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A Novel Cryptic t(2;3)(p21;q25) Translocation Fuses the *WWTR1* and *PRKCE* Genes in Uterine Leiomyoma With 3q- as the Sole Visible Chromosome Abnormality

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Abstract. *Background/Aim: Deletions in the q arm of chromosome 3 have been reported in uterine leiomyomas, also as sole anomalies. Because some neoplasia-associated deletions may give rise to tumorigenic fusion genes, we chose to investigate thoroughly one such tumor. Materials and Methods: A uterine leiomyoma obtained from a 45-year-old woman had the karyotype 46,XX,del(3)(q?)[11]. The tumor was further studied using array comparative genomic hybridization, RNA sequencing, reverse transcription polymerase chain reaction, Sanger sequencing, and fluorescence in situ hybridization methodologies. Results: The deletion was shown to be from 3q22.2 to 3q26.32. Unexpectedly, a cryptic balanced t(2;3)(p21;q25) translocation was also found affecting two otherwise normal chromosomes 2 and 3, i.e., the der(3)t(2;3) was not the deleted chromosome 3. The translocation generated two chimeras between the genes WW domain containing transcription regulator 1 (WWTR1) from 3q25.1 and protein kinase C epsilon (PRKCE) from 2p21. The WWTR1::PRKCE fusion would code for a chimeric serine/threonine kinase, whereas the reciprocal PRKCE::WWTR1 fusion would code for a chimeric transcriptional coactivator protein. Conclusion: Leiomyomas carrying a deletion on 3q may also have a balanced t(2;3)(p21;q25) leading to fusion of WWTR1 with PRKCE.*

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Key Words: Uterine leiomyoma, deletion 3q, cryptic translocation, t(2;3)(p21;q25), WW domain containing transcription regulator 1 (*WWTR1*), protein kinase C epsilon (*PRKCE*), *WWTR1::PRKCE*, *PRKCE::WWTR1*, chimeric serine/threonine kinase, chimeric transcriptional coactivator.

Uterine leiomyomas or fibroids are the most common neoplasms found in more than 70% of women during their reproductive age and later (1, 2). In 30% to 50% of patients, the tumors may cause menorrhagia (heavy menstrual bleeding), pelvic pain/pressure, infertility and other morbidities (1) that affect the quality of life (3, 4). Uterine leiomyomas are benign monoclonal tumors (5) arising from a single myometrial stem cell that, by acquiring somatic mutations, has been transformed into a neoplastic stem cell capable of development into a leiomyoma (5-7).

Cytogenetic examinations of uterine leiomyomas have revealed that 25-40% of them carry nonrandom chromosome aberrations, the most common of which is the translocation t(12;14)(q14~15;q23~24) found in 15-20% of karyotypically abnormal leiomyomas (8-27). A small number of uterine leiomyomas are cytogenetically characterized by a deletion of the q arm of chromosome 3 (10, 11, 15, 21, 22, 28). We present here our molecular findings in a leiomyoma carrying a deletion on 3q.

Materials and Methods

Ethics statement. The study was approved by the Regional committee for medical and health research ethics. Written informed consent was obtained from the patient for publication of the case details. The ethics committee's approval included a review of the consent procedure. All patient information has been de-identified.

Tumor description and Immunohistochemistry. A 45-year-old woman underwent surgery because of a clinical diagnosis of leiomyoma. The

weight of the hysterectomy specimen, with attached adnexae, was 1.9 kg. A 17 cm in diameter leiomyomatous tumor was seen in the uterus. Microscopic evaluation showed a smooth muscle tumor (Figure 1A) with no evidence of atypia (Figure 1B), increased mitotic activity or necrosis. Some areas with degenerative changes were seen. Desmin immunostaining was performed using a monoclonal mouse antibody (clone D33) from Dako/Agilent (Glostrup, Denmark) using the Dako EnVision Flex + System (K8012; Dako). A 1:30 dilution with antigen retrieval at HpH was applied. Immunohistochemical staining showed expression of desmin (Figure 1C). The tumor was diagnosed as leiomyoma.

G-banding and karyotyping. Fresh tissue from the tumor was minced using scalpels into 1-2 mm fragments, enzymatically disaggregated using collagenase II (Worthington, Freehold, NJ, USA), and the resulting cells were cultured, harvested, and processed for cytogenetic examination (29). Chromosome preparations were G-banded with Wright's stain (Sigma-Aldrich; St Louis, MO, USA) and examined (29). The karyotype was written according to The International System for Human Cytogenomic Nomenclature (ISCN) 2016 guidelines (30).

Array comparative genomic hybridization (aCGH). Genomic DNA was extracted from the tumor using the Maxwell RSC Instrument and Maxwell RSC Tissue DNA Kit (Promega, Madison, USA). The concentration was measured with the Quantus Fluorometer and the QuantiFluor ONE dsDNA System (Promega). Promega's human genomic female DNA was used as reference DNA. aCGH was performed using CytoSure array products (Oxford Gene Technology, Begbroke, Oxfordshire, UK) according to the company's protocols. Thus, the CytoSure Genomic DNA Labelling Kit was used for the labelling of 1 μg of each of tumor and reference DNA and the CytoSure Cancer +SNP array for hybridization. The slides were scanned in an Agilent SureScan Dx microarray scanner using Agilent Feature Extraction Software (version 12.1.1.1). Data were analyzed with the CytoSure Interpret analysis software (version 4.9.40). Annotations are based on human genome build 19.

RNA sequencing. Total RNA was extracted from a frozen (-80 °C) part of the specimen adjacent to areas used for cytogenetic analysis and histologic examination using miRNeasy Mini Kit (Qiagen, Hilden, Germany). One μg of total RNA was sent to the Genomics Core Facility at the Norwegian Radium Hospital, Oslo University Hospital for high-throughput paired-end RNA-sequencing and a total of 185×10^6 101-bp-length-reads were obtained. The FASTQC software was used for quality control of the raw sequence data (available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Fusion transcripts were found using the FusionCatcher software (31, 32).

Reverse transcription (RT) PCR and Sanger sequencing. In order to confirm the existence of the fusion transcripts (see below), RT-PCR and Sanger sequencing analyses were performed. The primers used for PCR amplifications and Sanger sequencing are shown in Table I. Detailed information on synthesis of cDNA, PCR amplification, and Sanger sequencing methodologies were given elsewhere (33).

The sequences obtained by Sanger sequencing were compared with the NCBI reference sequences NM_015472.4 [WW domain containing transcription regulator 1 (*WWTR1*), transcript variant 1, mRNA] and NM_005400.3 [protein kinase C epsilon (*PRKCE*), mRNA] using the Basic Local Alignment Search Tool (BLAST)

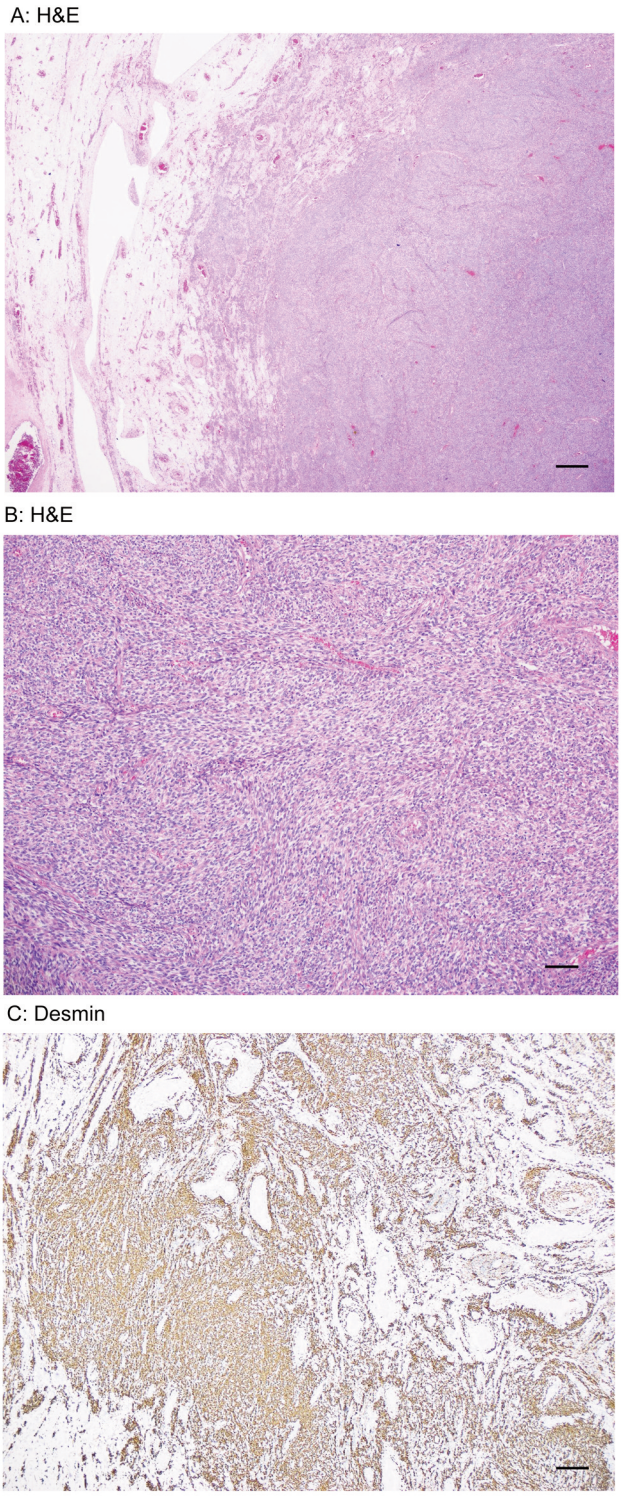

Figure 1. *Microscopic examination of the uterine leiomyoma. (A) Hematoxylin and eosin (H&E) stained section showing a smooth muscle tumor with varying degree of cellularity; scale bar represents 100 μm. (B) H&E staining showing smooth muscle cells with no evidence of atypia or increased mitotic activity; scale bar represents 50 μm. (C) Immunohistochemical staining showing expression of desmin in the uterine leiomyoma; scale bar represents 100 μm.*

Table I. *Designation, sequence (5'->3'), and position in reference sequences of the forward (F) and reverse (R) primers of the WW domain containing transcription regulator 1 (WWTR1) and the protein kinase C epsilon (PRKCE) genes which were used for polymerase chain reaction (PCR) amplification and Sanger sequencing analyses. For Sanger sequencing analyses the forward primers WWTR1-1002F1 and PRKCE-710F1 had the M13 forward primer sequence TGTAAAACGACGGCCAGT at their 5'-end. The reverse primers PRKCE-862R1 and WWTR1-1171R1 the M13 reverse primer sequence CAGGAAACAGCTATGACC had at their 5'-end.*

Designation	Sequence (5'->3')	Reference sequence: Position
WWTR1-996F1	TGA GTA TGC CCA ATG CGC TGA CCA	NM_015472.4: 996-1019
WWTR1-1002F1	TGC CCA ATG CGC TGA CCA CTC A	NM_015472.4: 1002-1023
PRKCE-904R1	ACC TGA TGG ACC CTG CGC CTG AC	NM_005400.3: 926-904
PRKCE-862R1	GCC GCA TGC GTT CCC TGA ACA C	NM_005400.3: 883-862
PRKCE-700F1	GAC GAC TTC GTG GCC AAC TGC	NM_005400.3: 700-720
PRKCE-710F1	TGG CCA ACT GCA CCA TCC AGT	NM_005400.3: 710-730
WWTR1-1188R1	GGA TCT CAT GTC TGG GGT CAT CG	NM_015472.4: 1210-1188
WWTR1-1171R1	ATC GTG GGT GGG TTG ACA GCA	NM_015472.4: 1191-1171

(34). They were also aligned on the Human GRCh37/hg19 assembly using the BLAST-like alignment tool (BLAT) and the human genome browser hosted by the University of California, Santa Cruz (35, 36).

Fluorescence in situ hybridization (FISH). Both metaphase plates and interphase nuclei were examined using the ZytoLight SPEC *WWTR1* Dual Color Break Apart Probe (Zytovision, Bremerhaven, Germany). The probe is designed for detection of translocations involving the chromosomal area 3q25.1 harboring the *WWTR1* gene and is a mixture of green and orange fluorochrome-labeled probes that hybridize to proximal and distal parts of the *WWTR1* gene, respectively. According to the human genome assembly GRCh37/hg19, the green-labeled part hybridizes to chr3:148533200-149234601 whereas the orange-labeled part hybridizes to chr3:149430325-149933565. Also, a homemade double fusion FISH probe was used to detect the fusion of *WWTR1* with *PRKCE*. BAC probes were purchased from the BACPAC Resource Center operated by BACPAC Genomics, Emeryville, CA (https://bacpacresources.org/) (Table II). The FISH probes were prepared from bacteriophage Phi29 DNA polymerase amplified BAC DNAs using previously described methodology (37) and kits for DNA isolation, amplification, labelling and hybridization, all according to the manufacturers' recommendations. In brief, single isolated bacterial colonies were grown in 5 ml culture overnight and BAC DNA was purified from them using High Pure Plasmid Isolation Kit (Roche Diagnostics, Mannheim, Germany). Following purification, BAC DNAs were isothermally amplified with Phi29 DNA polymerase using the GenomiPhi V2 DNA Amplification Kit (Cytiva, Marlborough, MA, USA). Finally, amplified BAC DNAs were labelled and hybridized using Abbott's nick translation kit (Abbott Molecular, Des Plaines, IL, USA). The probes for *WWTR1* were labelled with Texas Red-5-dCTP (PerkinElmer, Boston, MA, USA) to obtain a red signal. The probes for *PRKCE* were labelled with fluorescein-12-dCTP (PerkinElmer) to obtain a green signal. Mapping of the clones on normal controls was performed to confirm their chromosomal location. The probe for *PRKCE* was found to cross-hybridize to the p arm of chromosome 16. However, this cross-hybridization did not interfere with the interpretation. Detailed information on the FISH procedure has been given elsewhere (33, 38). Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

Results

Cytogenetics and aCGH analyses. The initial G-banding analysis revealed a deletion on the long arm of chromosome 3 in all eleven examined metaphases but neither the breakpoint(s) region nor band(s) could be identified. Thus, the karyotype was described as 46,XX,del(3)(q?)[11]. In order to map the deletion on 3q, aCGH was performed that showed a 44 Mbp interstitial deletion from q22.2 to q26.32 (Figure 2A). On 3q22.2, the deletion started within intron 1 of the EPH receptor B1 (*EPHB1*) gene (Figure 2B) whereas it ended on 3q26.32 between the two genes potassium calcium-activated channel subfamily M regulatory beta subunit 2 (*KCNMB2*), which was included in the deletion, and zinc finger matrin-type 3 (*ZMAT3*) which was distal to the deletion (Figure 2C). The deleted region includes more than 150 genes, among them *WWTR1* (chr3:149,235,022-149,375,812) (Figure 2A).

RNA sequencing, RT-PCR, and Sanger sequencing analyses Analysis of raw sequencing data using FusionCatcher detected reciprocal in-frame fusion transcripts of *WWTR1* from chromosome subband 3q25.1 with *PRKCE* from chromosome band 2p21. In the *WWTR1::PRKCE* transcript, exon 4 of *WWTR1* (nucleotide 1111 in sequence NM_015472.4) fused with exon 2 of *PRKCE* (nucleotide 775 in sequence NM_005400.3): GAGAGAAAGGATTCG AATGCGCCAAGAGGAGCTCATGAGGCAG*ATTGATCT GGAGCCAGAAGGAAGAGTGTATGTGATCATCGATC. In the *PRKCE::WWTR1* transcript, exon 1 of *PRKCE* (nucleotide 774 in sequence NM_005400.3) fused to exon 5 of *WWTR1* (nucleotide 1112 in sequence NM_015472.4): TGAGGAGCTGCTGCAGAACGGGAGCCGCCACTTCGA GGACTGG*GAAGCTGCCCTCTGTCGACAGCTCCCCAT GGAAGCTGAGACTC.

RT-PCR using WWTR1-996F1 and PRKCE-904R1 primer combinations amplified a 268 bp cDNA fragment (Figure

Table II. *BAC probes used for fluorescence in situ hybridization (FISH) experiments to detect the fusion genes. The position and the accession numbers of the PRKCE and WWTR1 genes are also given.*

BAC clones	Accession number	Chromosome mapping	Targeted gene	Position on GRCh38/ hg38 assembly	Labelling
RP11-576F1	AC008179.2	2p21	*PRKCE*	chr2:45351841-45533385	Green
RP11-425J3	AC012072.2	2p21	*PRKCE*	chr2:45533386-45638871	Green
RP11-119J12	AC092600.2	2p21	*PRKCE*	chr2:45583894-45757779	Green
	NM_005400.3 (*PRKCE*)	2p21	*PRKCE*	chr2:45651675-46187990	
RP11-457N9	AC017078.8	2p21	*PRKCE*	chr2:45813135-46006913	Green
RP11-110G2	AC017006.4	2p21	*PRKCE*	chr2:46024428-46216180	Green
RP11-130P22	AC016696.8	2p21	*PRKCE*	chr2:46216181-46323611	Green
				chr2:46216181- 46325611	
RP11-278L15	AC108751.10	3q25.1	*WWTR1*	chr3:149374155-149504048	Red
RP11-255N4	AC012014.20	3q25.1	*WWTR1*	chr3:149504049-149701558	Red
	NM_015472.6 (*WWTR1*)	3q25.1	*WWTR1*	chr3:149517235-149703273	
RP11-94F21	AC108672.13	3q25.1	*WWTR1*	chr3:149583325-149752254	Red
RP11-621D6	AC069216.13	3q25.1	*WWTR1*	chr3:149718036-149866181	Red

3A) which by Sanger sequencing was shown to confirm the *WWTR1::PRKCE* fusion transcript detected by RNA sequencing/FusionCatcher analysis (Figure 3B). RT-PCR with PRKCE-700F1 and WWTR1-1188R1 primers amplified a 174 bp cDNA fragment (Figure 3C) which by Sanger sequencing was shown to confirm the *PRKCE::WWTR1* fusion transcript detected by RNA sequencing/FusionCatcher (Figure 3D).

FISH analyses. Using the commercially available *WWTR1* dual color break apart probe on metaphase spreads, the proximal green-labeled part of the probe hybridized on the q arm of a seemingly normal chromosome 3 whereas the distal orange-labeled part of the probe hybridized on the p arm of a seemingly normal chromosome 2 (Figure 3E). Orange/green fusion signal representing normal (non-rearranged) *WWTR1* on 3q25 was absent, corresponding to the deletion seen by G-banding and aCGH.

Hybridization with a homemade double fusion *WWTR1*::*PRKCE* FISH probe detected a green signal corresponding to *PRKCE* on chromosome band 2p21 and two green/red fusion signals on seemingly normal chromosomes 2 and 3 corresponding to the *WWTR1::PRKCE* (on 2p21) and *PRKCE::WWTR1* (on 3q25.1) reciprocal fusion genes (Figure 3F). The red signal corresponding to the *WWTR1* normal locus on 3q25.1 was absent (Figure 3F). Two green signals on the p arms of both chromosomes 16, highlighted by cross-hybridization of the *PRKCE* probe, were also seen (Figure 3F). This cross-hybridization did not interfere with data interpretation.

Corrected karyotype. Based on the findings in the above-mentioned experiments, the new and corrected karyotype of the examined uterine leiomyoma became: 46,XX,t(2;3)(p21;q25), del(3)(q22q26).

Discussion

In the present study, we genetically analyzed a uterine leiomyoma which, in the initial cytogenetic examination, had a deletion of the q arm of one chromosome 3. The deletion's exact position and extent could not be determined by G-banding. The rationale behind our decision to examine the tumor further was that deletions sometimes generate fusion genes (33, 39).

By aCGH, the deletion was found to correspond to a del(3)(q22.2q26.32) that did not result in any fusion gene but caused allelic loss of more than 150 genes, among them *WWTR1* (Figure 2). Further examinations by a combination of RNA sequencing, RT-PCR/Sanger sequencing, and FISH methodologies then revealed a cryptic, balanced t(2;3)(p21;q25) translocation affecting the two seemingly normal (i.e., non-deleted) chromosomes 2 and 3 (Figure 4). This translocation resulted in the fusion of the 5'-part of *WWTR1* (exons 1 to 4) with the 3'-part of *PRKCE* (exons 2 to 15) on der(2)t(2;3)(p21;q25) and fusion of the 5'-part of *PRKCE* (exon 1) with the 3'-part of *WWTR1* (exons 5 to 7) on der(3)t(2;3)(p21;q25) (Figure 4). The t(2;3)(p21;q25) translocation is, to the best of our knowledge, novel; at least it is not listed among the chromosome aberrations of 72718 karyotypically abnormal neoplasms registered in the "Mitelman database of chromosome aberrations and gene fusions in cancer" (last updated on June 6, 2022) (40). Searching the same database and the literature on leiomyoma cytogenetics, we found, in addition to the present case, twelve more uterine leiomyomas reported to carry a deletion in the

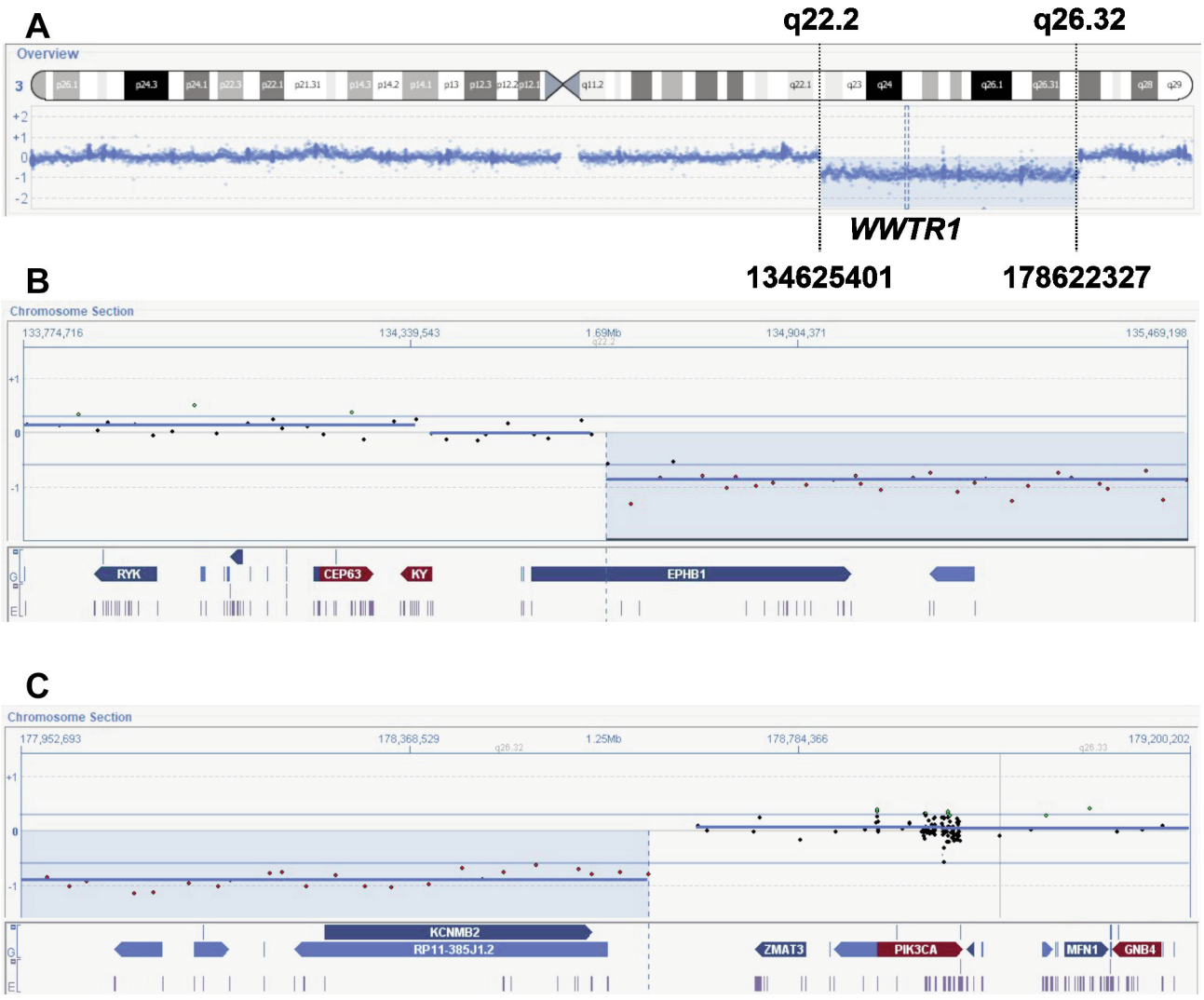

Figure 2. *Array comparative genomic hybridization examination of the uterine leiomyoma. (A) Genetic profile of whole chromosome 3 showing the deletion in the q arm of chromosome 3. Based on the hg19 assembly, the deletion started at position Chr3:134625401 on subband q22.2 and ended at Chr3:178622327 on subband q26.32. (B) The deletion started within intron 1 of the EPH receptor B1 (EPHB1) gene. (C) The deletion ended between the two genes of potassium calcium-activated channel subfamily M regulatory beta subunit 2 (KCNMB2), included in the deletion, and zinc finger matrin-type 3 (ZMAT3), distal to the deletion.*

3q arm (Table III) (10, 11, 15, 16, 21, 22, 41, 42). Partial karyotypes of the seemingly normal, non-deleted chromosome 3 together with 3q- were provided for some tumors, but because der(3)t(2;3)(p21;q25) is practically indistinguishable by inspection from a normal chromosome 3 even in good preparations, we cannot know whether a t(2;3)(p21;q25) existed in some of those cases (10, 16, 21, 41). It is therefore possible that at least some of the tumors carrying a deletion on 3q as what seemed to be a solitary aberration, were genetically similar to the tumor of the present study with loss of one *WWTR1* allele due to deletion occurring together with a cryptic t(2;3)(p21;q25) that gave rise to the two reciprocal fusion genes *WWTR1::PRKCE* and *PRKCE::WWTR1*. Alternatively, the simultaneous presence of both del(3q) and t(2;3) could be a fluke event without the existence of any contributory pathogenetic effect of one aberration on the other.

The *WWTR1* gene codes for a transcription factor which is involved in the Hippo signaling pathway (43, 44). The WWTR1 protein contains an N-terminal region which interacts with the TEAD family of transcription factors, a 14-3-3 binding site, a WW-domain, a transcription activation domain, and, at the C-terminal end, a conserved PDZ-domain (45-47). *WWTR1* has been reported to fuse, as a 5'-end partner in various tumors (48-54). In epithelioid hemangioendothelioma, *WWTR1* fuses with

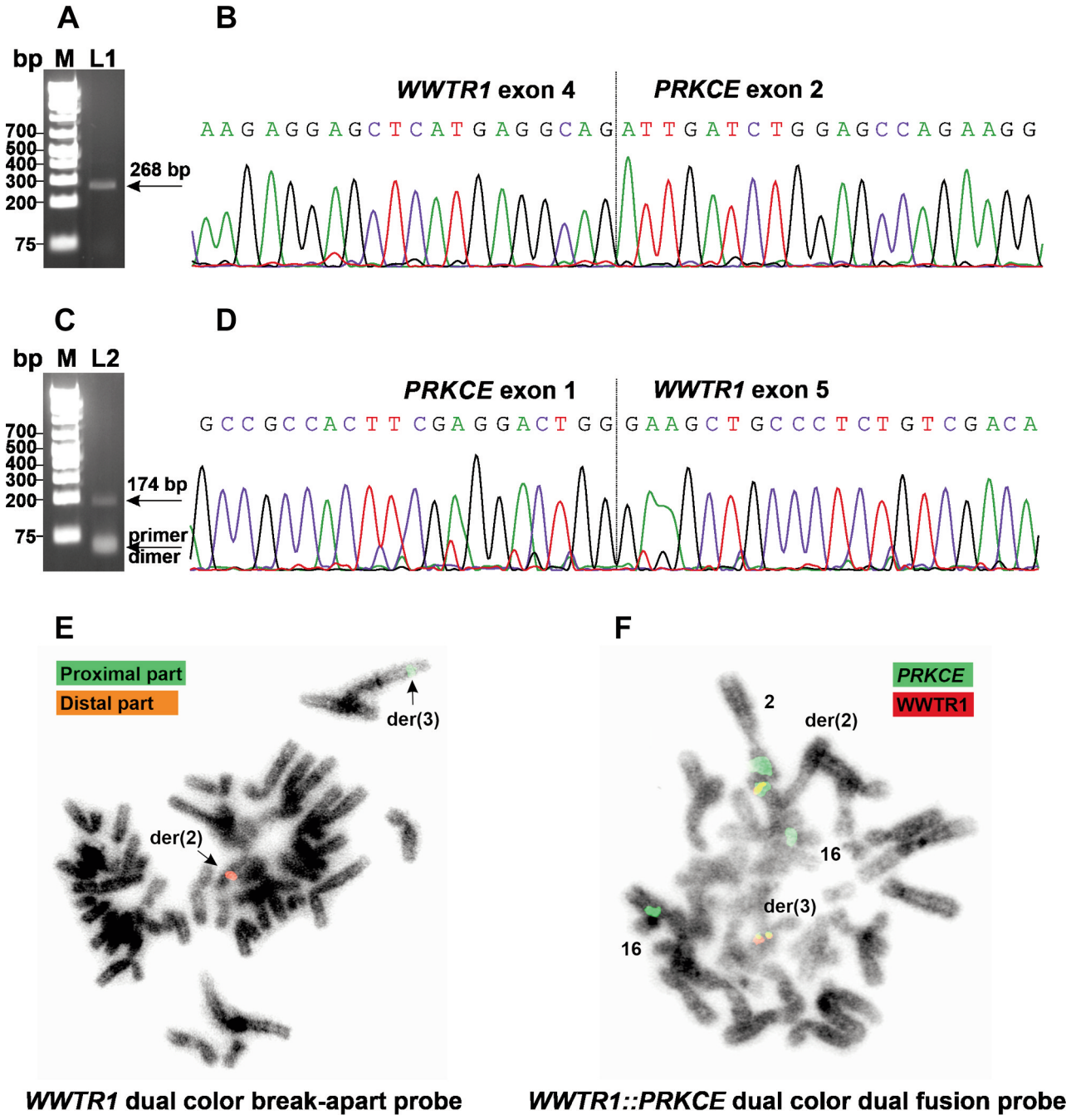

Figure 3. *Reverse transcription polymerase chain reaction (RT-PCR), Sanger sequencing, and fluorescence in situ hybridization (FISH) examination of the uterine leiomyoma. (A) Gel electrophoresis showing the amplified 268 bp cDNA fragment using the forward primer WWTR1-996F1 together with the reverse PRKCE-904R1 primer. (B) Partial Sanger sequencing chromatogram of the amplified 268 bp cDNA fragment showing the junction between exon 4 of WWTR1 and exon 2 of PRKCE. (C) Gel electrophoresis showing the amplified 174 bp cDNA fragment using the forward PRKCE-700F1 and reverse WWTR1-1188R1 primers. (D) Partial Sanger sequencing chromatogram of the amplified 174 bp cDNA fragment showing the junction between exon 1 of PRKCE and exon 5 of WWTR1. (E) FISH with a commercial WWTR1 dual color break-apart probe on a metaphase spread showing that the proximal part of the probe (green signal) hybridized to a seemingly normal chromosome 3 signed as der(3), whereas the distal part of the probe (red signal) hybridized to a seemingly normal chromosome 2, signed as der(2). The orange/green fusion signal, which indicates a normal WWTR1 locus, is absent. (F) FISH with a homemade WWTR1::PRKCE dual color dual fusion probe on a metaphase spread showing two red/green fusion signals, one on a der(2) and the other on der(3), indicating the presence of WWTR1::PRKCE and the reciprocal PRKCE::WWTR1 on those chromosomes, respectively. Three green signals for the PRKCE are also shown: one on the p arm of normal chromosome 2 representing the PRKCE locus, and two cross-hybridized signals on chromosomes 16. The red signal corresponding to the normal WWTR1 locus on 3q25.1 is absent.*

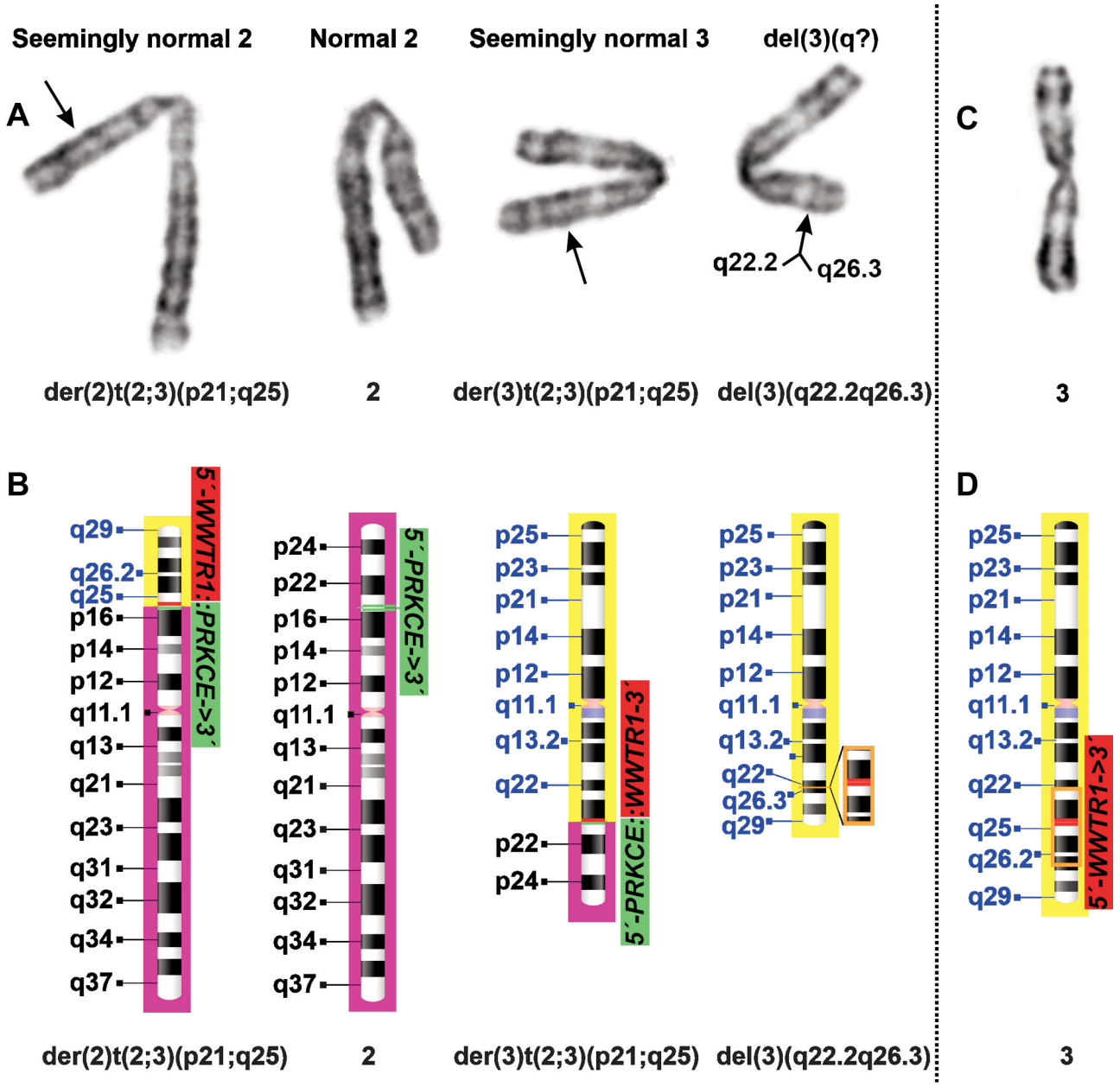

Figure 4. *Chromosomal aberrations detected in uterine leiomyoma. (A) Partial karyotype showing the seemingly normal chromosome 2 which was actually a der(2)t(2;3)(p21;q25), the normal chromosome 2, the seemingly normal chromosome 3, which was actually a der(3)t(2;3)(p21;q25), and the chromosome 3 carrying the deletion on its q arm eventually identified as del(3)(q22q26). Arrows indicating breakpoints were placed upon re-evaluation of the karyotype based on G-banding, aCGH, RNA sequencing, RT-PCR/Sanger sequencing, and FISH results. (B) Ideogram showing a der(2)t(2;3)(p21;q25) carrying the 5'-WWTR1::PRKCE-3' fusion gene, a normal chromosome 2, a der(3)t(2;3)(p21;q25) carrying the 5'-PRKCE::WWTR1-3' gene, and a del(3)(q22.2q26.3). (C) A normal chromosome 3 from a uterine leiomyoma. (D) Ideogram of a normal chromosome 3 showing the position of WWTR1 on subband 3q25.1 (red line) and the q22q26 part (orange box).*

the genes calmodulin binding transcription activator 1 (*CAMTA1* on 1p36), actin like 6A (*ACTL6A* on 3q26), and mastermind like transcriptional coactivator 2 (*MAML2* on 11q21) (48-50). In epithelioid hemangioma (50, 51) and pseudomyogenic hemangioendothelioma (52), it fuses with FosB proto-oncogene and AP-1 transcription factor subunit (*FOSB* on 19q13). In poroma, *WWTR1* fuses with NUT midline carcinoma family member 1 (*NUTM1* on 15q14) (53) and in an

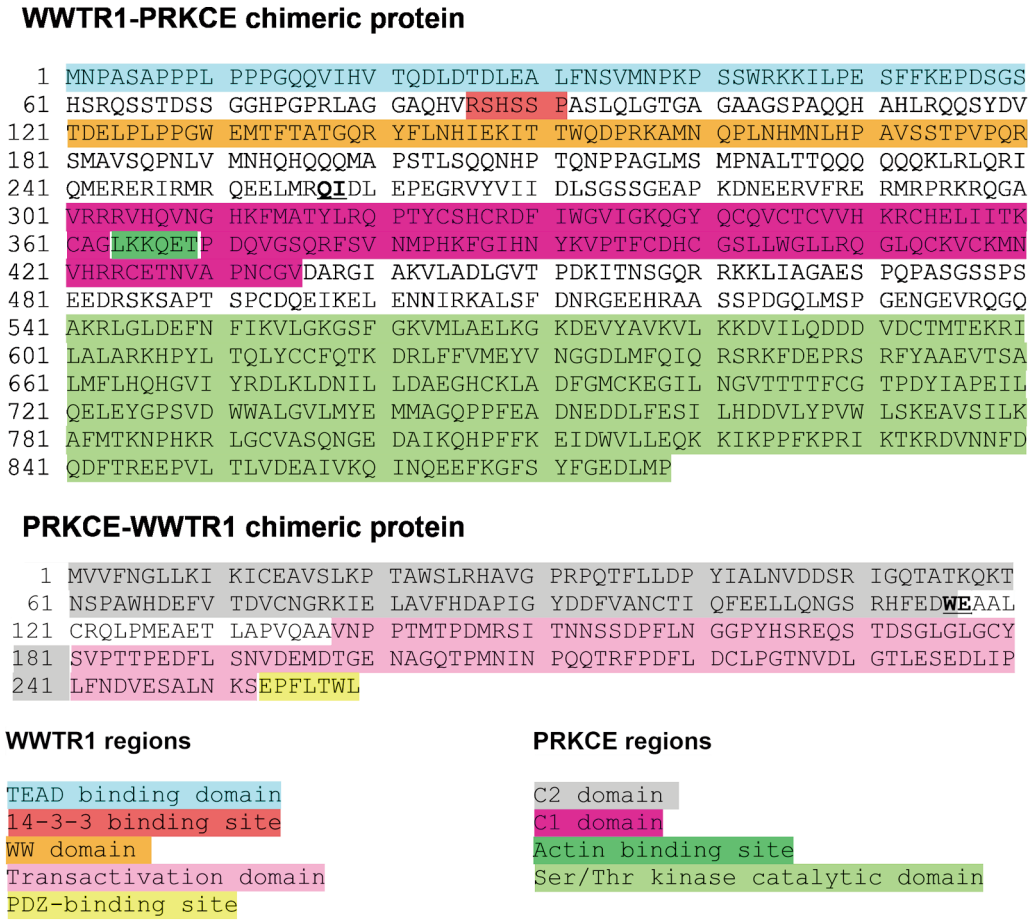

Figure 5. *The chimeric WWTR1::PRKCE and PRKCE::WWTR1 proteins. The fusion point is in bold and underlined. The different domains and binding sites are colored.*

Table III. *Published uterine leiomyomas carrying deletion on q arm of chromosome 3 and present case. Age of patients and abnormal karyotypes were obtained from Mitelman database of chromosome aberrations and gene fusions in cancer.*

Reference	Case	Age	Karyotype
Mark *et al*., 1990 (10)	17A	46	46,XX,del(3)(q21)
Nilbert *et al*., 1990 (11)	18	62	85-86,XXXX,add(1)(p36)x2,-2,-2,del(3)(q13q26)x2,-4,-4,t(11;17)(q13;q21)x2,t(12;14)(q14-15;q23-24)x2,-13,-13,-14,-14,add(14)(q24)x2,add(15)(q26)x2,-16,-16,-17,der(17)t(11;17)(q13;q21),-18,-18,+4mar
Nilbert *et al*., 1990 (11)	32	44	46,XX,del(3)(q24)
Nilbert *et al*., 1990 (11)	42	47	47,XX,del(1)(q32),del(3)(q26),+add(6)(q23),del(7)(q32),add(8)(q24),-10,+r
Pandis *et al*., 1990 (15)	47	49	46,XX,der(1)r(1)(p36q32)trp(1)(p22p36)/92,idemx2/46,XX,del(7)(q21q31)/46,XX,del(3)(q21q36),add(6)(p11),del(7)(q21q31),del(11)(q14),add(21)(q22)
Rein *et al*., 1991 (16)	3A	NA	46,XX,?inv(1)(q31q43),t(1;13)(q31;q21),del(3)(q21)
Ozisik *et al*., 1993 (41)	4	40	46,XX,del(7)(q22q32)/46,XX,del(7)(q22)/46,XX,del(3)(q23q26)
Dal Cin *et al*., 1995 (21)	1	46	46,XX,del(3)(q13q27)
Dal Cin *et al*., 1995 (21)	2	50	46,XX,del(3)(q12q24)
Dal Cin *et al*., 1995 (21)	3	29	46,XX,del(3)(q21q27)
Xing *et al*., 1997 (22)	16	NA	46,XX,del(3)(q23q26)
Christacos *et al*., 2006 (42)	9	NA	40,XX,del(1)(p11-12),del(3)(q12),add(4)(q?31),add(7)(q36),-9,-9,del(10)(q23),-14,-15,-19,-22
Panagopoulos *et al*., 2022 (present case)	1	45	46,XX,t(2;3)(p21;q25),del(3)(q22q26)

NA, Not available.

intra-abdominal sarcoma associated with endometriosis it fuses with AF4/FMR2 family member 2 (*AFF2* on Xq28) (54).

PRKCE codes for a serine- and threonine-specific protein kinase which is a member of the protein kinase C (PKC) family (55, 56). According to the human protein atlas, *PRKCE* is expressed in many tissues with high levels in the brain and lung (https://www.proteinatlas.org/). It plays a role in a number of biological processes including apoptosis and cardioprotection from ischemia (56, 57) (see also https://www.ncbi.nlm.nih.gov/gene/5581). In cancer, *PRKCE* was found to behave as an oncogene in tumor invasion and metastasis (58, 59). The PRKCE protein has an N-terminal region, a hinge, and a C-terminal region (56). The N-terminal region is the regulatory part of the protein. It contains a calcium-independent C2 domain (location on NP_005391.1: 3-135) (60) which binds to phospholipid activators, RACK scaffolding protein, and the HSP90 chaperone protein. It also contains a C1 domain (location on NP_005391.1: 161-295) (61) which binds to second messengers diacylglycerol, fatty acids, and phorbol esters as well as to filamentous actin (56, 57). The C-terminal part is the catalytic domain of the serine/threonine kinase having an ATP binding site and the activation loop (location on NP_005391.1: 412-732) (56, 57).

Thus, the *WWTR1::PRKCE* fusion transcript would code for an 878 amino acid long chimeric serine/threonine kinase. The first 257 amino acids would contain the N-terminal region interacting with the TEAD family of transcription factors, the 14-3-3 binding region, and the WW domain from the WWTR1 protein, whereas the last 621 amino acids of the chimeric protein contain the C1 domain and the catalytic domain of the serine/threonine kinase of PRKCE (Figure 5). The reciprocal *PRKCE::WWTR1* fusion transcript would encode a chimeric transcriptional coactivator protein containing the N-terminal C2 domain of PRKCE and the transcription activation domain and conserved PDZ-domain of WWTR1 (Figure 5). Because the examined tumor does not have any normal *WWTR1* allele - one is fused to the *PRKCE* locus whereas the second allele is lost through del(3)(q22q26) -, the involvement of WWTR1 in the hippo pathway must be exclusively via the two fusion proteins WWTR1::PRKCE and PRKCE::WWTR1 resulting in the pathway's deregulation (Figure 5) (43, 47). In genetic terms, we seem to be facing an unusual situation in which tumorigenesis comes across as a recessive trait at the cellular level, at least as far as the hippo pathway is concerned. The alternative, less exciting possibility would be that the del(3q) was a coincidental change adding little or nothing from the pathogenetic point of view. Only future studies of tumors having these genomic changes, 3q- and/or the 2;3-translocation, can clarify their relative tumorigenic importance.

In conclusion, by combining analyses by G-banding, aCGH, RNA sequencing, RT-PCR, Sanger sequencing, and FISH methodologies a leiomyoma whose only genomic change initially seemed to be an interstitial deletion on the long arm of chromosome 3, turned out to carry also a novel cryptic balanced t(2;3)(p21;q25) which generated reciprocal *WWTR1::PRKCE* and *PRKCE::WWTR1* chimeras. The karyotype of the leiomyoma was corrected to 46,XX,t(2;3)(p21;q25), del(3)(q22q26). We believe that at least some of the reported leiomyomas with del(3q) in the cytogenetic literature are genetically similar to the case just described.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest.

Authors' Contributions

IP designed and supervised the research, performed molecular genetic experiments, bioinformatics analysis, and wrote the manuscript. KA performed fluorescence in situ hybridization, molecular genetic methods and interpreted the data. LG performed cytogenetic analysis. BD performed the pathological examination. FM evaluated the data. SH assisted with experimental design and writing of the manuscript. All Authors read and approved of the final manuscript.

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