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Typical Renal-coloboma Syndrome Phenotype in a Patient with a Submicroscopic Deletion of the *PAX2* Gene

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Abstract

We present a patient with optic nerve hypoplasia, secondary strabismus, mild deafness, abnormal external ear helices and renal hypoplasia. The clinical phenotype was consistent with renal-coloboma syndrome, but no point mutation in the *PAX2* gene could be identified. High resolution array Comparative Genomic Hybridization (aCGH) analysis showed that this patient has a submicroscopic deletion on chromosome 10, affecting the entire coding region of the *PAX2* gene. This finding provided the molecular confirmation of the patient's clinical diagnosis and showed that, in addition to point mutations, deletions of the *PAX2* gene contribute to the etiology of the renal-coloboma syndrome.

Keywords

deletion; *PAX2* gene; renal-coloboma syndrome; haploinsufficiency

INTRODUCTION

Renal-coloboma syndrome (papillorenal syndrome) is an autosomal-dominant disorder characterized by hypodysplastic kidneys and optic nerve abnormalities (OMIM 120330). Renal and/or ocular anomalies are found in all patients, but the type and the severity of these abnormalities can vary markedly. Commonly observed manifestations that affect the kidneys include renal hypoplasia and vesicoureteric reflux, which in almost all affected patients leads to the end stage renal disease. The most common eye abnormalities are optic nerve

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(disc) dysplasia (excavated optic nerve with the vessels entering from the periphery of the widened optic disc) and staphyloma (cystic dilatation of the optic nerve posterior to the globe observed by CT or MRI). Less common findings include high-frequency hearing loss, soft skin, and ligamentous laxity [Schimmenti, 2011].

Heterozygous mutations in the *PAX2* gene were first described in the patients with Renal coloboma syndrome in 1995 [Sanyanusin et al., 1995; Schimmenti et al., 1995]. *PAX2* is the only gene known to be associated with Renal coloboma syndrome; however, with current techniques mutations in *PAX2* can only be detected in approximately 50% of the affected individuals [Schimmenti, 2011], while in the remaining patients the molecular cause of the disease stays unknown.

PAX2 encodes a transcription factor that belongs to the "paired-box" family of transcriptional regulators. Studies with Pax2 knockout mice have revealed that homozygous deletions lead to early postnatal death with absence of the kidneys and ureters, and the eyes that have failed optic fissure closure [Torres et al., 1995]. The human *PAX2* gene is located on chromosome 10, at band 10q24.1. It comprises 12 exons, with exons 2, 3, and 4 encoding a highly conserved 128 – amino acid DNA binding domain [Sanyanusin et al., 1996]. During embryogenic development *PAX2* is abundantly expressed in the kidney, eye, cochlea, pancreas, and central nervous system [Tellier et al., 2000], and is involved in the regulation of several genes, such as *WT1* [Discenza et al., 2003], *MYCN*, and *TP53* [McConnell et al., 1997].

PAX2 mutations causing the renal-coloboma syndrome have been identified in the exons encoding the DNA binding domain (exons 2, 3 and 4), the octapeptide domain (exon 5), the homeodomain (exon 7), and the transactivation domain (exons 8 and 9). Various mutation types have been detected in *PAX2*, including missense, nonsense and frameshift mutations [Fletcher et al., 2005; Schimmenti 2011; Bower et al., 20011]. There is also a single reported case of the renal-coloboma syndrome due to a *de novo* translocation interrupting the *PAX2* coding region [Narahara et al., 1997]. Initial genotype-phenotype correlation studies suggested that *PAX2* mutations in exons that encode the DNA binding (paired) domain (exons 2,3 and 4) are associated with both the ocular and renal manifestations, while mutations in exons 7 and 9 are typically associated with renal hypoplasia/dysplasia and milder ocular manifestations [Porteous et al., 2000; Nishimoto et al., 2001]. In contrast, a recent large study on 173 affected individuals seems to indicate that regardless of the type or location within the gene, *PAX2* mutations lead to a highly penetrant and highly variable phenotype involving abnormalities of both the kidneys and optic nerve [Bower et al., 20011].

Only two cases of the *PAX2* gene deletions have been described to date, both associated with large, cytogenetically visible deletions of the long arm of chromosome 10 [Reddy et al., 2011 ; Benetti et al., 2007]. We describe the first patient with renal-coloboma syndrome due to a submicroscopic deletion of the *PAX2* gene.

CLINICAL REPORT

The patient was born at 40 weeks of gestation after an uneventful pregnancy, with Apgar scores of 9 at 1 and 5 minutes. Both parents were healthy and there was no family history of renal or eye disease.

Dysplasia of both external ears was noted at birth; this prompted audiologic studies which showed high-frequency hearing loss on both sides.

Ophthalmologic investigation was performed at the age of 3 months and showed dysplasia of the right optic nerve with localized excavation of the optic disc. The right eye showed no pupil reaction and had no preserved vision. The patient also had strabismus convergens. No structural abnormalities were found in the left eye. The eyesight in the left eye was re-examined at 8 and 14 years of age, showing mild to moderate myopia.

Proteinuria was first detected accidentally when the patient was 1-year-old. Low-grade proteinuria (0.3-1 g/l) persisted. Ultrasound scan was performed and showed hypoplastic kidneys with hyperechogenicity. The kidney function continued to deteriorate, so that at the age of 8 the patient had gross proteinuria (>3g/l) in repeated urine samples and was admitted to the pediatric nephrology department of the Kaunas University of Medicine (Lithuania) for further studies. Renal function was investigated by glomerular filtration rate (GFR), showing 65,67 ml/min/1,73 m². Ultrasound scan identified hypoplasia and hyperechogenicity of both kidneys. The size of the right kidney was 5.6 cm x 2.8 cm, and of the left kidney was 6.0 cm x 3.8 cm, with the thickness of the parenchyma at 1.4 cm. The renal biopsy identified ten enlarged glomeruli and signs of secondary segmental and focal glomerulosclerosis (Fig 1A, 1B).

The patient is currently on symptomatic treatment. Her renal function is slowly decreasing. She has second-stage chronic kidney disease, with GFR of 67 ml/min/1.73 m². She is being treated with Angiotensin Converting Enzyme (ACE) inhibitors to control proteinuria and is followed by a nephrologist every three months to monitor renal function and blood pressure. She is also followed by an ophthalmologist to monitor vision in the left eye. The most recent ultrasound scan of the kidneys shows bilateral hypoplasia and nephrosclerosis (Fig 2).

MATERIALS AND METHODS

The patient was enrolled in the ongoing research study “Genetics of Microphthalmia, Anophthalmia and Coloboma” at the University of Minnesota, Department of Pediatrics, Division of Genetics and Metabolism, and a written informed consent was obtained from the patient and her parents. High resolution aCGH analysis was performed as a part of this large study, in order to investigate the frequency of genomic copy number abnormalities in individuals with anophthalmia, microphthalmia and coloboma [Raca et al., 2011]. The aCGH project was approved by the Institutional Review Boards at the University of Minnesota and the University of Wisconsin-Madison. The patient’s previous molecular testing included screening for point mutations in the *PAX2* gene by single-strand conformation polymorphism (SSCP) and targeted Sanger sequencing, which were reported as normal.

A commercially available HD2 human whole-genome CGH array (Roche NimbleGen Systems Inc., Madison, WI), was used for aCGH analysis. This array contains the total of ~2 million probes at a median interprobe distance of approximately 1169bp, and can detect small genomic imbalances (deletions and duplications) at the resolution of individual genes (~5–10 kb). Specimen labeling, array hybridization, washing and scanning were performed at NimbleGen service laboratory in Iceland. Data analysis was performed at the UW Cytogenetic Services Laboratory at the University of Wisconsin-Madison, using the NimbleScan and SignalMap softwares from Roche NimbleGen and OneClickCGH software from Infoquant. Regions with copy number changes detected by aCGH were compared against the database of Genomic Variants (<http://projects.tcag.ca/variation/>), which catalogues known benign copy number polymorphisms (CNPs) in the human genome. The detected deletion was confirmed by a separate aCGH experiment, using a commercially available 385,000 probe array (Roche NimbleGen Systems Inc., Madison, WI). Further confirmatory testing was performed on an independent peripheral blood sample using a custom array designed for clinical testing by the Baylor College of Medicine Medical Genetic Laboratories and manufactured by Agilent Technologies (Agilent Technologies, Santa Clara, CA). This array contains approximately 180,000 oligonucleotide probes and provides coverage of the whole genome at the average resolution of 30Kb, tilling coverage of all exons for 1,714 genes and it also covers 700 microRNAs, and the entire mitochondrial genome. Data analysis was performed using Agilent Feature Extraction software (Agilent Technologies, Santa Clara, CA) and CytoSure Interpret software (Oxford Gene Technology, Oxford, UK). Parental samples were tested together with the repeat sample from the proband, using the same custom array designed by the Baylor College of Medicine Medical Genetic Laboratories.

RESULTS

aCGH analysis showed the presence of a ~240kb deletion on chromosome 10 at cytogenetic location 10q24.31 in the patient relative to a normal control. The breakpoints were at positions 102,437,000 - 102,677,000 based on the Human Genome March 2006 (hg18 assembly, NCBI build 36.1). The deletion included the entire *PAX2* coding region and a portion of the *FAM178A* (C10orf6) gene (Fig 3). Review of the region in the UCSC genome browser, showed no presence of repetitive elements (LINE, SINE or segmental duplications) that could have played a role in mediating the observed rearrangement. Testing of the parental samples revealed no copy number abnormalities, showing that the deletion most likely occurred *de novo* in the patient, although the possibility of germline mosaicism in one of the parents could not be excluded.

DISCUSSION

We describe a patient with typical clinical features of the renal-coloboma syndrome due to a microdeletion of the *PAX2* gene detected by high resolution aCGH analysis. The deletion also included a portion of the *FAM178A*(C10orf6) open reading frame, which codes for a hypothetical protein of unknown function. The partial deletion of the *FAM178A* gene is unlikely to contribute to the patient's phenotype. The patient had classic features of the renal-coloboma syndrome, including optic nerve hypoplasia, secondary strabismus, mild

deafness, abnormal external ear helices and renal hypoplasia. *PAX2* gene mutation screening and targeted sequencing had been performed previously, but failed to identify any molecular abnormalities in the patient's sample. This is not surprising, since traditional Sanger sequencing cannot detect partial or complete gene deletions, because normal sequence is obtained from the non-deleted copy of the gene of interest. Our case therefore illustrates that without including deletion testing, molecular evaluation for the renal-coloboma syndrome may fail to detect the underlying mutation in some patients. Systematic evaluation of large numbers of affected individuals will be necessary to assess the contribution microdeletions in causing *PAX2* haploinsufficiency. Using current diagnostic approaches causative mutations in the *PAX2* gene are detected in approximately 50% of individuals with renal-coloboma syndrome [Schimmenti, 2011], and it is likely that microdeletions account for some of the remaining 50% of the cases.

Only two cases of the chromosome 10 deletions encompassing the *PAX2* gene have been reported in the literature. Benetti et al. [2007] described a patient with a cytogenetically visible deletion of the long arm of chromosome 10. aCGH analysis showed that the deletion spanned 7.9 megabases and affected 90 genes including *PAX2*. The patient's phenotype included a hypoplastic left kidney, ultrasound abnormalities of the right kidney, developmental delay and mild dysmorphic features, but no optic coloboma or any other eye anomaly.

Reddy et al. [2011] reported a patient with multiple congenital anomalies, including dysplastic kidneys resulting in chronic kidney disease, growth deficiency, global developmental delay and dysmorphic facial features. aCGH testing in the patient and the parents revealed that the patient had a de novo 8.3-Mb interstitial deletion on chromosome 10. The deleted region contained a total of 101 genes of which at least 14 (including *PAX2*) were associated with genetic diseases in humans.

Both previously described deletions of the 10q region were large, and affected many genes in addition to *PAX2*. The patients' phenotype included abnormalities associated with loss of other dosage sensitive genes in the deleted regions, making it impossible to determine which abnormalities were specifically caused by the absence of one copy of the *PAX2* gene. Interestingly, eye anomalies were not described in either of the previously reported 10q deletion patients, although optic nerve dysplasia is considered a hallmark of the renal-coloboma syndrome. The absence of eye malformations may have simply been a manifestation of the documented variability in the clinical findings of the renal-coloboma syndrome, with some patients having only renal or only ocular manifestations and others having both. However, the limited phenotype in the cases reported by Benetti et al. [2007] and Reddy et al. [2011] may have also been caused by the absence of the mutated *PAX2* allele due to complete gene deletion. Benetti et al. [2007] proposed that the lack of an eye phenotype in their patient with 10q deletion may suggest the origin of eye anomalies in the renal-coloboma syndrome from a dominant-negative effect of the mutated allele, rather than solely from the decreased level of the *PAX2* protein. The typical phenotype observed in our patient (including optic nerve hypoplasia) argues against the hypothesis of dominant-negative effects, and confirms haploinsufficiency as the pathogenetic mechanism of the renal-coloboma syndrome.

In summary, we report on the first case of a submicroscopic deletion affecting the coding region of the *PAX2* gene in a patient with classic phenotype of the renal-coloboma syndrome. No other genes likely to contribute to the patient's phenotype were affected by the rearrangement. This finding confirms decreased level of the functional *PAX2* protein as the molecular mechanism causing renal-coloboma syndrome, and emphasizes the importance of incorporating deletion/duplication testing together with the *PAX2* gene sequencing into molecular diagnostics of this disorder.

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Figure 1A, 1B.

Renal biopsy showing enlarged glomeruli and signs of secondary segmental and focal glomerulosclerosis.

Figure 2.
The ultrasound of the kidneys illustrating bilateral hypoplasia and nephrosclerosis.

Figure 3.

Array CGH results from confirmatory testing with 180,000-probe clinical array from Baylor College of Medicine, showing the *PAX2* deletion in the patient's sample. Panel A is showing the close view of the deleted segment from the Cytosure Interpret software. X-axis is representing genomic positions of individual probes on chromosome 10, while the Y axis is showing log2 values for signal intensity ratios between the patient's DNA and a control DNA. Each red dot represents one probe. Red box is indicating a 240kb region where statistically significant reduction in log2 ratios for multiple consecutive probes indicated the presence of a deletion in the patient relative to a normal control. Panel B is a screenshot from the UCSC genome browser of the deleted region between bases 102,437,000 and 102,677,000 on chromosome 10 (based on the Human Genome March 2006 (hg18) assembly). Top of the panel represents the ideogram of chromosome 10, with the red vertical bar showing the approximate position of the microdeletion within the band 10q24.1. The bottom of the panel indicates the positions of the affected genes *PAX2* and *FAM178A* relative to the deletion breakpoints.