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SHORT REPORT

Wilms' tumor in patients with 9q22.3 microdeletion syndrome suggests a role for *PTCH1* in nephroblastomas

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Nephroblastoma (Wilms' tumor; WT) is the most common renal tumor of childhood. To date, several genetic abnormalities predisposing to WT have been identified in rare overgrowth syndromes. Among them, abnormal methylation of the 11p15 region, GPC3 and DIS3L2 mutations, which are responsible for Beckwith–Wiedemann, Simpson–Golabi–Behmel and Perlman syndromes, respectively. However, the underlying cause of WT remains unknown in the majority of cases. We report three unrelated patients who presented with WT in addition to a constitutional 9q22.3 microdeletion and dysmorphic/overgrowth syndrome. The size of the deletions was variable (ie, from 1.7 to 8.9 Mb) but invariably encompassed the *PTCH1* gene. Subsequently, we identified a somatic *PTCH1* nonsense mutation in the renal tumor of one patient. In addition, by array comparative genomic hybridization method, we analyzed the DNA extracted from the blood samples of nine patients with overgrowth syndrome and WT, but did not identify any deleterious chromosomal imbalances in these patients. These findings strongly suggest that patients with constitutional 9q22.3 microdeletion have an increased risk of WT, and that *PTCH1* have a role in the pathogenesis of nephroblastomas.
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INTRODUCTION

Nephroblastoma (Wilms' tumor, WT), the most common renal cancer of childhood, is an embryonic tumor that is believed to result from malignant transformation of abnormally persistent renal progenitors.[1] The oncogenic transformation of these progenitors is driven by various genetic events that affect the normal development of the embryonic kidney. Interestingly, some of these genetic alterations, somatically acquired in sporadic WTs, are also encountered at the germline level as the causative event of various congenital syndromes are associated with an increased risk of WT. For instance, point-mutations in the *WT1* gene at 11p13 are responsible for Denys–Drash syndrome (OMIM 194080), which is characterized by predisposition to WT, genital abnormalities and development of an early nephropathy.[2] Similarly, abnormal methylation at the imprinted 11p15 region, a frequent feature of the embryonic histotype of WT, is also evidenced in the germline of patients with WT,[3] and is in particular responsible for Beckwith–Wiedemann syndrome, an overgrowth disease which is associated with an increased risk of nephroblastoma.[4] Likewise, the Simpson–Golabi–Behmel overgrowth syndrome (OMIM 312870) is caused by mutations in *GPC3*[5] and is also believed to enhance the risk of WT. This might reflect a role of *GPC3* in the oncogenesis of some nephroblastomas. Finally, Perlman syndrome (OMIM 267000) is another condition with an increased risk of WT. This syndrome is characterized by prenatal macrosomia with polyhydramnios, visceromegaly, facial dysmorphism, developmental delay, renal dysplasia, WT and a high mortality in infancy. Recently, mutations in the *DIS3L2* gene have been identified in patients with Perlman syndrome.[6] Nephroblastomas have also been reported in a context of larger chromosomes abnormalities, such as deletion at 2q37, 7q31q32, 11q22.3 and 22q13.[7] More broadly, the intriguing wide association observed between WT and various malformative syndromes strongly suggests that many other developmental genes might have a driving role in the oncogenesis of nephroblastomas.

In the present manuscript, we report three unrelated patients affected by nephroblastoma and sharing some dysmorphic and overgrowth features. All patients were presented with a heterozygous constitutional 9q22.3 microdeletion, which we therefore consider as a new predisposition syndrome for WT.

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PATIENTS AND METHODS

Method of patient recruitment

Among the 2230 unrelated patients with learning disability and/or congenital anomalies analyzed in our diagnostic laboratory, only two patients with a 9q22.3 deletion have been identified. Both patients presented with a WT. No patients with a 9q22.3 deletion but without WT have been identified in our series. The third patient was collected through the French array comparative genomic hybridization method (array-CGH) network by recruiting patients with a 9q22.3 deletion and a WT. In addition, we collected DNA samples from nine patients with WT and macrosomia at birth and normal 11p15 methylation.

Clinical description

Patient 1, a female, was the first child born to unrelated healthy parents, with no relevant family medical history. She was born at term following a pregnancy marked by polyhydramnios. Sinusoidal cardiotocograph led to emergency cesarean section at 38 weeks gestation. The weight, length and occipitofrontal circumference (OFC) of the girl were 2860 g (−1.1 SD), 47 cm (−1 SD) and 38 cm (+3.2 SD), respectively. At birth, physical examination showed a posterior cleft palate. She was hypotonic and presented with major feeding difficulties and respiratory distress. Enteral nutrition was required for two months. Psychomotor delay was obvious as she could sit at 2 years of age and walk at $4^{6/12}$ years of age. Speech delay was also noted as she was not able to speak a single word at 2 years of age. She was treated for recurrent middle ear and upper respiratory tract infections.

At 30 months of age, a bilateral WT with mixed histology was diagnosed. Conventional treatment led to a complete remission with 11 years of follow-up. Beckwith–Wiedemann syndrome was considered but 11p15 methylation (assessed as previously described[8]) was normal.

At last evaluation, at the age of $12^{6/12}$ years of age, her weight, height and OFC were 30 kg (−2 SD), 131.5 cm (−3 SD) and 56.5 cm (+2.5 SD), respectively. She was able to construct sentences and could also read and write. Physical examination showed facial dysmorphism with dolichocephaly, large front, synophrys, low set ears and a high arched palate (Figure 1). Fingers were long and thin. Chest examination showed moderate pectus excavatum. Cardiac echography was normal.

Patient 2, a female, was the first child born to unrelated healthy parents with no relevant family medical history. At birth, the weight, length and OFC of the girl were 4610 g (+3.5 SD), 58.5 cm (+4.7 SD) and 38 cm (+3.2 SD), respectively. Physical examination showed macroglossia and hypotonia. Beckwith–Wiedemann syndrome was considered but 11p15 methylation was normal. She could sit at 14 months of age and walking was acquired at 30 months of age. Speech was also delayed as she could construct simple sentences at the age of $3^{6/12}$ years of age. She required special education and is now able to read and write.

At 20 months of age, her weight, length and OFC were 14 kg (+2.7 SD), 90 cm (+3 SD) and 51.5 cm (+2.5 SD), respectively. At 4 years of age, she developed a right stage 2 nephroblastoma. After conventional chemotherapy, the patient is in complete remission with 12 years of follow-up. At 14 years of age, she underwent surgery for mandibular cysts. At last evaluation, at $8^{1/12}$ years of age, her weight, height and OFC were 29 kg (+2.5 SD), 131.0 cm (+1 SD) and 56.5 cm (+3.3 SD), respectively.

Patient 3, a female, was the first child born to unrelated parents. The mother of patient 3 showed macrocephaly (OFC = 58 cm, +2 SD). She showed psychomotor delay as she walked at 27 months of age and had learning difficulties. She is now able to write and read. Surgery was required at 6 months of age and 4 years of age for cardiac fibromas. She also showed odontogenic keratocysts and a breast basocellular carcinoma, surgically removed at the age of 29 years of age. Cranial tomodensitometry showed calcifications of the falx cerebri. Patient 3 was born at term following an uneventful pregnancy. Cesarean section was undertaken at 38 weeks gestation for macrosomia. Her weight, length and OFC were 4800 g (+3 SD), 54 cm (−0.7 SD) and 42 cm (+5 SD), respectively. She presented with hypotonia and respiratory distress. Psychomotor delay was noted as she could sit at 19 months of age and walk at $3^{1/12}$ years of age. Speech delay was also noted as she started to make sentences at the age of 4 years.

At 4 years old, the weight, length and OFC of the girl were 23.6 kg (+5 SD), 110 cm (+3 SD) and 60 cm (+4.5 SD), respectively. Facial dysmorphy was noted with a large forehead, hypertelorism, a small nose and an open mouth with a protruding tongue and a high arched palate. Physical examination also showed thoraco–lumbar scoliosis. Sotos syndrome (OMIM 117550) was considered but no *NSD1* mutation could be identified.

At the age of 7 years old, she was diagnosed with a unilateral stage 3 nephroblastoma. The patient was further treated by chemotherapy and radiotherapy. Complete remission is maintained after 6 years of follow-up.

At the age of 8 years of age, she showed walking difficulties with pes cavus. Electromyogram showed an axonal neuropathy. At the last evaluation, at the age of $12^{9/12}$ years of age, her weight and height were 41.6 kg (−0.3 SD) and 161 cm (+1.5 SD), respectively.

Constitutional cytogenetic and molecular genetic analyses

Informed consent for genetic analyses was obtained from the patients or their parents according to local ethical guidelines. Karyotyping was performed using standard methods on metaphase spreads from peripheral blood of the patients. Genomic DNA was extracted from peripheral blood using standard protocols. Array-CGH experiments were performed using Agilent Human Genome CGH oligonucleotide arrays (44 K using the catalog design, 60 and 180 K using the ISCA designs (www.iscaconsortium.org)). The arrays were analyzed with the Agilent scanner (Agilent, Santa Clara, CA, USA) and the Feature Extraction software (Agilent, v.10.5.1.1). Graphical overview was obtained using the Genomic Workbench software (Agilent, v.5.0). The positions of the deletions' breakpoints were mapped to the UCSC genome browser, hg19. Fluorescence *in situ* hybridization (FISH) with RP11-565E09 probe was used to confirm the deletions and for parental inheritance in all patients.

Genetic analysis of the tumors

Tissues from the left and right nephroblatomas of patient 1 were analyzed, whereas no tumor tissue was available for patients 2 and 3. DNA and RNA were extracted using Qiagen Dneasy and Rneasy kits (Qiagen, Valencia, CA, USA), respectively. Array-CGH was performed on homemade BAC-arrays as

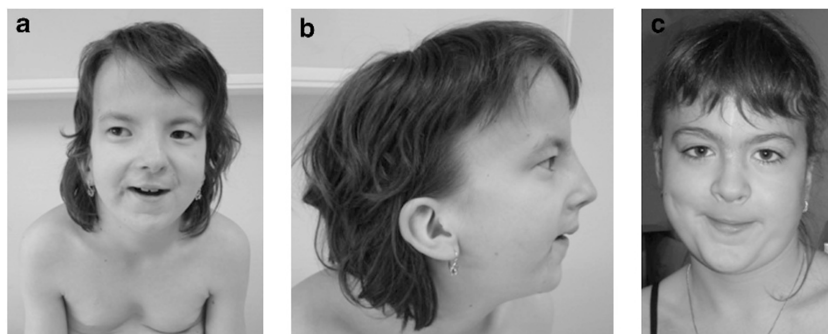

Figure 1 (**a**) and (**b**) Photographs showing facial dysmorphic features with prominent forehead, high frontal hairline, low set and posteriorly rotated ears in patient 1 at 13 years of age. (**c**) Photograph of the face of patient 2 at 14 years of age (photographs reproduced with patients' permission).

previously described.[9] The coding exons and the exon–intron boundaries of the *PTCH1* (NM_000264.3) and *FANCC* (NM_000136.2) genes were sequenced by Sanger technology in the two nephroblastomas of patient 1 (primer sequences available upon request). *PTCH1* transcripts were sequenced in the two nephroblastomas of patient 1. Expression analysis for *FANCC* was performed using total RNA extracted from the same two tumors of patient 1. Total RNA obtained from normal kidney tissue (Clontech, Palo Alto, CA, USA) was used for normalization. Real-time quantitative reverse transcription PCR was performed using the $\Delta\Delta$Ct method, two different sets of primers located in the coding sequence of *FANCC* and two housekeeping genes, *ACTIN* and *CYCLOPHILIN*.

RESULTS

Constitutional 9q22.3 deletions

Following normal standard karyotyping, array-CGH revealed a heterozygous constitutional 9q22.3 microdeletion in the three patients. No other genomic imbalances were identified. All deletions were confirmed by FISH. Parental analyses demonstrated *de novo* deletions in patients 1 and 2. Cytogenetic analysis performed by FISH identified the 9q22.32 deletion in the mother of patient 3 in a mosaic state (1/30 mitosis; 15% of the nuclei carrying the deletion). The sizes of the deletions were variable, ranging from 1.7 to 8.9 Mb. The size of the shortest region of overlap (SRO) was 1.7 Mb and encompassed 10 RefSeq genes and six miRNA (Figures 2a and b).

9q22.3 deletions and other genomic alterations in nephroblastomas

The two nephroblastomas from patient 1 were independently analyzed by array-CGH (Figure 2c). In addition to the common 9q22.3 deletion, they displayed distinct profiles, demonstrating that these two tumors are two independent cancers.

PTCH1 and *FANCC* analyses in the tumors

The SRO of the deletions contained two known tumor suppressor genes: *PTCH1* and *FANCC*. Following the Knudson 'two-hits' hypothesis, we looked for putative mutations in the remaining allele of both genes in the two nephroblastomas of patient 1. Sequencing of *PTCH1* led to the identification of a nonsense mutation (c.403C>T(p.Arg135X)) in the left kidney tumor, not found in the right kidney nor in the peripheral blood of the patient. We hypothesized that a cryptic intronic mutation could inactivate the remaining allele in the right kidney tumor as described in other cancer types; we therefore sequenced the *PTCH1* transcript in this tumor and showed that the *PTCH1* gene was expressed and harbored a wild-type sequence (Figure 2d). We also demonstrated that the genomic sequence of *FANCC* was normal in the two tumors of patient 1. Finally, we performed expression analysis for *FANCC* using RNA extracted from the two nephroblastomas of patient 1. We did not observe any significant differences between the expressions of *FANCC* in the right tumor or in the left tumor compared with the expression of *FANCC* in normal kidney used as a reference tissue (data not shown). The two housekeeping genes, *ACTIN* and *CYCLOPHILIN*, gave similar results.

Patients with macrosomia at birth and nephroblastoma

We performed array-CGH analysis in the nine patients with WT and macrosomia at birth and normal 11p15

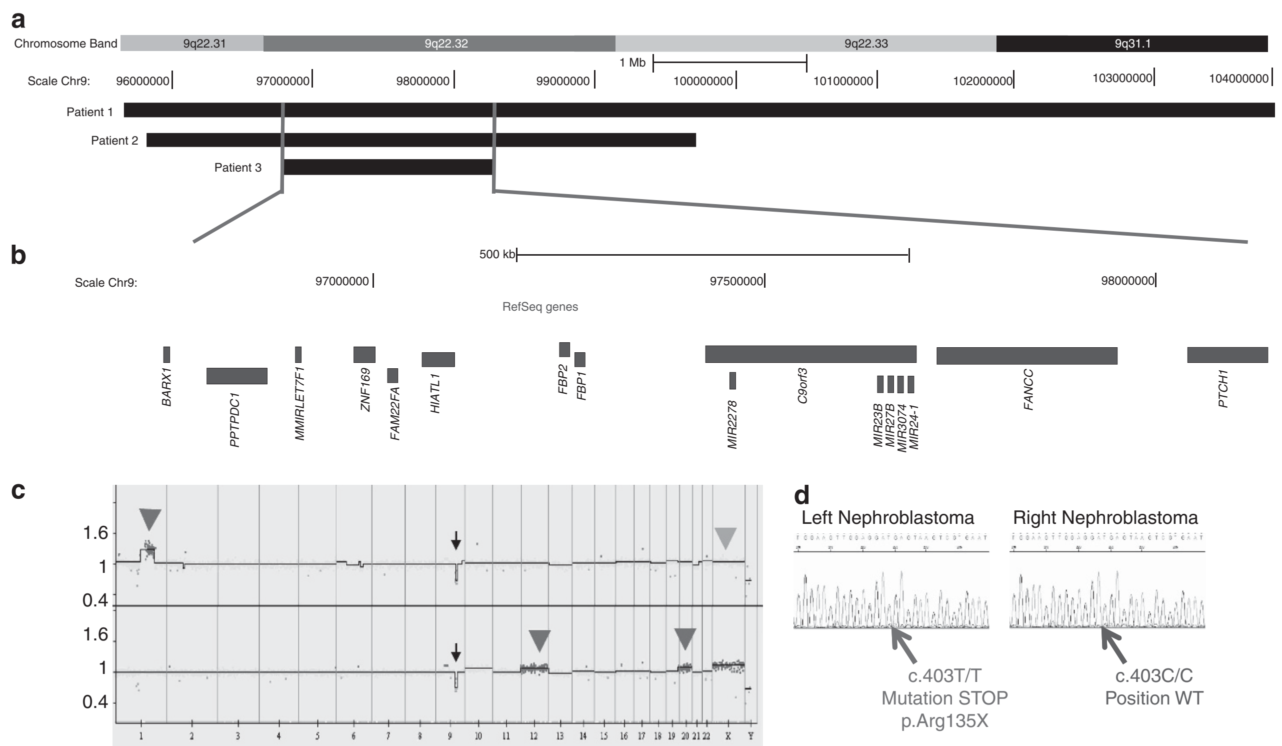

Figure 2 (**a**) Map of the deletions in chromosomal band 9q22.3 identified by array-CGH. Black horizontal bars indicate the deletions in the three patients. The RefSeq genes located in the genomic region are indicated. The vertical region shaded in red indicates the SRO shared by the three patients. (**b**) Detailed map of the SRO which contains 11 candidate genes and 6 miRNAs. (**c**) array-CGH on the left and right nephroblastomas of patient 1 showing clearly distinct profiles; black thin arrows show the common 9q deletion; red and green arrows show, respectively, the gains and losses of chromosome arms that distinguished the two tumors. (**d**) *PTCH1* mutation (c.403C>T(p.Arg135X)) identified in the left kidney tumor from patient 1.

methylation. No deleterious genomic imbalance was identified in the nine patients.

DISCUSSION

To date, 27 patients with 9q22.3 microdeletion have been reported. These patients present with craniofacial anomalies, metopic craniosynostosis, hydrocephalus, macrosomia and learning disability.[10] The size of the 9q22.3 microdeletions is variable among patients, ranging from 352 Kb to 20.5 Mb. To investigate genotype–phenotype correlations, Muller *et al*[10] studied ten individuals with 9q22.3 microdeletion, using array-CGH to precisely map the chromosomal imbalances. The authors identified overlapping critical regions of 929 Kb, 1.08 and 1.84 Mb for metopic craniosynostosis, obstructive hydrocephalus and macrosomia, respectively, but the deleted genes causing these clinical features still remain unknown. Among all previously published patients, only one patient developed malignancies including nephroblastoma.[11] The three patients described in this study raise to 4/30 the number of patients with 9q22.3 microdeletion and nephroblastoma.

The 9q22.3 deletion carried by all the patients who developed a WT invariably encompasses the *PTCH1* gene that encodes a receptor for sonic hedgehog. Haploinsufficiency of the *PTCH1* gene is responsible for basal cell nevus syndrome (BCNS), also known as Gorlin syndrome (OMIM 109400). BCNS is a malformation syndrome associated with cancer predisposition. Patients with point-mutation in *PTCH1* have an increased risk of childhood medulloblastomas (primitive neuroectoderm tumor), lymphomesenteric or pleural cysts, and ovarian or cardiac fibromas. Thus far, no WT have been reported in such patients. The nonsense mutation identified in one of the tumors from patient 1 suggests that a total loss of *PTCH1* might have a role in the oncogenesis of nephroblastoma. However, the absence of identified mutation in the second tumor of this patient and the lack of available DNA for the two other patients did not allow to fully confirm this hypothesis.

The discrepancy between the absence of WT in patients with BCNS and the presence of WT in some patients with 9q22.3 microdeletion is striking. The nephroblastoma might be caused by the haploinsufficiency of neighboring gene(s), in addition to *PTCH1* haploinsufficiency in patients with 9q22.3 microdeletion. Interestingly, the SRO in our three patients also contained another tumor suppressor gene, *FANCC*. However, the absence of mutation on the remaining allele did not argue in the favor of *FANCC*, having by itself a major role in the oncogenesis of WT.

Deletions of the 9q region are unfrequent in sporadic nephroblastomas.[12] One could therefore postulate that, if *PTCH1* inactivation has a critical role in the oncogenesis of nephroblastoma, it might remain restricted to a small subset of cases. Consistently, a high percentage of mutations evidenced by exome sequencing in pediatric cancers are found in a single tumor or have a low incidence – the so-called 'private mutations'-, although they have a genuine driver role.[13,14] Hence, it is plausible that 'private' PTCH1 mutations may be genuine driver events that are encountered in only a few cases of nephroblastoma. Alternatively, the SRO contains nine other coding genes and six miRNA (Figures 2a and b), among which one or more might have an active role in the tumor formation. miRNA in particular are widely involved in cancer, and although the ones contained in the SRO are not known to have a role in malignancies so far, they might deserve closer attention.

Interestingly, the three patients were presented with overgrowth or macrocephaly. Overgrowth is a frequent feature observed in patients with 9q22.3 deletion but also in Perlman syndrome. Perlman syndrome (OMIM 267000) is another condition with an increased risk of WT, which was considered in patients 1 and 2. Recently, compound heterozygous or homozygous mutations in the *DIS3L2* gene have been identified in patients with Perlman syndrome.[6] We did not sequence this gene in the nine patients with WT and macrosomia at birth, as they presented the cardinal features of the 9q22.3 deletion and did not fulfill the criteria for Perlman syndrome. The array-CGH performed in those nine patients did not lead to the identification of any deleterious genomic imbalances.

In conclusion, this report suggests that 9q22.3 deletions could be another rare molecular mechanism leading to overgrowth and nephroblastoma. Our results strongly suggest that 9q22.3 deletion is associated with an increased risk of WT and should aware clinicians to look for 9q22.3 deletion in children presenting with syndromic WT. In addition to the constitutional deletion of one copy of the *PTCH1* gene in the three studied patients, presence of a nonsense mutation in the remaining allele in the WT of one patient suggests a role for the *PTCH1* gene in the pathogenesis of nephroblastoma.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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