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Original Research

Dynamic clonal remodelling in breast cancer metastases is associated with subtype conversion

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Abbreviations: BC, Breast cancer; CCF, Cancer cell fraction; CNA, Copy number alteration; CS, Clinical subtype; ER, Oestrogen receptor; HER2, Human epidermal growth factor receptor 2; HER2−, Human epidermal growth factor receptor 2 negative; HER2+, Human epidermal growth factor receptor 2 positive; HR, Hormone receptor; HR−, Hormone receptor negative; HR+, Hormone receptor positive; IS, Intrinsic subtype; PR, Progesterone receptor; TN, Triple-negative; VAF, Variant allele frequency.

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Abstract ***Background:*** Changes in the clinical subtype (CS) and intrinsic subtype (IS) between breast cancer (BC) metastases and corresponding primary tumours have been reported. However, their relationship with tumour genomic changes remains poorly characterised. Here, we analysed the association between genomic remodelling and subtype conversion in paired primary and metastatic BC samples.
Methods: A total of 57 paired primary and metastatic tumours from GEICAM/2009–03 (ConvertHER, NCT01377363) study participants with centrally assessed CS (n = 57) and IS (n = 46) were analysed. Targeted capture and next-generation sequencing of 202 genes on formalin-fixed paraffin-embedded samples was performed. The cancer cell fraction (CCF) of mutations in primary and metastatic pairs was estimated as a surrogate of tumour clonal architecture. Changes in mutation CCF between matched primary and metastatic tumours were analysed in the presence or absence of subtype conversion.
Findings: CS conversion occurred in 24.6% and IS conversion occurred in 36.9% of metastases. Primary tumours and metastases had a median of 11 (range, 3–29) and 9 (range, 1–38) mutations, respectively ($P = 0.05$). Overall, mutations in metastases showed a higher estimated CCF than in primary tumours (median CCF, 0.51 and 0.47, respectively; $P = 0.042$), consistent with increased clonal homogeneity. The increase in mutation CCF was significant in CS-converted ($P = 0.04$) but not in IS-converted ($P = 0.48$) metastases. Clonal remodelling was highest in metastases from hormone receptor–positive and human epidermal growth factor 2 (HER2)–positive tumours ($P = 0.006$).
Conclusions[1]***:*** Mutations in BC metastases showed significantly higher estimated CCF than primary tumours. CCF changes were more prominent in metastases with CS conversion. Our findings suggest that changes in BC subtypes are linked to clonal remodelling during BC evolution.

1. Introduction

In breast cancer (BC), hormone receptors (HRs) and human epidermal growth factor receptor 2 (HER2) are the most relevant biomarkers in clinical practice; however, several studies have shown discordances between primary and metastatic tumours regarding HR expression, *HER2* amplification [1–3], intrinsic subtype (IS) classification [4] and other biomarkers such as *PIK3CA* mutations and PTEN expression [5,6].

Understanding the genomic landscape of BC and its evolution is of importance, not only from a biological perspective but also for its implications in personalised medicine. Landmark studies have revealed many of the genomic underpinnings of early BC [7–9], while later efforts have focused on characterising the genomic alterations present in advanced disease [10–12]. Further studies have addressed the genomic events that appear during disease progression and metastasis [13–17]. BC presents significant intertumour and intratumour genomic heterogeneity [18,19], which extends beyond localised disease and is fuelled by complex mechanisms of tumour dissemination [20,21]. Importantly, the relationship between BC subtypes and its genomic features during BC evolution remains poorly understood.

We have conducted a comprehensive, next-generation, sequencing-based analysis in paired primary and metastatic tumours from the GEICAM/2009–03 (ConvertHER) study [2]. Exploiting the high coverage provided by targeted sequencing of cancer genes, we have explored the variations in clonal architecture between primary tumours and their metastatic lesions in the context of clinical subtype (CS) and/or IS conversion [22].

[1] Results of this study were presented at the 2014 San Antonio Breast Cancer Symposium, San Antonio, TX, USA and at the 2017 ASCO Annual Meeting, Chicago, Illinois, USA.

2. Methods

2.1. Study design

The ConvertHER trial was a multicentre, prospective, observational study coordinated by the GEICAM Spanish Breast Cancer Group [2]. Blocks from formalin-fixed paraffin-embedded primary BC and biopsies of recurrent or metastatic tumours were obtained from all participants.

2.2. Clinical and intrinsic BC subtypes

Histological oestrogen receptor (ER), progesterone receptor (PR) and HER2 diagnoses were centrally and prospectively assessed by 2 pathologists. Tumours were classified into CSs: HR+/HER2-, HR+/HER2+, HR-/HER2+ and triple-negative (TN) [2]. ISs were determined by gene expression profiling using the PAM50 classifier [4]. CS conversion refers to the changes in immunohistochemical expression levels of HRs or HER2 between primary tumours and their metastases. IS conversion refers to a change in PAM50 classification between a primary tumour and its metastasis. Circos plots were generated using a publicly available online interface [23].

2.3. Genomic profiling and mutation calling

Comprehensive genome profiling was performed in 61 formalin-fixed paraffin-embedded samples using a target capture next-generation sequencing assay (T-200) developed at MD Anderson Cancer Center (MDACC) Institute of Personalized Cancer Therapy, as previously described (Supplementary Material and Supplementary Table S1) [24]. The T-200 target capture deep sequencing data were aligned to the human reference assembly hg19 using BWA [25], and duplicated reads were removed using Picard [26]. Single-nucleotide variants and small indels were called using an in-house developed analysis pipeline, which classified variants into 3 categories: somatic, germline and loss of heterozygosity based on variant allele frequencies in the tumour and the matched normal tissues [27]. Copy number alteration (CNA) was called using a previously published algorithm [28], which reports gain or loss status of each exon. Variants were annotated using variant effect predictor (VEP), Annovar and CanDra, and their functional impact was assessed using dbSNP, COSMIC [29] and the cancer genome atlas (TCGA) databases.

2.4. Estimating the cancer cell fraction of mutations

The variant allele frequency (VAF) of individual mutations was computed from the number of sequencing reads mapping to both the reference and the alternative allele, and cancer cell fraction (CCF) was estimated following a previously described rationale [30]. In brief, we estimated the CCF of mutations from their VAF values after filtering of potential VAF artifacts. First, VAF filtering was performed by discarding mutations with a VAF>0.75 (considered as potential germline or loss-of-heterozygosity false positive calls), as well as mutations mapping to amplified or homozygously deleted genomic segments. Second, the VAF of mutations in genomic regions with heterozygous deletion was divided by 2. Finally, the VAF was corrected by the maximum VAF observed across mutations remaining after the aforementioned filtering steps. We used this maximum VAF as a proxy to the tumour cell content (i.e. purity) of each sample. A detailed explanation on the CCF estimation (including relevant equations) can be found in Supplementary Methods. Changes in estimated CCF distribution (Mann–Whitney test) and mutation clonal status (Fisher's exact test) between primary tumours and metastases were compared based on subtype conversion status.

3. Results

3.1. Patient and tumour characteristics

DNA for next-generation sequencing was available in 57 case pairs. The median time from initial diagnosis to

Table 1
Patient characteristics at baseline.

Patient information (n = 57)	
Age at diagnosis (years)	
Median	61
Range	37–85
Stage at presentation	n (%)
I	7 (13.7)
II	24 (42.1)
III	13 (23.0)
IV	7 (12.2)
Unknown	6 (10.5)
Histological grade	n (%)
Gx	11 (19.3)
G1	5 (8.8)
G2	12 (21.1)
G3	29 (50.9)
Node affection	n (%)
N-	47 (82.5)
N+	10 (17.5)
Metastasis biopsy site	n (%)
Locoregional	**13 (22.8)**
Nodes	7 (12.3)
Chest wall	6 (10.5)
Distant	**44 (77.2)**
Bone	7 (12.3)
Brain	2 (3.5)
Distant node	3 (5.3)
Skin or subcutaneous tissue	15 (26.3)
Visceral	10 (17.5)
Other	7 (12.3)
Total	**57 (100)**

metastatic biopsy was 4.3 years (range, 0–19.2). Baseline characteristics of patients and sites of metastasis are described in Table 1. The most common CS in both primary and metastatic tumours was HR+/HER2- (64.9% and 54.4%, respectively). Luminal A (37%) and HER2-Enriched (HER2-E) (30.4%) were the most common IS in primary tumours and in metastases, respectively.

There were 14 (24.6%) CS conversions and 17 (37.0%) IS conversions (Fig. 1). There was a moderate agreement (50%) between CS and IS classification of primary and metastatic tumours (Supplementary Table S2). HER2 positivity was preserved in all metastases from HER2+ primary tumours, irrespective of HR status. There was no correlation between CS and IS conversion. Of 39 (68%) case pairs in which the CS was preserved, only 14 (35.9%) presented IS conversion, whereas of the 30 (65%) cases in which the IS was preserved, only 5 (10.9%) presented CS conversion (Supplementary Fig. S1A).

3.2. Mutation detection and CCF estimation in primary tumours and metastases

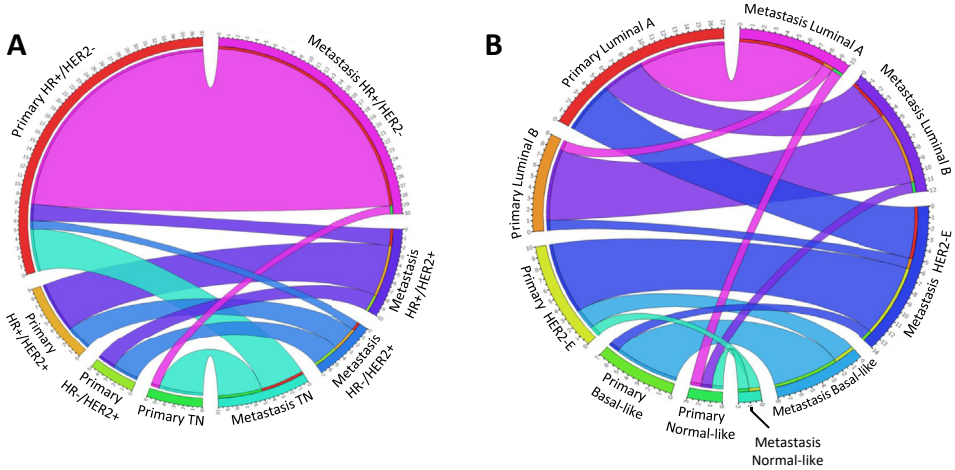
Among the 57 case pairs analysed, 554 somatic mutations have been identified in 151 genes. Fig. 2 summarises the mutations and CNAs found in cancer drivers [31] (Supplementary Table S3 lists the mutations and CNAs found). All tumours bore at least one mutation. The median number of mutations was 11 (range, 3–29) in primary tumours and 9 (range, 1–38) in metastases (Fig. 3). The most frequent mutations in each BC subtype are summarised in Supplementary Table S4.

CCF was calculated for 86.4% of mutations (85.7% in primary tumours and 87% in metastases). The CCF obtained using the maximum VAF as a surrogate of tumour purity significantly correlated with the CCF computed using pathology-based tumour cell content ($r = 0.84$; $P < 0.001$; Supplementary Figs. S1B–D). Mutations found in metastases had higher estimated CCF compared with those mutations in primary tumours (median CCF values of 0.57 and 0.47, respectively; $P = 0.042$) (Supplementary Figs. S2A and B), suggesting that mutations found in metastases were more clonal.

The analysis of the mean CCF of primary tumours and metastasis according to the tumour size and histological grade at diagnosis did not show significant differences (Supplementary Figs. S2C and D).

3.3. Clonal remodelling and subtype conversion

Significant changes in CCF between primary tumours and their metastases were found in some instances. This was more apparent in cases with subtype conversion. Extensive clonal remodelling in a HR+/HER2- (PAM50 Luminal A) primary tumour that switches to a TN (PAM50 HER2-E) phenotype in metastasis was observed (Fig. 4, left). However, highly concordant CCFs in another HR+/HER2- tumour without subtype conversion were observed (Fig. 4, right). In the first example, the most likely explanation of its clonal



Primary	Metastasis			
	HR+/HER2-	HR+/HER2+	HR-/HER2+	TN
HR+/HER2-	29 (78.4%)	2 (0.05%)	1 (0.03%)	5 (13.5%)
HR+/HER2+	0	6 (66.6%)	3 (33.3%)	0
HR-/HER2+	0	2 (40%)	3 (60%)	0
TN	1 (16.7%)	0	0	5 (83.3%)

Primary	Metastasis				
	Luminal A	Luminal B	HER2-E	Basal-like	Normal-like
Luminal A	8 (47%)	4 (23.5%)	5 (29.4%)	0	0
Luminal B	1 (11.1%)	7 (77.8%)	1 (11.1%)	0	0
HER2-E	0	0	7 (70%)	2 (20%)	1 (10%)
Basal-like	0	0	1 (14.3%)	6 (85.7%)	0
Normal-like	1 (33.3%)	1 (33.3%)	0	0	1 (33.3%)

Fig. 1. **Circos plots displaying the conversion of clinical (A) and intrinsic (B) subtypes in breast cancer metastases**. The subtype of primary lesions (left area of the plot) and of the corresponding metastasis (right area of the plot) is represented. Outer segments are labelled according to the different subtypes. Paired specimens are connected by ribbons. TN, triple-negative; HER2, human epidermal growth factor receptor 2; HR, hormone receptor.

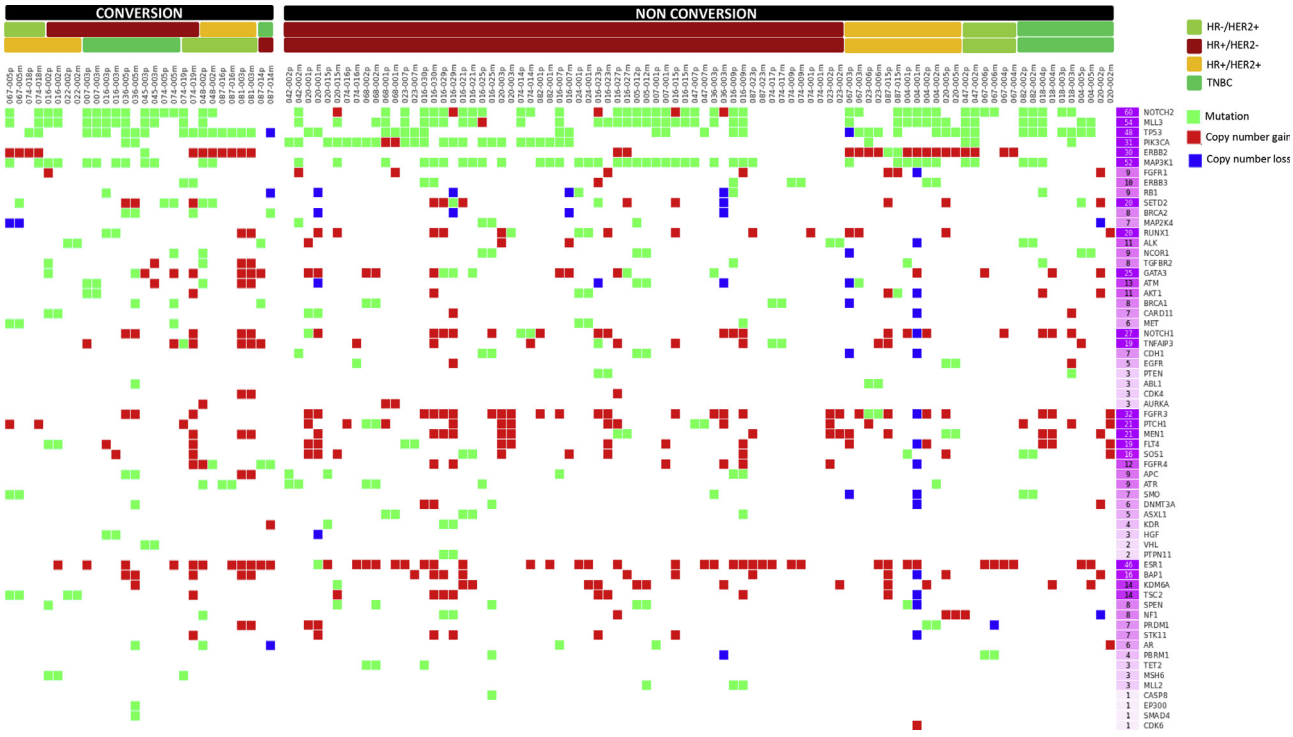


Fig. 2. **Gene alterations in both primary tumours and metastases**. Only relevant cancer genes [30,39] are represented. HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer.

remodelling includes a single surviving clone in the metastasis, with the mutations close to the diagonal (considering those with CCF close to 1 an error of copy number estimation). An alternative explanation (if the copy number of all mutant sites were correctly estimated) would be that 2 clones survived from the primary to the metastatic tumour. The same 2 explanations could explain the clonal remodelling observed in the second example.

Differences in CCF distribution between primary tumours and metastases were significant in cases with CS conversion ($P = 0.04$; Fig. 5A). On the other hand, CCF distribution showed significant changes only in instances in which the IS was preserved in metastases ($P = 0.02$; Fig. 5B). Moreover, primary and metastatic tumours that presented CS conversion had significantly lower estimated CCF ($P = 0.0003$ and $P = 0.01$, respectively; Fig. 5C) than those without CS conversion

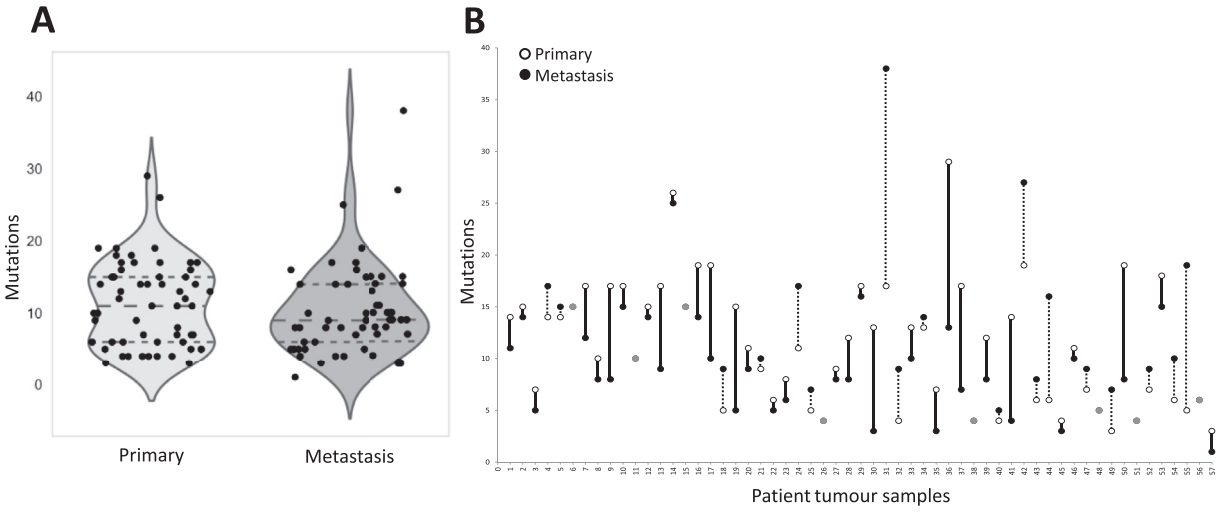


Fig. 3. **Number of mutations in primary tumours and metastases**. (A) Violin plot showing mutation distribution in primary vs metastatic tumours. (B) Scatter plot showing the mutation number for each case pair. White dots represent the number of mutations in the primary tumours, black dots represent the number of mutations in metastatic lesions, and grey dots represent the cases where the number of mutations was the same in both the primary tumour and its metastatic counterpart.

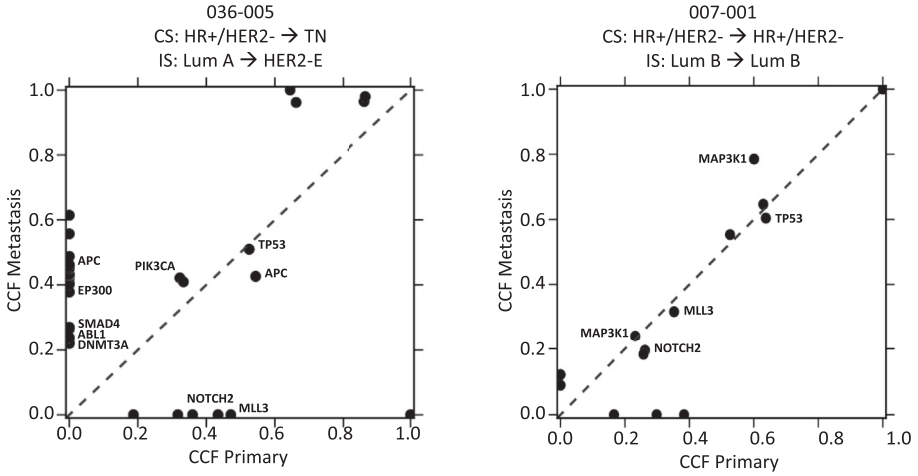


Fig. 4. **Mutation CCF according to clinical and intrinsic subtype conversion**. The increase in mutation CCF between primary tumours and their corresponding metastases reveals the existence of clonal remodelling during the process of breast cancer dissemination. Two opposite examples are shown. Left panel: Change in mutation CCF detected in an HR+/HER2- primary tumour which on metastasis switched to a triple-negative breast phenotype. Several mutations detected in the primary tumour are undetectable in the metastatic partner, including three mutations in NOTCH1, NOTCH2 and MLL3. On the other hand, several mutations undetected in the primary tumour appear in the metastasis, including mutations of DNMT3A, ABL1, SMAD4, EP300 and APC. Mutations close to the diagonal in the plot are those that remain at approximately the same CCF in both the primary and the metastatic lesion. Right panel: Change of CCF of mutations between the primary tumour and metastasis of a concordant HR+/HER2- tumour. Changes of clonal architecture in this case are subtler because CCFs of detected mutations have similar values in the primary tumour and the metastasis. For example, NOTCH2 (diploid) Pro6AgrfsX27 mutation estimated CCF in the primary tumour is 0.2619 and 0.1951 in the metastasis; MAP3K1 (diploid) Arg763-CysfsX35 mutation estimated CCF in the primary tumour is 0.2319 and 0.2405 in the metastasis. These two mutations, despite the variability of their estimated CCF, probably support the same clone, rather than two different clones. CCF, cancer cell fraction; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TN, triple-negative; CS, clinical subtype; IS, intrinsic subtype.

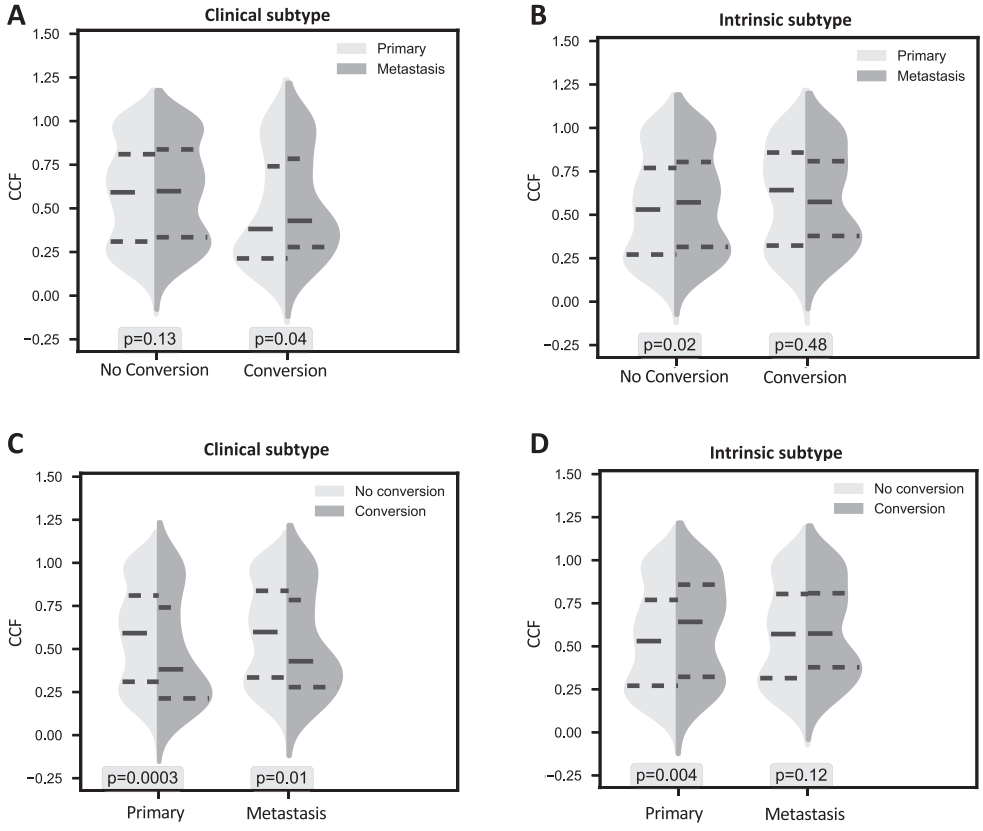


Fig. 5. **Distribution of mutation CCF according to clinical or intrinsic subtype conversion**. Violin plot showing changes in CCF distribution between primary tumours and metastases in the presence or absence of clinical (A) and intrinsic (B) subtype conversion and between converted vs non-converted primary tumours and metastases (C and D). CCF, cancer cell fraction.

(Fig. 5D). In contrast, IS conversion was associated with higher estimated CCF in primary tumours ($P = 0.004$; Fig. 5D). Remarkably, the 3 cases in the HR+/HER2+ subtype that switched to HR-/HER2+ in metastases showed a significant increase in estimated CCF values ($P = 0.006$). Significant CCF differences were only observed in those HER2-E primary tumours, in which the IS was preserved in the metastases ($P = 0.001$; Fig. 6A).

The association between conservation of mutation clonality and subtype conversion was consistent with previous results. The proportion of mutations whose clonal status changed between primary tumours and metastases was higher in cases with CS conversion ($P < 0.01$; Table 2A), whereas no association was found for IS conversion ($P = 0.59$; Table 2B).

Table 2
Changes in mutation clonal status according to clinical (A) or intrinsic (B) subtype conversion.

A		
Clinical subtype conversion	Clonality	
	Conserved	Non-conserved
No	214 (44.4%)	268 (55.6%)
Yes	59 (30.3%)	136 (69.7%)
		p < 0.01
B		
Intrinsic subtype conversion	Clonality	
	Conserved	Non-conserved
No	151 (42.2%)	207 (57.8%)
Yes	85 (45%)	104 (55%)
		p = 0.586

3.4. Potential clinical and pathological surrogates of clonal remodelling

Primary tumours from case pairs without CS conversion had higher estimated CCF, suggesting lower clonal heterogeneity. The HR + subset showed subtype conversion in 23.9% (11/46) of cases. Interestingly, we found that primary tumours that underwent a CS conversion presented significantly lower levels of ERs (70% vs 95%; $P = 0.008$) and PRs (15% vs 70%; $P = 0.018$) (Fig. 6B). Moreover, having ≤90% ER or ≤40% PR expression was significantly associated with CS conversion ($P = 0.03$ and $P = 0.01$, respectively), and this association was stronger when both features were present ($P = 0.002$; data not shown). As expected, primary HR + conversion

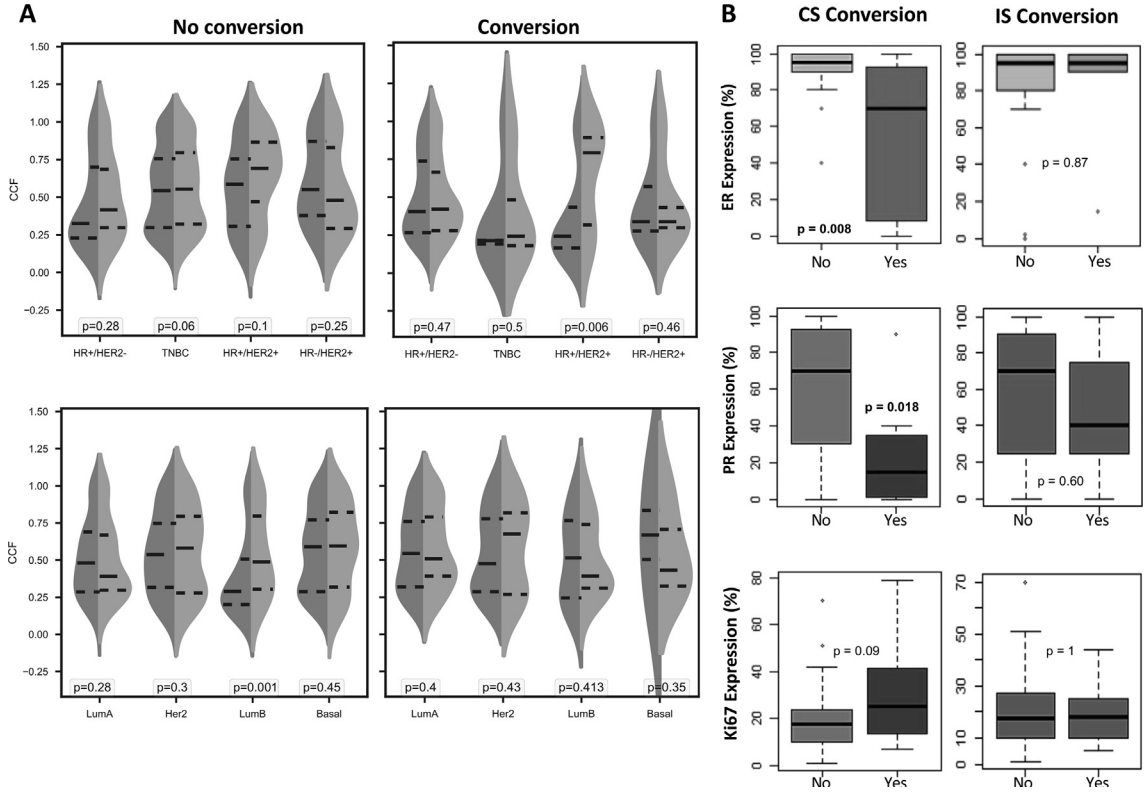


Fig. 6. (A) CCF changes between primary tumours and metastases according to the primary tumour subtype. Changes in the distribution of mutation CCF between primary tumours (left) and metastases (right) according to the clinical subtype (CS) (top) or intrinsic subtype (IS) (bottom) of the primary tumours. (B) Status of histopathological markers in primary tumours according to subtype conversion. Percentage of ER, PR and Ki67 positivity in primary tumours that underwent CS or IS conversion in their corresponding metastases. CCF, cancer cell fraction; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; TNBC, triple negative breast cancer.

to TN in the metastases had the lowest levels of ER expression, although this difference was not statistically significant (Supplementary Figs. S3A and B). In contrast, HR status was not associated with IS conversion, and Ki67 bore no relationship with CS or IS conversion ($P = 0.09$ and $P = 1$, respectively; Fig. 6B). Tumour size and nodal positivity do not influence CCF in primary tumours (Supplementary Fig. S4 A-C and Supplementary Results). No differences were found in the time to metastasis when stratifying the patients by differences in CCF between the primary tumours and their metastases (Supplementary Fig. S4D). Of note, both relapse-free survival and overall survival of the patients is maintained, regardless of the CS and IS conversion (Supplementary Figs. S4E and S5).

4. Discussion

In this study, we provide evidence of clonal remodelling between primary tumours and metastases from a prospective cohort of patients with BC [2]. Furthermore, we report a significant association between clonal remodelling and CS conversion in BC metastasis. Our data also indicate that HER2 positivity is preserved in metastases from HER2+ primary tumours, independently of HR expression, while undergoing significant changes in clonal architecture. Moreover, high expression of HRs in primary tumours may be associated with a lower rate of subtype conversion in the metastases.

The study is based on samples prospectively collected and analysed within the ConvertHER [2] study. The status of HR and HER2 expression was centrally assessed. We observed changes in ER, PR and HER2 expression between primary tumours and metastases in 15.8%, 17.5% and 5.3% cases, respectively. These rates are slightly less than what is commonly reported [1,3]. Remarkably, none of the HER2+ primary tumours lost their HER2 positivity at metastasis. Regarding IS, Luminal A showed the highest rate of conversion (52.9%), whereas basal-like showed the lowest (14.3%). This is consistent with the results on IS changes between primary and matched metastatic BC samples that also included patients from the ConvertHER cohort [4].

Our results lend further support to the hypothesis put forward by others [32] that significant clonal remodelling occurs during the process of BC dissemination. Taking advantage of the deep sequencing assay, we estimated the CCF of the different mutations in each sample, thus obtaining a measure of tumour clonal heterogeneity. Despite using a somewhat restrictive pipeline, it was possible to obtain the CCF of most mutations detected in our study population. Interestingly, we observed higher estimated CCF values in metastases compared with primary tumours. This is in concordance with a recent study that used whole-genome sequencing [33] and suggests that primary tumours are more clonally heterogeneous than individual metastasis. Recent reports suggest that the acquisition of novel genomic traits (and thus the development of tumour clonal diversity) is directly correlated with time [21,34]. In our study, metastatic sampling was performed at the first diagnosis of spread disease. Therefore, it is plausible that the time for clonal diversity to develop in the metastasis may have been limited, which would potentially explain the globally higher estimated CCF (i.e. homogeneity) found in metastases.

Significant changes in CCF distribution and in mutation clonal status were confined to case pairs that showed CS conversion. Furthermore, primary tumours from converted pairs had lower estimated CCF values (i.e. were more heterogeneous) than their non-converted counterparts, and a similar observation was made for converted metastases. This heterogeneity could be due to a longer time of tumour growth before detection and sampling, for both the primary tumour and the converted metastasis. However, neither primary tumour size, node stage or grade nor time to relapse or treatment regime (except for CS conversion in therapy after biopsy characterisation, $P = 0.038$) correlated with subtype conversion or with CCF changes in our cohort (Supplementary Fig. S4, Supplementary Results and Supplementary Table S5). An interesting finding was the significant clonal remodelling found in HR+/HER2+ tumours. As stated previously, the three metastases in this group that underwent a conversion presented a HR-/HER2+ phenotype, and their mutations exhibited significantly higher estimated CCF values than those of their corresponding primary tumours. This could be due to clonal selection of HER2+ cells in the metastasis, although these results should be interpreted with caution owing to the small sample size.

The relationship found between CS and CCF variation was different than the relationship between IS conversion and CCF variation. One explanation could be that CS and IS in BC portray different biological aspects of the tumour and that these do not necessarily overlap [35]. Alternatively, because gene expression (on which the PAM50 classifier is based) may be affected by CNAs [36], the withdrawal of mutations in amplified and deleted regions for CCF calculation may explain the observed lack of association between clonal remodelling and IS changes in our study. In turn, CNA may be less concordant between primary and metastatic tumours than mutations, as previously reported [24]. Furthermore, our results must be interpreted taking into account that there were around 20% fewer patients for which the IS was available and that the IS changed more frequently than the CS (36.9% vs 24.6%, respectively).

Recent studies that analysed matched primary BC and metastasis from distinct clinical populations reported high (>80%) concordance rates of mutations between primary and metastatic sites using different

genomic platforms [13,24,37]. Consistently, we found the most frequently mutated genes in primary tumours to be also present in metastases, independently of the tumour subtype (Supplementary Table S4). Interestingly, we observed a high prevalence of alterations in the components of the *NOTCH* gene family of transmembrane receptors. The NOTCH pathway is an evolutionarily conserved signalling pathway involved in cell fate determination, and its alteration has been associated with BC carcinogenesis and patient prognosis. Our findings suggest that NOTCH signalling is relevant in both primary tumours and metastases, thus supporting the development of specific NOTCH inhibitors in BC [38]. Other relevantly altered genes in our study include *PIK3CA*, *TP53, MAP3K1, GATA3* and *MLL3*, which have been extensively described in BC [7,8,13]. Finally, we found alterations in less well-known genes such as *CRIPAK* and *HYDIN*, the relevance of which warrants further study.

Summarising, we show a significant association between subtype conversion and clonal remodelling in BC metastases. Whether variation in mutation clonality shapes the BC phenotype or vice versa warrants further investigation.

5. Conclusions

Through deep sequencing of selected genes in a prospective cohort of patients with BC, we found that there was an increase in the CCF of mutations between primary tumours and corresponding metastases, revealing the existence of clonal remodelling during BC dissemination. Primary tumours that underwent CS conversion in their metastases had significantly lower estimated CCF values than their non-converted counterparts. In contrast, IS conversion was associated to higher estimated CCF values in primary tumours when compared with those without IS conversion. Furthermore, IS conversion was not linked to changes in mutation clonal status. These results suggest that the CS and IS portray somewhat different biologic traits of BC.

Author contribution

A.L., A.M.G.-A., D.C., A.G.-Z., G.B.M., E.C., R.C., F.R., A.G.-P., F.M.-B. and J.A. contributed to study concepts. A.L., A.M.G.-A., D.C., A.G.-Z., G.B.M., E.C., R.C., F.R., A.G.-P., F.M.-B. and J.A. contributed to study design. A.K.E., X.Z., S.L., K.C., A.L., A.M.G.-A., E.M.D., A.G.-Z., J.I.C., S.A., I.B., J.A. and A.P. contributed to data acquisition. G.B.M., F.M.-B., A.K.E., X.Z., S.L., K.C. and A.G.-P. contributed to quality control of data and algorithms. All authors contributed to data analysis and interpretation. D.C., G.B.M., F.M.-B., A.K.E., X.Z., S.L., K.C. and A.G.-P. contributed to statistical analysis. All authors contributed to manuscript preparation. D.C., P.L.-S., R.C., A.G.-P. and J.A. contributed to manuscript editing. All authors contributed to manuscript review.

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Conflict of interest statement

The authors report no conflict of interests related to this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.07.003.

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