Fibroepithelial Breast Lesion: When Sequencing Can Help to Make a Clinical Decision. A Case Report

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Clinical Practice Points

- Breast fibroepithelial lesions (FELs) comprise a wide spectrum of tumors ranging from indolent fibroadenomas (FAs) to malignant phyllodes tumors (PTs). The histologic distinction between the 2 on core needle biopsy can be challenging.
- There is a need for molecular markers to better discriminate subclasses of breast FELs on core needle biopsy to tailor clinical management.
- Recently, TERT promoter mutations and gene amplifications have been used to differentiate FAs from PTs.
- This report describes a 59-year-old woman diagnosed with an FEL of the left breast who refused surgery and presented 13 months later with a disfiguring borderline PT that required mastectomy. Sequencing of the early FEL and of the PT showed MED12 hotspot (c.131G>A) and TERT promoter (c.-124C>T) somatic mutations, suggesting that the lesion at time of the initial biopsy was already a PT.
- The characterization of somatic mutations on core needle biopsy can help to better characterize FELs and therefore increase diagnostic accuracy.

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Introduction

Breast fibroepithelial lesions (FELs) are composed of biphasic proliferation of both epithelial and stromal elements and comprise a wide spectrum of tumors ranging from indolent fibroadenomas (FAs) to malignant phyllodes tumors (PTs).[1] Although FAs are common benign tumors and are usually managed conservatively, PTs are rare (accounting for ~2.5% of all mammary FELs and for 1% of all breast cancers) and may recur locally or even metastasize to distant sites.[1]

The histologic distinction between FAs and PTs on core needle biopsy (CNB) can be challenging.[2] Pathologists may designate a FEL and add a comment of concern such as "cannot rule out phyllodes" or "increased stromal cellularity" if features of phyllodes are present but not definitive.[3] However, the exact characterization of a FEL is clinically important as the management may range from observation to wide surgical resection (≥ 1 cm margin).[2,4] As it may be impossible to distinguish FA from benign or borderline PT on CNB owing to overlapping histologic features,[2,3,5] excision is recommended in many patients, resulting in potential overtreatment.[2,5,6] A selective surgical approach based on clinical, radiologic, and pathologic features is therefore recommended.[4,7]

FAs and PTs share not only histologic similarities, but also genetic features.[8] Recurrent somatic mutations affecting exon 2 of *MED12* have been identified in both lesions.[9-12] Additionally, recent genomic analyses of FAs and PTs[12] demonstrated that PTs are genetically more advanced and display a higher mutational burden than FAs.[9]Furthermore, there have been reports of progression from FAs to PTs,[8,13,14] with *MED12* mutation as the founder genetic event and the subsequent acquisition of additional somatic genetic alterations such as *TERT* promoter mutations in the PTs.[8]

Here, we describe the case of a 59-year-old woman initially diagnosed with a FEL with a *TERT* promoter mutation who refused surgery and presented 13 months later with a disfiguring borderline PT.

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Case Report

In May 2016, a 59-year-old woman with no personal history of breast or ovarian cancer was diagnosed with polymyositis. Clinical examination revealed an asymmetry of the left breast and a ~6-cm palpable mass on the upper external quadrant (Figure 1A) but no lymphadenopathies. Mammogram (Figure 1B) and ultrasound confirmed a 5.5 × 5-cm mass in the upper external quadrant of the left breast and a 0.6 × 0.7-cm mass in the upper external quadrant of the right breast. CNB of both lesions was performed for diagnostic purposes. Histologic examination revealed a FA on the right side (classified as B2-lesion) (Figure 1C) and a FEL with increased stromal cellularity on the left side (classified as B3-lesion) (Figure 1D). Surgical excision of the FEL lesion was scheduled, but shortly before surgery, the patient canceled the operation and was lost to follow-up.

Thirteen months later (June 2017), the patient presented to the emergency department with disfiguring swelling of the left breast (Figure 2A). On physical examination, the left breast was massively enlarged and tender with a flattened nipple, engorged superficial vessels, and deeply erythematous skin (Figure 2A). Breast ultrasound and magnetic resonance imaging (Figure 2B) showed a 16-cm heterogeneous mass filling the entire left breast with an extensive central fluid collection without suspected lymphadenopathies (Figure 2B). A diagnostic puncture of 1200 mL of bloody fluid was sent for cytology and microbiological tests that revealed no bacterial growth and no cellular atypia. CNB showed a myofibroblastic proliferation without an epithelial component. Differential diagnosis included a PT (stromal component only) or a non-specific reactive inflammatory process. After a multidisciplinary discussion, owing to the large size of the tumor, mastectomy was performed. Based on the presence of focal stromal overgrowth, increased mitoses (7 per 10 high powered field) and focally invasive tumor borders (Figure 2C,D), the diagnosis of a borderline PT was made on final histology. The patient recovered well, without complications. Thirteen months after treatment, the patient continued to do well with no evidence of disease recurrence.

To correlate the genomic profile of the tumor with its clinical evolution, massively parallel sequencing of 32 genes commonly altered in breast cancer (see Supplemental Table 1 in the online version) was performed and analyzed as previously described,[15] on tissues from the FA (right breast), the FEL, and the PT (left breast). No somatic mutations were identified in any of the genes recurrently mutated in breast cancer. Subsequently, all 3 lesions were subjected to Sanger sequencing for the *MED12* hotspot and for *TERT* promoter somatic mutations. Again, no mutation was identified in the FA in the right breast (Figure 3A). However, we identified a *MED12* hotspot (c.131G>A) and *TERT* promoter (c.-124C>T) mutations in both the early B3 lesion and in the subsequent lesion after 13 months, suggesting the progression of the early lesion. This observation was further supported by their shared

Figure 1 **Clinical, Radiologic, and Histologic Images at First Presentation. A, Photo of the Breast at First Presentation. B, Mammogram Showing a 55 × 50-mm Mass on the Left Breast and a 6 × 7-mm Mass on the Right Breast. C, Core Needle Biopsy of the Right Breast Lesion Showing a Fibroepithelial Lesion Well Demarcated From the Surrounding Tissue With Low Stromal Cellularity, Consistent With Fibroadenoma (Magnification 40×, Hematoxylin and Eosin). D, Core Needle Biopsy of the Left Breast Lesion Showing a Fibroepithelial Lesion With Mildly Increased Stromal Cellularity but No Stromal Cell Atypia (Magnification 100×, Hematoxylin and Eosin)**

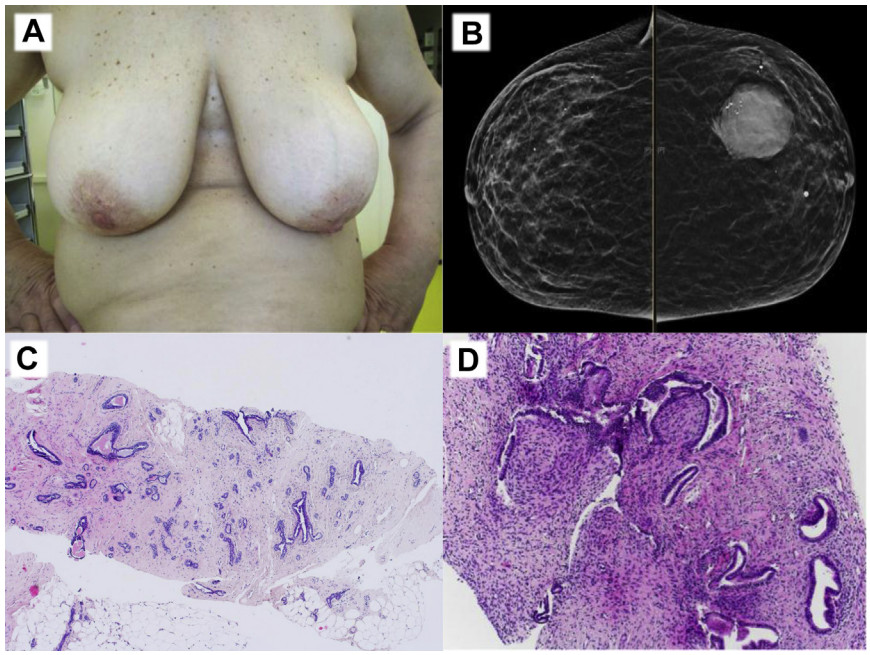


Figure 2 Clinical, Radiologic, and Histologic Images 13 Months After the First Presentation. A, Photo of the Breast After 13 Months. B, Left: Axial Fluid Sensitive T2-Weighted Magnetic Resonace Image. Right: Axial Contrast-Enhanced T1-Weighted Fat-Suppressed Magnetic Resonance Image. Both Images Show a 16-cm Heterogeneous Mass Filling the Entire Left Breast With an Extensive Central Fluid Collection. C, Surgical Specimen (Mastectomy) of the Left Breast: Showing a Borderline Phyllodes Tumor With Typical Intracanalicular "Leaf-Like" Growth Pattern and Mildly Increased, Variable Stromal Cellularity (Magnification 40×, Hematoxylin and Eosin). D, Surgical Specimen (Mastectomy) of the Left Breast: Areas Reminiscent of a Cellular Fibroadenoma Without Stromal Cellatypia (Magnification 100×, Hematoxylin and Eosin)

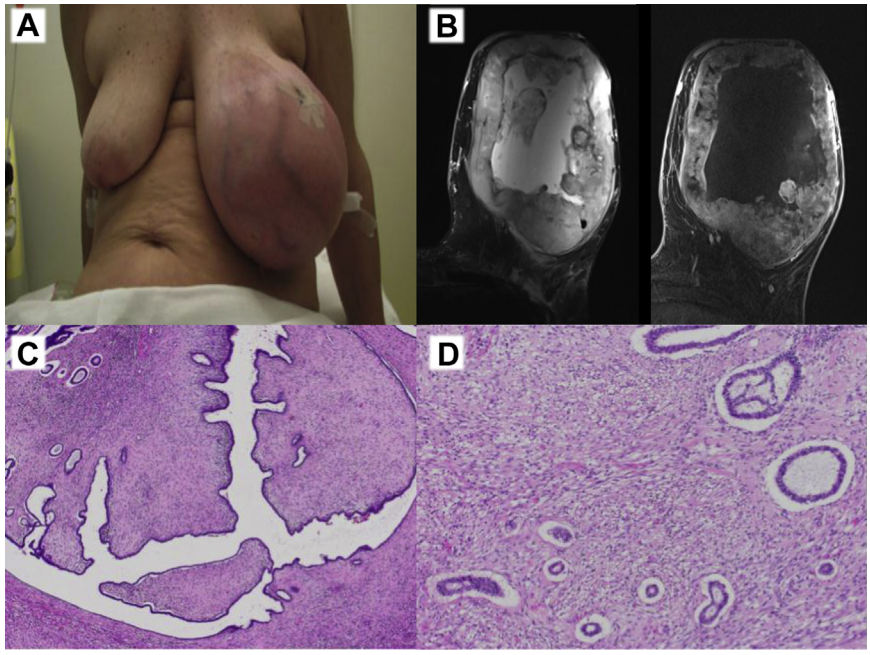
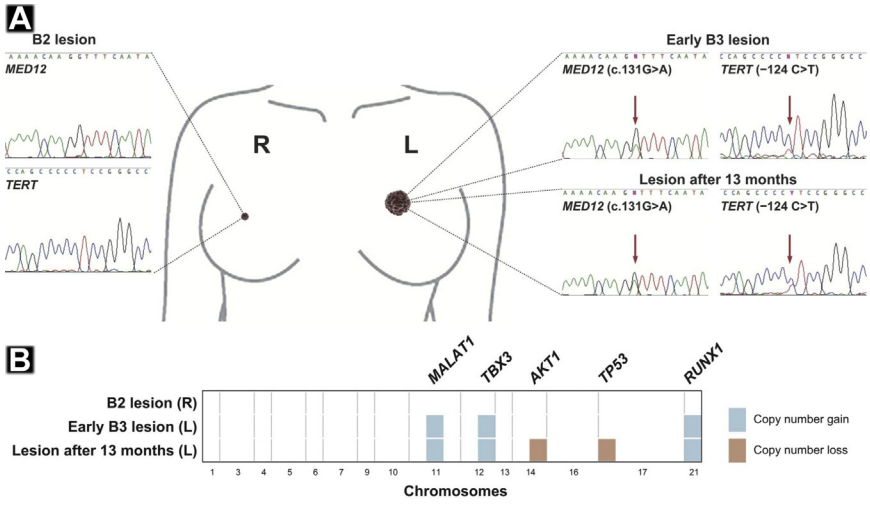


Figure 3 Genetic Profiling of the Lesions. A, Sanger Sequencing for the MED12 Hotspot and for TERT Promoter Mutations. No Somatic Mutation Was Identified in the Fibroadenoma (Right Breast) Whereas MED12 Hotspot (c.131G>A) and TERT Promoter (c.-124C>T) Mutations Were Identified in Both the Early B3 Lesion and in the Phyllodes Tumor (Left Breast). B, Copy Number Profiling of the 29 Complete Coding Genes Included in the Targeted Sequencing Panel. No Copy Number Alteration Was Identified in the Fibroadenoma (Right Breast). Gains of MALAT1, TBX3, and RUNX1 Were Found in Both the Early B3 Lesion and in the Phyllodes Tumor (Left Breast). Addition Losses of AKT1 and TP53 Were Found Only in the Lesion After 13 Months



copy number alterations on chromosomes 11, 12, and 21, and the subsequent acquisition of copy number loss of *AKT1* and *TP53* in the later lesion (Figure 3B).

Discussion

This 59-year-old woman was diagnosed with a FEL with increased stromal cellularity (B3-lesion). The uncertain malignant potential was discussed with the patient in detail, and surgical excision was scheduled. Given the absence of "cancer cells" on the CNB, the patient perceived the lesion as not dangerous. The patient canceled the scheduled operation, and follow-up was lost. The disfiguring evolution of the breast lesion and the inconclusive diagnosis on the second CNB 13 months later led to a mastectomy without reconstruction.

Currently, there are no absolute criteria to recommend the excision of FELs when definitive diagnosis of a PT is not rendered after CNB.[2,7] Many authors advocate reliance on pathologists' comments and concordance assessment as important considerations to guide clinical management of FELs.[3,16] Van Osdol et al recently reported a sensitivity and specificity of 82% and 93%, respectively, for a pathologist comment of concern.[7] Nevertheless, only 18% to 38% of patients who received excision for a FEL had a PT on definitive histology.[3,17,18] The sometimes extreme difficulty of distinguishing PTs from cellular FA, particularly in a limited tissue sample of a CNB, has also been acknowledged in the latest World Health Organization classification,[1] which states that in inconclusive cases, the diagnosis of a cellular fibroepithelial neoplasm may be appropriate, recognizing the inability to classify these lesions correctly.[19]

Recently, it has been suggested that *TERT* alterations may assist in the differential diagnosis between FAs and PTs, as *TERT* vpromoter mutations and gene amplifications are frequent in PTs but are absent in FAs, and their frequency increases according to the grade of the PT (18% in benign, 57% in borderline, and 64% in malignant PTs).[8,20] Piscuoglio et al concluded that sequencing and gene copy number analysis differentiate FAs from PTs with a 100% specificity and 100% positive predictive value.[8] The finding of a *TERT* promoter mutation in the early B3 FEL on the left side would suggest it was also a PT.

Genomic profiling has also furthered our understanding of the etiopathology of PTs. Pareja et al[21] have recently studied PTs with and without FA-like areas and hypothesized that borderline and malignant PTs might follow 2 distinct evolutionary pathways, according to *MED12* status. In the *MED12*-mutant pathway, *MED12* exon 2 mutations are posited to lead to the development of a benign FEL, which, upon the occurrence of additional genetic alterations affecting *TERT* and/or other cancer gene, may progress to a borderline or malignant PT. In the *MED12*-independent pathway, borderline or malignant PTs might arise de novo, through acquisition of genetic alterations targeting cancer genes such as *TERT* and/or *EGRF* and other tumor suppressor genes.[21] The presence of both the *MED12* and *TERT* mutations in our current case would suggest it likely occurred through the *MED12*-mutant pathway, followed by the subsequent acquisition of the *TERT* promoter mutation, as well as additional copy number alterations.

The evolution from FA to PTs rarely occurs and has been reported in the literature as a slow process.[22,23] On the other hand, PTs are often reported as rapidly growing.[24] Whether growth rate differs according to the expression of MED12 is unknown. In the present case, the patient presented with a 5-cm FEL, with *MED12* and *TERT* promoter mutations, which rapidly evolved into a disfiguring borderline PT, which needed mastectomy.

Conclusion

Recent studies have suggested that *TERT* promoter hotspot mutation and/or amplification may drive PT progression from a benign lesion such as FA, which represents an example of how genomic profiling may inform the clinical management of FELs. The current case demonstrates the natural course of a borderline PT after refusal of surgery. Knowing that the lesion had a *TERT* mutation and therefore a high likelihood of being a PT might have had encouraged the patient to undergo surgical excision and thus would have spared her the burden of a mastectomy. Further studies are needed to evaluate the impact of massively parallel sequencing on the outcome of patients with FELs.

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental table accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2018.10.007.

References

1. Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. *WHO Classification of Tumours of the Breast*, Vol 4. Lyon: IARC Press; 2012.
2. Karim RZ, O'Toole SA, Scolyer RA, et al. Recent insights into the molecular pathogenesis of mammary phyllodes tumours. *J Clin Pathol* 2013; 66:496-505.
3. Resetkova E, Khazai L, Albarracin CT, Arribas E. Clinical and radiologic data and core needle biopsy findings should dictate management of cellular fibroepithelial tumors of the breast. *Breast J* 2010; 16:573-80.
4. National Comprehensive Cancer Network. NCCN guidelines for Phylloides tumors, Available at: http://www.ncnn.org 2017. Accessed: May 19, 2018.
5. Tan PH, Ellis IO. Myoepithelial and epithelial-myoepithelial, mesenchymal and fibroepithelial breast lesions: updates from the WHO Classification of Tumours of the Breast 2012. *J Clin Pathol* 2013; 66:465-70.
6. Jacobs TW, Connolly JL, Schnitt SJ. Nonmalignant lesions in breast core needle biopsies: to excise or not to excise? *Am J Surg Pathol* 2002; 26:1095-110.
7. Van Osdol AD, Landercasper J, Andersen JJ, et al. Determining whether excision of all fibroepithelial lesions of the breast is needed to exclude phyllodes tumor: upgrade rate of fibroepithelial lesions of the breast to phyllodes tumor. *JAMA Surg* 2014; 149:1081-5.
8. Piscuoglio S, Geyer FC, Burke KA, et al. Massively parallel sequencing analysis of synchronous fibroepithelial lesions supports the concept of progression from fibroadenoma to phyllodes tumor. *NPJ Breast Cancer* 2016; 2:16035.
9. Lim WK, Ong CK, Tan J, et al. Exome sequencing identifies highly recurrent MED12 somatic mutations in breast fibroadenoma. *Nat Genet* 2014; 46:877-80.
10. Piscuoglio S, Murray M, Fusco N, et al. MED12 somatic mutations in fibroadenomas and phyllodes tumours of the breast. *Histopathology* 2015; 67:719-29.
11. Cani AK, Hovelson DH, McDaniel AS, et al. Next-gen sequencing exposes frequent MED12 mutations and actionable therapeutic targets in phyllodes tumors. *Mol Cancer Res* 2015; 13:613-9.

12. Piscuoglio S, Ng CK, Murray M, et al. Massively parallel sequencing of phyllodes tumours of the breast reveals actionable mutations, and TERT promoter hotspot mutations and TERT gene amplification as likely drivers of progression. *J Pathol* 2016; 238:508-18.
13. Noguchi S, Yokouchi H, Aihara T, et al. Progression of fibroadenoma to phyllodes tumor demonstrated by clonal analysis. *Cancer* 1995; 76:1779-85.
14. Kuijper A, Buerger H, Simon R, et al. Analysis of the progression of fibroepithelial tumours of the breast by PCR-based clonality assay. *J Pathol* 2002; 197:575-81.
15. Ng CKY, Di Costanzo GG, Tosti N, et al. Genetic profiling using plasma-derived cell-free DNA in therapy-naive hepatocellular carcinoma patients: a pilot study. *Ann Oncol* 2018; 29:1286-91.
16. Landercasper J, Linebarger JH. Contemporary breast imaging and concordance assessment: a surgical perspective. *Surg Clin North Am* 2011; 91:33-58.
17. Schoonjans JM, Brem RF. Fourteen-gauge ultrasonographically guided large-core needle biopsy of breast masses. *J Ultrasound Med* 2001; 20:967-72.
18. Gould DJ, Salmans JA, Lassinger BK, et al. Factors associated with phyllodes tumor of the breast after core needle biopsy identifies fibroepithelial neoplasm. *J Surg Res* 2012; 178:299-303.
19. Tan PH, Tse G, Lee A, Simpson JF, Hanby AM. Fibroepithelial tumours. In: Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ, eds. *WHO Classification of Tumours of the Breast*. 4th ed. Lyon, France: IARC Press; 2012.
20. Yoshida M, Ogawa R, Yoshida H, et al. TERT promoter mutations are frequent and show association with MED12 mutations in phyllodes tumors of the breast. *Br J Cancer* 2015; 113:1244-8.
21. Pareja F, Geyer FC, Kumar R, et al. Phyllodes tumors with and without fibroadenoma-like areas display distinct genomic features and may evolve through distinct pathways. *NPJ Breast Cancer* 2017; 3:40.
22. Abe M, Miyata S, Nishimura S, et al. Malignant transformation of breast fibroadenoma to malignant phyllodes tumor: long-term outcome of 36 malignant phyllodes tumors. *Breast Cancer* 2011; 18:268-72.
23. Sanders LM, Daigle ME, Tortora M, Panasiti R. Transformation of benign fibroadenoma to malignant phyllodes tumor. *Acta Radiol Open* 2015; 4, 2058460115592061.
24. Telli ML, Horst KC, Guardino AE, Dirbas FM, Carlson RW. Phyllodes tumors of the breast: natural history, diagnosis, and treatment. *J Natl Compr Canc Netw* 2007; 5:324-30.

Supplemental Data

Supplemental Table 1 List of Genes Included in the Targeted Sequencing Panel

Gene Name	Chromosome	Start	End	Cytoband	Remarks
ARID1A	1	27022524	27108595	p36.11	Complete coding region
NRAS	1	115247090	115259515	p13.2	Hotspot residues 12, 13, and 61 only
SETD2	3	47057919	47205457	p21.31	Complete coding region
PIK3CA	3	178865902	178957881	q26.32	Complete coding region
FBXW7	4	153242410	153457253	q31.3	Complete coding region
MAP3K1	5	56111401	56191979	q11.2	Complete coding region
PIK3R1	5	67511548	67597649	q13.1	Complete coding region
ARID1B	6	157099063	157531913	q25.3	Complete coding region
EGFR	7	55086714	55324313	p11.2	Complete coding region
KMT2C	7	151832010	152133090	q36.1	Complete coding region
PTPRD	9	8314246	10612723	p23	Complete coding region
GATA3	10	8095567	8117161	p14	Complete coding region
PTEN	10	89622870	89731687	q23.31	Complete coding region
HRAS	11	532242	537287	p15.5	Hotspot residues 12, 13, and 61 only
NEAT1	11	65190245	65213011	q13.1	Complete coding region
MALAT1	11	65265233	65273940	q13.1	Complete coding region
ATM	11	108093211	108239829	q22.3	Complete coding region
KRAS	12	25357723	25403870	p12.1	Hotspot residues 12, 13, and 61 only
ERBB3	12	56473641	56497289	q13.2	Complete coding region
TBX3	12	115108059	115121969	q24.21	Complete coding region
RB1	13	48877887	49056122	q14.2	Complete coding region
FOXA1	14	38059189	38069245	q21.1	Complete coding region
AKT1	14	105235686	105262088	q32.33	Complete coding region
CBFB	16	67063019	67134961	q22.1	Complete coding region
CTCF	16	67596310	67673086	q22.1	Complete coding region
CDH1	16	68771128	68869451	q22.1	Complete coding region
TP53	17	7565097	7590856	p13.1	Complete coding region
MAP2K4	17	11924141	12047147	p12	Complete coding region
NCOR1	17	15932471	16121499	p11.2	Complete coding region
NF1	17	29421945	29709134	q11.2	Complete coding region
ERBB2	17	37844167	37886679	q12	Complete coding region
RUNX1	21	36160098	37376965	q22.12	Complete coding region