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NDRG1-PLAG1 and *TRPS1-PLAG1* Fusion Genes in Chondroid Syringoma

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Abstract. *Background/Aim: Chondroid syringoma is a rare benign tumor emanating from sweat glands. Although rearrangements of the pleomorphic adenoma gene 1 (PLAG1) have been reported in such tumors, information on PLAG1 fusion genes is very limited. Materials and Methods: Cytogenetic, fluorescence in situ hybridization, RNA sequencing, array comparative genomic hybridization, reverse transcription polymerase chain reaction, and Sanger sequencing analyses were performed on two chondroid syringoma cases. Results: Both tumors had structural rearrangements of chromosome 8. An NDRG1-PLAG1 transcript was found in the first tumor in which exon 3 of PLAG1 was fused with exon 1 of NDRG1. A TRPS1-PLAG1 chimeric transcript was detected in the second chondroid syringoma in which exon 2 or exon 3 of PLAG1 was fused with exon 1 of TRPS1. Conclusion: The NDRG1-PLAG1 and TRPS1-PLAG1 resemble other PLAG1 fusion genes inasmuch as the expression of PLAG1 comes under the control of the NDRG1 or TRPS1 promoter.*

Chondroid syringoma, also known as mixed tumor of the skin, is a rare benign tumor emanating from sweat glands and able to display a wide array of histological patterns (1-5). It may have histological similarities with another benign exocrine gland tumor, pleomorphic adenoma or mixed tumor of the salivary glands, something that may cause differential diagnostic difficulties (3, 5, 6). The name chondroid syringoma was coined by Hirsch and Helwing (3) because of the invariable presence in the tumor of sweat gland elements (syringoma) and because cartilage-like material (chondroid) was also present in most tumors they studied (3). Chondroid syringoma most commonly occurs in the head-and-neck region of middle-aged males (3, 5, 7-9) but can also be found in the axilla and on the anterior chest, trunk, extremities, and scrotum (3, 7, 10-19).

We studied genetically two chondroid syringomas finding fusion of the pleomorphic adenoma gene 1 (*PLAG1*) at 8q12.2 in one with the gene N-myc downstream regulated 1 (*NDRG1*), which maps to chromosome subband 8q24.22, and in the second with the gene transcriptional repressor GATA binding 1 (*TRPS1*), which maps to 8q23.3.

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Key Words: Chondroid syringoma, *PLAG1*, fusion genes, *NDRG1-PLAG1*, *TRPS1-PLAG1*, chromosome 8.

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 patients. The ethics committee's approval included a review of the consent procedure. All patient information has been de-identified.

Case description

Case 1. A 60-year-old male without any relevant medical previous history was surgically treated for a tumor located at the left ankle. The tumor was found to be consistent with a chondroid syringoma. Surgical margins were doubtful. Six years later re-excision was performed. Macroscopically the tumor was lobulated, but well demarcated. The cut section was soft and gelatinous intermingled with harder areas. Microscopically the tumor was composed of small cystic spaces, areas with myxoid tissue and areas with solid growth of cubic epithelium (Figure 1A and B).

Case 2. A 59-year-old male was surgically treated for a tumor on the left thigh. The tumor was located in the subcutaneous adipose tissue, measuring 18 mm in greatest diameter. Macroscopically the

tumor was white and with rubbery turgor. Microscopically the tumor was solid with cribriform growth of cubic cells separated by collagen bundles and small areas with myxoid material, intermingled with loose arranged spindle cells (Figure 1C and D).

G-banding and karyotyping. Fresh tissue from a representative area of the tumors was analyzed cytogenetically as previously described (20).

Fluorescence in situ hybridization (FISH) analysis. In order to characterize the ring and the der(15) chromosome of case 1 (see below), FISH was performed on metaphase spreads using whole chromosome painting probes for chromosomes 8 and 15 (Cytocell, Oxford Gene Technology, Begbroke, Oxfordshire, UK).

The BAC probes were purchased from BACPAC Resource Center located at the Children's Hospital Oakland Research Institute (Oakland, CA) (https://bacpacresources.org/) (Table I). Detailed information on the FISH procedure was given elsewhere (21).

DNA was extracted and probes were labelled and hybridized using Abbott's nick (Abbott Molecular, Des Plaines, IL, USA) translation kit according to the manufacturer's recommendations. The *PLAG1* probe was labelled with Texas Red-5-dCTP (PerkinElmer, Boston, MA, USA) in order to obtain a red signal. The probes for *NDRG1* and *TRPS1* were labelled with fluorescein-12-dCTP (PerkinElmer, Boston, MA, USA) in order to obtain green signals. FISH mapping of the probes on normal controls was performed to confirm their chromosomal location. Chromosome preparations were counterstained with 0.2 μg/ml DAPI and overlaid with a 24×50 mm^2 coverslip. Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

Array comparative genomic hybridization (aCGH) analysis. Genomic DNA was extracted using the Maxwell RSC Instrument and the Maxwell RSC Tissue DNA Kit (Promega, Madison, USA). The concentration was measured using the Quantus Fluorometer and the QuantiFluor ONE dsDNA System (Promega, Madison, WI, USA). Promega's human genomic female DNA was used as reference DNA. For aCGH, CytoSure array products were used (Oxford Gene Technology) according to the company's protocols. Thus, the CytoSure Genomic DNA Labelling Kit was used for labelling of 1 μg of each patient and reference DNAs and the CytoSure Cancer +SNP array for hybridization. The slides were scanned in an Agilent scanner using the Agilent Feature Extraction Software (version 10.7.3.1). Data were analysed with the CytoSure Interpret analysis software (version 4.9.40). The genomic imbalances were identified using the Circular Binary Segmentation (CBS) algorithm (22) and added a custom-made aberration filter defining a copy number aberration (CNA) as a region with a minimum five probes gained/lost. Annotations are based on human genome build 19.

RNA sequencing. Total RNA was extracted from frozen (–80˚C) tumor tissue adjacent to that used for cytogenetic analysis and histologic examination using miRNeasy Mini Kit (Qiagen, Hilden, Germany). For case 1, one μg of total RNA from the tumor was sent to the Genomics Core Facility at the Norwegian Radium Hospital, Oslo University Hospital (http://genomics.no/oslo/) for high-throughput paired-end RNA-sequencing according to the Illumina TruSeq Stranded mRNA protocol. The softwares FusionCatcher, deFuse, TopHat-Fusion, and FuSeq were used to find fusion transcripts (23-28). In addition, the "grep" command was used to search the fastq files of the sequence data for *PLAG1* sequence. The principle of this approach has been described elsewhere (29, 30). The search term was the 20-nucleotide-sequence (nt) "ATTGGCCAAAATGGGAAGGA" which corresponds to the first 20 nt in exon 3 of *PLAG1* or nt 286-305 in the *PLAG1* reference sequence (accession number: NM_002655.2).

Reverse transcription (RT) PCR and Sanger sequencing analyses. The primers used for PCR amplifications and Sanger sequencing analyses are shown in Table II. One μg of total RNA was reverse-transcribed in a 20 μl reaction volume using iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA).

For amplification of the *NDRG1-PLAG1* fusion transcript, the primer combinations were NDRG1-1F1/PLAG1-498R1 and NDRG1-23F1/PLAG1-458R1. For amplification of the *TRPS1-PLAG1* fusion transcript, the primer combinations were TRPS1-200F1/PLAG1-498R1 and TRPS1-318F1/PLAG1-458R1. All PCR amplifications were performed in 25 μl reaction volume which contained 12.5 μl Premix Ex Taq™ DNA Polymerase Hot Start Version (Takara Bio Europe, Saint-Germain-en-Laye, France), 1 μl cDNA template, and 0.4 μM of each of the forward and reverse primers. PCR amplifications were run on a C-1000 Thermal cycler (Bio-Rad) and the cycling was at 94˚C for 30 s, followed by 35 cycles of 7 s at 98˚C, 30 s at 60˚C, 30 s at 72˚C, and a final extension for 5 min at 72˚C. Three μl of the PCR products were stained with GelRed (Biotium, Fremont, California, USA), analyzed by electrophoresis through 1% agarose gel, and photographed. The remaining PCR products were purified using the MinElute PCR Purification Kit (Qiagen) and direct sequenced using the dideoxy procedure with the BigDye terminator v1.1 cycle sequencing kit (ThermoFisher Scientific, Waltham, Massachusetts, USA) on the Applied Biosystems SeqStudio Genetic Analyzer system. The basic local alignment search tool (BLAST) software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for computer analysis of sequence data (31).

Results

Case 1. The G-banding analysis supported by whole chromosome paint FISH results yielded the karyotype 50,XY,+5,r(8),+9,+14,+15,der(15)t(8;15)(?;?q15)x2, der(19)t(15;19)(q22;q13), add(21)(p13) (Figure 2A and B).

Examination of the RNA sequencing data with four different programs did not identify any fusion genes related to chromosome 8 (data not shown). Using the "grep" command and a search term corresponding to the first 20 nt in the exon 3 of *PLAG1* on the raw sequencing data, which were in the text-based fastq format, only 5 unique sequences were extracted (Table III). Using the BLAST algorithm on the NCBI National Canter for Biotechnology Information database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) we aligned each of the above-mentioned sequences with the human genomic plus transcript database. The alignment showed that 3 sequences were from the *PLAG1* gene whereas 2 sequences were hybrids containing sequences from exon 3 of *PLAG1* (sequence with accession number NM_002655.2) and exon 1 of *NDRG1* (sequence with accession number NM_006096.4) (Table III).

RT-PCR with the primer combinations NDRG1-1F1/PLAG1-498R1 and NDRG1-23F1/PLAG1-458R1 amplified a 352 bp

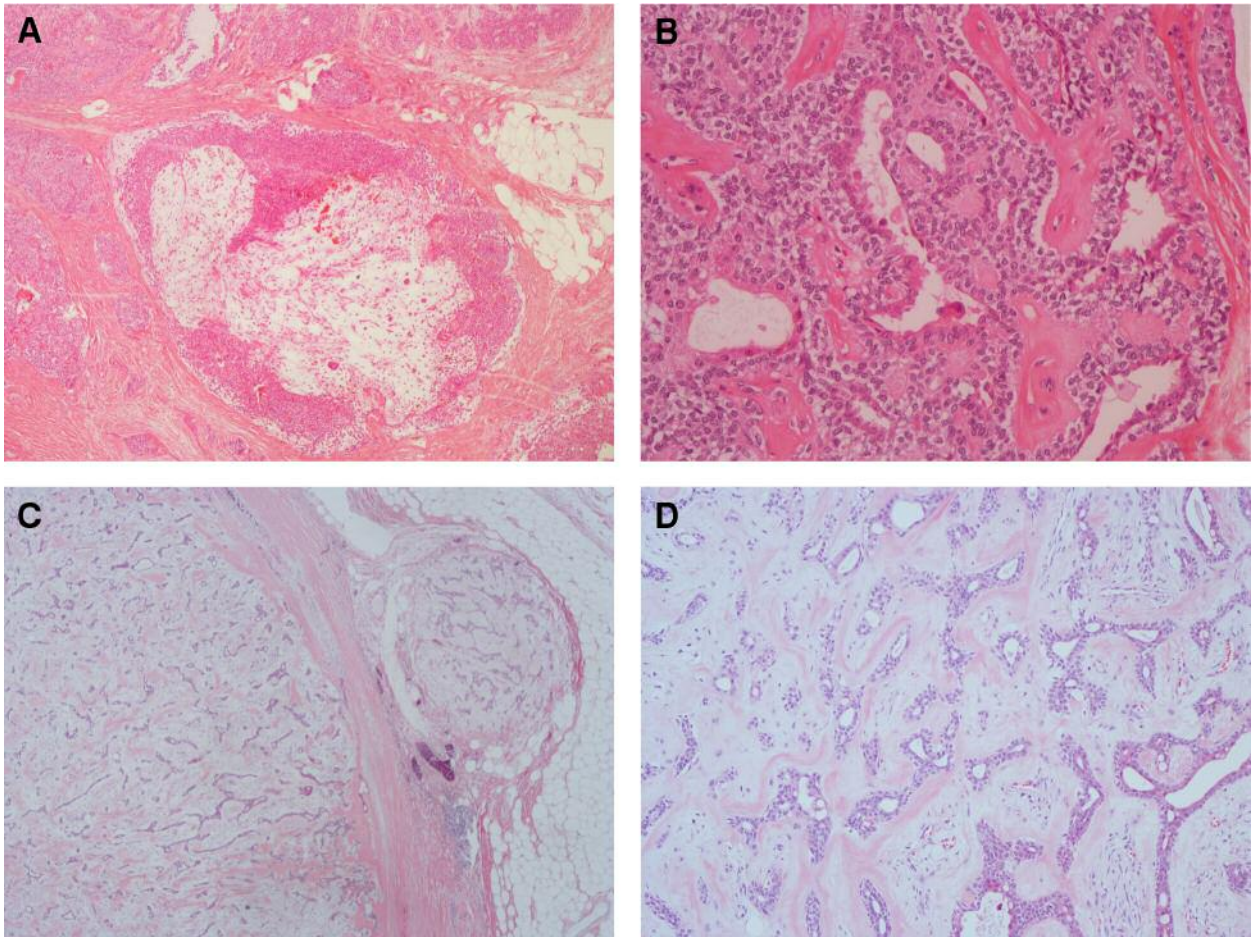

Figure 1. *Microscopic examination of chondroid syringoma from case 1 (A and B) and case 2 (C and D). (A) Tumor with myxoid areas, ×40. (B) Ductal structures embedded in solid growth of cubic epithelial cells, intermingled with collagen band, ×200. (C) Partly capsulated tumor with slit like ductal spaces embedded in chondromyxoid stroma, ×20. (D) High magnification of the capsulated tumor (from C), ×100.*

Table I. *BAC probes used for FISH experiments.*

BAC clones	Chromosome mapping	Targeted gene	Position on GRCh38/hg38 assembly	Labelling
RP11-22E14	8q12.1	*PLAG1*	chr8:55956731-56135453	Red
RP11-446E9	8q12.1	*PLAG1*	chr8:55870288-56054628	Red
RP11-1145H17	8q24.22	*NDRG1*	chr8:133321482-133454497	Green
RP11-167A6	8q24.22	*NDRG1*	chr8:133368156-133568465	Green
RP11-17O18	8q23.3	*TRPS1*	chr8:115692191-115877698	Green

Table II. *Primers used for PCR amplification and Sanger sequencing analyses.*

Name	Sequence (5'->3')	Position	Reference sequence	Gene
PLAG1-458R1	TTGTTGGACACTTGGGAACTGCC	480-458	NM_002655.2	*PLAG1*
PLAG1-498R1	GGAATGACAGTGGCCATCGCA	518-498	NM_002655.2	*PLAG1*
NDRG1-1F1	AAACCTCGCCTGGCTCCCAG	1-20	NM_006096.4	*NDRG1*
NDRG1-23F1	GAAGCTCGTCAGTTCACCATCCG	29-51	NM_006096.4	*NDRG1*
TRPS1-200F1	ACCGGGCACATCCTTGCTCTATT	200-222	NM_014112.5	*TRPS1*
TRPS1-318F1	CCCCCGCAATAATCCAAGATCA	318-339	NM_014112.5	*TRPS1*
TRPS1-353F1	CAGAAGACGGTTCATGGCTTTGG	353-375	NM_014112.5	*TRPS1*

and a 286 bp cDNA fragment, respectively (Figure 3A). Direct sequencing of them showed that they were *NDRG1-PLAG1* chimeric cDNA fragments (Figure 3B). The fusion point was identical to that found by the analysis of the RNA sequencing data. Thus, the non-coding sequence exon 1 of *NDRG1* (nt 119 in sequence with accession number NM_006096.4) was fused to exon 3 of *PLAG1* (nt 286 in NM_002655.2) (Figure 3B).

FISH analysis on metaphase spreads showed that the *NDRG1-PLAG1* fusion gene was on the ring chromosome (Figure 3C).

Case 2. The G-banding analysis yielded the karyotype 46,XY,del(8)(q12q23)[10] (Figure 4A). aCGH also detected a large deletion in the q arm of chromosome 8 (Figure 4B). Based on the hg19 assembly, the deletion started at position Chr8:57120365 in intron1 of *PLAG1* and ended at Chr8:116661489 in exon 1 of *TRPS1* (Figure 4B). Thus, aCGH data were in agreement with the results of G-banding analysis and suggested that a *TRPS1-PLAG1* fusion gene had been formed as a result of the deletion.

RT-PCR with the primer combinations TRPS1-200F1/PLAG1-498R1 amplified two, a 570 bp and a 465 bp, cDNA fragments (Figure 4C). Direct sequencing of these fragments showed that both were *TRPS1-PLAG1* chimeric cDNA fragments. In the 570 bp long fragment, exon 1 of *TRPS1* was fused to exon 2 of *PLAG1* whereas in the 465 bp fragment, exon 1 of *TRPS1* was fused to exon 3 of *PLAG1* (Figure 4D).

RT-PCR with the primer combinations TRPS1-318F1/PLAG1-458R1 also amplified two, a 414 bp and a 309 bp, cDNA fragments (Figure 4C). The sequence of these fragments confirmed the presence of the above-mentioned *TRPS1-PLAG1* fusion transcripts (Figure 4D).

FISH analysis on metaphase spreads showed that the *TRPS1-PLAG1* fusion gene was on the del(8)(q12q23) chromosome (Figure 4E).

Discussion

We report the identification of two fusion genes, *NDRG1-PLAG1* and *TRPS1-PLAG1*, in two chondroid syringomas. To the best of our knowledge, the *NDRG1-PLAG1* fusion gene is reported here for the first time. However, the *TRPS1-PLAG1* gene fusion was recently reported in a myoepithelial tumor of soft tissue and was also found to be recurrent in a subset of uterine myxoid leiomyosarcomas with *PLAG1* rearrangements (Table IV) (32, 33).

Chondroid syringoma and its more aggressive counterpart, malignant chondroid syringoma, are included among cutaneous myoepithelial neoplasms (34-38). Recently, we reported a malignant chondroid syringoma which had a t(X;6)(p11;p21) as the sole karyotypic aberration and demonstrated that the molecular consequence of that translocation was fusion of the PHD finger protein 1 (*PHF1*) gene from 6p21 with the transcription factor binding to IGHM enhancer 3 gene (*TFE3*) from Xp11 (39). In the present study, we found *NDRG1-PLAG1* and *TRPS1-PLAG1* in chondroid syringomas. Thus, although the data are extremely limited, malignant chondroid syringoma and benign chondroid syringoma seem to be developed through different pathogenetic mechanisms.

Genetic studies of both cutaneous and soft tissue myoepithelial neoplasms have demonstrated considerable genetic heterogeneity (40-46). In some tumors, rearrangements of the Ewing sarcoma breakpoint region 1 gene (*EWSR1*) leading to the fusion genes *EWSR1-ZNF444*, *EWSR1-PBX1*, *EWSR1-PBX3* or *EWSR1-POU5F1* were found (40, 43-45, 47). In benign myoepithelial tumors, rearrangements of *PLAG1* have been found (41, 42, 48, 49). Matsuyuama *et al*. (48) studied 16 cutaneous mixed tumors and found that all of them expressed PLAG1, predominantely in cells with myopithelial or chondroid differentiation. Because they did not detect the fusion genes *CTNNB1-PLAG1*, *LIFR-PLAG1*, *CHCHD7-PLAG1* which have been reported in pleomorphic adenomas of the salivary gland, they concluded that the mechanism of *PLAG1* overexpression in cutaneous mixed tumors may be different from that in pleomorphic adenomas (48, 50-52). Bahrami and co-workers (42) found *PLAG1* rearrangements in 8 out of 11 (73%) benign myoepitheliomas/mixed tumors of the skin and soft tissue. Antonescu and co-workers (41) detected *PLAG1* rearrangements in 13 out of 35 (37%) myoepithelial tumors lacking *EWSR1* and *FUS* rearrangements and identified a *LIFR-PLAG1* fusion in one case (Table IV). Recently, Russell-Goldman and co-workers (49) showed that PLAG1 is expressed in skin mixed tumors referred to as being of the apocrine type but not in eccrine-type tumors. The conclusion from the above-mentioned studies was that a subset of benign myoepitheliomas/mixed tumors of the skin and soft tissue exists that are genetically related to their salivary gland counterparts (41, 42, 46, 48, 49). *PLAG1* together with *PLAGL1* (at chromosome subband 6q24.2) and *PLAGL2* (at 20q11.21) constitute the *PLAG* gene family coding for zing finger proteins (53-55). All three members of the family are implicated in neoplasia (54, 55). *PLAG1* was shown to be rearranged in pleomorphic adenomas of the salivary glands, lipoblastomas, as well as other tumors *via* chromosomal translocations targeting 8q12 (50-52, 56, 57) (Table IV). *PLAGL1* functions as a suppressor of cell growth and is often deleted or methylated and silenced in cancer cells (58-62). A *MYB-PLAGL1* fusion was reported in three acute lymphoblastic leukemias (63-65). *PLAGL2* is a proto-oncogene more structurally and functionally similar to *PLAG1* than is *PLAGL1* (53-55, 66-68). Overexpression of *PLAGL2* was seen in acute myeloid leukemia, bladder urothelial carcinoma, and colorectal cancer (67-71).

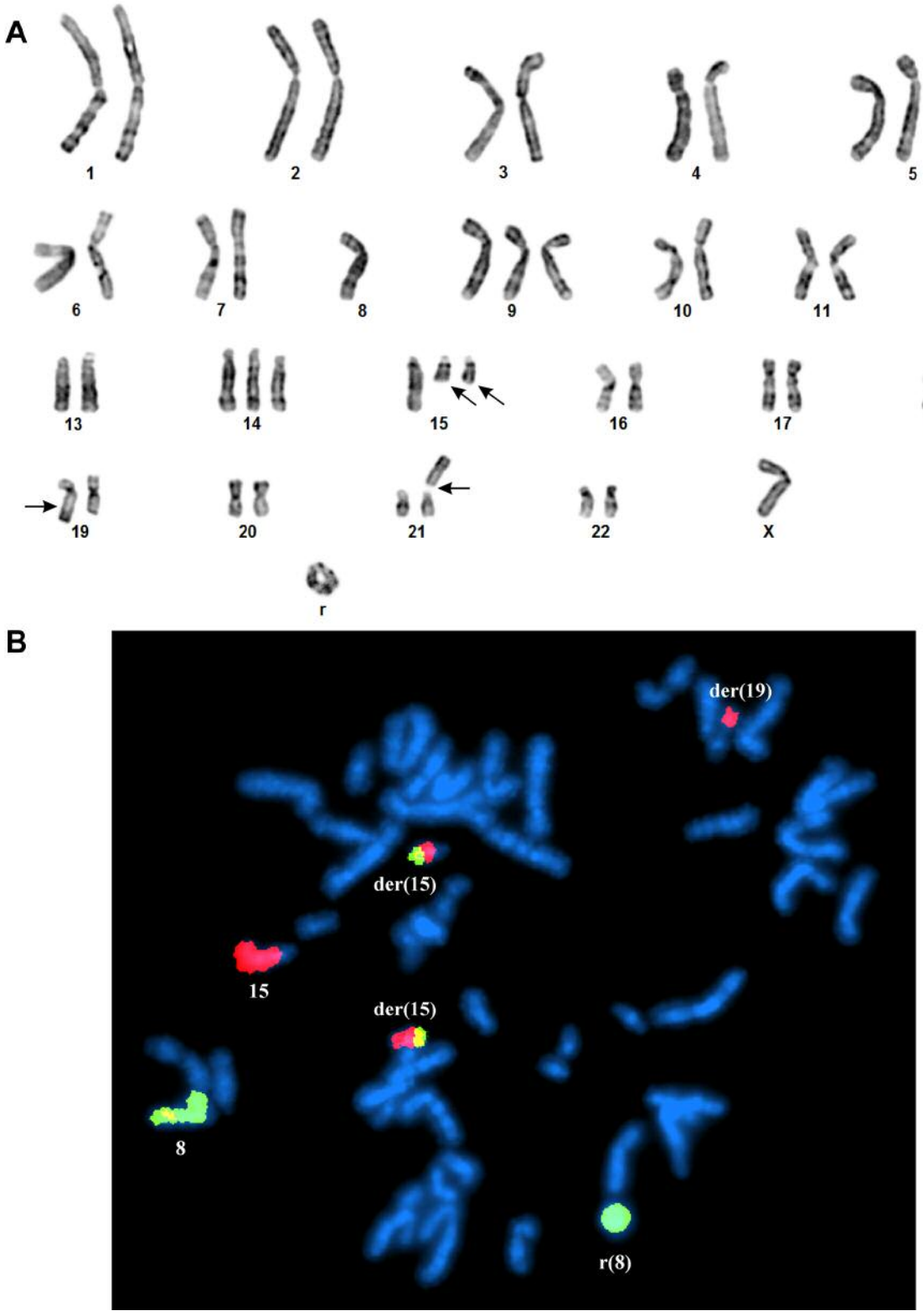

Figure 2. *G-banding and FISH analyses of the case 1 of chondroid syringoma. (A) Representative karyogram demonstrating the chromosome aberrations (breakpoints are shown by arrows). (B) FISH using whole chromosome painting probes for chromosome 8 (green signal) and chromosome 15 (red signal) shows the normal chromosomes 8 and 15, the ring chromosome consisting of chromosome 8 material, the two der(15)t(8;15), and the der(19)t(15;19).*

Table III. *The retrieved sequences from the fastq file of the RNA sequencing using the "grep" command and the search term "ATTGGCCAAAATGGGAAGGA" which is the first 20 nt in the exon 3 of PLAG1 to nt 286-305 in the PLAG1 reference sequence with the accession number NM_002655.2.*

Sequence	Gene
ACCGGTTCCATTTAGAGGATAGTCCATTTTCACTTTTTTTTCATAGATTGGCCAAAATGGGAAGGATTGGATTCC	*PLAG1*
CTTTCTCCCTCGCGTTAGATTGGCCAAAATGGGAAGGATTGGATTCCACTCTCTTCCACGAAGAGTCAATGGGAC	*NDRG1-PLAG1*
CCTCGCGTTAGATTGGCCAAAATGGGAAGGATTGGATTCCACTCTCTTCCACGAAGAGTCAATGGGACTGGCTAA	*NDRG1-PLAG1*
CCCAGATTGGCCAAAATGGGAAGGATTGGATTCCACTCTCTTCCACGAAGAGTCAATGGGACTGGCTAAGATCAA	*PLAG1*
GTTAGATTGGCCAAAATGGGAAGGATTGGATTCCACTCTCTTCCACGAAGAGTCAATGGGACTGGCTAAGATCAA	*PLAG1*

Table IV. *The PLAG1 fusion genes which are currently reported in various neoplasias. PLAG1 maps to chromosome subband 8q12.1, its position is chr8:56,160,909-56,211,273 (based on GRCh38/hg38 assembly) and its orientation is from telomere (tel) to centromere (cen).*

Cytogenetic map location of 5'partner gene	Position of 5'partner gene on GRCh38/hg38 assembly	Orientation of 5'partner gene	5'partner gene	Neoplasia	Reference
1p35.2	chr1:30,931,506-31,065,717	Cen->tel	*PUM1*	Mesenchymal tumor	(80)
2q32.2	chr2:188,974,320-189,012,746	Cen->tel	*COL3A1*	Lipoblastoma	(81)
3p22.1	chr3:41,194,741-41,239,949	Tel->cen	*CTNNB1*	Pleomorphic salivary gland adenoma	(51)
5p13.1	chr5:38,474,963-38,595,404	Cen->tel	*LIFR*	Pleomorphic salivary gland adenoma and soft tissue myoepithelial tumor	(41, 52)
7q21.3	chr7:94,394,561-94,431,232	Cen->tel	*COL1A2*	Lipoblastoma	(57)
8p11.23	chr8:38,411,139-38,468,834	Cen->tel	*FGFR1*	Pleomorphic salivary gland adenoma	(82)
8q11.23	chr8:53,966,556-54,022,448	Tel->cen	*TCEA1*	Pleomorphic salivary gland adenoma	(50, 56)
8q12.1	chr8:56,211,789-56,218,809	Cen->tel	*CHCHD7*	Pleomorphic salivary gland adenoma	(50)
8q12.1	chr8:60,516,987-60,623,644	Cen->tel	*RAB2A*	Lipoblastoma	(81)
8q23.3	chr8:115,408,496-115,669,001	Tel->cen	*TRPS1*	Soft tissue myoepithelial tumor, uterine myxoid leiomyosarcoma, and chondroid syringoma	(32, 33), Present study
8q24.13	chr8:121,612,116-121,641,440	Tel->cen	*HAS2*	Lipoblastoma	(57)
8q24.22	chr8:133,237,176-133,297,586	Tel->cen	*NDRG1*	Chondroid syringoma	Present study
14q24.1	chr14:67,865,032-68,683,118	Cen->tel	*RAD51B*	Lipoblastoma	(83)

The characteristics of the *PLAG1*-reported fusion genes found in pleomorphic adenomas of the salivary glands, lipoblastomas, and other tumors are that the chromosome rearrangements result in fusion of the 5'-non-coding region of *PLAG1* with the partner gene's 5'-non-coding region exchanging the two genes' regulatory elements. Promoter swapping between *PLAG1* and the fusion partner thus takes place and the expression of *PLAG1* comes under the control of the other gene's promoter leading to overexpression or ectopic activation of *PLAG1* (51, 52, 54, 56, 57). Overexpression or ectopic activation of *PLAG1* in its turn leads to deregulation of *PLAG1*-target genes and tumor formation (72-75).

In vitro studies showed that ectopic expression of *PLAG1* in NIH3T3 cells results in loss of cell–cell contact inhibition and anchorage-independent growth. *PLAG1*-expressing NIH3T3 cells induce tumors in nude mice (66). In transgenic mice, targeted *PLAG1* overexpression in salivary or mammary glands resulted in tumor development (76, 77). *PLAG1* was found to regulate the expression of the *IGF2* gene coding for insulin-like growth factor 2, to upregulate genes which are associated with IGF and WNT signaling, and to deregulate a plethora of long non-coding RNAs (54, 66, 72-75, 78, 79).

NDRG1-PLAG1 and *TRPS1-PLAG1* share characteristics of other *PLAG1* fusion genes. In the first tumor we analyzed, the untranslated exon 3 of *PLAG1* fused with exon 1 of *NDRG1*. Thus, *PLAG1* expression came under the control of *NDRG1* promoter. *NDRG1* is ubiquitously expressed and codes for a cytoplasmic protein involved in stress responses, hormone responses, cell growth, and differentiation (https://www.ncbi.nlm.nih.gov/gene/10397#gene-expression). In the second tumor, exon 2 or exon 3 of *PLAG1* fused with exon 1 of *TRPS1* and the expression of *PLAG1* came under control of the *TRPS1* promoter. *TRPS1* is also ubiquitously expressed and codes for

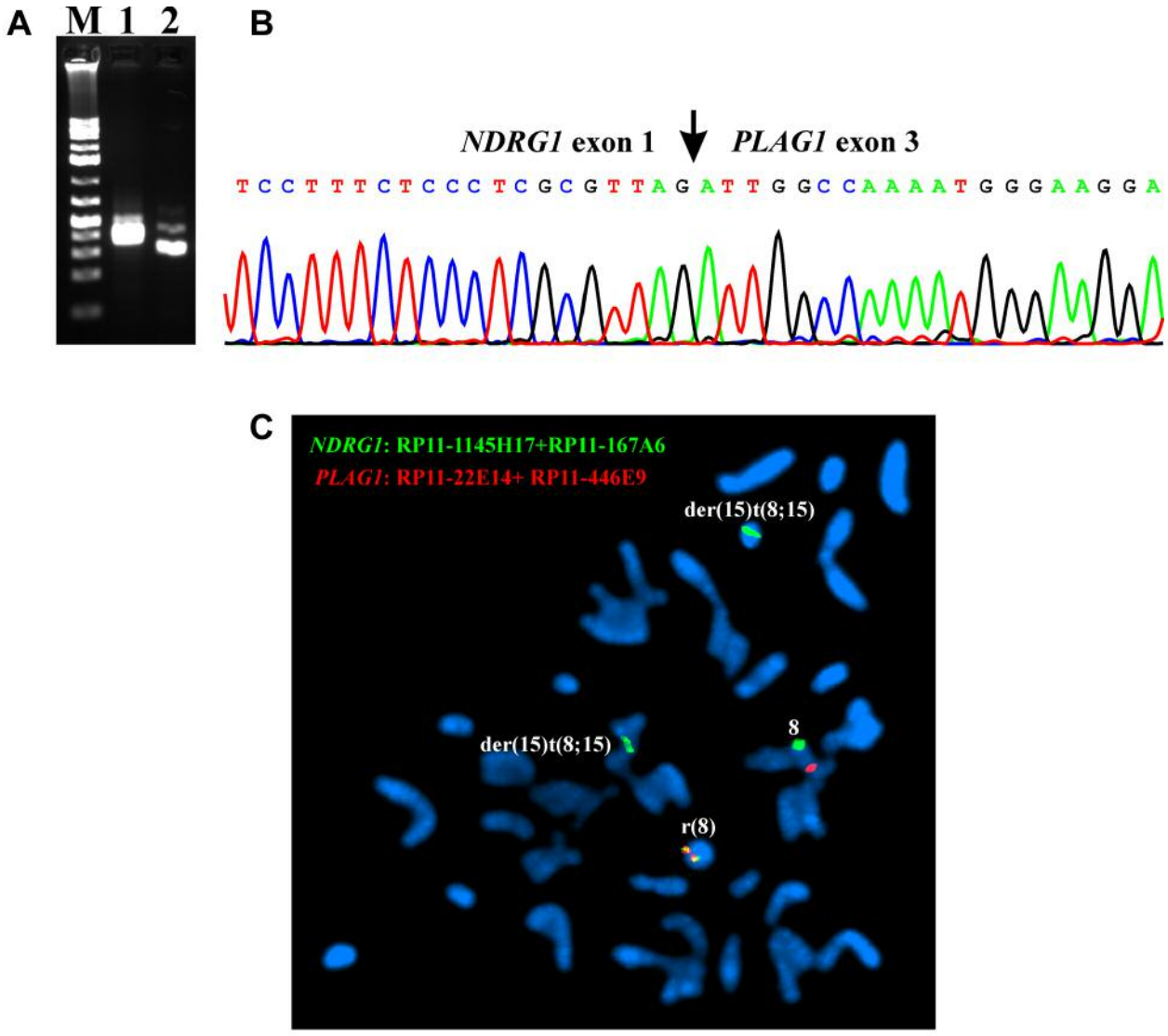

Figure 3. *RT-PCR, Sanger sequencing, and FISH analyses of the case 1 of chondroid syringoma. (A) Gel electrophoresis showing the amplified NDRG1-PLAG1 cDNA fragments using the primer combinations NDRG1-1F1/PLAG1-498R1 (lane 1) and NDRG1-23F1/PLAG1-458R1 (lane 2). M, GeneRuler 1 Kb Plus DNA ladder (ThermoFisher Scientific). (B) Partial sequence chromatograms of the cDNA amplified fragment showing the junction position of exon 1 of NDRG1 with exon 3 of PLAG1. (C) FISH analysis on metaphase spreads with PLAG1 probe (red signal) and NDRG1 probe (green signal) showing that the NDRG1-PLAG1 fusion gene was on the ring chromosome 8 (yellow signal). One copy of PLAG1 (red signal) is on chromosome 8. NDRG1 probe (green signal) hybridized also to chromosome 8 and the two der(15)t(8;15) chromosomes.*

a transcription factor that represses GATA-regulated genes and binds to a dynein light-chain protein (https://www.ncbi.nlm.nih.gov/gene/7227#gene-expression).

The number of *PLAG1* fusion genes in various neoplasias until now, including the two present ones, is thirteen (Table IV). Worthy of mention is that in seven of them, the 5' fusion partner maps to chromosome 8: one to the p arm and six to the q arm. Evidently, recombinations involving chromosome 8, particularly the long arm, are for some reason particularly common events in *PLAG1* activating fusions.

Conflicts of Interests

No potential conflicts of interest exist.

Authors' Contributions

IP designed and supervised the research, performed molecular genetic experiments, bioinformatics analysis, and wrote the article. LG performed cytogenetic analysis and evaluated the FISH data. KA performed molecular genetic experiments, FISH analysis, and evaluated the data. ML-I performed the pathological examination.

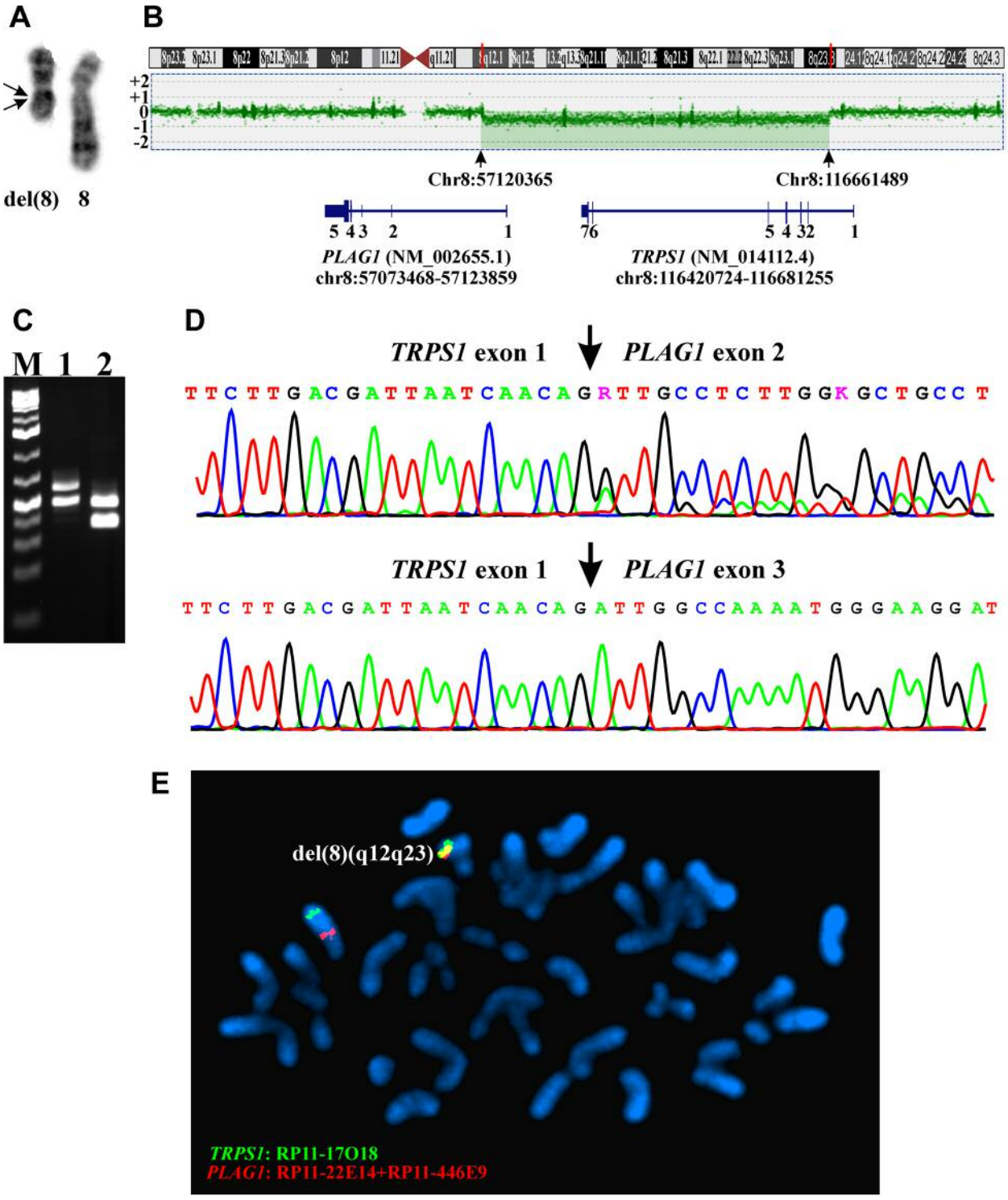

Figure 4. *Genetic analyses of the case 2 of chondroid syringoma. (A) Partial karyotype showing the del(8)(q12q23) and the normal chromosome 8 (breakpoints are shown by arrows). (B) aCGH showing the deletion in the q arm of chromosome 8. Based on the hg19 assembly, the deletion started at position Chr8:57120365 in intron1 of PLAG1 and ended at Chr8:116661489 in exon 1 of TRPS1. (C) Gel electrophoresis showing the amplified TRPS1-PLAG1 fragments using the primer combinations TRPS1-200F1/PLAG1-498R1 (lane 1) and TRPS1-318F1/PLAG1-458R1 (lane 2). (D) Partial sequence chromatograms of the cDNA amplified fragment showing the junction positions of exon 1 of TRPS1 with exon 2 of PLAG1 and exon 1 of TRPS1 with exon 3 of PLAG1. E) FISH analysis on metaphase spreads with PLAG1 probe (red signal) and TRPS1 probe (green signal) showing that the TRPS1-PLAG1 fusion gene was on the del(8)(q12q23) (yellow signal). A copy of PLAG1 (red signal) and TRPS1 (green signal) is on chromosome 8.*

IL performed the pathological examination. FM supervised the research. SH assisted with experimental design and writing of the article. All Authors read and approved the final article.

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