9I8A0_CHICK    YQCDFKDCERRFSRSD Q LKRHQRRHTGVKPFQCKTCQRKFSR
tr|P79958|P79958_XENLA    YQCDFKDCERRFSRSD Q LKRHQRRHTGVKPFQCKTCQRKFSR
tr|Q9PUT7|Q9PUT7_BRARE    YQCDFTDCGRRFSRSD Q LKRHQRRHTGVKPFQCETCQRKFSR
sp|P50902|WT1_ALLMI       YQCDFKDCERRFSRSD Q LKRHQRRHTGVKPFQCKTCQRKFSR
tr|O93433|O93433_FUGRU    YQCDVTECGRRFSRSD Q LKRHQLRHTGVKSFECETCQRRFAR
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(C)

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Sequence: WT1 Zinc finger 2 wild type
                 *
YQCDFKDCERRFSRSDQLKRHQRRH

-------HHHHH-HHHHHHHH----  alpha helix

------------------------  beta strand

Sequence: Gln369His (our patient)
                 *
YQCDFKDCERRFSRSDHLKRHQRRH

-------HHHH--H-HHHHHHH---  alpha helix

----------E--------------  beta strand

Sequence: Gln369Arg [8]
                 *
YQCDFKDCERRFSRSDRLKRHQRRH

-------HHHHH-HHHHHHHHH---

------------------------
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Fig. 2. (**A**) Sequencing chromatogram of the reverse strand from exon 8. The nucleotide exchange is marked by an arrow and the corresponding amino-acid change is shown. (**B**) Comparison of a part from the WT1 protein sequence between different species. The affected glutamine (Q) is highlighted by a box. Xenla, *Xenopus laevis*; BRARE, zebrafish; ALLMI: *Alligator mississippiensis*; FUGRU, Fugu. (**C**) Secondary sequence prediction from zinc finger 2 of the wild type WT1, of our patient and the patient from Ohta *et al.* [8] generated using the sequNNPREDICT programme (www.cmpharm.ucsf.edu/~nomi/nnpredict.html).

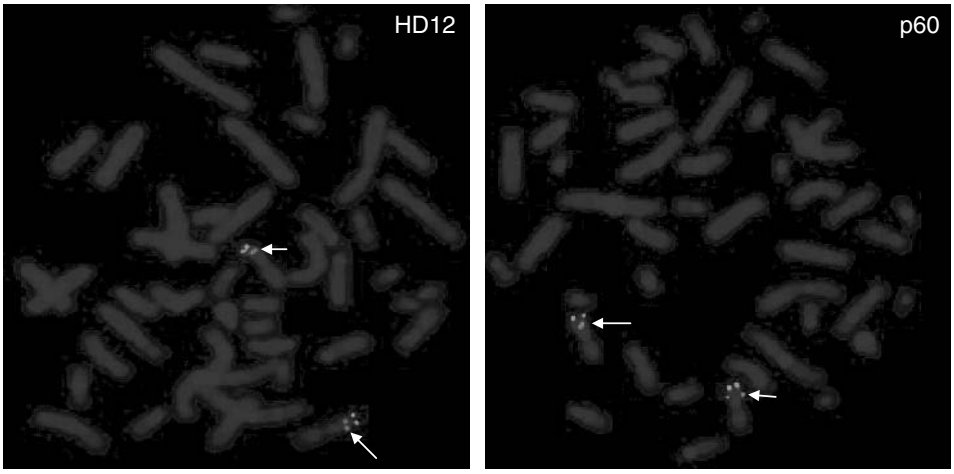

Fig. 3. Simultaneous fluorescence *in situ* hybridization with two probes from chromosome 11p13 and 11p15. The 11p13 probes (HD12 and p60) are marked with a white arrow. The 11p15 control probe is not marked.

histidine causes major changes in the secondary structure of zinc finger 2 (Figure 2C), which may result in loss of DNA-binding capacities. None of the parents was carrying the mutation, indicating a *de novo* mutation.

Since the patient presented with a complex phenotype, especially with psychomotor delay, we asked whether additional cytogenetic changes could account for these symptoms. However, high-resolution karyotyping (500 bands) remained without pathological findings (data not shown). In addition, we screened for submicroscopic deletions in the vicinity of the *WT1* gene as this region is known to be deleted in patients with WAGR syndrome, presenting with a high risk for Wilms' tumours, aniridia, genital abnormalities in males and mental retardation. For this, we performed, as previously described [7], a fluorescence *in situ* hybridization with one probe covering the 3' region of the *WT1* gene (HD12) and another one covering the region located between the *WT1* gene and the aniridia gene *Pax6* (p60). Thereby, we could exclude the presence of submicroscopic deletions in this region (Figure 3).

Discussion

The prime role of WT1 in the pathogenesis of several disorders like DDS, Frasier syndrome and WAGR syndrome is substantiated by the demonstration of mutations or deletions in the *WT1* gene. Here, we present a case of DDS with a novel germline heterozygous *WT1* mutation. The gene product is predicted to have lost its capacity for DNA binding due to changes in the secondary protein structure. Ohta *et al.* [8] have found the same amino acid (Gln 369) to be affected in a DDS patient. However, in their case, glutamine was replaced by arginine. Interestingly, their patient showed only a mild renal phenotype, with nephrotic syndrome first diagnosed at the age of 5 years and only a slight progression over the course of 18 years. The prediction of the secondary protein structure showed less pronounced changes compared with the mutation in our patient (Figure 2C).

Our patient shows features not typically found in DDS. First, he shows normal male genitalia and complete absence of testicular tissue. The fact that the child did not display hypospadia might point at the presence of disappearing testosterone-producing tissue during development ('vanishing testes'). Second, he presents with a horseshoe kidney. Until now it is not known whether *WT1* mutations account for this abnormality, but interestingly, an increased incidence of Wilms' tumour has been noted in patients with a horseshoe kidney. As 10–15% of all Wilms' tumours are caused by *WT1* mutations, this mutation could be responsible for a horseshoe kidney. Third, the patient showed cerebral abnormalities. It is currently unknown to what extent *WT1* mutations may also account for a phenotype in other tissues expressing *WT1*, such as the central nervous system. During mouse and rat development, *WT1* is found to be expressed in ependymal cells of the spinal cord and in the area postrema of the brain, where expression continues postnatally, suggesting a role throughout lifetime [3,4]. Furthermore, recent findings indicate that *WT1* deficient mice have thinner retinas with apoptotic loss of a large fraction of retinal ganglion cell precursors [9]. Together, these observations suggest that WT1 may also play a role in the development of neural structures and may be required for neuronal differentiation. This is also supported by the observation that PC19 embryonal carcinoma cells, induced with retinoic acid, turn on *WT1* expression and differentiate into glial and neuronal cells [10]. Only little is known about *WT1* expression in human brain. It was found to be expressed in different areas of the adult brain, such as occipital lobe, frontal lobe, hind brain, cerebellum and pons [11]. In addition, there is evidence that WT1 is associated with neuronal degeneration [12].

Although *WT1* is expressed in the brain, patients with *WT1* mutations have not been reported to show neurological symptoms. Our DDS case presented here showed psychochomotor delay and cortical cerebral atrophy preceding the onset of renal disease. In the literature, there are only two reports on DDS combined with neurological abnormalities: one patient with psychomotor delay and cerebral atrophy; the other with gross motor delay [13]. As we could exclude cytogenetic changes and small deletions in the WAGR region, the question arises whether *WT1* mutations may account for the neurological phenotype. In other words, cerebral atrophy in these cases could be a sign of a deficient neural development caused by mutation in the *WT1* gene. We suggest therefore that the diagnosis of *WT1* mutations should always be accompanied by in-depth neurological examination.

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Conflict of interest statement. None declared.

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