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Fusion of the *HMGA2* and *BNC2* Genes in Uterine Leiomyoma With t(9;12)(p22;q14)

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Abstract. *Background/Aim: The translocation t(9;12)(p22;q14~15) has been reported in lipomas, pleomorphic adenomas, a myolipoma, two chondroid hamartomas, and two uterine leiomyomas. In lipomas and pleomorphic adenomas, the translocation fuses HMGA2 (12q14) with the NFIB gene from 9p22; in myolipoma, it fuses HMGA2 with C9orf92 from 9p22; and in chondroid hamartomas, fluorescence in situ hybridization (FISH) investigations showed the chromosomal aberration to cause intragenic rearrangement of HMGA2. The translocation's molecular consequence in a uterine leiomyoma is described here. Materials and Methods: A typical leiomyoma was investigated using banding cytogenetics, FISH, RNA sequencing, reverse transcription polymerase chain reaction and Sanger sequencing. Results: A single translocation, t(9;12)(p22;q14) leading to an HMGA2::BNC2 chimera, was found in tumor cells. A sequence of the untranslated part of exon 5 of HMGA2 (nucleotide 1035 in the NCBI reference sequence NM_003483.4) had fused with a sequence from the untranslated part of exon 7 of BNC2 from 9p22 (nucleotide 9284 in reference sequence NM_017637.6). Conclusion: At the molecular level, the t(9;12)(p22;q14~15) found in several benign tumors appears to be heterogeneous fusing HMGA2 with either BNC2, C9orf92 or NFIB which all three map close to one another within a 3 Mbp region in 9p22. Because the fusion point in HMGA2 in the present tumor lays downstream from the first Let-7 miRNA consensus binding site, we conclude that deletion of the first Let-7 miRNA binding site is not important for the transcriptional upregulation of HMGA2 caused by the genomic rearrangement.*

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Key Words: Uterine leiomyoma, chromosomal aberration, high mobility group AT-hook 2 (*HMGA2*) gene, fusion transcript.

Uterine leiomyomas are benign neoplasms found in most middle-aged and older women (1, 2). They presumably arise from a single myometrial stem cell which, by acquiring a suitable somatic mutation, has been transformed neoplastically (3-5). The most common tumorigenic events (70%) seem to be point mutations targeting exons 1 and 2 of the mediator complex subunit 12 gene which maps to chromosome sub-band Xq13.1 and codes for a subunit of the multiprotein transcriptional regulator mediator complex (6). At a higher level of genomic organization, cytogenetic examinations of uterine leiomyomas have detected nonrandom chromosome aberrations in 25-40% of tumors subjected to banding analysis (7-14). By far the most common clonal abnormality thus detected is the translocation t(12;14)(q14~15;q23-24), seen in 15-20% of karyotypically abnormal leiomyomas (15, 16). Other rearrangements targeting 12q14~15 are also occasionally seen, including t(9;12)(p22;q14~15) that was hitherto been reported in two uterine leiomyomas (17, 18). None of these two leiomyomas has been analyzed molecularly. Here, we describe another uterine leiomyoma that carried a t(9;12)(p22;q14) chromosome translocation and include a full description of the molecular consequences of that cytogenetic aberration.

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). Written informed consent was obtained from the patients for publication of the case details.

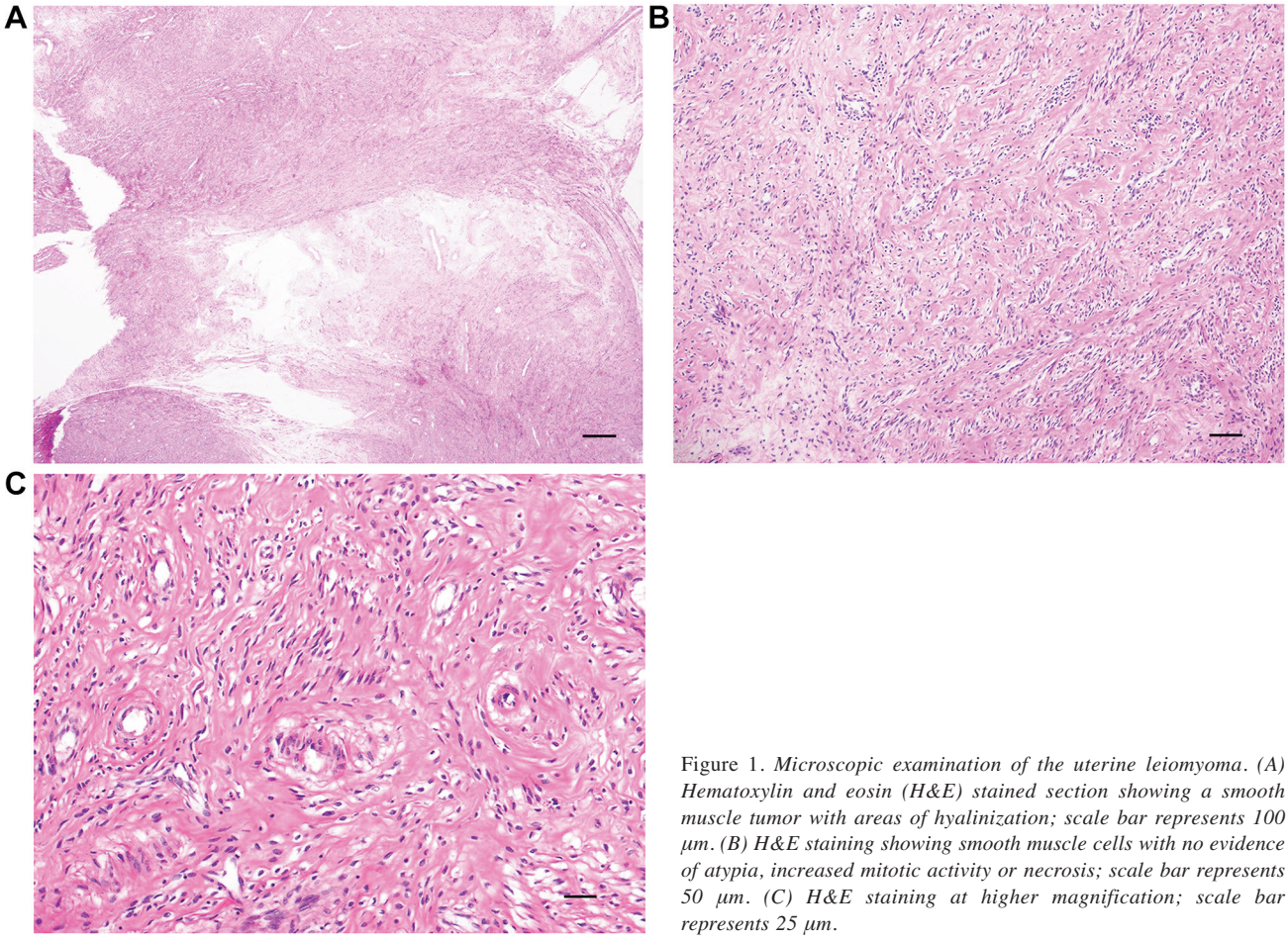

Figure 1. *Microscopic examination of the uterine leiomyoma. (A) Hematoxylin and eosin (H&E) stained section showing a smooth muscle tumor with areas of hyalinization; scale bar represents 100 μm. (B) H&E staining showing smooth muscle cells with no evidence of atypia, increased mitotic activity or necrosis; scale bar represents 50 μm. (C) H&E staining at higher magnification; scale bar represents 25 μm.*

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

Patient. The patient was a 45-year-old woman who underwent hysterectomy because of uncertainty as to whether her uterine tumors were benign or malignant. Several leiomyomatous tumors were found in the uterus, the largest, which was subsequently examined genetically, had a diameter of 24 cm. Microscopic evaluation showed a smooth muscle tumor with no evidence of atypia, increased mitotic activity or necrosis (Figure 1). Areas with degenerative changes in the form of hyalinization were seen (Figure 1A). The diagnosis was leiomyoma.

G-banding, karyotyping and fluorescence in situ hybridization (FISH). A piece of tumor tissue was mechanically and enzymatically disaggregated and the resulting cells were short-term cultured (7 days), harvested, and processed for cytogenetic examination as described in detail previously (18). Chromosome preparations were G-banded using Wright's stain (Sigma Aldrich; St Louis, MO, USA) and examined. The subsequent cytogenetic analysis and karyotype description followed the recommendations of the International System for Human Cytogenomic Nomenclature (ISCN) 2020 guidelines (19).

FISH analysis was performed on metaphase plates using an in-house prepared *HMGA2* break-apart probe as previously described (20-22). The centromeric (proximal) part of the probe (red signal) was constructed from a pool of clones RP11-185K16, RP11-30I11, and RP11-662G15. The telomeric (distal) part of the probe (green signal) was constructed from a pool of the clones RP118B13, RP11-745O10, and RP11-263A04 (20-22).

RNA sequencing. Total RNA was extracted from frozen (-80 ˚C) tumor material adjacent to that used for cytogenetic analysis and histologic examination using the 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 (http://genomics.no/oslo/) for high-throughput paired-end RNA-sequencing. The FASTQC software was used for quality control of the raw sequence data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Fusion transcripts were detected using the FusionCatcher software (23).

Reverse transcription (RT) PCR and Sanger sequencing analyses. In order to confirm the existence of the fusion transcripts (see below), RT-PCR and Sanger sequencing analyses were performed. The primers are listed in Table I. The methods for cDNA synthesis,

Table I. *Designation, sequence (5´->3´), and position in reference sequences of the forward (F) and reverse (R) primers of the high mobility group AT-hook 2 (HMGA2) and the basonuclin 2 (BNC2) genes, used for polymerase chain reaction (PCR) amplification and Sanger sequencing analyses.*

Designation	Sequence	Reference sequence: Position
HMGA2-929F1	ACC GGT GAG CCC TCT CCT AAG AG	NM_003483.4: 929-951
HMGA2-1012F1	AGC AGA AGC CAC TGG AGA AAA AC	NM_003483.4: 1012-1034
BNC2-9409R1	TTT GAT GGC CAA GTG CTG AAC TG	NM_017637.6: 9431-9409
BNC2-9443R1	AAG GGG ATA AAC AGG CAA TTA CGC	NM_017637.6: 9466-9443

RT-PCR amplification, and Sanger sequencing are described elsewhere (22, 24-26). For the first, outer PCR the primers HMGA2-929F1 and BNC2-9443R1 were used, whereas the primer combinations HMGA2-1012F1 and BNC2-9409R1 were used for the second, inner PCR. The sequences obtained by Sanger sequencing were aligned to the NCBI reference sequences NM_003483.4 [high mobility group AT-hook 2 (*HMGA2*), transcript variant 1, mRNA] and NM_017637.6 [basonuclin 2 (*BNC2*), transcript variant 1, mRNA] using the Basic Local Alignment Search Tool (BLAST) (27).

Results

The G-banding analysis of tumor cells yielded a karyotype with a single chromosome abnormality in all twelve examined metaphases: 46,XX,t(9;12)(p22;q14)[12] (Figure 2A). FISH analysis on metaphase spreads showed that the distal part of the *HMGA2* probe hybridized to the p22 band of der(9), whereas the proximal part of the probe hybridized to the q14 band of der(12) (Figure 2B).

Analysis of raw sequencing data using FusionCatcher detected a fusion transcript in which a sequence of the untranslated part of exon 5 of *HMGA2* from 12q14.3 (nucleotide 1221 in the NCBI reference sequence NM_003483.4) had fused with a sequence of the untranslated part of exon 7 of *BNC2* from 9p22 (nucleotide 9284 in reference sequence NM_017637.6): AGCAGTTGGATCTTTT GAAGGGAGAAGACACTGCAGTGACCACTTATTCT*::TA ATGTGAACGCTGCTGACAAGACTGTCACACTAACAGC AGACAACACCC.

Nested PCR with the inner primer combination HMGA2-1012F1 and BNC2-9409R1 amplified a 358 bp fragment (Figure 2C), which by Sanger sequencing was shown to be a chimeric *HMGA2::BNC2* cDNA fragment with a fusion point identical to that obtained by RNA sequencing (Figure 2D). The chimeric *HMGA2::BNC2* cDNA fragment has been registered in GenBank with accession number ON989351.

Discussion

The centerpiece of the present study was a uterine leiomyoma with t(9;12)(p22;q14) as the sole cytogenetic abnormality. The gene-level consequence of the translocation was fusion of *HMGA2* from 12q14 with *BNC2* from 9p22 (Figure 2D-F). This is the first time that the latter gene is demonstrated to be an *HMGA2* fusion partner. The *BNC2* fusion point in the chimeric *HMGA2::BNC2* was in the last exon (exon 7 in sequence with accession no NM_017637.6) within the 3´-untranslated region that has many binding miRNA sites (28-30).

BNC2 codes for one of the most evolutionarily conserved DNA-binding zinc finger proteins and is highly expressed in the uterus (31). The gene has multiple promoters, many alternative spliced exons, and also other genetic features giving it the potential to generate 90000 mRNA alternative transcripts coding for more than 2000 protein isoforms (32). Interestingly, *BNC2* has been implicated in ovarian carcinogenesis (33-35).

The general pattern of *HMGA2* involvement in tumorigenesis is that disruption of the locus results in the gene's upregulation and expression (36). The main mechanism appears to be truncation of *HMGA2*, resulting in physical separation of exons 1-3, that code for three AT-hook domains, from the 3´-untranslated region of the gene which regulates *HMGA2* transcription (20-22, 26, 37-39). The 3´-untranslated region contains seven Let-7 miRNA consensus binding sites which suppress *HMGA2* expression. Substitution or deletion of them leads to upregulation of *HMGA* expression (38, 39).

In the present tumor, the fusion point in *HMGA2* was found in the last (fifth) exon, after the gene's stop codon, or 52 nucleotides downstream from the first Let-7 miRNA consensus binding site (38, 39). Fusion points downstream from the first Let-7 binding site were previously reported in two lipomas (40), an aggressive angiomyoxoma (41), uterine leiomyomas (42, 43), a pleomorphic adenoma cell line with t(1;12)(p22;q15) (43, 44), a polycythemia vera carrying a balanced t(3;12)(q26;q14) which fused *HMGA2* with a sequence from intron 9 of *TNIK* (45), in paroxysmal nocturnal hemoglobinuria (46), and in a myeloproliferative neoplasm (47) (Table II). These data indicate that deletion of the first Let-7 miRNA consensus binding site is not important in transcriptional upregulation of *HMGA2*.

Apart from leiomyomas, the translocation t(9;12)(p22;q14~15), or variants thereof, have also been reported

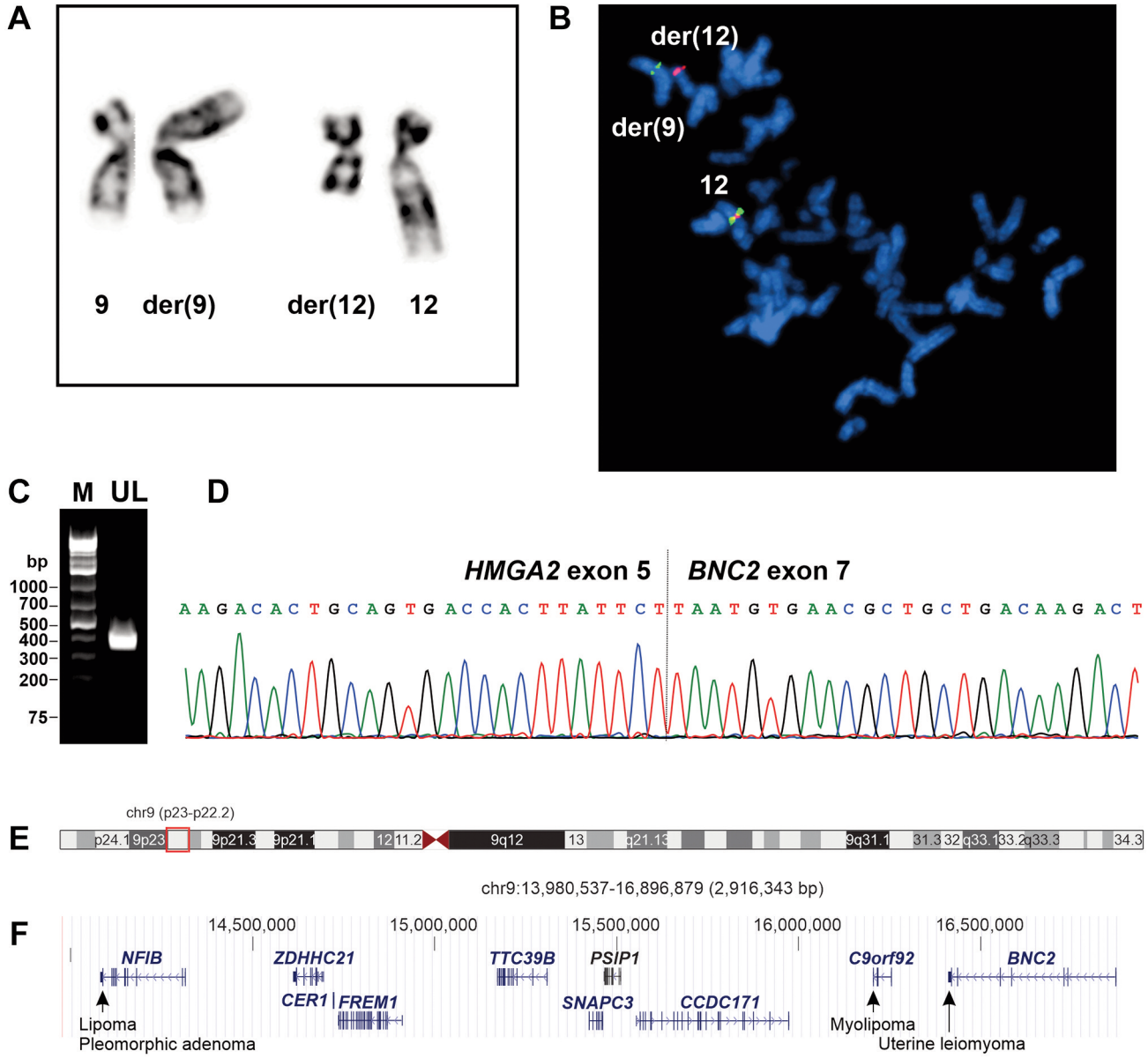

Figure 2. *Genetic examination of the uterine leiomyoma. (A) Partial G-banded karyotype showing the der(9)t(9;12)(p22;q14) and der(12)t(9;12)(p22;q14) together with the corresponding normal chromosome homologs. (B) FISH on a metaphase spread with the high-mobility group AT-hook 2 (HMGA2) break-apart probe showing a yellow normal signal on chromosome 12, a red signal on der(12), and a green signal on der(9), suggesting rearrangement of the HMGA2 gene. (C) Gel electrophoresis showing the amplified 358 bp cDNA fragment (UL) using the primer combination HMGA2-1012F1 and BNC2-9409R1. M: GeneRuler 1 kb Plus DNA ladder (ThermoFisher Scientific). (D) Partial Sanger sequencing chromatogram of the amplified fragment showing the junction between exon 5 of HMGA2 and exon 7 of BNC2. (E) Chromosome 9 ideogram showing the 9p22 region where the breakpoints were found in lipomas, pleomorphic adenomas, myolipoma, and the present uterine leiomyoma carrying t(9;12)(p22;q14~15). (F) Diagram showing the fusion points of HMGA2 with NFIB in lipomas and pleomorphic adenomas, C9orf92 in myolipoma, and BNC2 in uterine leiomyoma.*

in lipomas (48-50), pleomorphic adenomas (51, 52), chondroid hamartomas (53) and a myolipoma (20). In lipomas and pleomorphic adenomas, t(9;12)(p22;q14~15) fuses *HMGA2* with the nuclear factor I B (*NFIB*) gene (48-51) (Figure 2E and F). In the reported fusion transcripts, exon 3 or 4 of *HMGA2* recombined with the last exon of *NFIB* (exon 9 in reference sequence NM_005596.3) giving rise to a stop codon shortly after the fusion point (48-51). In chondroid hamartomas, the t(9;12)(p22;q14~15) resulted in intragenic rearrangement of *HMGA2* (53). In myolipoma

Table II. *Reported tumors in which HMGA2-chimeras had fusion points downstream of the first Let-7 binding site of HMGA2. In the reference sequence of HMGA2 with accession number NM_003483.4, the first Let-7 binding site (CGCCAACGTTCGATTTCTACCTCA) is found between nucleotides 1146 and 1169. The second Let-7 binding site (AGACCTGAATACCACTTACCTCA) is found between nucleotides 2232 and 2254. In fusion sequences, HMGA2 sequences are written with upper case and fusion partner genes with lower case letters.*

Tumor	Cytogenetic aberration	Fusion point on sequence NM_003483.4	3´- fusion partner	Fusion sequence	Sequence accession number/ References
Uterine leiomyoma	t(9;12)(p22;q14)	1221	*BNC2*	CTGCAGTGACCACTTATTCT::taatgtgaacgctgctgaca	ON989351/Present
Lipoma	add(12)(q24.1)	1247	Intergenic sequence 12q15	CCATGGTCTTTCCACTTTCA::gtgctggggttacaggcatg	FJ469145/(36)
Lipoma	inv(12)(q13q15)	1238	Intergenic sequence 12q21.33	TCTGTATTGCCATGGTCTTT::gagtactctatttatttcct	FJ469146/(36)
Aggressive angiomyxoma	t(1;12)(p32;q15)	1221	Intergenic sequence 1p32.2	CTGCAGTGACCACTTATTCT::ctgtccttgtcagtgaagct CTGCAGTGACCACTTATTCT::gtgaagaaaccaaggcaaca	EU004591 & EU004592/(37)
Uterine leiomyoma	t(12;14)(q15;q24)	1913	*RAD51B*	TTGTTTTCAGGACAACACTT::agcaacaggattgtcacaaa	AF533653/(38)
Uterine leiomyoma	t(12;14)(q15;q24)	1221	*RAD51B*	CTGCAGTGACCACTTATTCT::gttatcttgacgaatcagat	AY138860/(38)
Pleomorphic adenoma	t(1;12)(p22;q15)	1234	*CDC14A*	TTATTCTGTATTGCCATGGT::aatctgaaatccaaaatatc	U29111/(39, 40)
Uterine leiomyoma	t(8;12)(q23;q14)	1765	*LINC00536*	AATGCTGATGTATCCTTTCA::aagtcaagagacagaacaga	U29110/(39)
Polycythemia vera	t(3;12)(q26;q14)	1866	*TNIK*	TGGATATCACACATATCAG::ggctttgtagtgtcactgt	None/(41)
Paroxysmal nocturnal hemoglobinuria	t(12;12)(q13;q15)	2360	Intergenic sequence 12q15	TCTTCATTCAAACTGCACTT::gaatttgaatgttggcctgt	None/(42)
Paroxysmal nocturnal hemoglobinuria	ins(12)(p12~13q13q12)	1729	Intergenic sequence 12q14.3	TGATGATTTTTAACTTTTTA::tcatcaatcctaatgataaa	None/(42)
Myeloproliferative neoplasm	t(12;22)(q14;q13)	2114	*EFCAB6*	ATCTAAATTTCTTTTGCTAT::tggccaacatggtgaaacct	None/(43)

with t(9;12), finally, exon 4 of *HMGA2* fused to exon 4 of *C9orf92* (fusion between nucleotide 1093 of the *HMGA2* reference sequence NM_003483.4 with nucleotide 276 from the *C9orf92* reference sequence NR_171034.1) as a result of the translocation. A truncated form of HMGA2 protein was generated containing amino acid residues 1-94 from HMGA2 and 6 amino acid residues from *C9orf92* (20) (Figure 2E and F).

Thus, cytogenetically identical translocations t(9;12)(p22;q14~15) can be remarkably heterogeneous at the molecular level fusing *HMGA2* with either *BNC2*, *C9orf92* or *NFIB*, which all map within 3 Mbp in chromosome band 9p22. Regardless of the exact mechanism, transcriptional upregulation of *HMGA2* seems to be the important result achieved by the removal of the gene's 3´-untranslated region which contains Let-7 binding sites and other sequences which normally regulate *HMGA2* transcription (37, 54). Our results demonstrate that deletion of the first Let-7 miRNA binding site is not important for the transcriptional upregulation of *HMGA2*.

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 analyses, and wrote the manuscript. KA performed molecular genetic experiments and interpreted the data. LG performed the 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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References

1 Stewart EA, Cookson CL, Gandolfo RA and Schulze-Rath R: Epidemiology of uterine fibroids: a systematic review. BJOG *124(10)*: 1501-1512, 2017. PMID: 28296146. DOI: 10.1111/1471-0528.14640

2 Hervé F, Katty A, Isabelle Q and Céline S: Impact of uterine fibroids on quality of life: a national cross-sectional survey. Eur J Obstet Gynecol Reprod Biol *229*: 32-37, 2018. PMID: 30099225. DOI: 10.1016/j.ejogrb.2018.07.032

3 Mas A, Cervelló I, Gil-Sanchis C, Faus A, Ferro J, Pellicer A and Simón C: Identification and characterization of the human leiomyoma side population as putative tumor-initiating cells. Fertil Steril *98(3)*: 741-751.e6, 2012. PMID: 22633281. DOI: 10.1016/j.fertnstert.2012.04.044

4 Holdsworth-Carson SJ, Zaitseva M, Vollenhoven BJ and Rogers PA: Clonality of smooth muscle and fibroblast cell populations isolated from human fibroid and myometrial tissues. Mol Hum Reprod *20(3)*: 250-259, 2014. PMID: 24243625. DOI: 10.1093/molehr/gat083

5 Patterson AL, George JW, Chatterjee A, Carpenter TJ, Wolfrum E, Chesla DW and Teixeira JM: Putative human myometrial and fibroid stem-like cells have mesenchymal stem cell and endometrial stromal cell properties. Hum Reprod *35(1)*: 44-57, 2020. PMID: 31913469. DOI: 10.1093/humrep/dez247

6 Mehine M, Mäkinen N, Heinonen HR, Aaltonen LA and Vahteristo P: Genomics of uterine leiomyomas: insights from high-throughput sequencing. Fertil Steril *102(3)*: 621-629, 2014. PMID: 25106763. DOI: 10.1016/j.fertnstert.2014.06.050

7 Heim S, Nilbert M, Vanni R, Floderus UM, Mandahl N, Liedgren S, Lecca U and Mitelman F: A specific translocation, t(12;14)(q14-15;q23-24), characterizes a subgroup of uterine leiomyomas. Cancer Genet Cytogenet *32(1)*: 13-17, 1988. PMID: 3355995. DOI: 10.1016/0165-4608(88)90305-6

8 Nilbert M, Heim S, Mandahl N, Flodérus UM, Willén H and Mitelman F: Karyotypic rearrangements in 20 uterine leiomyomas. Cytogenet Cell Genet *49(4)*: 300-304, 1988. PMID: 3248388. DOI: 10.1159/000132682

9 Nilbert M, Heim S, Mandahl N, Flodérus UM, Willén H and Mitelman F: Characteristic chromosome abnormalities, including rearrangements of 6p, del(7q), +12, and t(12;14), in 44 uterine leiomyomas. Hum Genet *85(6)*: 605-611, 1990. PMID: 2227952. DOI: 10.1007/BF00193583

10 Nilbert M, Heim S, Mandahl N, Flodérus UM, Willén H and Mitelman F: Trisomy 12 in uterine leiomyomas. A new cytogenetic subgroup. Cancer Genet Cytogenet *45(1)*: 63-66, 1990. PMID: 2302686. DOI: 10.1016/0165-4608(90)90067-k

11 Pandis N, Heim S, Bardi G, Flodérus UM, Willén H, Mandahl N and Mitelman F: Chromosome analysis of 96 uterine leiomyomas. Cancer Genet Cytogenet *55(1)*: 11-18, 1991. PMID: 1913597. DOI: 10.1016/0165-4608(91)90229-n

12 Dal Cin P, Moerman P, Deprest J, Brosens I and Van den Berghe H: A new cytogenetic subgroup in uterine leiomyoma is characterized by a deletion of the long arm of chromosome 3. Genes Chromosomes Cancer *13(3)*: 219-220, 1995. PMID: 7669743. DOI: 10.1002/gcc.2870130313

13 Panagopoulos I, Gorunova L, Andersen K, Lobmaier I and Heim S: Several fusion genes identified in a spermatic cord leiomyoma with rearrangements of chromosome arms 3p and 21q. Cancer Genomics Proteomics *18(4)*: 531-542, 2021. PMID: 34183386. DOI: 10.21873/cgp.20278

14 Panagopoulos I, Andersen K, Gorunova L, Davidson B, Micci F and Heim S: 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. Cancer Genomics Proteomics *19(5)*: 636-646, 2022. PMID: 35985686. DOI: 10.21873/cgp.20348

15 Nibert M and Heim S: Uterine leiomyoma cytogenetics. Genes Chromosomes Cancer *2(1)*: 3-13, 1990. PMID: 2278965. DOI: 10.1002/gcc.2870020103

16 Sandberg AA: Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: leiomyoma. Cancer Genet Cytogenet *158(1)*: 1-26, 2005. PMID: 15771900. DOI: 10.1016/j.cancergencyto.2004.08.025

17 Kiechle-Schwarz M, Sreekantaiah C, Berger CS, Pedron S, Medchill MT, Surti U and Sandberg AA: Nonrandom cytogenetic changes in leiomyomas of the female genitourinary tract. A report of 35 cases. Cancer Genet Cytogenet *53(1)*: 125-136, 1991. PMID: 2036633. DOI: 10.1016/0165-4608(91)90124-d

18 Kataoka S, Yamada H, Hoshi N, Kudo M, Hareyama H, Sakuragi N and Fujimoto S: Cytogenetic analysis of uterine leiomyoma: the size, histopathology and GnRHa-response in relation to chromosome karyotype. Eur J Obstet Gynecol Reprod Biol *110(1)*: 58-62, 2003. PMID: 12932873. DOI: 10.1016/s0301-2115(03)00075-7

19 McGowan-Jordan J, Hastings RJ and Moore S: ISCN 2020: An International system for human cytogenomic nomenclature. Basel, Karger, pp. 164, 2020.

20 Panagopoulos I, Gorunova L, Agostini A, Lobmaier I, Bjerkehagen B and Heim S: Fusion of the *HMGA2* and *C9orf92* genes in myolipoma with t(9;12)(p22;q14). Diagn Pathol *11*: 22, 2016. PMID: 26857357. DOI: 10.1186/s13000-016-0472-8

21 Panagopoulos I, Gorunova L, Andersen HK, Pedersen TD, Lømo J, Lund-Iversen M, Micci F and Heim S: Genetic characterization of myoid hamartoma of the breast. Cancer Genomics Proteomics *16(6)*: 563-568, 2019. PMID: 31659109. DOI: 10.21873/cgp.20158

22 Panagopoulos I, Gorunova L, Andersen K, Lund-Iversen M, Hognestad HR, Lobmaier I, Micci F and Heim S: Chromosomal translocation t(5;12)(p13;q14) leading to fusion of high-mobility group AT-hook 2 gene with intergenic sequences from chromosome sub-band 5p13.2 in benign myoid neoplasms of the breast: A second case. Cancer Genomics Proteomics *19(4)*: 445-455, 2022. PMID: 35732319. DOI: 10.21873/cgp.20331

23 Nicorici D, Satalan H, Edgren H, Kangaspeska S, Murumagi A, Kallioniemi O, Virtanen S and Kikku O: FusionCatcher – a tool for finding somatic fusion genes in paired-end RNA-sequencing data. bioRxiv, 2014. DOI: 10.1101/011650

24 Panagopoulos I, Gorunova L, Lund-Iversen M, Bassarova A and Heim S: Fusion of the genes *PHF1* and *TFE3* in malignant chondroid syringoma. Cancer Genomics Proteomics *16(5)*: 345-351, 2019. PMID: 31467228. DOI: 10.21873/cgp.20139

25 Panagopoulos I, Gorunova L, Andersen K, Lund-Iversen M, Lobmaier I, Micci F and Heim S: *NDRG1-PLAG1* and *TRPS1-PLAG1* fusion genes in chondroid syringoma. Cancer Genomics Proteomics *17(3)*: 237-248, 2020. PMID: 32345665. DOI: 10.21873/cgp.20184

26 Panagopoulos I, Gorunova L, Andersen K, Lobmaier I, Micci F and Heim S: Fusion of high mobility group AT-hook 2 gene (*hmga2*)

with the chromosome 12 open reading frame 42 gene (*C12orf42*) in an aggressive angiomyxoma with del(12)(q14q23) as the sole cytogenetic anomaly. Cancer Genomics Proteomics *19(5)*: 576-583, 2022. PMID: 35985684. DOI: 10.21873/cgp.20342

27 Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ: Basic local alignment search tool. J Mol Biol *215(3)*: 403-410, 1990. PMID: 2231712. DOI: 10.1016/S0022-2836(05)80360-2

28 Majoros WH and Ohler U: Spatial preferences of microRNA targets in 3´ untranslated regions. BMC Genomics *8*: 152, 2007. PMID: 17555584. DOI: 10.1186/1471-2164-8-152

29 Gu S, Jin L, Zhang F, Sarnow P and Kay MA: Biological basis for restriction of microRNA targets to the 3´ untranslated region in mammalian mRNAs. Nat Struct Mol Biol *16(2)*: 144-150, 2009. PMID: 19182800. DOI: 10.1038/nsmb.1552

30 Yang Y, Li D, Yang Y and Jiang G: An integrated analysis of the effects of microRNA and mRNA on esophageal squamous cell carcinoma. Mol Med Rep *12(1)*: 945-952, 2015. PMID: 25823933. DOI: 10.3892/mmr.2015.3557

31 Vanhoutteghem A and Djian P: Basonuclin 2: an extremely conserved homolog of the zinc finger protein basonuclin. Proc Natl Acad Sci U.S.A. *101(10)*: 3468-3473, 2004. PMID: 14988505. DOI: 10.1073/pnas.0400268101

32 Vanhoutteghem A and Djian P: The human basonuclin 2 gene has the potential to generate nearly 90,000 mRNA isoforms encoding over 2000 different proteins. Genomics *89(1)*: 44-58, 2007. PMID: 16942855. DOI: 10.1016/j.ygeno.2006.07.006

33 Winham SJ, Armasu SM, Cicek MS, Larson MC, Cunningham JM, Kalli KR, Fridley BL and Goode EL: Genome-wide investigation of regional blood-based DNA methylation adjusted for complete blood counts implicates BNC2 in ovarian cancer. Genet Epidemiol *38(5)*: 457-466, 2014. PMID: 24853948. DOI: 10.1002/gepi.21815

34 Cesaratto L, Grisard E, Coan M, Zandonà L, De Mattia E, Poletto E, Cecchin E, Puglisi F, Canzonieri V, Mucignat MT, Zucchetto A, Stocco G, Colombatti A, Nicoloso MS and Spizzo R: *BNC2* is a putative tumor suppressor gene in high-grade serous ovarian carcinoma and impacts cell survival after oxidative stress. Cell Death Dis *7(9)*: e2374, 2016. PMID: 27899818. DOI: 10.1038/cddis.2016.278

35 Buckley MA, Woods NT, Tyrer JP, Mendoza-Fandiño G, Lawrenson K, Hazelett DJ, Najafabadi HS, Gjyshi A, Carvalho RS, Lyra PC Jr, Coetzee SG, Shen HC, Yang AW, Earp MA, Yoder SJ, Risch H, Chenevix-Trench G, Ramus SJ, Phelan CM, Coetzee GA, Noushmehr H, Hughes TR, Sellers TA, Goode EL, Pharoah PD, Gayther SA, Monteiro ANA and Ovarian Cancer Association Consortium: Functional analysis and fine mapping of the 9p22.2 ovarian cancer susceptibility locus. Cancer Res *79(3)*: 467-481, 2019. PMID: 30487138. DOI: 10.1158/0008-5472.CAN-17-3864

36 Unachukwu U, Chada K and D'Armiento J: High mobility group AT-Hook 2 (*HMGA2*) oncogenicity in mesenchymal and epithelial neoplasia. Int J Mol Sci *21(9)*: 3151, 2020. PMID: 32365712. DOI: 10.3390/ijms21093151

37 Borrmann L, Wilkening S and Bullerdiek J: The expression of *HMGA* genes is regulated by their 3´UTR. Oncogene *20(33)*: 4537-4541, 2001. PMID: 11494149. DOI: 10.1038/sj.onc.1204577

38 Lee YS and Dutta A: The tumor suppressor microRNA let-7 represses the *HMGA2* oncogene. Genes Dev *21(9)*: 1025-1030, 2007. PMID: 17437991. DOI: 10.1101/gad.1540407

39 Mayr C, Hemann MT and Bartel DP: Disrupting the pairing between *let-7* and *Hmga2* enhances oncogenic transformation. Science *315(5818)*: 1576-1579, 2007. PMID: 17322030. DOI: 10.1126/science.1137999

40 Wang X, Hulshizer RL, Erickson-Johnson MR, Flynn HC, Jenkins RB, Lloyd RV and Oliveira AM: Identification of novel *HMGA2* fusion sequences in lipoma: evidence that deletion of *let-7* miRNA consensus binding site 1 in the *HMGA2* 3´ UTR is not critical for *HMGA2* transcriptional upregulation. Genes Chromosomes Cancer *48(8)*: 673-678, 2009. PMID: 19431195. DOI: 10.1002/gcc.20674

41 Medeiros F, Erickson-Johnson MR, Keeney GL, Clayton AC, Nascimento AG, Wang X and Oliveira AM: Frequency and characterization of *HMGA2* and *HMGA1* rearrangements in mesenchymal tumors of the lower genital tract. Genes Chromosomes Cancer *46(11)*: 981-990, 2007. PMID: 17654722. DOI: 10.1002/gcc.20483

42 Quade BJ, Weremowicz S, Neskey DM, Vanni R, Ladd C, Dal Cin P and Morton CC: Fusion transcripts involving *HMGA2* are not a common molecular mechanism in uterine leiomyomata with rearrangements in 12q15. Cancer Res *63(6)*: 1351-1358, 2003. PMID: 12649198

43 Schoenmakers EF, Wanschura S, Mols R, Bullerdiek J, Van den Berghe H and Van de Ven WJ: Recurrent rearrangements in the high mobility group protein gene, *HMGI-C*, in benign mesenchymal tumours. Nat Genet *10(4)*: 436-444, 1995. PMID: 7670494. DOI: 10.1038/ng0895-436

44 Geurts JM, Schoenmakers EF and Van de Ven WJ: Molecular characterization of a complex chromosomal rearrangement in a pleomorphic salivary gland adenoma involving the 3´-UTR of *HMGIC*. Cancer Genet Cytogenet *95(2)*: 198-205, 1997. PMID: 9169041. DOI: 10.1016/s0165-4608(96)00411-6

45 Storlazzi CT, Albano F, Locunsolo C, Lonoce A, Funes S, Guastadisegni MC, Cimarosto L, Impera L, D'Addabbo P, Panagopoulos I, Specchia G and Rocchi M: t(3;12)(q26;q14) in polycythemia vera is associated with upregulation of the *HMGA2* gene. Leukemia *20(12)*: 2190-2192, 2006. PMID: 17024113. DOI: 10.1038/sj.leu.2404418

46 Inoue N, Izui-Sarumaru T, Murakami Y, Endo Y, Nishimura J, Kurokawa K, Kuwayama M, Shime H, Machii T, Kanakura Y, Meyers G, Wittwer C, Chen Z, Babcock W, Frei-Lahr D, Parker CJ and Kinoshita T: Molecular basis of clonal expansion of hematopoiesis in 2 patients with paroxysmal nocturnal hemoglobinuria (PNH). Blood *108(13)*: 4232-4236, 2006. PMID: 16940417. DOI: 10.1182/blood-2006-05-025148

47 Martin SE, Sausen M, Joseph A, Kingham BF and Martin ES: Identification of a *HMGA2-EFCAB6* gene rearrangement following next-generation sequencing in a patient with a t(12;22)(q14.3;q13.2) and JAK2V617F-positive myeloproliferative neoplasm. Cancer Genet *205(6)*: 295-303, 2012. PMID: 22749035. DOI: 10.1016/j.cancergen.2012.03.006

48 Nilsson M, Panagopoulos I, Mertens F and Mandahl N: Fusion of the *HMGA2* and *NFIB* genes in lipoma. Virchows Arch *447(5)*: 855-858, 2005. PMID: 16133369. DOI: 10.1007/s00428-005-0037-9

49 Italiano A, Ebran N, Attias R, Chevallier A, Monticelli I, Mainguené C, Benchimol D and Pedeutour F: *NFIB* rearrangement in superficial, retroperitoneal, and colonic lipomas with aberrations involving chromosome band 9p22.

Genes Chromosomes Cancer *47(11)*: 971-977, 2008. PMID: 18663748. DOI: 10.1002/gcc.20602

50 Pierron A, Fernandez C, Saada E, Keslair F, Hery G, Zattara H and Pedeutour F: *HMGA2-NFIB* fusion in a pediatric intramuscular lipoma: a novel case of *NFIB* alteration in a large deep-seated adipocytic tumor. Cancer Genet Cytogenet *195(1)*: 66-70, 2009. PMID: 19837271. DOI: 10.1016/j.cancergencyto.2009.06.009

51 Geurts JM, Schoenmakers EF, Röijer E, Aström AK, Stenman G and van de Ven WJ: Identification of *NFIB* as recurrent translocation partner gene of *HMGIC* in pleomorphic adenomas. Oncogene *16(7)*: 865-872, 1998. PMID: 9484777. DOI: 10.1038/sj.onc.1201609

52 Afshari MK, Fehr A, Nevado PT, Andersson MK and Stenman G: Activation of *PLAG1* and *HMGA2* by gene fusions involving the transcriptional regulator gene *NFIB*. Genes Chromosomes Cancer *59(11)*: 652-660, 2020. PMID: 32654217. DOI: 10.1002/gcc.22885

53 Kazmierczak B, Meyer-Bolte K, Tran KH, Wöckel W, Breightman I, Rosigkeit J, Bartnitzke S and Bullerdiek J: A high frequency of tumors with rearrangements of genes of the *HMGI(Y)* family in a series of 191 pulmonary chondroid hamartomas. Genes Chromosomes Cancer *26(2)*: 125-133, 1999. PMID: 10469450.

54 Kristjánsdóttir K, Fogarty EA and Grimson A: Systematic analysis of the *Hmga2* 3′ UTR identifies many independent regulatory sequences and a novel interaction between distal sites. RNA *21(7)*: 1346-1360, 2015. PMID: 25999317. DOI: 10.1261/rna.051177.115

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