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Concurrent Reduced Expression of Contiguous *PKD1*, *TSC2* and *NTHL1* Leading to Kidney Diseases and Multiple Diverse Renal Cancers

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Abstract. *Background/Aim: Several cases of concurrent reduction of expression of polycystin 1 (PKD1) and Tuberous Sclerosis Complex 2 (TSC2) that are contiguous in chromosome 16p13 have been previously reported. This study newly addresses the concurrent reduction of expression of PKD1, TSC2 and NTHL1, which is adjacent to TSC2 and is a tumor suppressor gene. Materials and Methods: We investigated the mRNA expression levels of PKD1, TSC2, PKD2, TSC1 and NTHL1 in blood and renal cell carcinoma (RCC) tissues in a proband with autosomal dominant polycystic kidney disease (ADPKD), tuberous sclerosis complex (TSC) and multiple pathologically diverse RCCs, including clear cell, papillary and chromophobe types. Additionally, we investigated germline variants in blood using whole exome sequencing (WES) in the proband and her four siblings. Results: mRNA expression levels of PKD1, TSC2 and NTHL1 were reduced in the proband's blood and RCCs, compared with control groups. WES identified one novel variant with amino acid changes in the PKD1 exon in the three subjects with ADPKD, including the proband. Moreover, two variants in the TSC2 intron specific to the proband were also identified. Conclusion: In this study, we report a novel pathogenic variant in the PKD1 exon which likely led to ADPKD, and two variants in the TSC2 intron, which might have led to reduction in the expression of both TSC2 and NTHL1, consequently leading to TSC and multiple pathologically diverse RCCs.*

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Key Words: PKD1, *TSC2*, *NTHL1*, autosomal dominant polycystic kidney disease, tuberous sclerosis, renal cell carcinoma.

Autosomal dominant polycystic kidney disease (ADPKD), which is one of the most well-known hereditary diseases, is characterized by the development of multiple cysts in the kidney, leading to progressive kidney dysfunction (1). ADPKD commonly results from a mutation of either the *PKD1* gene on chromosome 16p13 or the *PKD2 gene* on chromosome 4q21-q23 (2-4), and as recently reported, also from chromatin methylation abnormalities (5). More than 2000 mutations of *PKD1* and *PKD2* have been reported and described in the Autosomal Dominant Polycystic Kidney Disease Mutation Database (PKDB; http://pkdb.mayo.edu/). Moreover, in addition to single mutations in the *PKD1* gene, the concurrent mutation of *PKD1* and *TSC2*, which is a gene adjacent to *PKD1*, has been reported (6, 7).

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem disorder characterized by hamartomas in various parts of the body, that results from a mutation of either *TSC1* on chromosome 9q34 or *TSC2* on chromosome 16p13 (8). A concurrent mutation of *PKD1* and *TSC2* causes reduction in the expression of both these genes and leads to both ADPKD and TSC (6). Further, besides this condition, many other types of contiguous gene disorders have also been reported globally, such as the Prader-Willi syndrome, Angelman syndrome and DiGeorge syndrome, which can contain concurrent reduction of expression of three or more genes(9-11).

NTHL1 is a gene that is also contiguous with *PKD1* on chromosome 16p13 (12). *NTHL1* is a base excision repair gene that plays an important role in preventing the development of cancer (13, 14). While there have been several reports about reduction in expression of *NTHL1* causing different types of cancer (12, 15), concurrent

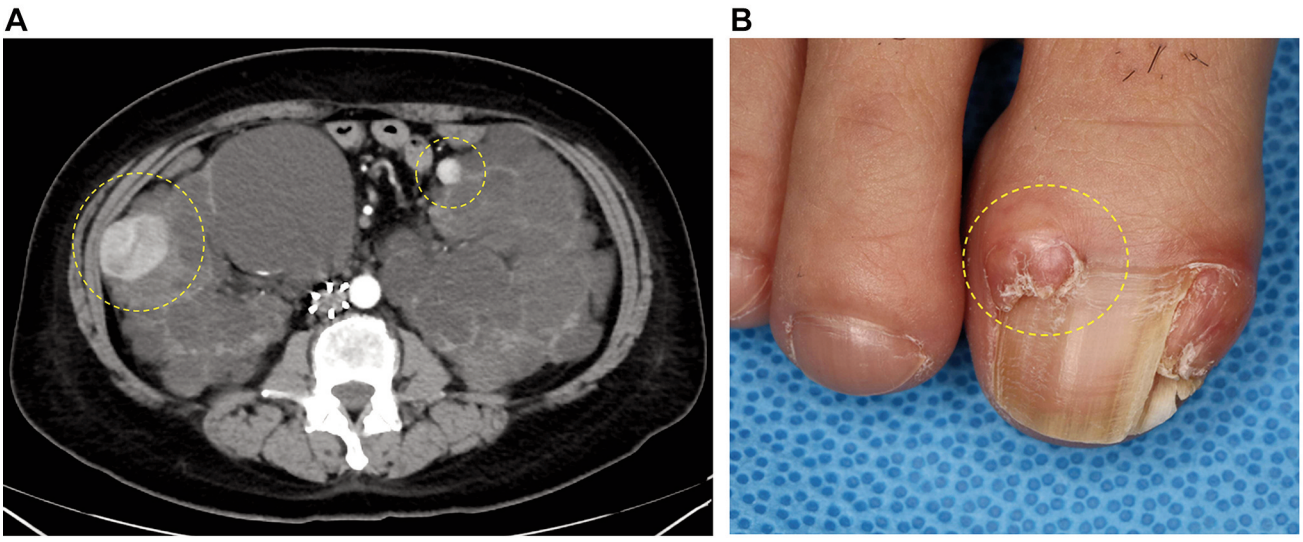

Figure 1. *Computed tomography image of tumors in the kidneys and a representative ungual fibroma in the proband. (A) Preoperative contrast-enhanced computed tomography identified two tumors (yellow dotted circles) in the proband's kidneys. (B) The yellow dotted circle shows a representative ungual fibroma on the proband's right first toe.*

reduction in expression of *NTHL1* in addition to *PKD1* and *TSC2* has so far never been reported.

In this study, we report a case of a proband with ADPKD, TSC and multiple pathologically diverse renal cell carcinomas (RCCs), who belonged to a family in which several of the members had ADPKD. In this case, real-time polymerase chain reaction (PCR) revealed the concurrent reduction of expression of three contiguous genes on chromosome 16p13: *PKD1*, *TSC2* and *NTHL1*. Moreover, using whole exome sequencing (WES), we identified a novel pathogenic variant in the *PKD1* exon and two variants with possible effects on expression of both *TSC2* and *NTHL1*.

Materials and Methods

Presentation of the proband. A 44-year-old Japanese woman presented with hematuria. She had a history of ADPKD and had been on hemodialysis for four years. Contrast-enhanced computed tomography (CECT) revealed multiple cysts in both kidneys and bilateral renal tumors (Figure 1A). Since both tumors were hypervascular on CECT and were suspected to be RCCs, she underwent open bilateral total nephrectomy. The tumor observed by preoperative CECT in the right kidney was pathologically diagnosed as a chromophobe RCC, and sporadic angiomyolipoma was also identified (Figure 2). The tumor observed by preoperative CECT in the left kidney was also pathologically diagnosed as a chromophobe RCC. Additionally, papillary RCC, clear cell RCC and sporadic angiomyolipoma were identified in the left kidney (Figure 3). Since there were many angiomyolipomas scattered sporadically in both kidneys, she was suspected to have TSC. Physical examination revealed two or more ungual fibromas on her foot (Figure 1B), leading to a definitive clinical diagnosis of TSC according to the diagnostic criteria (16). Her postoperative course was uneventful, without any signs of cancer recurrence over a follow-up period of two years.

Family members of the proband. Figure 4 shows her family pedigree depicting the inheritance of ADPKD. Five members of her family, including the proband, had ADPKD, although only the proband had a history of TSC and RCCs. With their consent, we performed whole-body CECT, blood examination including tumor markers (CA19-9, CEA, and CA15-3) and whole-body examination in the five family members with ADPKD, including the proband and her four sisters [Subjects 1, 2, 3, 4 and 5 (the proband) in Figure 4]. These examinations revealed that four subjects, except the proband (Subjects 1-4) did not have presentations of TSC or any cancer, including RCC. Moreover, the proband did not present any other cancer besides RCCs.

Real-time PCR. Total RNA was prepared from the peripheral blood of the proband only (Subject 5 in Figure 4) using a QIAamp® RNA blood mini kit (QIAGEN, Venlo, the Netherlands) according to the manufacturer's instructions. As a control, total RNA was also prepared from the peripheral blood of a subject who was not part of the proband's family and who did not have any disease, including ADPKD, TSC or any type of cancer. Single-standard cDNA was synthesized using a SuperScript® III First-Standard Synthesis System for RT-PCR (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The PCR reagents used for *PKD1* (Hs00947402_g1), *TSC2* (Hs01020404_g1), *NTHL1* (Hs00267385_m1), *PKD2* (Hs00960946_m1) and *TSC1* (Hs01060648_m1) were purchased from Applied Biosystems (Waltham, MA, USA). A 40-cycle PCR with denaturation at 95˚C, annealing at 55˚C and extension at 72˚C was performed using a StepOne™ Real-Time PCR System (Applied Biosystems). The data were standardized using β-actin reagent as the internal control (Applied Biosystems).

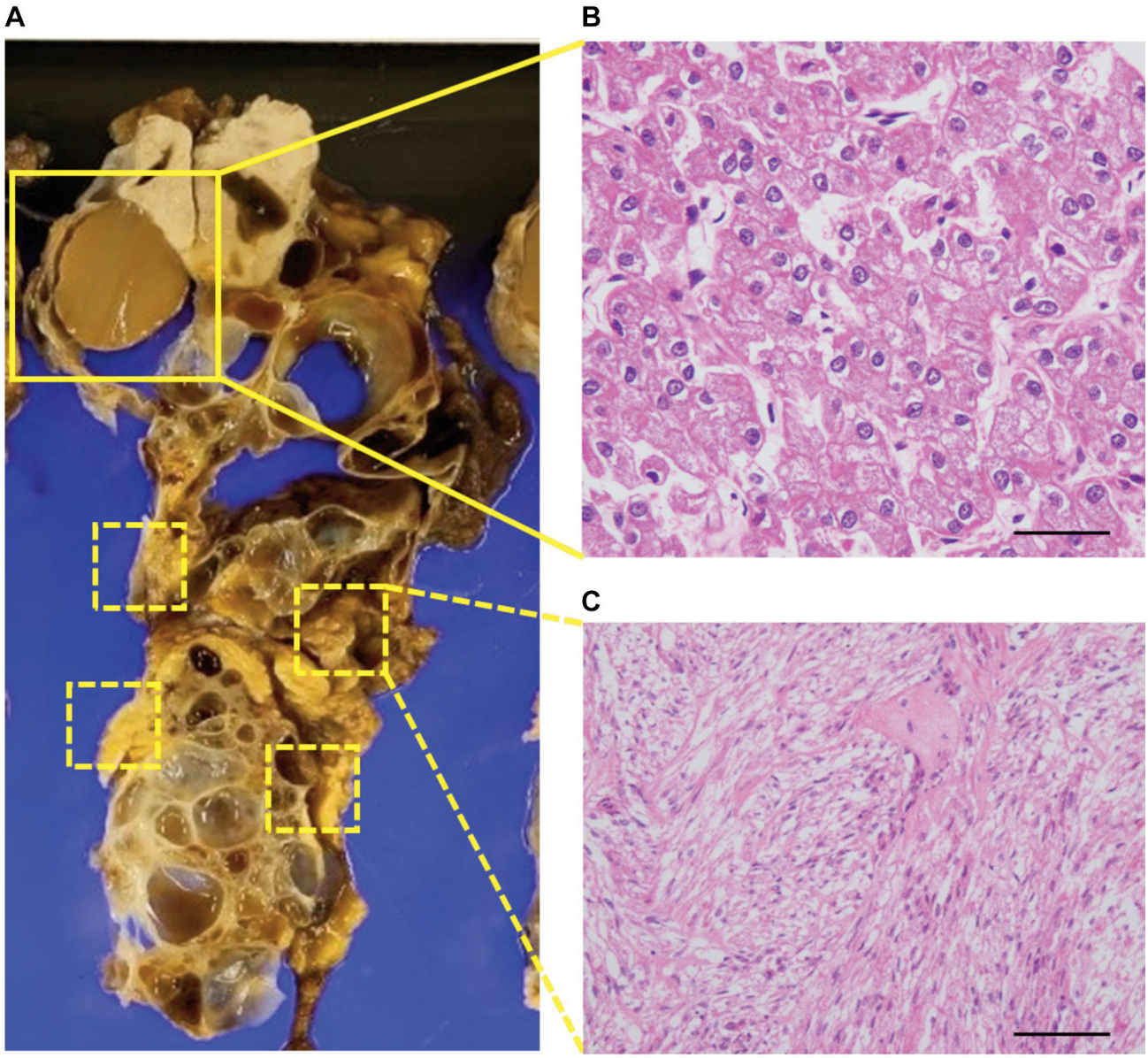

Figure 2. *Right kidney of the proband. (A) Resected right kidney. The yellow square shows the chromophobe renal cell carcinoma (RCC). The yellow dotted squares show multiple angiomyolipomas. Microscopic view of the (B) chromophobe RCC and (C) angiomyolipomas Scale bars, 50 μm.*

Moreover, total RNA from the kidneys of the proband was also prepared using an RNeasy® FFPE kit (QIAGEN) according to the manufacturer's instructions. The proband's kidneys that had been removed during nephrectomy were used for tissue samples. Cancer tissues from the tumors that were pathologically diagnosed as papillary RCC in the left kidney and chromophobe RCC in the right kidney were used for extraction of RNA. RNA could not be extracted from the clear cell RCC because it did not form a tumor mass, and only a small amount of clear cell RCC was identified pathologically. As a control group, papillary and chromophobe RCC tissues from several other patients were used for the extraction of RNA. Moreover, several kidneys from other people that had been removed during nephroureterectomy for a diagnosis of lower ureter cancer but whose parenchyma was intact, were also used as normal controls. cDNA synthesis and real-time PCR were performed by the same method as described above.

Whole exome sequencing. DNA was purified from the peripheral blood of the five subjects, including the proband (Figure 4), using a QIAamp® DNA blood mini kit (QIAGEN) according to the manufacturer's instructions. WES was outsourced to Novogene Corp. (Sacramento, CA, USA), and the purified DNA was sequenced on the Illumina NovaSeq6000 system (2×100 bp, paired-end) using a Sure Select Human All Exon V6 (58 Mb) enrichment kit (Agilent

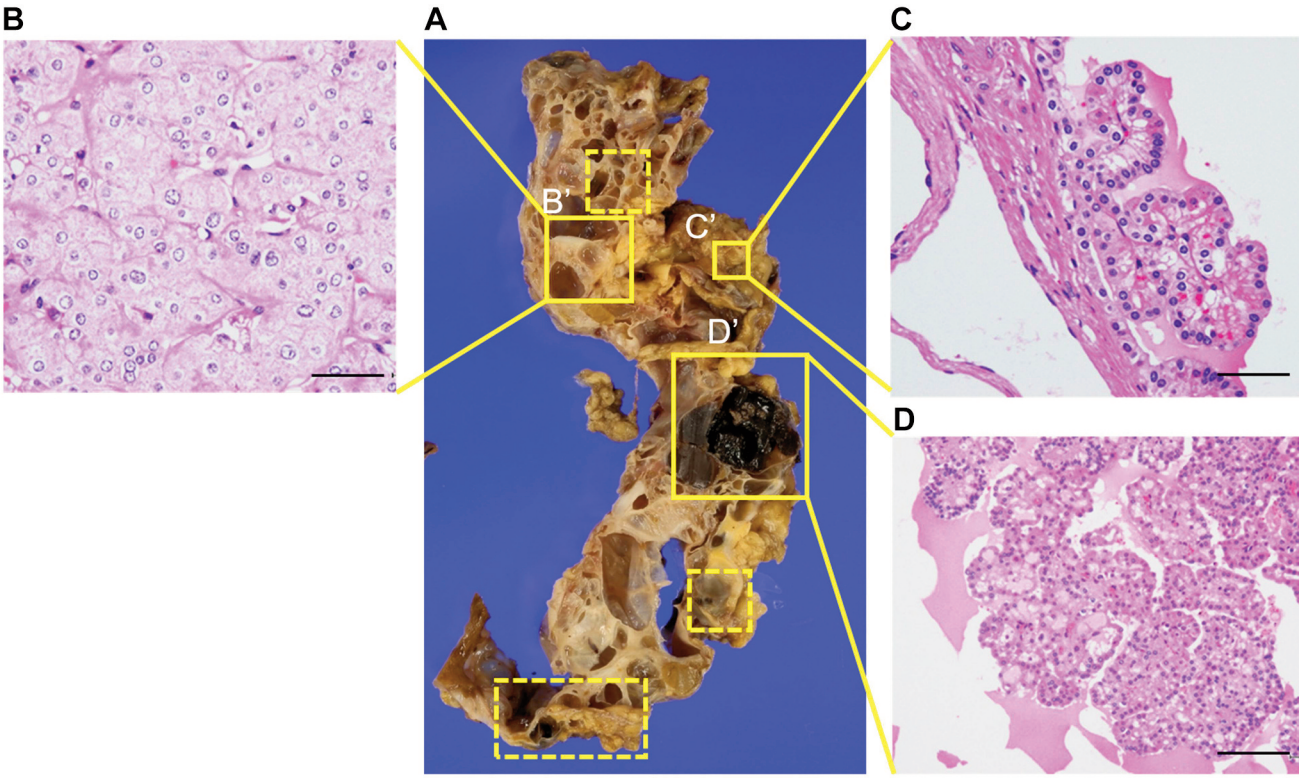

Figure 3. *Left kidney of the proband. (A) Resected left kidney. The yellow squares show the chromophobe renal cell carcinoma (RCC) (B'), clear cell RCC (C') and papillary RCC (D'). The yellow dotted squares show multiple angiomyolipomas. Microscopic view of the chromophobe RCC (B), clear cell RCC (C) and papillary RCC (D). Scale bar, 50 μm.*

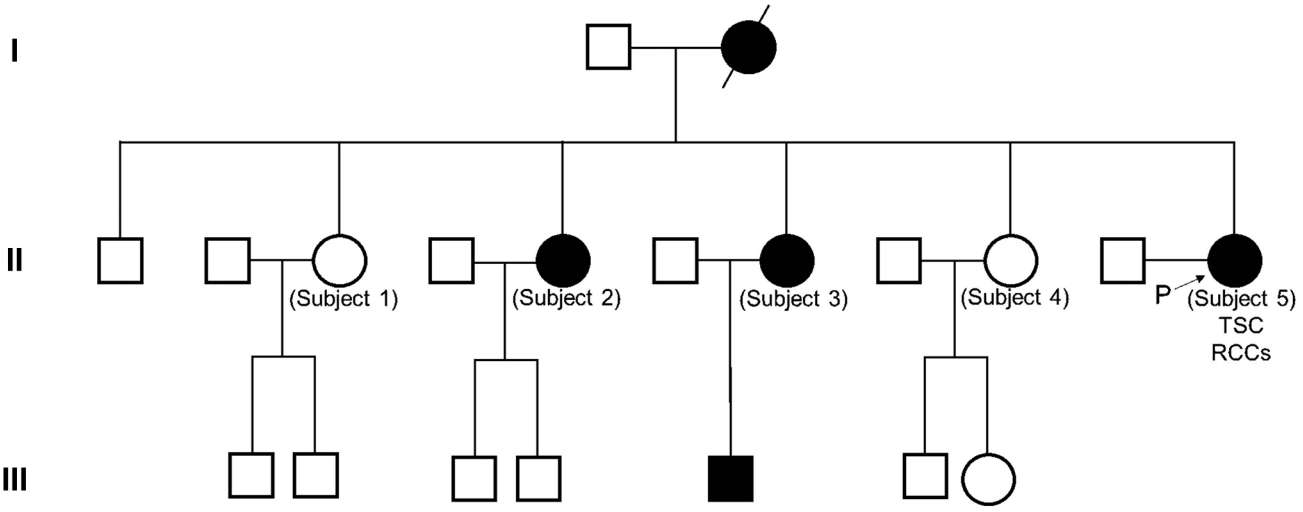

Figure 4. *Family pedigree depicting inheritance of autosomal dominant polycystic kidney disease (ADPKD). Five siblings in a family had ADPKD (subjects 1-5). Subject 5 was the proband in this study and also had tuberous sclerosis complex (TSC) and renal cell carcinomas (RCCs).*

Technologies, Santa Clara, CA, USA). A Burrows-Wheeler aligner was utilized to map the paired-end clean reads versus the human reference genome (hg38). For quality control, paired-end reads were discarded if: one read contained adapter contamination, more than 10% of bases were uncertain in either read, or the proportion of low-quality bases was more than 50% in either of the reads.

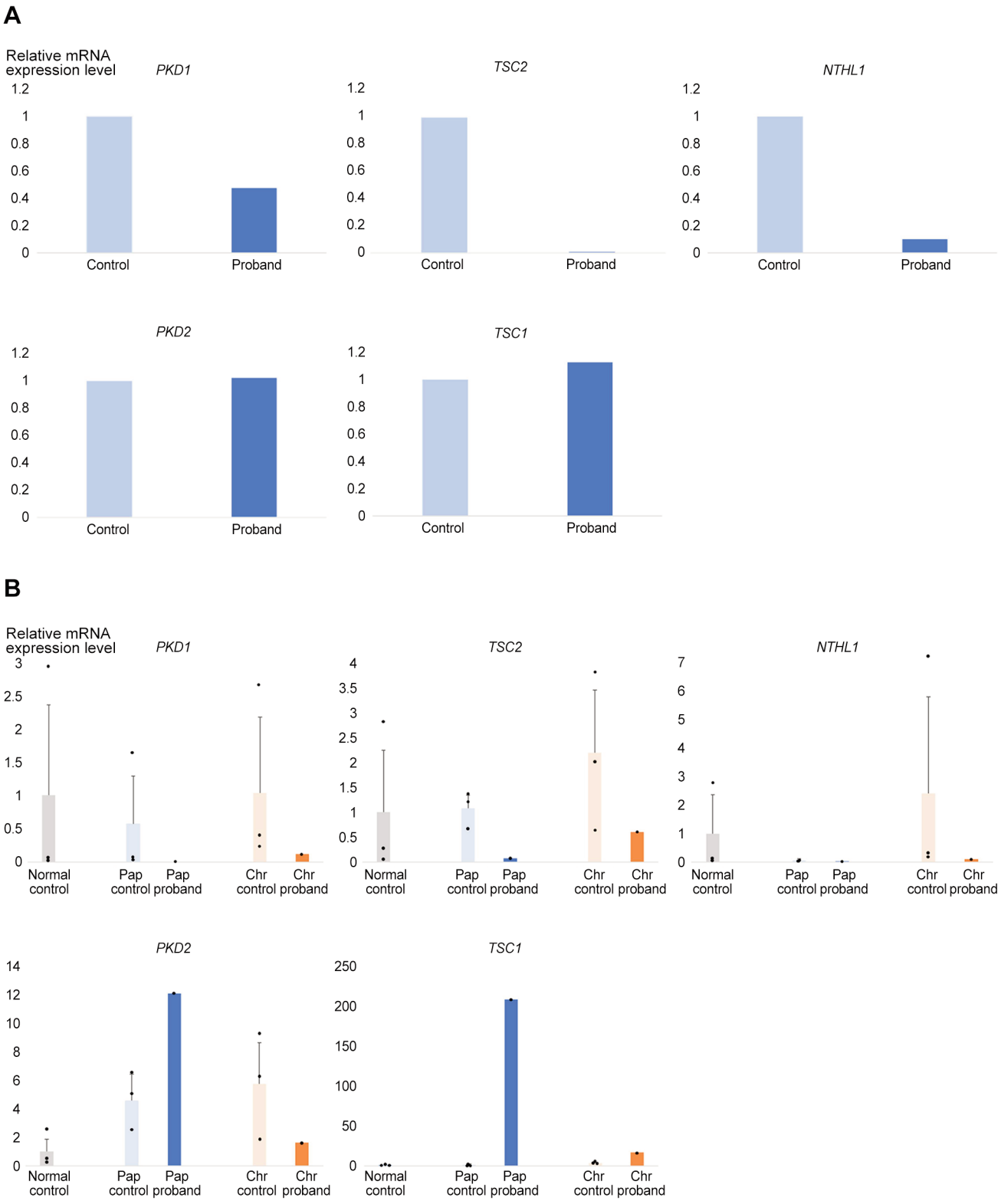

Figure 5. *Relative mRNA expression levels of several targeted genes in the proband's blood and renal cell carcinomas (RCCs) compared to controls. (A) In the proband's blood, relative mRNA expression levels of PKD1, TSC2 and NTHL1 were reduced compared to controls (Each control, n=1). (B) In the proband's papillary and chromophobe RCCs, relative mRNA expression levels of PKD1, TSC2 and NTHL1 were reduced compared to normal or cancer control groups (all control groups, n=3). Pap, Papillary renal cell carcinoma; Chr, chromophobe renal cell carcinoma.*

Table I. *Germline variants in PKD1 and TSC2 in the five subjects.*

Subject	Phenotype	Gene	Location	Mutation	Amino acid change	Type of allele	Type of mutation	Known/ Novel
1	–	PKD1	Intron 1	c.216-2372insG	–	Biallelic	Splice	Known
1	–	TSC2	Intron 9	c.975+183insTT	–	Biallelic	Splice	Known
2	ADPKD	PKD1	Exon 23	c.8731delC	p.Leu2911fs	Uniallelic	Frameshift	Novel
2	ADPKD	PKD1	Intron 12	c.2986-15G>A	–	Uniallelic	Splice	Known
2	ADPKD	PKD1	Intron 22	c.8161+38C>T	–	Biallelic	Splice	Known
2	ADPKD	TSC2	Intron 9	c.976-100C>G	–	Biallelic	Splice	Known
2	ADPKD	TSC2	Intron 10	c.1120-93T>C	–	Biallelic	Splice	Known
2	ADPKD	TSC2	Intron 16	c.1717-55T>C	–	Uniallelic	Splice	Known
2	ADPKD	TSC2	Intron 17	c.1839+422A>C	–	Biallelic	Splice	Known
2	ADPKD	TSC2	Intron 19	c.2098-67G>C	–	Uniallelic	Splice	Known
2	ADPKD	TSC2	Intron 40	c.5161-9C>T	–	Uniallelic	Splice	Known
3	ADPKD	PKD1	Exon 23	c.8731delC	p.Leu2911fs	Uniallelic	Frameshift	Novel
3	ADPKD	PKD1	Intron 12	c.2986-15G>A	–	Biallelic	Splice	Known
3	ADPKD	PKD1	Intron 22	c.8161+21A>G	–	Uniallelic	Splice	Known
3	ADPKD	PKD1	Intron 22	c.8161+42C>G	–	Uniallelic	Splice	Known
3	ADPKD	PKD1	Intron 42	c.11710-88A>G	–	Biallelic	Splice	Known
3	ADPKD	TSC2	Intron 9	c.975+183insT	–	Biallelic	Splice	Known
3	ADPKD	TSC2	Intron 9	c.976-100C>G	–	Biallelic	Splice	Known
3	ADPKD	TSC2	Intron 10	c.1120-93T>C	–	Biallelic	Splice	Known
3	ADPKD	TSC2	Intron 16	c.1716+183A>G	–	Biallelic	Splice	Known
3	ADPKD	TSC2	Intron 16	c.1717-55T>C	–	Uniallelic	Splice	Known
3	ADPKD	TSC2	Intron 19	c.2098-67G>C	–	Uniallelic	Splice	Known
3	ADPKD	TSC2	Intron 40	c.5161-9G>A	–	Uniallelic	Splice	Known
4	–	TSC2	Intron 3	c.225+124insT		Uniallelic	Splice	Known
4	–	TSC2	Intron 9	c.975+183insT	–	Biallelic	Splice	Known
4	–	TSC2	Intron 9	c.976-100C>G	–	Biallelic	Splice	Known
4	–	TSC2	Intron 10	c.1120-93T>C	–	Biallelic	Splice	Known
4	–	TSC2	Intron 16	c.1716+183A>G	–	Biallelic	Splice	Known
4	–	TSC2	Intron 16	c.1717-55T>C	–	Uniallelic	Splice	Known
4	–	TSC2	Intron 19	c.2098-67G>C	–	Uniallelic	Splice	Known
4	–	TSC2	Intron 40	c.5161-9G>A	–	Uniallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	PKD1	Exon 23	c.8731delC	p.Leu2911fs	Uniallelic	Frameshift	Novel
5 (The proband)	ADPKD, TSC and RCCs	PKD1	Intron 12	c.2986-15G>A	–	Biallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 3	c.225+112delT	–	Uniallelic	Splice	Novel
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 7	c.649-111C>T	–	Uniallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 9	c.975+183insTT	–	Biallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 9	c.976-100C>G	–	Biallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 10	c.1120-93T>C	–	Biallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 16	c.1716+183A>G	–	Biallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 16	c.1717-55T>C	–	Uniallelic	Splice	Known
5 (The proband)	ADPKD, TSC and RCCs	TSC2	Intron 40	c.5161-9G>A	–	Uniallelic	Splice	Known

ADPKD, Autosomal dominant polycystic kidney disease; TSC, tuberous sclerosis complex; RCC, renal cell carcinoma.

Informed consent. This study was approved by the ethics committee of our institution (approval number 2019-286). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and its later amendments. Informed consent was obtained from the five study subjects.

Results

mRNA expression levels of targeted genes in the proband's blood and RCC tissues. As shown in Figure 5A, relative mRNA expression levels of *PKD1*, *TSC2* and *NTHL1* were reduced in the proband's blood compared to controls. On the other hand, relative mRNA expression levels of *PKD2* and *TSC1* were slightly increased (Figure 5A). Moreover, as shown in Figure 5B, relative mRNA expression levels of *PKD1*, *TSC2* and *NTHL1* in the proband's RCC tissues, including papillary and chromophobe RCC, were all reduced compared to normal or cancer control groups. In contrast, relative mRNA expression levels of *PKD2* and *TSC1* in the RCCs were not consistently reduced when compared to control groups. Conversely, in the papillary RCC, relative

Table II. *Overview of whole exome sequencing statistics*.

	Subject				
	1	2	3	4	5 (The proband)
Total bases sequenced (Gb)	14.4	13.2	12.1	12.5	13.3
Clean reads in all raw reads (%)	98.9	99.1	99.4	99.1	99.1
The average error rate of all bases (%)	0.03	0.03	0.03	0.03	0.03
Bases with phred-scaled quality scores greater than 30 (%)	93.7	97.4	97.3	97.3	97.4
The total number of clean reads (n) (%)	95,157,424 (100)	87,045,252 (100)	80,386,248 (100)	82,640,134 (100)	87,958,410 (100)
The number of duplicated reads (n) (%)	23,799,862 (25.0)	19,307,065 (22.2)	18,554,635 (23.1)	18,844,725 (22.8)	21,615,555 (24.6)
The number of reads that mapped to the reference genome (n) (%)	95,090,747 (99.9)	86,961,528 (99.9)	80,310,211 (99.9)	82,567,880 (99.9)	87,877,123 (99.9)
The number of reads that mapped to the reference genome and within the expected insert size (n) (%)	93,524,876 (98.3)	85,635,458 (98.4)	79,240,606 (98.6)	81,282,298 (98.4)	86,575,692 (98.4)
The number of pair-end reads that mapped to the reference genome (n) (%)	95,031,220 (99.9)	86,886,682 (99.8)	80,243,836 (99.8)	82,504,336 (99.8)	87,803,916 (99.8)
The number of reads with mate reads mapped to different chromosomes (n) (%)	1,346,550 (1.4)	1,120,616 (1.3)	879,958 (1.1)	1,095,270 (1.3)	1,107,006 (1.3)
The number of bases in the target region (n)	60,250,365	60,250,365	60,250,365	60,250,365	60,250,365
The number of bases in the target region or flanking region of the target (n)	135,737,108	135,737,108	135,737,108	135,737,108	135,737,108
The data size of the effective reads mapped to the reference genome (Mb)	14,226.0	13,007.3	12,013.3	12,352.1	13,144.5
The data size of the reads mapped to the target region (Mb)	10,533.7	9,505.1	8,562.7	8,996.5	9,564.0
Bases mapped to the target region in all bases mapped to the reference genome (%)	74.0	73.1	71.3	72.8	72.8
Bases mapped to the target or flanking region in all bases mapped to the reference genome (%)	95.1	95.1	95.0	95.1	95.1
The average sequencing depth in the target region	174.8	157.8	142.1	149.3	158.7
The number of the bases covered in the target region (n)	59,945,125	59,938,140	59,938,646	59,929,124	59,929,782
The coverage of target regions (%)	99.5	99.5	99.5	99.5	99.5
Bases with depth >100× in target regions (%)	73.1	69.1	64.4	66.4	69.4
Bases with depth >50× in target regions (%)	89.8	88.3	87.0	87.5	88.4
Bases with depth >20× in target regions (%)	96.7	96.2	96.0	96.1	96.2
Bases with depth >10× in target regions (%)	98.3	98.1	98.1	98.1	98.1
Bases with depth >4× in target regions (%)	99.1	99.1	99.1	99.1	99.1

Table III. *Specific germline variants in PKD1 among the three subjects [Subject 2, 3 and 5 (proband)] with autosomal dominant polycystic kidney disease (ADPKD).*

Specific in Subject 2,3 and 5 (with ADPKD)							
Phenotype	Gene	Location	Mutation	Amino acid change	Type of allele	Type of mutation	Known/Novel
ADPKD	PKD1	Exon 23	c.8731delC	p.Leu2911fs	Uniallelic	Frameshift	Novel
ADPKD	PKD1	Intron 12	c.2986-15G>A	–	Biallelic	Splice	Known

Table IV. *Specific germline variants in TSC2 in the proband (subject 5) with autosomal dominant polycystic kidney disease (ADPKD), tuberous sclerosis complex (TSC) and renal cell carcinomas (RCCs).*

Specific in Subject 5 (the proband) (with ADPKD, TSC and RCC)							
Phenotype	Gene	Location	Mutation	Amino acid change	Type of allele	Type of mutation	Known/Novel
ADPKD, TSC and RCCs	TSC2	Intron 3	c.225+112delT	–	Uniallelic	Splice	Novel
ADPKD, TSC and RCCs	TSC2	Intron 7	c.649-111C>T	–	Uniallelic	Splice	Known

mRNA expression levels of both *PKD2* and *TSC1* were increased compared to control groups, possibly to compensate for the reduced *PKD1* and *TSC2* expression, respectively.

Variants. WES revealed germline variants in *PKD1* and *TSC2* in the five subjects (Table I). An overview of sequencing statistics is shown in Table II. Among the three subjects with ADPKD, including the proband [Subject 2, 3 and 5 (proband) in Figure 4], there was a novel frameshift variant with an amino acid change in the *PKD1* exon (c.8731delC, p.Leu2911fs). The specific variants in these three subjects are shown in Table III. Besides the above variant in the *PKD1* exon, there was one common variant in the *PKD1* intron (c.2986-15G>A). The specific variants in the proband with ADPKD, TSC and RCC are shown in Table IV. There were two variants in the *TSC2* intron (c.225+112delT; c.649-111C>T) specific to the proband. However, there were no variants in *NTHL1* in any of the five subjects.

Discussion

In this study, in the proband with ADPKD, TSC and RCCs, relative mRNA expression levels of three contiguous genes on chromosome 16p13, *i.e.*, *PKD1*, *TSC2* and *NTHL1*, were concurrently reduced in both blood and RCC tissues compared to control subjects. Moreover, through WES, we identified two variants in *PKD1* specific to the three subjects with ADPKD, including the proband, and two variants in *TSC2* specific to the proband with ADPKD, TSC and RCCs. Of the two variants in *PKD1* specific to the three subjects with ADPKD, we regarded the variant in the *PKD1* exon (c.8731delC) as being pathogenic, rather than the variant in the *PKD1* intron (c.363+5C>T), because the variant in the *PKD1* exon showed evidence of amino acid exchange.

The proband had RCCs in addition to two inherited diseases, ADPKD and TSC. Since there were multiple pathologically diverse RCCs in both kidneys in the proband, a genetic cause was suspected. In fact, both ADPKD and TSC have been reported to be associated with a high risk for development of RCC (17, 18). However, to the best of our knowledge, there have been no reports of multiple pathologically diverse RCCs in patients with ADPKD or TSC, as in this case. Therefore, we focused on another gene that was adjacent to *TSC2* and is known to be associated with carcinogenesis: *NTHL1* (13, 14). Biallelic *NTHL1* mutations have been reported to predispose to the development of cancers such as colorectal and breast cancer, and also those in genitourinary organs, *e.g.*, urothelial cell and prostate cancer (15). However, there has been no report about RCC caused by *NTHL1* mutations. In the proband of this study, expression of *NTHL1* was reduced in the blood and two types of RCCs. Therefore, reduction of expression of *NTHL1* might have affected the development of the pathologically diverse RCCs.

Interestingly, in terms of the *TSC2* and *NTHL1* regions in the proband, only the two variants in the *TSC2* intron (c.225+112delT; c.649-111C>T) were specific to the proband, while there was no variant in *NTHL1*. Although the proband had a pathogenic variant in the *PKD1* exon (c.8731delC), this variant was not specific to the proband, and two subjects (Subject 2 and 3) without TSC or RCC also had this variant. Therefore, the *PKD1* variant was not likely to have been the cause of the TSC and RCC phenotypes in

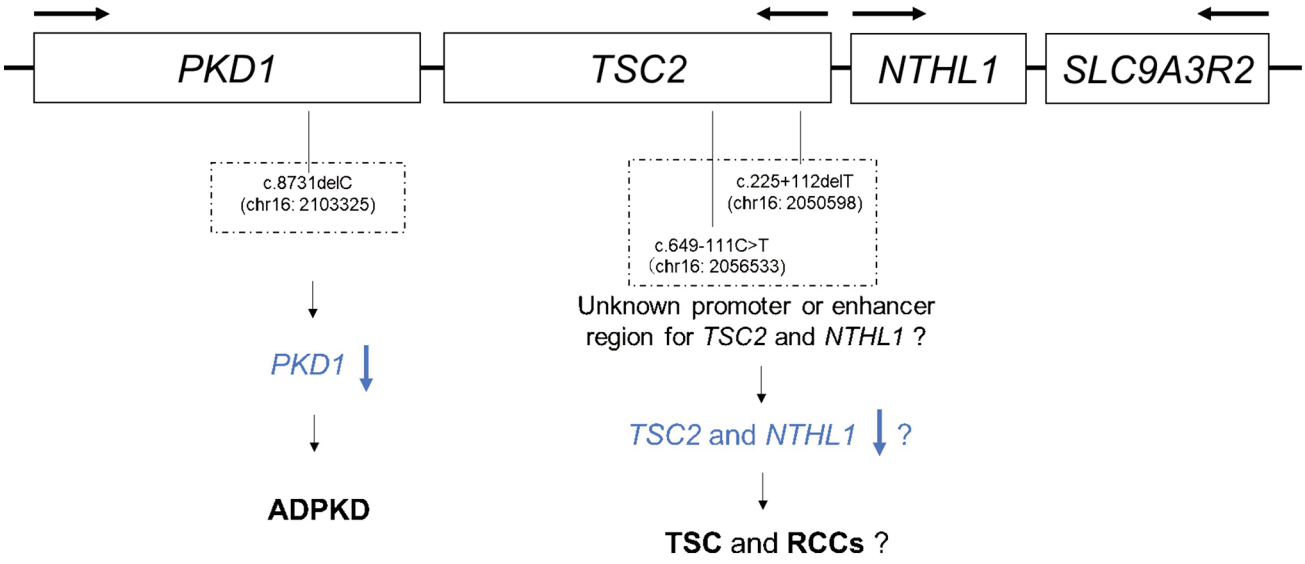

Figure 6. *Hypothesis on the pathophysiological mechanisms related to chromosome 16p13 variants in the proband of this study. While autosomal dominant polycystic kidney disease (ADPKD) was caused by the variant in the PKD1 exon, tuberous sclerosis complex (TSC) and renal cell carcinomas (RCCs) might have been caused by one or both of the variants in the TSC2 intron.*

the proband. On the other hand, the two variants in the *TSC2* intron were specific to the proband. From previous studies, even a single nucleotide polymorphism (SNP) in the intron has the potential to affect phenotypes, such as eye color and breast cancer (19, 20). Moreover, in another report, an SNP in promotor and enhancer function regions in the intron affected their activity through non-code RNA as a risk variant (21).

A promotor is a DNA sequence that directs the accurate initiation of transcription and regulates gene expression (22, 23), while an enhancer is a DNA sequence involving multiple binding sites for a variety of transcription factors that is able to activate transcription (24, 25). Referring to the Gene Cards human gene database (https://www.genecards.org/), there are several known promotor and enhancer regions in *TSC2* targeting both *TSC2* and *NTHL1*, although the promotor and enhancer regions involving the sites of the two variants in the *TSC2* intron in this case have not been mentioned. Moreover, there were no other variants specific to the proband in several genes surrounding NTHL1, including *SLC9A3R2,* which is adjacent to NTHL1. Therefore, as shown in Figure 6, we hypothesized that one or both of the variants in the *TSC2* intron might have affected the expression of both *TSC2* and *NTHL1* in an unknown promotor or enhancer region, and consequently might have led to the phenotypes of TSC and RCCs. In other words, unlike previously reported cases that involved a large deletion over several genes (6, 7), one or both of the *TSC2* intron variants might have caused TSC and RCCs, while the variant in the *PKD1* exon caused ADPKD. Generally, however, reduction of expression of *NTHL1* is caused by biallelic mutations (12), although the two variants in the *TSC2* intron in this case were uniallelic. Therefore, how the uniallelic variants in the *TSC2* intron effected biallelic expression of *NTHL1* was unclear, suggesting that there might have been some other additional causes for this, including an epigenetic factor.

Several limitations must be considered in this study. First, the proband's clear cell RCC could not be used for analysis of mRNA expression and, therefore, not all types of RCC were examined. Second, regarding *TSC2* and *NTHL1*, we only identified the DNA variants and examined mRNA expression, and did not investigate an intermediate series or a causal relationship between the two phenomena. Therefore, we need to conduct further research with greater focus on the *TSC2* and *NTHL1* regions to elucidate the mechanisms of the phenotypes in this case.

Conclusion

In conclusion, we reported a novel pathogenic variant in the *PKD1* exon in a family, and two variants in the *TSC2* intron with possible effect on the expression of both *TSC2* and *NTHL1* in the proband. In the proband, while ADPKD was caused by the variant in the *PKD1* exon, TSC and the multiple pathologically diverse RCCs might have been caused by one or both the variants in the *TSC2* intron. Since the detailed mechanisms of this phenomenon are, however, unclear, further research focusing on the *TSC2* and *NTHL1* regions, including epigenetic functions, should be conducted.

Database Information

Accession number and URLs for the data in this article are as follows: ADPKD Mutation Database: https://pkdb.mayo.edu/ (for variants in *PKD1*); Gene Cards human gene database: https://www.genecards.org/ (for promoters or enhancers of *TSC2* and *NTHL1*).

Conflicts of Interest

The Authors have no conflicts of interest and received no additional funding for this work.

Authors' Contributions

Koguchi Tomoyuki, Yusuke Hakozaki, Akifumi Onagi, Kanako Matsuoka, Seiji Hoshi, Junya Hata, Yuichi Sato, Hidenori Akaihata, Masao Kataoka and Soichiro Ogawa performed the experiments and evaluated the data. Satoru Meguro drafted the manuscript. Supervision and project administration was performed by Yoshiyuki Kojima.

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