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MED12 somatic mutations in fibroadenomas and phyllodes tumors of the breast

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Abstract

Aims—Somatic mutations in exon 2 of the *MED12* gene have been identified in 60% of breast fibroadenomas (FAs). The aim of this study was to define whether phyllodes tumors (PTs) would harbor *MED12* somatic mutations in a way akin to FAs.

Methods and results—A collection of 73 fibroepithelial tumors (including 26 FAs, 25 benign PTs, 9 borderline PTs and 13 malignant PTs) from 64 patients was retrieved from the authors' institution. Sections from FFPE blocks were microdissected to ensure an enrichment in neoplastic stromal elements of >70%. DNA samples extracted from tumor and matched normal tissues were subjected to Sanger sequencing of exon 2 of the *MED12* gene. *MED12* exon 2 somatic mutations, including 28 somatic single nucleotide variants and 19 insertions and deletions, were found in 65%, 88%, 78% and 8% of FAs, benign PTs, borderline PTs and malignant PTs, respectively. Malignant PTs significantly less frequently harbored *MED12* exon 2 somatic mutations than FAs, benign and borderline PTs.

Conclusions—Although *MED12* exon 2 somatic mutations likely constitute the driver genetic event of most FAs, benign and borderline PTs, our results suggest that the majority of malignant PTs may be driven by other genetic/epigenetic alterations.

Keywords

fibroepithelial tumors; breast; *MED12*; sequencing; somatic mutations

Introduction

Fibroadenomas (FAs) and phyllodes tumors (PTs) are fibroepithelial tumors of the breast composed of biphasic proliferations of both epithelial and stromal elements.[1] FAs are benign

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and constitute the most frequent type of fibroepithelial tumors,[2,3] being characterized by an admixture of epithelial and stromal components that can be arranged in two growth patterns: pericanalicular, characterized by a circumferential stromal proliferation around ductal structures, and intracanalicular, where the proliferating stromal cells compress the ductal structures into clefts.[1] PTs are rarer than FAs, accounting for approximately 2.5% of all fibroepithelial tumors of the breast. Although in the majority of cases PTs develop *de novo* from intralobular or periductal stroma of the breast, there are reports of progression from FAs to PTs and of examples of FAs found in the proximity of PTs.[1,4] Histologically, PTs have a cellular stroma and display an intracanalicular growth pattern, which is often more exuberant than that found in FAs and results in leaf-like projections protruding into the dilated luminal spaces of ductal structures.[1] PTs are classified as benign, borderline or malignant based on specific histologic criteria,[1] however these form a continuum, and grading these lesions is often challenging.[5]

The Mediator Complex Subunit 12 (*MED12*) gene (Xq13.1) encodes a member of the Multiprotein mediator complex. MED12 plays role in the regulation of transcription of all RNA polymerase II-dependent genes.[6] The kinase/CDK8 module formed by MED12 together with CDK8/CDK19, Cyclin C, and MED13 is often associated with transcriptional repression and functions as a stimulus-specific positive coregulator within the p53 transcriptional program.[7] Recent studies have demonstrated that up to 60% of FAs, 80% of benign and borderline PTs, and 40% of malignant PTs harbor somatic mutations in the exon 2 of the *MED12* gene.[8,9] Transcriptomic analysis of FAs provided additional evidence to support the contention that *MED12 exon* 2 somatic mutations likely constitute driver genetic alterations in FAs.[8] In addition, *MED12* somatic mutations have been reported in several tumor types, including hormone-associated lesions, such as uterine leiomyomas, uterine leiomyosarcomas, pelvic/retroperitoneal smooth muscle tumors, prostate cancer and adrenocortical cancer.[10-14]

Given the histologic similarities between FAs and PTs, and the evidence to suggest that at least a subset of PTs may originate from FAs,[1,4] we sought to define whether PTs would harbor mutations affecting exon 2 of the *MED12* gene in a way akin to FAs. Furthermore, as a hypothesis generating aim, we characterized the repertoire of *MED12* exon 2 mutations in distinct fibroepithelial tumors from patients with multiple lesions to define their potential clonal relatedness.

Materials and Methods

Cases

The files of the Department of Pathology of Memorial Sloan Kettering Cancer Center (MSKCC) were searched for FAs and PTs diagnosed and surgically removed at our Institution between January 1996 and October 2014. The diagnostic slides and tissue blocks of 26 FAs, 25 benign PTs, 9 borderline PTs and 13 malignant PTs from 64 female patients were retrieved. Samples were anonymized prior to analysis, and the study was approved by the MSKCC Institutional Review Board. All cases were independently reviewed by three pathologists (MM, FLL and EB), and classified and graded according to the latest World

Health Organization classification (WHO) criteria,[1] which are detailed in the Supplementary Methods.

Microdissection and DNA extraction

Representative 8μm-thick sections from formalin-fixed paraffin-embedded histologic blocks of FAs and PTs were subjected to microdissection of tumor and matched normal tissue with a sterile needle under a stereomicroscope as previously described.[15,16] Tumor areas were microdissected to enrich for neoplastic cells from the stromal elements (>70%) of the FAs and PTs analyzed. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) according to manufacturer's guidelines.

PCR amplification and Sanger sequencing

The entire *MED12* exon 2 was amplified from 10ng of genomic DNA using the AmpliTaq 360 master mix (Life Technologies) as previously described.[16] For this, a primer pair that amplifies a 373bp fragment encompassing exon 2 of the *MED12* gene was employed (5′-TGTTCTACACGGAACCCTCCTC-3′ (forward) and 5′-CTGGGCAAATGCCAATGAGAT-3′ (reverse)) as described by Lim *et al.*[8] (Supplementary Figure 1). Sequencing reactions were performed on an ABI3730 capillary sequencer using the ABI BigDye Terminator chemistry (v3.1, Life Technologies) according to manufacturer's instructions. To confirm that the *MED12* mutations identified in FAs and PTs were somatic events, matched normal DNA was sequenced for all cases included in this study. Sequences of the forward and reverse strands were analyzed using MacVector software (MacVector Inc).[16] All analyses were performed in duplicate. Insertions and deletion were manually annotated. Mutation function prediction was performed as previously described[17] (Supplementary Methods).

Statistical analysis

The Chi-square test (χ^2 test) and the Fisher's exact test were employed for the comparison of non-parametric variables. All statistical analyses were carried out using IBM SPSS Statistics v.20 (IBM). Two-tailed *P* values <0.05 were considered significant.

Results

Seventy-three tumors from 64 female patients were subjected to Sanger sequencing analysis for the presence of *MED12* exon 2 somatic mutations. According to the WHO criteria,[1] 26 tumors were classified as FAs (36%), 25 benign PTs (34%), 9 borderline PTs (12%) and 13 malignant PTs (18%; Figure 1, Table 1 and Supplementary Tables 1 and 2).

High frequency of *MED12* somatic mutations in FAs, benign and borderline PTs but not in malignant PTs

MED12 exon 2 somatic mutations were found in 65% of FAs (17/26), 88% of benign PTs (22/25), 78% (7/9) of borderline PTs and 8% (1/13) of malignant PTs. Six distinct somatic single nucleotide variants and nineteen distinct insertions and deletions affecting *MED12* were identified in 28 and 19 cases, respectively (Figures 2 and 3, Table 1, Supplementary Table 1). All single nucleotide variants and insertions and deletions were predicted to be

pathogenic by multiple mutation effect predictors (Supplementary Table 1). No nonsense single nucleotide variants were found. No associations between the presence of *MED12* mutations and age of the patient or tumor size were observed (Student's *t*-test, $P>0.1$, Table 2).

The prevalence of *MED12* exon 2 somatic mutations in FAs (Table 1, Figure 3, Supplementary Table 1) was similar to that recently described by Lim *et al.*[8] In FAs, *MED12* exon 2 mutations were found to be similarly frequent in intracanalicular-type (8/8, 100%) and mixed-type FAs (3/3, 100%), but were significantly less prevalent in pericanalicular-type FAs (6/15, 40%) than in intracanalicular-type FAs or the group of intracanalicular-type or mixed-type FAs (Table 3). Of the 17 *MED12*-mutant FAs, 9 (53%) harbored hotspot point mutations in codon 44 (3 p.G44D, 2 p.G44V, 2 p.G44S, 1 p.G44R and 1 p.G44C). Eight (47%) *MED12*-mutant FAs displayed insertions or deletions, of which 2 were frameshift and 6 were in-frame (Figure 3, Table 1, Supplementary Table 1).

Twenty-two (88%) benign PTs and 7 (78%) borderline PTs harbored *MED12* exon 2 somatic mutations. Of the 22 *MED12*-mutant benign PTs, 13 (59%) displayed hotspot point mutations in codon 44 (7 p.G44S, 3 p.G44D, 2 p.G44V and 1 p.G44C), 9 (41%) had in-frame insertions or deletions (Table 1, Figure 3, Supplementary Table 1). In the 7 *MED12*-mutant borderline PTs, point mutations in codon 44 (1 p.G44D, 1 p.G44S, 2 p.G44V and 1 p.G44A) were found in 5 cases (71%) and in-frame insertions or deletions were found in 2 cases (29%) (Table 1, Figure 3, Supplementary Table 1). By contrast, only 1 of the 13 malignant PTs analyzed (8%) was found to harbor a single nucleotide variant affecting codon 44 (p.G44D), which was considered as likely pathogenic by mutation function predictors.

Although a similarly high prevalence of *MED12* exon 2 mutations was found in FAs, benign and borderline PTs ($P>0.1$, Fisher's exact test), malignant PTs were found to display *MED12* exon 2 somatic mutations significantly less frequently than FAs, benign or borderline PTs ($P<0.001$, Fisher's exact test, Figure 4). These results suggest that whilst the majority of FAs, benign PTs and borderline PTs harbored *MED12* exon 2 mutations, only a minority of malignant PTs display these mutations.

MED12 mutational status in different tumors affecting the same patients

Four of the 64 women part of the study had multiple mammary fibroepithelial lesions (Table 1, Supplementary Table 1). We therefore compared the *MED12* mutational status in multiple FAs and/or PTs from the same patient to define whether the lesions would be clonally related. In patient 04, the FAs displayed distinct *MED12* exon 2 somatic mutations (p.G44V, p.G44D and p.G44C), the benign PT displayed a p.G44V mutation and the malignant PT harbored a p.G44D mutation. Interestingly, the malignant PT and one of the intracanalicular FAs harbored the p.G44D *MED12* mutation, whereas the benign PT and another intracanalicular FA displayed the p.G44V *MED12* mutation. In patient 09, two p.G44S and two p.G44D were found in the 4 benign PTs in the right breast, demonstrating that some of the PTs were likely not clonally related. Patients 53 and 56 presented with bilateral FAs; in patient 53, a p.G44S mutation was found in the FA of the right breast, whereas the left breast FA harbored a p.N47_E55del in-frame deletion. In patient 56, the left breast FA harbored a

p.N40_V51del in-frame deletion, whereas the right breast FA was *MED12* wild-type (Table 1, Supplementary Table 1). Taken together, these results demonstrate that fibroepithelial lesions arising in patients with multiple FAs and/or PTs do not necessarily harbor identical *MED12* somatic mutations, even when the lesions are ipsilateral, suggesting that some of these lesions likely originated independently. Furthermore, the presence of a p.G44D in a FA and in a malignant PT affecting the same breast of patient 4 may be interpreted as supportive of a clonal relationship between these two lesions and raises the possibility that in this case the FA may have constituted the substrate for the development of the malignant PT.

Discussion

Somatic mutations in *MED12* exon 2 have been recently reported in benign indolent types of stromal tumors, including the majority of leiomyomas,[18-23] FAs[8] and benign and borderline PTs[9], and in a subset of malignant PTs.[9] In FAs, which are biphasic tumors with epithelial components, these mutations have been found to be restricted to the stromal elements.[8] Here we provide an independent validation of the results reported by Lim *et al.*[8] and Cani *et al.*[9] as we have also identified *MED12* exon 2 mutations in 65%, 88% and 78% of FAs, benign and borderline PTs, respectively. In contrast to the high prevalence of *MED12* somatic mutations found in FAs, benign and borderline PTs, *MED12* exon 2 somatic mutations were found to be significantly less frequent in malignant PTs, being found in only in 8% (1/13) of cases. These observations are consistent with those reported by Cani *et al.*,[9] who observed *MED12* somatic mutations in 40% (2/5) of malignant PTs, but in 80% of benign (4/5) and borderline (4/5) PTs.

There is burgeoning evidence to demonstrate that *MED12* exon 2 mutations likely constitute genomic drivers of FAs.[8] The first 100 amino acids of *MED12*, which include its entire exon 2, have been shown to be essential for the interaction of MED12 with Cyclin C, and somatic mutations in *MED12* exon 2 have been reported to be tumorigenic, decreasing the interaction between MED12 and Cyclin C-CDK8/CDK19 and resulting in loss of Mediator-associated CDK activity[24] and in RAD51B overexpression.[25] These mutations have also been shown to impact on estrogen signaling. Not only does the Mediator complex interact with estrogen receptors α and β,[26] but also *MED12* exon 2-mutated FAs have been shown to display dysregulated estrogen signaling.[8] Given that breast and uterine stromal cells and uterine smooth muscle cells are estrogen responsive, it is plausible that the high frequency of *MED12* exon 2 somatic mutations in these tumors and their relative rarity in stromal tumors originating in other non-estrogen responsive anatomical sites stems from the impact of *MED12* mutations on estrogen signaling.[8]

MED12 somatic mutations appear to be less frequent in malignant than in benign tumors.[13,20,27-29] We have observed that *MED12* exon 2 somatic mutations were significantly more frequently found in FAs, benign and borderline PTs than in malignant PTs. Consistent with these observations, leiomyosarcomas have also been shown to harbor *MED12* mutations at significantly lower frequencies than leiomyomas.[29,30] These observations may be reflective of the fact that although some leiomyosarcomas and malignant PTs may originate from leiomyomas and FAs/benign PTs, respectively, which are estrogen dependent lesions, others may originate *de novo* through an estrogen independent

pathway. Consistent with this notion, the expression both ERβ (in the stromal component) and ERα (in the epithelial component) of fibroepithelial lesions of the breast has been reported to be inversely correlated with their degree of aggressiveness.[31,32] On the other hand, 60% of the malignant PTs analyzed by Cani *et al.* lacked *MED12* somatic mutations,[9] and these cases harbored *TERT* gene amplification. Further studies are required to define fully the mechanistic interactions between *MED12* exon 2 mutations and estrogen signaling in *MED12* mutant lesions and the alternative driver genetic alterations in *MED12* wild-type malignant PTs.

The genotyping of multiple fibroepithelial lesions occurring in 4 patients constitutes a unique and intriguing aspect of our study. The identification of ipsilateral fibroepithelial lesions harboring distinct *MED12* mutations is consistent with the notion that multiple coexisting ipsilateral fibroepithelial tumors can arise independently. The analysis of multiple ipsilateral FAs, benign and malignant PTs also revealed that in one case, identical *MED12* mutations were found in a FA and in a malignant PT in the same breast, an observation that is consistent with a clonal relationship between the two lesions and highlights the possibility that, in this case, the FA may have constituted the substrate for the development of the malignant PT.

This study has several limitations. First, the sample size is small, in particular, the cohort of borderline PTs comprises only 9 tumors; despite the small sample size, we have documented a significantly lower prevalence of *MED12* exon 2 mutations in malignant PTs than in FAs and benign and borderline PTs. This observation, however, should be considered as hypothesis-generating and warrants the analysis of independent cohorts of malignant PTs. Second, we have deliberately focused on somatic mutations affecting exon 2 of the *MED12* gene. Although previous findings demonstrate that the vast majority of *MED12* somatic mutations in FAs affect exon 2, we cannot rule out the possibility of somatic mutations affecting other coding sequences of the *MED12* gene may be present in breast fibroepithelial lesions. Finally, we employed Sanger sequencing for the detection of *MED12* somatic mutations; given that all lesions included in this study were microdissected and considering the sensitivity of Sanger sequencing, subclonal *MED12* mutations present in <20% of the clones would not be accurately detected. It should be noted, however, that given the evidence that *MED12* somatic mutations are early driver events in fibroepithelial lesions, these mutations would likely be present in the modal populations of *MED12* mutant FAs and PTs.

In conclusion, our study demonstrates that somatic mutations in *MED12* exon 2 likely constitute driver genetic events of not only FAs but also of benign and borderline PTs. *MED12* exon 2 somatic mutations were found to be significantly less frequent in malignant PTs, indicating that genetic and/or epigenetic alterations other than *MED12* exon 2 mutations may constitute the driving genetic events in the pathogenesis of malignant PTs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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BW, EB and JSR-F conceived the study. MM and EB provided the samples. MM, FLL and EB performed the pathologic review. SP, NF, AMS, CM, LGM and MA carried out experiments and analyzed data. SP, BW and JSR-F wrote the first draft of the manuscript. All authors interpreted the data, and reviewed and approved the final version of the manuscript.

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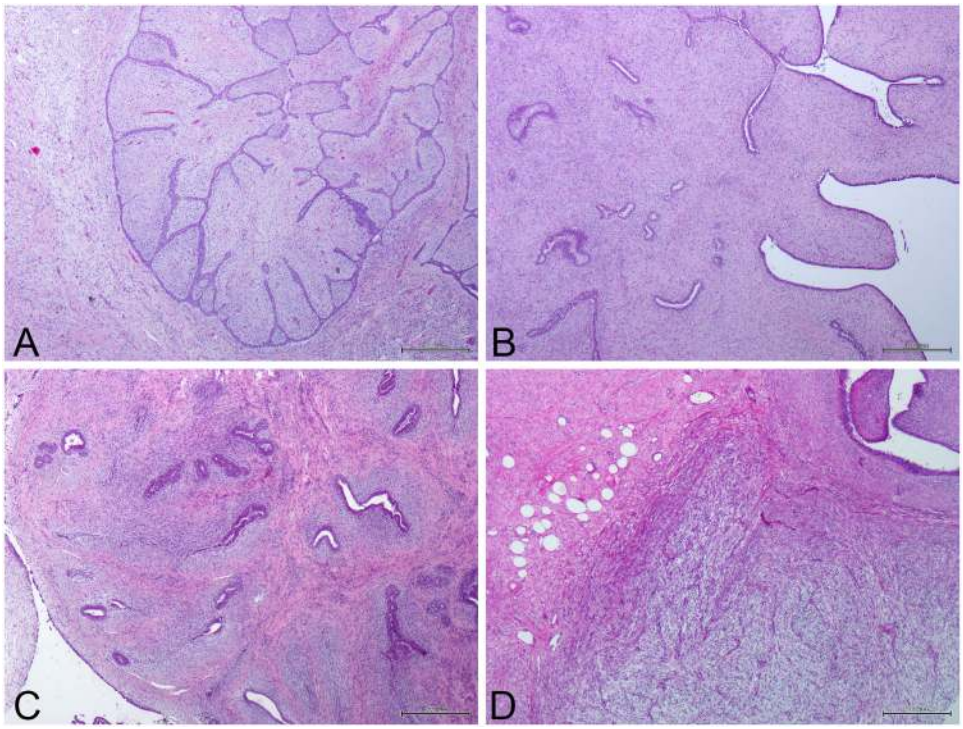

Figure 1. Representative micrographs of a fibroadenoma and phyllodes tumors of the breast included in this study

(A) Fibroadenoma. (B) Benign phyllodes tumors of the breast. (C) Borderline phyllodes tumors of the breast. (D) Malignant phyllodes tumors of the breast. Scale bar, 500 μM.

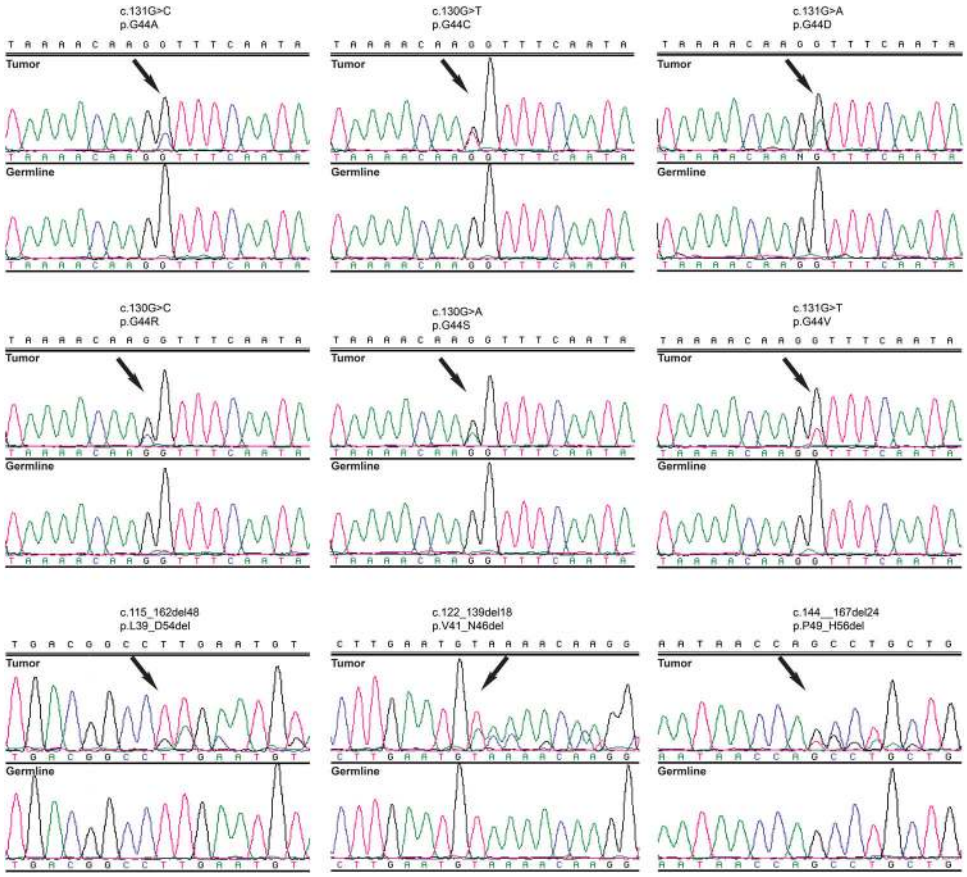

Figure 2. ***MED12*** **exon 2 mutations identified by Sanger sequencing analysis in fibroepithelial tumors**

Representative sequence electropherograms (tumor and matched germline) of the 6 single nucleotide variants, and of 3 of the 19 distinct insertions and deletions in exon 2 of *MED12* identified by Sanger sequencing in our cohort of 73 fibroepithelial tumors.

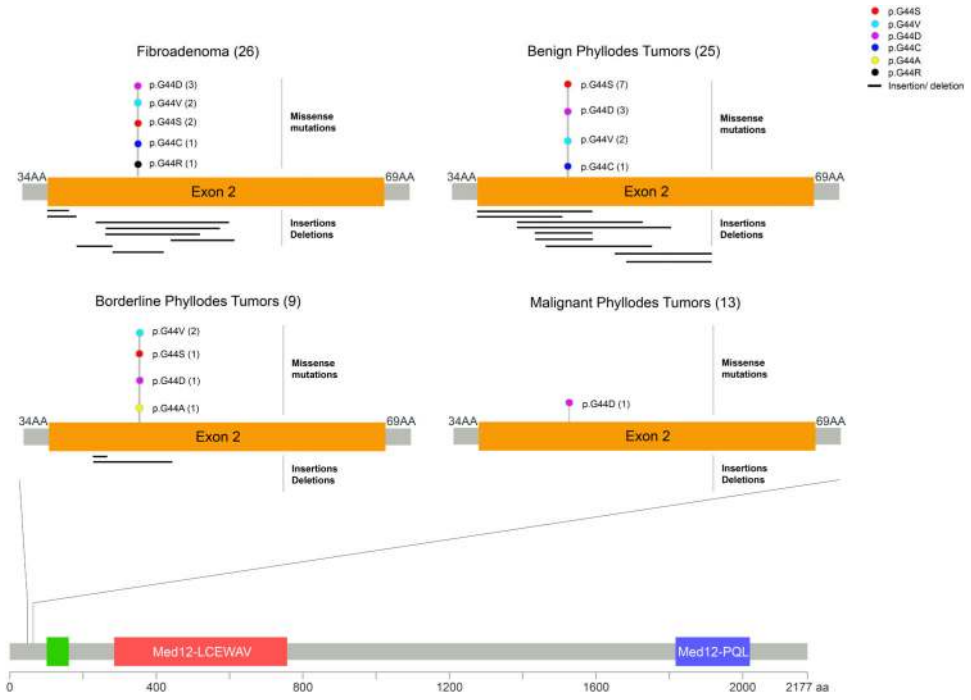

Figure 3. Distribution of *MED12* mutations in exon 2

Domain structure of the MED12 protein, and a close-up view of the amino acid residues in *MED12* exon 2 affected by alterations in the fibroadenomas and phyllodes tumors analyzed in this study. The frequency of each alteration is denoted in parentheses. AA, amino acid.

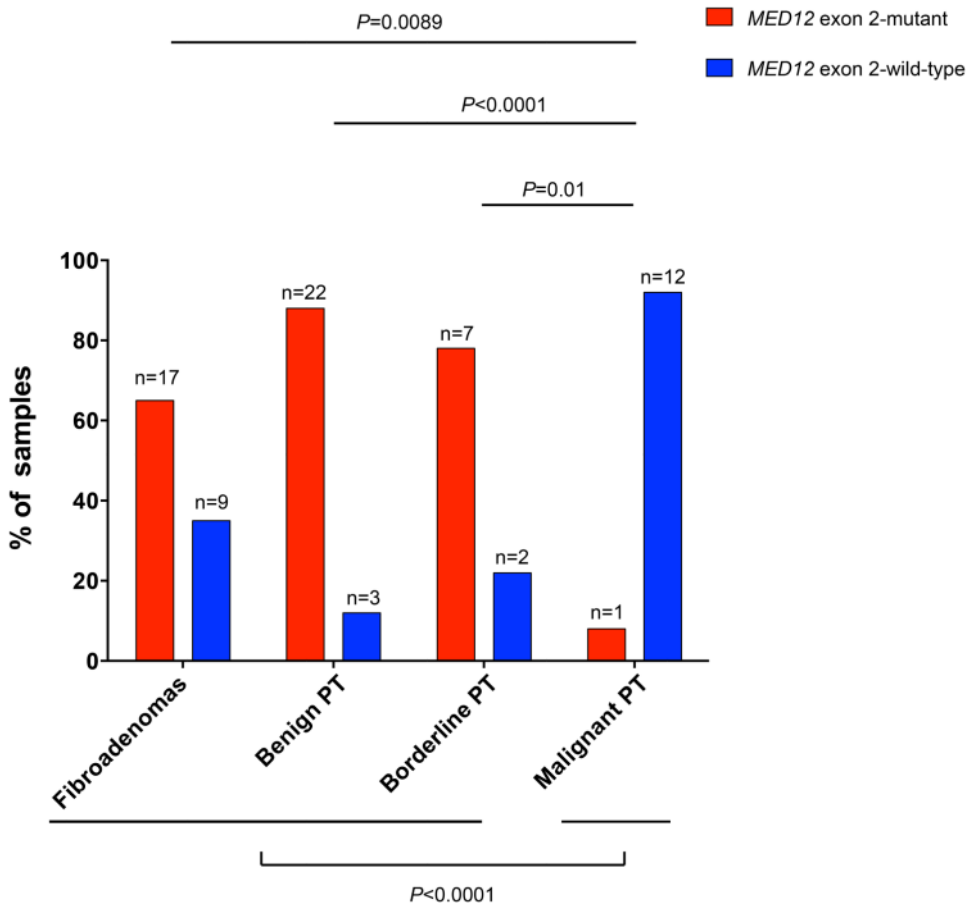

Figure 4. Frequencies of *MED12* exon 2 mutations in different fibroepithelial tumors of the breast

The percentages of cases with and without *MED12* exon 2 somatic mutations are depicted. The *MED12* exon 2 mutational frequencies in fibroadenomas, benign, borderline and malignant phyllodes tumors of the breast were compared using Fisher's exact tests. PT, phyllodes tumor.

Table 1

***MED12* exon 2 mutations identified in 73 fibroepithelial tumors of the breast from 64 female patients**

Patient ID	Sample ID	Ethnicity	Diagnosis	Laterality	Type of mutation	cDNA change	Protein change
Patient 01	MD-01	Black	Malignant PT	Left	wild-type	wild-type	wild-type
Patient 02	MD-02	White	Malignant PT	Right	wild-type	wild-type	wild-type
Patient 03	MD-03	Unknown	Borderline PT	Right	wild-type	wild-type	wild-type
Patient 04	MD-04	White	Malignant PT	Right	SNV	c.131G>A	p.G44D
	MD-60a	White	Benign PT	Right	SNV	c.131G>T	p.G44V
	MD-60b	White	Fibroadenoma	Right	SNV	c.130G>T	p.G44C
	MD-60c	White	Fibroadenoma	Right	SNV	c.131G>T	p.G44V
	MD-60d	White	Fibroadenoma	Right	SNV	c.131G>A	p.G44D
Patient 05	MD-05 a	White	Malignant PT	Left	wild-type	wild-type	wild-type
Patient 06	MD-06	Asian	Borderline PT	Left	SNV	c.131G>A	p.G44D
Patient 07	MD-07	White	Malignant PT	Right	wild-type	wild-type	wild-type
Patient 08	MD-08	Asian	Malignant PT	Left	wild-type	wild-type	wild-type
Patient 09	MD-09 a	White	Benign PT	Left	SNV	c.130G>A	p.G44S
	MD-09 b	White	Benign PT	Left	SNV	c.130G>A	p.G44S
	MD-09 c	White	Benign PT	Left	SNV	c.131G>A	p.G44D
	MD-09 d	White	Benign PT	Left	SNV	c.131G>A	p.G44D
Patient 10	MD-10	Asian	Borderline PT	Right	wild-type	wild-type	wild-type
Patient 11	MD-11	Black	Borderline PT	Left	Insertion (frameshift)	c.112_113insAC	p.L39_N40fs
Patient 12	MD-12	White	Borderline PT	Right	Deletion (in-frame)	c.115_141del27	p.L39_N47del
Patient 13	MD-13	Black	Borderline PT	Left	SNV	c.131G>C	p.G44A
Patient 14	MD-14	White	Benign PT	Left	Deletion (in-frame)	c.122_139del18	p.V41_N46del
Patient 15	MD-15a	Asian	Borderline PT	Left	SNV	c.131G>T	p.G44V
Patient 16	MD-16	Asian	Borderline PT	Right	SNV	c.130G>A	p.G44S
Patient 17	MD-17	White	Borderline PT	Left	SNV	c.131G>T	p.G44V
Patient 18	MD-18	White	Benign PT	Left	Deletion (in-frame)	c.123_152del30	p.K42_V51del
Patient 19	MD-19	Unknown	Benign PT	Left	SNV	c.130G>A	p.G44S
Patient 20	MD-20	White	Benign PT	Left	SNV	c.130G>A	p.G44S

Patient ID	Sample ID	Ethnicity	Diagnosis	Laterality	Type of mutation	cDNA change	Protein change
Patient 21	MD-21	White	Benign PT	Right	SNV	c.130G>A	p.G44S
Patient 22	MD-22	White	Benign PT	Right	SNV	c.131G>T	p.G44V
Patient 23	MD-23	Black	Benign PT	Left	Deletion (in-frame)	c.141_167del27	p.Q48_H56del
Patient 24	MD-24	White	Benign PT	Left	wild-type	wild-type	wild-type
Patient 25	MD-25	White	Benign PT	Left	Deletion (in-frame)	c.144__167del24	p.P49_H56del
Patient 26	MD-26	White	Benign PT	Left	SNV	c.131G>A	p.G44D
Patient 27	MD-27	White	Benign PT	Right	SNV	c.130G>T	p.G44C
Patient 28	MD-28	White	Benign PT	Right	Deletion (in-frame)	c.100_129del30	p.D34_Q43del
Patient 29	MD-29	White	Benign PT	Left	wild-type	wild-type	wild-type
Patient 30	MD-30	White	Benign PT	Right	SNV	c.130G>A	p.G44S
Patient 31	MD-31	White	Benign PT	Right	SNV	c.130G>A	p.G44S
Patient 32	MD-32	Asian	Benign PT	Left	Deletion (in-frame)	c.114_149del36	p.L39_A50del
Patient 33	MD-33	White	Benign PT	Right	Deletion (in-frame)	c.121_138del18	p.V41_N46del
Patient 34	MD-34	White	Benign PT	Right	Deletion (in-frame)	c.100_138del39	p.D34_N46del
Patient 35	MD-35	Unknown	Benign PT	Right	Deletion (in-frame)	c.116_157del42	p.L39_S52del
Patient 36	MD-36	White	Benign PT	Right	wild-type	wild-type	wild-type
Patient 37	MD-37	White	Fibroadenoma	Left	Deletion (in-frame)	c.121_135del15	p. V41_F45del
Patient 38	MD-38	Unknown	Fibroadenoma	Right	Deletion (in-frame)	c.118_159del42	p.N40_G53del
Patient 39	MD-39	White	Fibroadenoma	Right	Deletion (frameshift)	c.100_112del13	p.D34_T37fs
Patient 40	MD-40	Unknown	Fibroadenoma	Left	wild-type	wild-type	wild-type
Patient 41	MD-41	White	Fibroadenoma	Right	SNV	c.130G>A	p.G44S
Patient 42	MD-42	White	Fibroadenoma	Right	Deletion (in-frame)	c.108_122del15	p.T37_V41del
Patient 43	MD-43	White	Fibroadenoma	Right	wild-type	wild-type	wild-type
Patient 44	MD-44	Black	Fibroadenoma	Left	Deletion (frameshift)	c.100_107del8	p.D34_L36fs
Patient 45	MD-45	White	Fibroadenoma	Right	wild-type	wild-type	wild-type
Patient 46	MD-46	Asian	Fibroadenoma	Right	SNV	c.131G>T	p.G44V
Patient 47	MD-47	Black	Fibroadenoma	Right	wild-type	wild-type	wild-type
Patient 48	MD-48	White	Fibroadenoma	Right	wild-type	wild-type	wild-type
Patient 49	MD-49	Unknown	Fibroadenoma	Right	wild-type	wild-type	wild-type

Patient ID	Sample ID	Ethnicity	Diagnosis	Laterality	Type of mutation	cDNA change	Protein change
Patient 50	MD-50	Unknown	Fibroadenoma	Right	wild-type	wild-type	wild-type
Patient 51	MD-51	White	Fibroadenoma	Right	wild-type	wild-type	wild-type
Patient 52	MD-52	White	Fibroadenoma	Left	Deletion (in-frame)	c.115_162del48	p.L39_D54del
Patient 53	MD-53	White	Fibroadenoma	Left	Deletion (in-frame)	c.138_161del24	p.N47_E55del
	MD-54	White	Fibroadenoma	Right	SNV	c.130G>A	p.G44S
Patient 54	MD-55	White	Fibroadenoma	Right	SNV	c.131G>A	p.G44D
Patient 55	MD-58	White	Fibroadenoma	Left	SNV	c.130G>C	p.G44R
Patient 56	MD-56	White	Fibroadenoma	Right	wild-type	wild-type	wild-type
	MD-57	White	Fibroadenoma	Left	Deletion (in-frame)	c.117_152del36	p.N40_V51del
Patient 57	MD-59	Unknown	Fibroadenoma	Right	SNV	c.131G>A	p.G44D
Patient 58	MD-61	White	Malignant PT	Right	wild-type	wild-type	wild-type
Patient 59	MD-62	Unknown	Malignant PT	Left	wild-type	wild-type	wild-type
Patient 60	MD-63	White	Malignant PT	Right	wild-type	wild-type	wild-type
Patient 61	MD-64	White	Malignant PT	Right	wild-type	wild-type	wild-type
Patient 62	MD-65	White	Malignant PT	Left	wild-type	wild-type	wild-type
Patient 63	MD-66	White	Malignant PT	Right	wild-type	wild-type	wild-type
Patient 64	MD-67	Asian	Malignant PT	Right	wild-type	wild-type	wild-type

PT, phyllodes tumor; SNV, single nucleotide variant.

Table 2

Correlation between *MED12* exon 2 mutation status and patient age and tumor size in patients with fibroepithelial tumors

	Age (Mean ± SD) *MED12* mutant	n	Age (Mean ± SD) *MED12* wild-type	n	*P* value
Fibroadenoma	31.8 ± 1.9	17	37.1 ± 3.7	9	0.158
Benign PT	42 ± 2.2	22	36.3 ± 8.1	3	0.404
Borderline PT	49.3 ± 4.5	7	44.5 ± 2.5	2	0.604
	Tumor size (cm) (Mean ± SD) *MED12* mutant	**n**	**Tumor size (cm) (Mean ± SD) *MED12* wild-type**	**n**	***P* value**
Fibroadenoma	1.7 ± 0.2	17	2.2 ± 0.3	9	0.168
Benign PT	2.3 ± 0.2	22	2.4 ± 1.3	3	0.899
Borderline PT	5.8 ± 1.2	7	5.8 ± 3.1	2	0.986

Student's t-tests, two-tailed. PT, phyllodes tumor; SD, standard deviation. Malignant PTs were excluded from this comparison due to small sample size and the low frequency of *MED12* mutations.

Table 3

***MED12* exon 2 mutation status according to growth patterns in fibroadenomas**

Growth pattern	n	*MED12* wild-type n (%)	*MED12* mutant n (%)	*P* value
Pericanalicular	15	9 (60%)	6 (40%)	0.007
Intracanalicular	8	0 (0%)	8 (100%)	
Intracanalicular	8	0 (0%)	8 (100%)	1.000
Mixed	3	0 (0%)	3 (100%)	
Pericanalicular	15	9 (60%)	6 (40%)	0.205
Mixed	3	0 (0%)	3(100%)	
Pericanalicular	15	9 (60%)	6 (40%)	0.002
Intracanalicular or mixed	11	0 (0%)	11 (100%)	