Several Fusion Genes Identified in a Spermatic Cord Leiomyoma With Rearrangements of Chromosome Arms 3p and 21q

IOANNIS PANAGOPOULOS1, LUDMILA GORUNOVA1, KRISTIN ANDERSEN1, INGVILD LOBMAIER2 and SVERRE HEIM1,3

1Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;
2Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;
3Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway

Leiomyomas are benign smooth-muscle tumors. They have been described in nearly every organ but are most common in the uterus (fibroids) (1-5).

Much is known about the genetics, and hence the pathogenesis, of uterine leiomyomas (6-9). In brief, most uterine leiomyomas are cytogenetically characterized by the presence of one or more of the following cytogenetic aberrations: t(12;14)(q15;q23–24); del(7)(q21.2q31.2); rearrangements involving 6p21, 10q22, and 1p; trisomy 12; deletions of 3q; and changes of the X chromosome (10, 11). These chromosomal aberrations rearrange and deregulate the following genes: High mobility group AT-hook 2 (HMG A2) on 12q14, RAD51 paralog B (RAD51B) on 14q24, cut-like homeobox 1 (CUX1) on 7q22, procollagen C-endopeptidase enhancer (PCOLCE) on 7q22, HMG A1 (on 6p21), lysine acetyltransferase 6B (KAT6B) on 10q22, and collagen type IV alpha 5 chain/collagen type IV alpha 6 chain (COL4A5/ COL4A6) on Xq22 (6-9). Additionally, point mutations of the mediator complex subunit 12 (MED12) gene (on Xq13) have also been reported to be frequent in uterine leiomyomas (12-15). Very little genetic information exists on leiomyomas of other organs, such as gastrointestinal (16-20) and retroperitoneal leiomyomas (21-23). A single para-testicular leiomyoma has also been genetically examined (24).

In the present study, we report rearrangements of the p arm of chromosome 3 in a leiomyoma of the spermatic cord leading to multiple fusion genes. We review the literature and conclude that such 3p aberrations may be characteristic of a leiomyoma subgroup.

Materials and Methods

Ethics statement. The study was approved by the regional Ethics Committee (Regional komité for medisinsk forskningsetikk Sør-Øst,
Norge, http://helseforskning.etikkom.no) and written informed consent was obtained from the patient. The Ethics Committee’s approval included a review of the consent procedure. All patient information has been de-identified.

**Case description.** A small lump was detected in the groin of a 60-year-old man. Core-needle biopsy showed a leiomyomatous tumor with uncertain malignant potential. The tumor was resected. The tumor consisted of bundles of smooth muscle cells without atypia (Figure 1). There was no mitotic activity nor necrosis. The final diagnosis was spermatic cord leiomyoma.

**G-Banding karyotyping and array comparative genomic hybridization (aCGH) analyses.** Fresh tissue from a representative area of the tumor was analyzed cyogenetically as previously described (25). Genomic DNA was extracted from the tumor using the Maxwell RSC Instrument and Maxwell RSC Tissue DNA Kit (Promega, Madison, WI, USA), the concentration was measured using Quantus Fluorometer and the QuantiFluor ONE dsDNA System (Promega), and aCGH was performed using CytoSure array products (Oxford Gene Technology, Begbroke, Oxfordshire, UK) as previously described (26).

**RNA sequencing.** Total RNA was extracted from frozen tumor tissue adjacent to that used for cytogenetic analysis and histological examination using miRNeasy Mini Kit (Qiagen, Hilden, Germany). One microgram of total RNA from the tumor was sent to the Genomics Core Facility at the Norwegian Radium Hospital, Oslo University Hospital for high-throughput paired-end RNA-sequencing. Fusion transcripts were found using FusionCatcher software (27, 28).

**Reverse transcription PCR (RT-PCR) and Sanger sequencing analyses.** The primers used for PCR amplification and Sanger sequencing analyses are shown in Table I. The primer combinations and the corresponding fusion transcripts are presented in Table II. The iScript Advanced cDNA Synthesis Kit for RT-PCR was used to reverse transcribe 1 μg of total RNA according to the manufacturer’s instructions (Bio-Rad, Hercules, CA, USA). cDNA corresponding to 20 ng total RNA was used as template in subsequent PCR assays. BigDye Direct Cycle Sequencing Kit was used to perform both PCR and cycle (Sanger) sequencing according to the company’s recommendations (ThermoFisher Scientific, Waltham, MA, USA). Sequencing was run on an Applied Biosystems SeqStudio Genetic Analyzer system (ThermoFisher Scientific). For computer analysis of sequence data, the basic local alignment search tool (BLAST) software was used (29).

**Results**

The G-banding analysis (Figure 2A) yielded the following karyotype:

48~50,XY,add(3)(p21),+4,+7,+8,+9,add(21)(q22)[cp9]/46,XY[2].

aCGH confirmed trisomy for chromosomes 4,7,8, and 9 (Figure 2B). In addition, multiple gains and losses were detected on the p arm of chromosome 3 (Figure 2C) and on the q arm of chromosome 21 (Figure 2D).

RNA sequencing analysis with FusionCatcher detected seven fusion genes (Table III, Figure 3A). One transcript was detected for each of the chimeras inositol 1,4,5-trisphosphate receptor divergent transcript–nuclear receptor subfamily 2 group C member 2 (ITPR1–DT–NR2C2), cytoplasmic linker associated protein 2–interleukin 17 receptor D (CLASP2–IL17RD), nuclear receptor subfamily 2 group C member 2–cilium and flagella-associated protein 410 (NR2C2–CFAP410), zinc finger protein 621–leucyl-tRNA synthetase 2, mitochondrial (ZNF621–LARS2), and contactin 4–ras homolog family member A (CNTN4–RHOA), whereas two chimeric transcripts were detected for the fusion genes Rho guanine nucleotide exchange factor 3–calcium voltage-gated channel auxiliary subunit alpha2delta 2 (ARHGEF3–CACNA2D2) and trafficking kinesin protein 1–TIMP metalloproteidase inhibitor 4 (TRAK1–TIMP4) (Table III). In six of the fusion genes, both the 5’-end and 3’-end fusion partner genes were mapped on chromosome arm 3p (Table III, Figure 3A). In the seventh, the NR2C2–CFAP410 chimera, the 5’-end partner gene, NR2C2, was from 3p25.1, whereas CFAP410 was from 21q22.3 (Tables I and III). All fusion transcripts were verified using RT-PCR and Sanger sequencing analyses (Figure 3B).
Discussion

Prior to the present study, to our knowledge, only a single para-testicular leiomyoma, located beneath the tunica albuginea, had been cytogenetically described, by Gorunova et al. (24). That tumor had an abnormal karyotype: 46,XY,der(5)(p15q15),der(14)(q15q24),del(12;14)(q15q24). The net imbalance of the structural rearrangements thus seemed to be the loss of 5q31ter and gain of 12q15qter. Molecular investigations were not performed, but strong nuclear immunostaining was found for the HMGA2 protein in the tumor cells, suggesting that the der(14)(q15q24) was a translocation event in uterine leiomyomas and leads to overexpression of HMGA2. Because 12q15q24 is the most frequent translocation in uterine leiomyomas, the authors concluded that a common pathogenetic pathway exists for both para-testicular (male) and uterine (female) leiomyomas (24).

The present leiomyoma was found in the spermatic cord and had different cytogenetic aberrations from those described by Gorunova et al. (24). Trisomies were found for chromosomes 4, 7, 8, and 9, whereas structural aberrations affected chromosome arms 3p and 21q (Figure 2A). The observed numerical aberrations have also been reported in leiomyomas (30): trisomies for chromosomes 4, 8, and 9 in uterine leiomyomas (16, 31-35) and trisomy 7 in uterine bladder, and gastric leiomyomas (16, 17, 31, 36). Rearrangements of the 3p arm were reported in 22 uterine leiomyomas (30). In 12 of them, the 3p aberration was found together with rearrangements of chromosome bands 12q14–15 or band 6p21. Leiomyoma aberrations in the region 12q14–15 target the HMGA2 gene, whereas aberrations in 6p21 target HMGAI (37, 38). Reports have been published on nine leiomyomas with aberration in the 3p arm of chromosome 3 without microscopically visible involvement of 12q14–15 or 6p21 (Table IV) (31, 32, 35, 39-42). Structural rearrangement of the 21q arm were reported in 12 uterine leiomyomas, always together with aberrations of 6p (often 6p21) or 12q14–15 (32, 38, 39, 43-46).

The aCGH investigation of the tumor in our case not only confirmed trisomies for chromosomes 4, 7, 8, and 9, but also showed multiple gains and losses of material from chromosome arms 3p and 21q (Figure 2B-D), suggesting that the rearrangements detected in 3p and 21q, add(3)(p21) and add(21)(q22) were more complex than those seen in G-banded preparations. RNA sequencing detected six fusion genes, ARHGEF3–CACNA2D2, TRAK1–TIMP4, ITPRI–DT–NR2C2, CLASP2–IL17RD, ZNF621–LARS2, and CNTN4–RHOA, with both 5’- and 3’-end fusion partner genes located on 3p (Table III and Figure 3A), and one fusion gene, NR2C2–CFAP410, with the 5’- end partner (NR2C2) from 3p and the 3’-end partner gene (CFAP410) from 21q (Table III). All of them were verified by RT-
and plays a role in many biological processes such as
promoter. NR2C2 protein belongs to the nuclear hormone
receptor family, acts as a ligand-activated transcription factor
coding part of first untranslated exon of NR2C2–CFAP410
in which 5'-end untranslated regions of the 5'-end fusion
transcript, the first type, illustrated by the
chimera of this tumor would also be expected to
lack the mitochondrion-target peptide and might interfere
with the CFAP410/NEK1 complex and DNA-damage repair.
The second type of chimera involves out-of-frame
transcripts. In the two detected ARHGEF3–CACNA2D2
transcripts, exon 1 or 2 of ARHGEF3 fused with exon 2 of
CACNA2D2, introducing a stop codon shortly after the
junctions. ARHGEF3 (also known as XPLN) codes for
guanine nucleotide exchange factor 3, which specifically
activates Rho GTPase family members RHOA and RHOB
(65, 66). The putative truncated form of ARHGEF3 would
lack the carboxyl terminal part of the ARHGEF3 protein
responsible for guanine nucleotide exchange factor activity
but retain the amino terminal part of the protein which
interacts with mammalian target of rapamycin complex 2
(mTORC2), inhibiting mTORC2 kinase activity (66, 67).
The presence of the truncated ARHGEF3 protein and its
possible functions cannot be determined without further
cellular studies. Nevertheless, the ARHGEF3–CACNA2D2

Table II. Primer combinations for polymerase chain reaction amplification to detect fusion transcripts.

| Primer combination | Fusion transcript (5'->3') | Type of fusion               |
|------------------|--------------------------|-----------------------------|
| ITPR1–AS1-F1/NR2C2-R1 | ITPR1–DT–NR2C2          | Promoter-replacement-chimera |
| ARHGEF3–F1/CACNA2D2-R1 | ARHGEF3–CACNA2D2        | Out-of-frame                |
| CLASP2–F1/IL17RD-R1 | CLASP2–II17RD           | In-frame                    |
| TRAK1–F1/TIMP4-R1   | TRAK1–TIMP4              | In-frame                    |
| TRAK1–F2/TIMP4-R1   | TRAK1–TIMP4              | In-frame                    |
| NR2C2–F1/C21orf2-R1 | NR2C2–CFAP410            | Promoter-replacement-chimera |
| ZNF621–F1/LARS2-R1  | ZNF621–LARS2             | In-frame                    |
| CNTN4–F1/RHOA-R1    | CNTN4–RHOA               | Promoter-replacement-chimera |

PCR/Sanger sequencing methodologies (Tables I and II; Figure 3B).

The data indicated that chromothripsis involving
chromosome arms 3p and 21q occurred in the tumor, causing
the generation of multiple fusion genes (47, 48). This
phenomenon, through some unusual, cataclysmic event, a
chromosome breaks into many fragments, whereupon these
fragments, or some of them, are randomly reassembled (47,
48). Some chromosome fragments are lost through this
process while some fusion genes with oncogenic potential
are generated at the junctions of the assembled fragments
(47, 48). Chromothripsis has been detected in uterine
leiomyomas which had neither mutations in MED12 or
fumarate hydratase genes, nor 12q14~15 or 6p21
leiomyomas which had neither mutations in
MTORC2, inhibiting mTORC2 kinase activity (66, 67).
The presence of the truncated ARHGEF3 protein and its
possible functions cannot be determined without further
cellular studies. Nevertheless, the ARHGEF3–CACNA2D2
transcript indicated further the dysfunction of RHOA signal transduction in this tumor.

The third type of chimera resulted in chimeric proteins. In the ZNF621–LARS2 transcript, exon 2 of ZNF621 fused in-frame to exon 5 of LARS2. In the chimeric ZNF621–LARS2, the first 121 amino acids of LARS2 are replaced by the first eight amino acids of ZNF621. The LARS2 gene codes for a mitochondrial leucyl-tRNA synthetase with a mitochondrial targeting signal within its first 50 amino acids (68). Thus, since the mitochondrial targeting signal is absent from the chimeric ZNF621–LARS2 protein, the protein would probably be unable to enter the mitochondrion from the cytosol.

The two in-frame TRAK1–TIMP4 fusion transcripts would also give rise to chimeric proteins. The ubiquitously expressed TRAK1 gene codes for trafficking kinesin-binding protein 1 which is involved in mitochondrial and endosome-lysosome trafficking (69-74). Based on the reference sequence NP_001036111.1, the 953 amino acids-long TRAK1 protein has the following regions: A huntingtin-associated protein 1 conserved region between amino acids 50-353 binding to huntingtin in a polyglutamine repeat-length-dependent manner; a region between amino acids 359-509 which interacts with hepatocyte growth factor-regulated tyrosine kinase substrate protein; a region named milton between 416-583 which recruits the heavy chain of kinesin to mitochondria enabling the motor movement function of kinesin; and a region between amino acids 658-672 which interacts with O-linked N-acetylglucosamine (GlcNAc) transferase protein (70-72, 75, 76).

Figure 2. Cytogenetic and array comparative genomic hybridization (aCGH) analyses of the spermatic cord leiomyoma. A: Partial karyotype showing the add(3) and add(21) chromosomes with corresponding normal chromosome homologs. B: aCGH showing gains of one copy of each of chromosomes 4, 7, 8, and 9, as well as multiple gains and losses of genomic material from the p arm of chromosome 3 and q arm of chromosome 21. C: aCGH showing multiple gains and losses of genomic material from the p arm of chromosome 3. D: aCGH showing the multiple gain and losses on the q arm of chromosome 21.
TIMP4, which codes for tissue inhibitor of metalloproteinase 4, is weakly expressed in testis (77) and inhibits many metalloproteinases (78-80). The inhibitory property of TIMP4 was shown to depend on its CACNA2D2 residens 1-127, which contains a site of interaction with the functional regions of TRAK1 and residues 1-127 of TIMP4.

Based on the reference sequences NM_015097.2/NP_055912.2 for CLASP2 and NM_017563.4/NP_060033.3 for IL17RD, the CLASP2–IL17RD chimeric transcript would be expected to consist of exons 1 to 34 of CLASP2 and exons 2 to 13 of IL17RD, be 12317 bp-long, and code for an 1,892 amino acid protein composed of amino acids 1 to 1,195 from CLASP2 and 43 to 739 from IL17RD. The CLASP2 gene codes for cytoplasmic linker protein-associating protein that is a microtubule plus end tracking protein (81, 82). CLASP2 is localized to the distal ends of microtubules and is involved in the regulation of microtubule dynamics (83). Microtubules switch between phases of growth and shrinkage through transitions known as microtubule catastrophe and rescue (84).
Figure 3. The detected chimeras in the spermatic cord leiomyoma. A: Diagram of the p arm of chromosome 3, showing the mapping positions of the genes contactin 4 (CNTN4); inositol 1,4,5-trisphosphate receptor divergent transcript ITPR1-DT; TIMP metalloproteinase inhibitor 4 (TIMP4); nuclear receptor subfamily 2 group C member 2 (NR2C2); cytoplasmic linker associated protein 2 (CLASP2); zinc finger protein 621 (ZNF621); trafficking kinesin protein 1 (TRAK1); leucyl-tRNA synthetase, mitochondrial (LARS2); ras homolog family member A (RHOA); calcium voltage-gated channel auxiliary subunit alpha2delta 2 (CACNA2D2); Rho guanine nucleotide exchange factor 3 (ARHGEF3); and interleukin 17 receptor D (IL17RD); as well as the chimeric genes CNTN4-RHOA, ITPR1-DT-NR2C2, TRAK1-TIMP4, CLASP2-IL17RD, ZNF621-LARS2, and ARHGEF3-CACNA2D2. The ↑ arrow next to a gene symbol indicates transcription of the gene from telomere to centromere. The ↓ arrow next to a gene symbol indicates transcription of the gene from centromere to telomere. B: Partial sequence chromatograms showing the junction positions in the chimeric transcripts CNTN4-RHOA, ITPR1-DT-NR2C2, NR2C2-CFAP410, CLASP2-IL17RD, ZNF621-LARS2, TRAK1-TIMP4 and ARHGEF3-CACNA2D2.
microtubule growth or shrinkage (85, 86). CLASP2 functions in various microtubule-dependent processes including cell division, cytoskeletal remodeling for cell migration, and vesicle transport between intracellular structures and the plasma membrane (87). CLASP2 protein has multiple HEAT repeats, a structural motif composed of two alpha helices linked by a short loop (88), two domains from tumour overexpressed gene that interact with α-tubulin, contributing to microtubule dynamics (89, 90), a region for interaction of microtubule-associated protein RP/EB family member 1 (MAPRE1, also known as EB1) (85, 91), and a carboxyl terminal region which interacts with CLIP1 protein and is required for the localization of CLASP2 protein to the Golgi apparatus and kinetochores (92).

The IL17RD gene codes for a single pass transmembrane protein mainly located in plasma membrane and regulating various signal pathways such as fibroblast growth factor and mitogen-activated protein kinase/extracellular signal-regulated kinase signal pathways (93-95). Murine models and expression patterns indicate that IL17RD is a tumor-suppressor gene (96-100). Thus, CLASP2–IL17RD chimeric transcript would produce a chimeric protein affecting microtubules and signaling pathways.

In summary, our data, together with those previously published, indicate the existence of a group of leiomyomas cytogenetically characterized by aberration of the p arm of chromosome 3 and the formation of fusion genes. A chromothripsis event seems to lie behind the pathogenetic changes that occurred in this para-testicular leiomyoma, resulting in multiple fusion genes.

Conflicts of Interest
No potential conflicts of interest exist.

Authors’ Contributions
IP designed and supervised the research, performed molecular genetic and bioinformatic analyses, and wrote the article. LG performed cytogenetic analyses. KA performed molecular genetic analyses and evaluated the data. IL performed the pathological examination. SH assisted with experimental design and writing of the article. All Authors read and approved of the final article.

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