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RESEARCH

The molecular genetic make-up of male breast cancer

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Abstract

Male breast cancer (MBC) is extremely rare and accounts for less than 1% of all breast malignancies. Therefore, clinical management of MBC is currently guided by research on the disease in females. In this study, DNA obtained from 45 formalin-fixed paraffin-embedded (FFPE) MBCs with and 90 MBCs (52 FFPE and 38 fresh-frozen) without matched normal tissues was subjected to massively parallel sequencing targeting all exons of 1943 cancer-related genes. The landscape of mutations and copy number alterations was compared to that of publicly available estrogen receptor (ER)-positive female breast cancers (smFBCs) and correlated to prognosis. From the 135 MBCs, 90% showed ductal histology, 96% were ER-positive, 66% were progesterone receptor (PR)-positive, and 2% HER2-positive, resulting in 50, 46 and 4% luminal A-like, luminal B-like and basal-like cases, respectively. Five patients had Klinefelter syndrome (4%) and 11% of patients harbored pathogenic *BRCA2* germline mutations. The genomic landscape of MBC to some extent recapitulated that of smFBC, with recurrent *PIK3CA* (36%) and *GATA3* (15%) somatic mutations, and with 40% of the most frequently amplified genes overlapping between both sexes. *TP53* (3%) somatic mutations were significantly less frequent in MBC compared to smFBC, whereas somatic mutations in genes regulating chromatin function and homologous recombination deficiency-related signatures were more prevalent. *MDM2* amplifications were frequent (13%), correlated with protein overexpression ($P = 0.001$) and predicted poor outcome ($P = 0.007$). In conclusion, despite similarities in the genomic landscape between MBC and smFBC, MBC is a molecularly unique and heterogeneous disease requiring its own clinical trials and treatment guidelines.

Key Words
- breast cancer
- male
- mutation
- copy number
- amplification
- genomic

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Introduction

Male breast cancer (MBC) is a rare disease accounting for about 1% of all breast cancers (Jemal *et al.* 2010, Giordano 2018). Five-year overall survival is lower compared to female patients, related to the older age at diagnosis and more advanced stage at presentation (Giordano *et al.* 2004, Stang & Thomssen 2008). Major genetic factors associated with an increased risk of MBC include *BRCA2* and *BRCA1* germline mutations, Klinefelter syndrome (KS) and family history. Additional suspected genetic factors include *CHEK2* and *PALB2* mutations, with less supporting evidence for *AR* and *FANCM* gene mutations, *CYP17* polymorphism and Cowden syndrome (Weiss *et al.* 2005, Silvestri *et al.* 2017, 2018). Epidemiologic risk factors include hormonal imbalance and radiation exposure (Thomas *et al.* 1994, Abdelwahab Yousef 2017). The majority of MBCs are invasive ductal carcinomas with high estrogen (ER) and progesterone (PR) receptor levels without overexpression of HER2 (Kornegoor *et al.* 2012*c*).

Because of its low incidence, MBC has not been studied as extensively as female breast cancer (FBC). Few retrospective studies have included more than 100 cases. Therefore, despite possible differences in pathogenesis, biology and genetics between both sexes, treatment strategies for MBC have largely been extrapolated from FBC. This, in combination with the older age at diagnosis, hampers improvement in outcome as has been seen in FBC over the last decades.

Recent large-scale cross-platform projects have provided a detailed characterization of the genomic landscape of FBC (Cancer Genome Atlas Network 2012, Curtis *et al.* 2012, Nik-Zainal *et al.* 2016, Pereira *et al.* 2016, Bailey *et al.* 2018, Berger *et al.* 2018). The Cancer Genome Atlas (TCGA) (Cancer Genome Atlas Network 2012) reported the enrichment of specific mutations in *PIK3CA* (47%), *GATA3* (14%), *MAP3K1* (13%), *TP53* (12%), *CDH1* (10%) and *MAP2K4* (7%) with the luminal A subtype and in *TP53* (31%), *PIK3CA* (32%) and *MAP3K1* (5%) within the luminal B subtype (Gao *et al.* 2015). Integrated genomic/transcriptomic analysis of breast cancers from METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) identified 40 putative mutation driver genes (Curtis *et al.* 2012, Pereira *et al.* 2016). Subsequent studies identified overlapping as well as new probable driver mutations (Nik-Zainal *et al.* 2016, Bailey *et al.* 2018, Berger *et al.* 2018).

Genomic data on MBC are scarce. On a molecular level, like FBC, MBC is a heterogeneous disease with differences in transcriptional (Callari *et al.* 2011, Johansson *et al.* 2012), copy number (Johansson *et al.* 2011, Tommasi *et al.* 2011, Biesma *et al.* 2015, Piscuoglio *et al.* 2016), microRNA (Fassan *et al.* 2009, Lehmann *et al.* 2010) and methylation (Kornegoor *et al.* 2012*a*, Johansson *et al.* 2015, Rizzolo *et al.* 2018) patterns. Genomic gains appear to be more common in MBC than in FBC and often involve whole chromosome arms, while genomic losses and high-level amplifications are less frequent (Johansson *et al.* 2011, Tommasi *et al.* 2011, Kornegoor *et al.* 2012*b*, Lacle *et al.* 2013, 2015, Biesma *et al.* 2015). Luminal A-like and luminal B-like MBC subtypes seem to exhibit remarkably similar copy number aberration profiles, in contrast to their distinct landscape of somatic mutations (Piscuoglio *et al.* 2016). Piscuoglio *et al.* recently studied mutations of 59 MBCs by massively parallel sequencing the exons of 241 genes and demonstrated that MBCs less frequently harbored *PIK3CA* (20%) and *TP53* mutations (8.5%) than subtype-matched (sm)FBC (Piscuoglio *et al.* 2016). The most frequently mutated genes in luminal A-like MBC included *PIK3CA* and *MAP3K1* (both at 12%), whereas the most frequently mutated genes in luminal B-like MBC were *PIK3CA* (24%) and *GATA3* (21%).

Thus, MBCs share many features with FBCs, but there is evidence for distinct differences with potential implications for clinical management. Here we (i) used targeted capture massively parallel sequencing of 1943 cancer-related genes to study the mutational and copy number landscape in a cohort of 135 MBCs, including 5 patients with KS and (ii) compared these profiles to those of smFBCs.

Materials and methods

Patients and samples

MBC samples were collected from ten pathology laboratories throughout The Netherlands. Patients were diagnosed between 1986 and 2011. Pathology reports were used to extract age, tumor size and lymph node status. Tumor slides were reviewed by three expert breast pathologists (PJvD, RK, MML) to confirm diagnosis and demarcate normal and tumor areas. Histological type (WHO) and grade were assessed as described previously (Elston & Ellis 1991, Van Diest *et al.* 1992). Patient and tumor characteristics are summarized in Table 1. All samples and databases were anonymized before use. The Dutch national ethical guidelines (www.federa.org) state that no ethical approval is required for the use of anonymous leftover tissue, and this is also part of the standard treatment agreement (Van Diest 2002).

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Table 1 Clinico-pathologic features and immunohistochemical subtypes of the 135 male breast cancers included in this study.

Clinico-pathologic features	*n* = 135	%	Actual %
Median age at diagnosis	67 (range 32–89)		
Median mitotic activity index	10 (range 0–56)		
Median tumor size (cm)	2.3 (range 0.8–11)		
Type			
Ductal	121	89.6	90.3
Lobular	2	1.5	1.5
Other	11	8.1	8.2
Missing	1	0.7	
Histologic grade[a]			
1	27	20.0	22.7
2	49	36.3	41.2
3	43	31.9	36.1
Missing	16	11.9	
Mitotic activity index/2 mm^2			
<8 mitoses	50	37.0	40.7
8–14 mitoses	35	25.9	28.5
>14 mitoses	38	28.1	30.9
Missing	12	8.9	
ER status			
Negative	6	4.4	4.5
Positive	128	94.8	95.5
Missing	1	0.7	
PR status			
Negative	45	33.3	33.8
Positive	88	65.2	66.2
Missing	2	1.5	
HER2 status			
Negative	129	95.6	97.7
Positive	3	2.2	2.3
Missing	3	2.2	
LN status			
Negative	48	35.6	41.4
Positive	68	50.4	58.6
Missing	19	14.1	
Ki-67 labeling index			
Low (<14%)	94	69.6	71.8
High (≥14%)	37	27.4	28.2
Missing	4	3.0	
Surrogate intrinsic subtype[b]			
Luminal A-like	66	48.9	50.4
Luminal B-like	60	44.4	45.8
HER2-driven	0	0.0	0.0
Basal-like	5	3.7	3.8
Missing	4	3.0	

[a]According to the Nottingham grading system (Elston & Ellis 1991); [b]defined by immunohistochemistry according to St. Gallen criteria (Goldhirsch *et al.* 2013).
ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; PR, progesterone receptor.

Immunohistochemistry was repeated to classify MBCs into the four different intrinsic subtypes according to the St. Gallen International criteria for FBC (Goldhirsch *et al.* 2013). Luminal A-like tumors were defined as ER-positive (1%) and PR-positive (≥20% positive cells), HER2-negative and Ki67 low (<14% positive cells), whereas the remaining ER-positive tumors were classified as luminal B-like. HER2-driven tumors were defined as ER/PR negative and HER2 overexpressed or amplified (ASCO/CAP guidelines; Wolff *et al.* 2013), and basal-like tumors as ER/PR/HER2 negative. The DNA extraction procedure is detailed in Supplementary methods (see section on supplementary data given at the end of this article). All tumor samples consisted of 60% or more neoplastic cells, as assessed by pathology review.

Targeted capture massively parallel sequencing

Sequencing libraries were prepared from 600 ng of sheared DNA as previously described with a few modifications (Harakalova *et al.* 2011). After end repair samples were A-tailed, followed by T-tailed adaptor ligation. Samples were pooled and subjected to hybrid capture enrichment using a custom SureSelect assay (Agilent) containing baits targeting all exons of 1943 genes including known oncogenes, tumor suppressor genes, all kinases and genes involved in important pathways related to tumorigenesis and anti-cancer treatment (e.g., angiogenesis; apoptosis; and EGFR, PIK3CA, TGFβ, mTOR and VEGF pathways were included) (Supplementary Table 1). Pre-barcoded individual libraries were pooled, enriched and subsequently sequenced on the SOLiD 5500 system (Thermo Fisher). Supplementary Table 2 summarizes sequencing statistics. All sequencing data have been deposited in EGA under accession number EGAS00001002683.

SOLiD data were processed with our in-house developed pipeline v1.2.1 (https://github.com/UMCUGenetics/IAP) by Genome Analysis Toolkit (GATK) v3.2.2 calling for all samples according to best practices guidelines, including somatic mutation analysis for matched pairs (Mckenna *et al.* 2010, Van der Auwera *et al.* 2013). Filtering was applied to select high quality variants (coverage ≥10, allele fraction ≥0.15) and to exclude germline variants (population frequency <5%), full details available in Supplementary methods.

Copy number alteration (CNA) analysis was performed using Codecz v1.0.1 with the appropriate design file kinome_design_SS_V2_110811. For FFPE samples all (normal) controls were merged into a control pool against which the tumors were analyzed. For FF samples, each sample was compared to the complete FF tumor data set as a means of normalization. Z-scores were calculated as described by Hoogstraat *et al.* (2015).

Mutational signature analysis was performed on MBCs with available ER and HER2 status (n = 129) and

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matched 1:2 with FBCs from TCGA with the same receptor status ($n=258$). Mutational signatures were defined by deconstructSigs using all SNVs for samples with ≥20 somatic SNVs, as previously described (Rosenthal *et al.* 2016, Mueller *et al.* 2018).

In addition, targeted next-generation sequencing (NGS) of the complete coding sequences of *BRCA1* and *BRCA2* was performed on normal tissue (germline) using the Oncomine BRCA FFPE gene panel (ThermoFisher) according to the manufacturer's instructions. In short, 20 ng FFPE DNA was used for library preparation with 24 PCR cycles. Emulsion PCR and enrichment were performed using the Ion PGM Template OT2 200 Template Kit and the Ion One Touch 2 instrument (ThermoFisher). Sequencing was performed using the Ion PGM Sequencing 200 kit v2 using the Ion 318 chip (ThermoFisher). Samples were run on the Ion Torrent PGM System (ThermoFisher). Sequencing data were analyzed using SeqNext (JSI medical systems).

Sanger sequencing

A selection of NGS variants was validated by Sanger sequencing. After Exonuclease I – Shrimp Alkaline Phosphatase enzymatic cleanup of amplified PCR products, BigDye Terminator v1.1 Cycle Sequencing (ThermoFisher) of 1 µL PCR product and Sephadex G-50 based sequencing reaction cleanup, sequencing products were analyzed on a 3730 DNA Analyzer (ThermoFisher). Primers are listed in Supplementary Table 3. Sanger sequencing validation is described in the Supplementary results section.

MDM2 immunohistochemistry

Tissue microarray (triplicate cores) or full 4 µm slides were deparaffinized, pretreated for 24 min in CC1 (EDTA pH8.0) and stained on a Ventana Benchmark XT autostainer using a mouse monoclonal anti-MDM2 antibody (ThermoFisher Scientific, clone IF2, dilution 1/25). All samples with sufficient tumor material ($n=106$) were scored as positive (nuclear staining in at least 5% positive tumor cells) or negative by consensus of two observers.

Statistics

Associations between gene copy number or mutation status and clinicopathological features were calculated using the Pearson chi-square test (or Fisher's exact test when appropriate) for categorical variables (IBM SPSS Statistics, version 21). Associations were evaluated for all genes and only significant associations were reported. Mitotic activity index (MAI) was dichotomized as ≤14 or >14 mitoses/2 mm^2, tumor size as ≤2 or >2 cm, age as ≤60 or >60 and histological grade as grade 1/2 versus grade 3 (Elston & Ellis 1991). Mutation load and CNA load were dichotomized at the median (7 and 62, respectively). Associations of mutational load with clinicopathological characteristics are described in the Supplementary results section. Cluster analysis for CNAs was performed in R using hclust (default) and heatmap.2. Five-year overall survival (OS) was analyzed with Kaplan–Meier plots/log-rank test. Backward LR Cox proportional hazards models were used to estimate risk of death adjusted for parameters significant or with trend in univariate analyses. Hazard ratios (HRs) were calculated with 95% confidence intervals (CIs). For multivariable analysis, P values >0.1 were removed from the model. MedCalc statistical software was used for comparison of SNV and CNA proportions to publicly available METABRIC and MBC data. Corrections for multiple comparisons were applied according to Holm–Bonferroni. For pathway analysis, PANTHER, version 11.1 was used (released 2016-10-24) (Mi *et al.* 2017).

Results

Characteristics of the cohort

From the 135 MBCs, 90% showed ductal histology, 96% were ER positive, 66% were PR positive and 2% HER2 positive, resulting in 50, 46 and 4% luminal A-like, luminal B-like and basal-like cases, respectively. None of the cases was HER2 driven (Table 1). Five patients had KS (4%) and 11% (5/44 with paired normal tissue) of patients harbored pathogenic *BRCA2* germline mutations. The median age at diagnosis was 67 years. Follow-up was available for 111/135 patients. Median follow-up was 52 months (range 1–243), and 59 patients were alive at the time of last follow-up. Tumor size and lymph node status were significant predictors of poor 5-year OS ($P=0.037$ and $P=0.019$, respectively). Luminal B-like ($P=0.122$), PR negative ($P=0.128$) and poorly differentiated ($P=0.094$) tumors tended to be associated with worse 5-year OS compared to luminal A-like, PR-positive and well-differentiated tumors, respectively. Luminal B-like MBCs were associated with high grade ($P=0.00004$), high MAI ($P=0.018$) and PR negativity ($P<0.00001$) when compared to luminal A-like MBCs.

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The majority of *BRCA2* germline mutation carriers presented with poorly differentiated tumors (4/5 were high grade; $P=0.149$) at a similar age as non-*BRCA2*-mutation carriers (median 71 (range 44–78) vs 69 (range 32–87) years, respectively). KS patients tended to be younger (3 of 5 were aged ≤60; $P=0.120$), with generally well-differentiated (5/5; $P=0.158$), PR-positive tumors (5/5; $P=0.166$).

Somatic mutations

Somatic mutations are listed in Supplementary Table 4. Overlapping FBC mutation-driver genes from four studies (Curtis *et al.* 2012, Nik-Zainal *et al.* 2016, Pereira *et al.* 2016, Bailey *et al.* 2018, Berger *et al.* 2018) were specifically compared to MBC. Of the 89 overlapping genes, 21 were not targeted and 1 (*HRAS*) was insufficiently covered by our panel, resulting in 67 interrogated genes. The remaining genes were mutated in 0–36% of MBC. Table 2 compares somatic mutation frequencies with FBC and MBC (Piscuoglio *et al.* 2016). Somatic mutations in *TP53* were rare in MBC (3%). In fact we detected only four (two in matched normal, four in the entire cohort) somatic mutations in *TP53*, two of them known from FBC and being likely oncogenic (OncoKB; Chakravarty *et al.* 2017), the other two unknown. None of the hotspots from FBC (R175, R248 and R273) were affected.

PIK3CA (36%), *KMT2C* (21%), *PBRM1* (20%) and *GATA3* (15%) were identified as most frequently mutated genes in this study (Fig. 1). The pattern of somatic mutations found in *PIK3CA* resembled that of FBC: all mutations found in MBC were missense mutations and 75–80% (12/15 in matched normal; 30/40 in the entire cohort) affected the FBC hotspots (H1047, E542 and E545; Supplementary Fig. 1). Besides *PIK3CA*, other recurrent mutations in MBC included a K358 frameshift *GATA3* mutation (4/25 *GATA3* mutations), the E17K *AKT1* mutation (3/8 *AKT1* mutations) and the Q26K *NCOR1* mutation (4/21 *NCOR1* mutations).

PIK3CA mutations were significantly more abundant in poorly differentiated ($P=0.013$; $n=96$) tumors with high mitotic activity ($P=0.014$; $n=99$) and in lymph node-positive ($P=0.006$; $n=94$) and younger patients ($P=0.046$; $n=108$). Somatic mutations in *PIK3CA* were associated with worse 5-year OS ($P=0.026$; $n=90$; Supplementary Fig. 2). In multivariable analysis, however, *PIK3CA* somatic mutations did not independently predict survival alongside tumor grade ($P=0.034$; HR 2.627) and size ($P=0.001$; HR 1.410).

ATM somatic mutations were associated with high tumor grade ($P=0.040$; $n=74$) and high mitotic counts ($P=0.014$; $n=77$). *NOTCH2* mutations were more frequent in poorly differentiated tumors ($P=0.017$; $n=100$) without lymph node metastases ($P=0.020$; $n=100$). *ATRX* and *CREBBP* somatic mutations were more frequently observed in KS patients ($P=0.001$ ($n=72$) and $P=0.030$ ($n=103$), respectively). *PTPRD* mutations were more abundant in Ki67-high tumors ($P=0.005$; $n=96$) and *MED12* mutations in luminal A-like tumors ($P=0.026$; $n=86$).

Compared to mutation frequencies in ER-positive FBC from METABRIC and TCGA, *TP53* (both $P<0.0001$), *MAP3K1* ($P=0.02$ and $P=0.05$, respectively) and *CDH1* ($P=0.007$ and $P=0.0002$, respectively) somatic mutations were significantly less frequent in MBC (Table 2), whereas particularly *PBRM1* (both $P<0.0001$), *NSD1* ($P<0.0001$ TCGA) and *SETD2* (both $P<0.0001$) mutations were more frequent. Overall, genes regulating chromatin function appeared more often affected in MBC (Fig. 2).

Mutational signature analysis revealed that 24% of MBCs have a dominant signature 3 associated with defective homologous recombination DNA repair, whereas only 13% of TCGA FBCs demonstrated a dominant signature 3 (Fig. 3). In addition, another 24% of MBCs have a dominant signature 8, as compared to 0.4% of FBCs. In a recent review by Nik-Zainal *et al.*, this signature 8 was reported to be increased in homologous recombination deficiency and late in cancer evolution (Nik-Zainal & Morganella 2017).

Copy number aberrations

CNA load

On average, each tumor harbored 138 gene CNAs (range 6–1230), including amplifications ($Z>2.80$) and homozygous deletions ($Z<-2.80$). We observed no correlation between CNA load and ER/PR status, tumor histology, lymph node status, Ki67 status or age (data not shown). CNA load was not associated with mutational load ($P=0.286$) or 5-year survival ($P=0.549$) and was similar between luminal A-like and luminal B-like tumors ($P=0.285$). CNA load was however higher in poorly differentiated ($P=0.012$) and larger tumors ($P=0.045$) with high MAI ($P=0.011$) (Supplementary Fig. 4). CNA load was significantly higher in tumors harboring mutations in *KDM6A* ($P=0.006$) and *NOTCH2* ($P=0.028$).

Figure 4 depicts a heatmap of all CNAs in the MBC cohort after unsupervised hierarchical cluster analysis. MBCs of the same surrogate intrinsic molecular subtype did not cluster on the basis of their CNA profile,

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Table 2 Comparison of somatic mutation frequency with female breast cancer and male breast cancer.

Gene	Studies where driver	*n*	MBC (*n* = 135), %	METABRIC ER+ FBC (*n* = 1825)[a], %	*P* value	TCGA ER+ FBC (*n* = 600)[b], %	*P* value	Berger BRCA (*n* = 982)[b], %	*P* value	Bailey BRCA (*n* = 779) , %	*P* value	Piscuoglio *et al.* (*n* = 59), %	*P* value
PIK3CA	1,2,3,4	108	36.1	44.5	0.0876	39.8	0.4688	34.7	0.7720	34.4	0.7280	20.3	0.0346
KMT2C	1,3,4	122	20.5	12.3	0.0087	8.8	0.0001	NA	NA	9.5	0.0003	5.1	0.0074
PBRM1	1,2,3	105	20.0	1.5	<0.0001	0.7	<0.0001	NA	NA	NA	NA	NA	NA
GATA3	1,2,3,4	132	15.2	14.4	0.8008	14.3	0.7903	12.8	0.4429	12.6	0.4113	15.3	0.9859
NSD1	3,4	126	15.1	NA	NA	1.0	<0.0001	NA	NA	NA	NA	NA	NA
NOTCH1	2,3	27	14.8	4.4	0.0101	0.3	<0.0001	NA	NA	NA	NA	NA	NA
SETD2	2,3	125	13.6	2.4	<0.0001	1.7	<0.0001	NA	NA	NA	NA	NA	NA
NF1	1,2,3,4	99	13.1	3.5	<0.0001	2.3	<0.0001	4.6	0.0004	3.9	0.0001	0.0	0.0038
ARID1A	1,2,3,4	123	13.0	5.1	0.0002	4.7	0.0005	4.3	<0.0001	3.3	<0.0001	1.7	0.0145
ATRX	2,3	72	12.5	NA	NA	1.8	<0.0001	NA	NA	NA	NA	0.0	0.0051
APC	2,3	108	12.0	1.9	<0.0001	1.2	<0.0001	NA	NA	NA	NA	1.7	0.0219
NOTCH2	2,3	116	11.2	NA	NA	2.3	<0.0001	NA	NA	NA	NA	NA	NA
NCOR1	1,2,3	120	10.8	4.0	0.0004	5.0	0.0144	NA	NA	4.7	0.0065	0.0	0.0090
CREBBP	2,3	103	10.7	NA	NA	1.2	<0.0001	NA	NA	NA	NA	NA	NA
KDM6A	1,2,3	51	9.8	1.7	<0.0001	1.7	0.0002	NA	NA	NA	NA	NA	NA
ATM	2,3,4	85	9.4	NA	NA	2.3	0.0005	3.3	0.0047	NA	NA	0.0	0.0157
USP9X	1,2,3	77	9.1	2.6	0.0008	1.0	<0.0001	NA	NA	NA	NA	NA	NA
ATR	2,3	99	8.1	3.9	0.0405	1.0	<0.0001	NA	NA	NA	NA	1.7	0.0946
AKT1	1,2,3,4	101	7.9	4.7	0.1505	3.2	0.0237	3.0	0.0103	3.2	0.0192	0.0	0.0272
KMT2D	2,3	115	7.8	6.8	0.6808	1.8	0.0003	NA	NA	NA	NA	3.4	0.2598
BRAF	2,3	65	7.7	0.6	<0.0001	0.5	<0.0001	NA	NA	NA	NA	3.4	0.3024
MED12	3,4	92	7.6	NA	NA	2.2	0.0042	NA	NA	NA	NA	3.4	0.2890
EGFR	2,3	108	6.5	1.4	0.0001	0.7	<0.0001	NA	NA	NA	NA	1.7	0.1669
STAG2	2,3	55	5.5	NA	NA	0.8	0.0022	NA	NA	NA	NA	NA	NA
TAF1	3,4	75	5.3	1.5	0.0114	1.7	0.0405	2.8	0.2197	NA	NA	NA	NA
RASA1	3,4	77	5.2	NA	NA	0.3	<0.0001	0.8	0.0004	NA	NA	NA	NA
BRCA2	1,2,3	78	5.1	1.5	0.0145	2.2	0.1255	NA	NA	NA	NA	0.0	0.0794
MAP3K1	1,2,3,4	123	4.9	11.5	0.0241	10.7	0.0480	9.1	0.1181	8.3	0.1925	8.5	0.3423
SMAD4	1,2,3	105	4.8	1.2	0.0022	1.0	0.0038	NA	NA	NA	NA	NA	NA
CIC	2,3	89	4.5	NA	NA	0.8	0.0038	NA	NA	NA	NA	NA	NA
MSH2	2,3	92	4.3	NA	NA	1.3	0.0383	NA	NA	NA	NA	0.0	0.1077
BRCA1	1,2,3,4	117	4.3	1.2	0.0055	1.8	0.0925	3.0	0.4460	3.1	0.4956	1.7	0.3722
ERBB3	2,3,4	117	4.3	2.6	0.2718	2.2	0.1871	NA	NA	NA	NA	1.7	0.3722
SMARCA4	2,3	74	4.1	NA	NA	0.8	0.0125	NA	NA	NA	NA	NA	NA
CASP8	2,3,4	112	3.6	1.1	0.0206	1.3	0.0807	2.1	0.3111	2.1	0.3212	NA	NA
CDH1	1,2,3,4	118	3.4	11.4	0.0069	16.8	0.0002	13.3	0.0019	13.1	0.0024	1.7	0.5217
TP53	1,2,3,4	125	3.2	20.6	<0.0001	20.8	<0.0001	34.7	<0.0001	33.8	<0.0001	8.5	0.1211
MEN1	1,2,3	102	2.9	1.1	0.1036	0.8	0.0609	NA	NA	NA	NA	NA	NA
PMS2	2,3	120	2.5	NA	NA	0.7	0.0706	NA	NA	NA	NA	0.0	0.2219
RB1	1,2,3,4	44	2.3	1.7	0.7620	1.5	0.6791	2.8	0.8436	2.7	0.8731	0.0	0.2440
AXIN1	2,3	91	2.2	NA	NA	0.5	0.0749	NA	NA	NA	NA	NA	NA
EP300	3,4	92	2.2	2.6	0.8134	1.0	0.3171	2.2	1.0000	NA	NA	NA	NA
GNAS	2,3	96	2.1	NA	NA	1.2	0.4741	NA	NA	NA	NA	NA	NA
PTEN	1,2,3,4	96	2.1	4.0	0.3490	5.3	0.1761	6.1	0.1081	5.8	0.1302	1.7	0.8615
CHEK2	1,3	53	1.9	0.8	0.3888	0.8	0.4139	NA	NA	NA	NA	0.0	0.2897

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TBL1XR1	1,3	115	1.7	0.7	0.2310	1.7	1.0000	NA	NA	NA	NA	NA	NA
MLH1	2,3	117	1.7	NA	NA	1.0	0.5096	NA	NA	NA	NA	1.7	1.0000
FBXW7	2,3,4	118	1.7	0.9	0.3920	0.8	0.3567	1.9	0.8799	1.8	0.9391	NA	NA
KRAS	1,2,3,4	118	1.7	0.5	0.0942	0.8	0.3567	0.8	0.3273	0.9	0.4172	0.0	0.3152
ASXL1	2,3	121	1.7	2.2	0.7147	0.2	0.0249	NA	NA	NA	NA	NA	NA
TBX3	1,2,3,4	70	1.4	5.8	0.1174	4.0	0.2776	3.6	0.3303	3.1	0.4217	1.7	0.8907
BAP1	1,3	76	1.3	1.0	0.7994	0.7	0.5726	NA	NA	NA	NA	NA	NA
ERBB2	1,2,3	91	1.1	2.6	0.3739	2.2	0.4910	NA	NA	2.6	0.2815	1.7	0.7553
CTNNB1	3,4	104	1.0	NA	NA	0.3	0.2988	0.7	0.7324	NA	NA	0.0	0.4424
RUNX1	1,2,3,4	112	0.9	35	0.1378	4.7	0.0629	4.8	0.0567	5.0	0.0501	0.0	0.4662
FGFR2	2,3	114	0.9	NA	NA	1.3	0.7233	NA	NA	NA	NA	3.4	0.2362
PIK3R1	1,2,3,4	117	0.9	2.3	0.3187	1.2	0.7809	3.5	0.1324	2.7	0.2419	0.0	0.4661
CDKN1B	1,2,3,4	117	0.9	1.3	0.7103	1.7	0.5245	1.4	0.6575	1.2	0.7777	1.7	0.6420
NRAS	2,3	127	0.8	0.1	0.0563	0.2	0.2655	NA	NA	NA	NA	0.0	0.4920
CCND1	3,4	116	0.0	NA	NA	0.2	0.6300	0.3	0.5549	NA	NA	NA	NA
CDKN2A	1,2,3	119	0.0	0.6	0.3970	0.3	0.5500	NA	NA	NA	NA	1.7	0.1549
ESR1	2,3	85	0.0	NA	NA	0.8	0.4083	NA	NA	NA	NA	0.0	1.0000
MAP2K4	1,2,3,4	91	0.0	3.9		4.7	0.0348	4.4	0.0412	4.0	0.0522	1.7	0.1549
NF2	2,3	102	0.0	0.7	0.3967	0.3	0.5799	NA	NA	NA	NA	0.0	1.0000
PPP2R1A	3,4	115	0.0	NA	NA	0.2	0.1353	0.8	0.3360	NA	NA	NA	NA
RHOA	2,3	118	0.0	NA	NA	0.8	0.3300	NA	NA	NA	NA	NA	NA
STK11	2,3	55	0.0	0.8	0.5056	0.0	1.0000	NA	NA	NA	NA	NA	NA
HRAS	2,3	Insufficient coverage		0.2	–	0.0	–	NA	NA	NA	NA	0.0	–
ARID5B	3,4	Not targeted		1.3	–	0.3	–	1.0	–	NA	NA	NA	NA
B2M	3,4	Not targeted		NA	NA	0.3	–	0.3	–	NA	NA	NA	NA
BCOR	2,3	Not targeted		NA	NA	0.8	–	NA	NA	NA	NA	NA	NA
CBFB	1,2,3	Not targeted		5.7	–	3.3	–	NA	NA	2.3	–	0.0	–
CHD4	3,4	Not targeted		NA	NA	3.0	–	3.3	–	2.6	–	1.7	–
CTCF	1,2,3,4	Not targeted		2.7	–	2.7	–	2.6	–	2.6	–	1.7	–
DNMT3A	2,3	Not targeted		NA	NA	0.8	–	NA	NA	NA	NA	NA	NA
FOXA1	2,3	Not targeted		NA	NA	4.0	–	NA	NA	3.5	–	1.7	–
FOXP1	1,2	Not targeted		0.9	–	1.0	–	NA	NA	NA	NA	NA	NA
GPS2	1,3	Not targeted		1.3	–	1.3	–	NA	NA	1.0	–	NA	NA
LATS1	3,4	Not targeted		NA	NA	0.7	–	1.1	–	NA	NA	NA	NA
MLLT4	1,2	Not targeted		2.6	–	1.8	–	NA	NA	NA	NA	NA	NA
NIPBL	3,4	Not targeted		NA	NA	1.7	–	3.0	–	NA	NA	NA	NA
PHF6	2,3	Not targeted		NA	NA	0.5	–	NA	NA	NA	NA	NA	NA
RNF43	3,4	Not targeted		NA	NA	0.8	–	1.0	–	NA	NA	NA	NA
RPL22	3,4	Not targeted		NA	NA	0.5	–	0.4	–	NA	NA	NA	NA
SF3B1	1,2,3	Not targeted		3.5	–	2.3	–	NA	NA	2.1	–	NA	NA
SPOP	3,4	Not targeted		NA	NA	0.5	–	0.9	–	NA	NA	NA	NA
TET2	2,3	Not targeted		NA	NA	0.8	–	NA	NA	NA	NA	NA	NA
ZFHX3	3,4	Not targeted		NA	NA	2.3	–	NA	NA	NA	NA	5,1%	–
ZFP36L1	1,2,3	Not targeted		1.0	–	1.2	–	NA	NA	NA	NA	NA	NA
PIK3CA	1,2,3,4	108	36.1	44.5	0.0876	39.8	0.4688	34.7	0.7720	34.4	0.7280	20.3	0.0346

Studies: 1 = METABRIC; 2 = Nik-Zainal *et al.* 2016; 3 = Bailey *et al.* 2018; 4 = Berger *et al.* 2018 PAN/GYN.
[a]METABRIC: Nature 2012 + Nature 2016; data accessed through cBioPortal dd March 15, 2019; [b]TCGA: Cell 2015; data accessed through cBioPortal dd March 15, 2019.
NA, not available.

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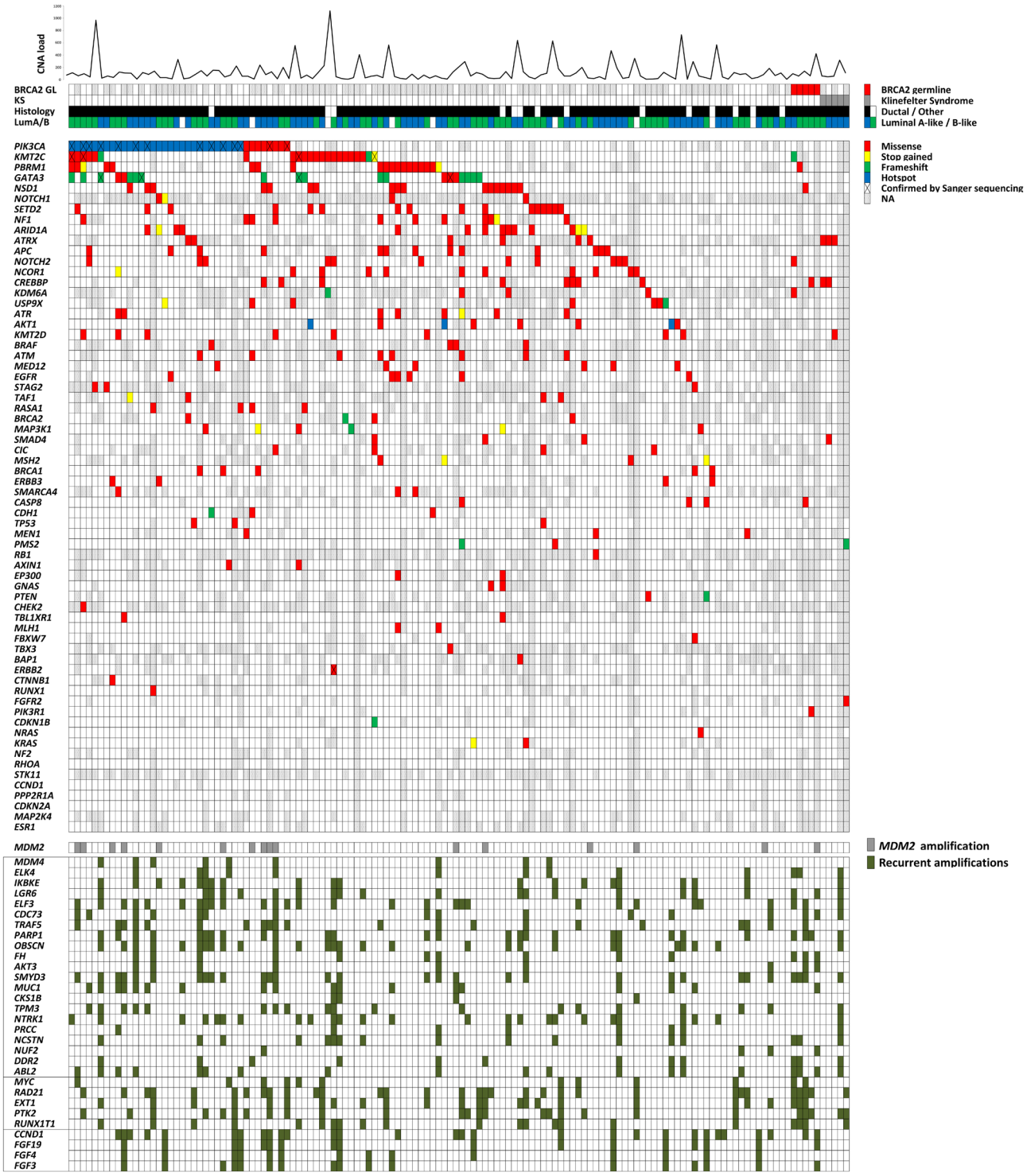

Figure 1
Somatic non-synonymous mutations and recurrent amplifications in 135 male breast cancers. Individual genes are represented as rows, and individual patients are represented as columns. Patients with pathogenic germline *BRCA2* mutations or Klinefelter syndrome are indicated, as well as tumor histology and the distribution of surrogate IHC subtype.

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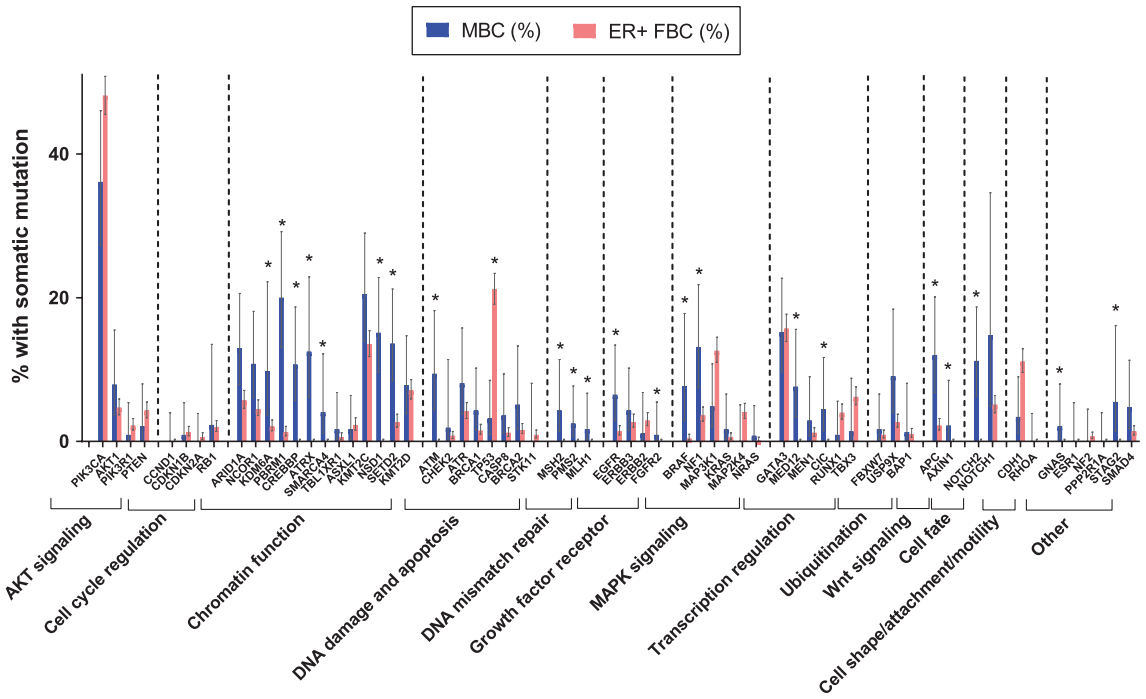

Figure 2
Comparison of MBC somatic mutation frequencies to METABRIC and TCGA ER-positive FBC. Genes have been sorted per function and 95% CI (http://vassarstats.net/prop1.html) have been indicated. * indicates a significant difference with METABRIC and/or TCGA.

whereas KS patients seemed to be subgrouped as a result of their CNA-naïve profile with chromosome X gains. In addition, a cluster of 17 mainly luminal A-like MBCs with high genomic instability was apparent. No correlation was observed with clinicopathological variables or survival when compared to the other MBCs but somatic mutation load tended to be slightly higher (P=0.075).

Amplifications

Analysis of CNAs revealed that MBCs harbored recurrent amplifications on 8p/8q, 16p, 20q and 1q. Supplementary Table 6 summarizes the associations of a set of 220 genes amplified in at least 10% of MBCs with intrinsic subtype, clinicopathological characteristics, SNV load and *PIK3CA* mutation status. Some of the most recurrently amplified genes in both cohorts (FF and FFPE) included *UBR5* (24%; 8q), *TSC2* (22%; 16p), *ANK1* (22%; 8p), *OBSCN* (21%; 1q) and *WHSC1L1* (21%; 8p). A comparison between amplification frequencies in MBC and smFBC is presented in the same table. Merely 86/220 (39%) frequently amplified genes in MBC were also amplified in more than 10% of ER-positive FBC. PANTHER reactome pathway analysis of these 220 genes demonstrated an overrepresentation of genes involved in extracellular matrix organization (three-fold enrichment; P=0.0004), chromatin organization (two-fold enrichment; P=0.0012) and STAT3 signaling (six-fold enrichment; P=0.046), whereas cell cycle-related genes were relatively underrepresented (0.4-fold enrichment; P=0.0012) (Mi *et al.* 2013). Based on The Drug Gene Interaction Database, 19% of these genes (41/220) are clinically actionable and for 26% (57/220), drug interactions have been described (Supplementary Table 6).

Individually, 7/220 genes were indicative of poor 5-year OS when amplified: *MDM2*, *PAK1*, *SCYL3*, *TGFB2*, *CLTC*, *SMYD3* and *ASH1L* (Supplementary Table 6). In multivariable analysis including clinical and pathological characteristics, the amplification status of *PAK1*, *SCYL3*, *MDM2*, *ASH1L*, *E2F7*, *CLTC* or *TGFB2* remained in the model. In multivariable analysis including all seven genes, *PAK1* (HR 3.65; P=0.011), *TGFB2* (HR 3.14; P=0.011) and *E2F7* (HR 2.98; P=0.017) remained. When all seven gene amplifications as well as *PIK3CA* mutations were included, only *ASH1L* (HR 2.44; P=0.057) and lymph node status (HR 2.44; P=0.087) remained in the model as independent predictors of survival (Supplementary Table 7).

Of particular interest was *MDM2*, amplified in as much as 13% of the cohort, and predictor of worse 5-year OS (P=0.007; Fig. 5) even after correction for other established prognostic variables (P=0.024; Supplementary Table 7). *MDM2* amplifications were more frequent in PR

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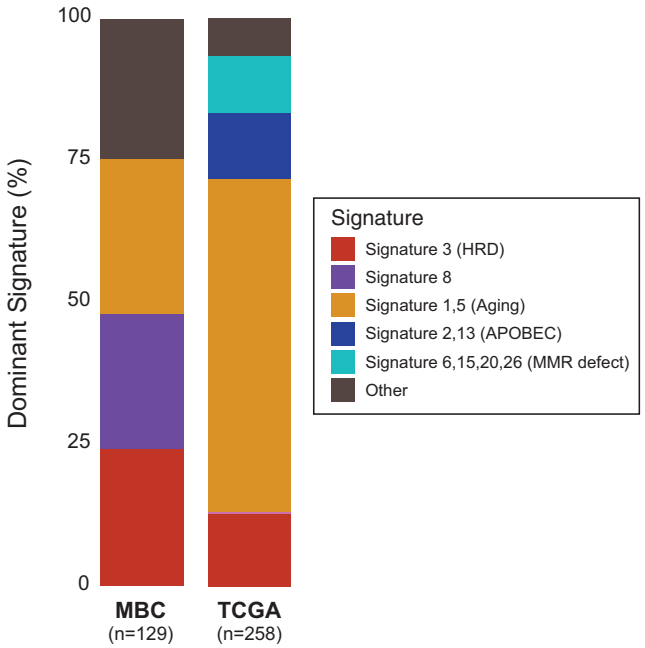

Figure 3
Mutational signature analysis in MBCs compared to ER/HER2- matched FBCs (TCGA). HRD, homologous recombination defect; MMR, mismatch repair

negative (P=0.004) and poorly differentiated (P=0.040) tumors (Supplementary Fig. 5). Co-amplifications with *COL1A1* (P=0.007), *HER2* (P=0.009), *DNAH11* (P=0.027), *PIK3C2B* (P=0.027) and *PPM1D* (P=0.043) were frequent. Tumors harboring *PIK3CA* mutations (P=0.005) or *NF1* mutations (P=0.026) were more likely to harbor *MDM2* amplifications (Fig. 1 and Supplementary Fig. 5). *MDM2* amplifications were significantly correlated with MDM2 protein overexpression (P=0.001), but protein overexpression did not predict poor outcome (P=0.337).

Germline mutations

With on average 1723 reads per normal tissue sample, we detected pathogenic *BRCA2* germline mutations in 5/44 (11%) matched normal samples using the IonTorrent Oncomine BRCA FFPE gene panel (library preparation failed for one sample; Supplementary Table 5). There was no indication for loss of heterozygosity of the WT *BRCA2* allele based on variant allele frequencies. No pathogenic *BRCA1* germline mutations were identified. Tumors from *BRCA2* germline mutation carriers harbored a similar amount of somatic mutations and total CNAs compared to other MBCs. *BRCA2*-related tumors appear to have increased CNA counts on chromosome 8q (P=0.056) accompanied by decreased counts on 8p (P=0.004). All five *BRCA2*-carriers demonstrated *UBR5* (8q; P=0.002), *ASAP1* (8q; P=0.001), *CSMD3* (8q; P=0.001) and *CCNE2* (8q; P=0.0002) amplification, but *DNAH11* (7p; P=0.003), *RRM2B* (8q; P=0.007), *FZD6* (8q; P=0.010), *RUNX1T1* (8q; P=0.023) and *SGK3* (8q; P=0.023) amplifications were also more frequently seen. We observed no enrichment for particular somatic mutations. None of the *BRCA2* germline mutation carriers harbored a *PIK3CA* or *GATA3* mutation (Fig. 1). Within *AR*, *CHEK2* and *PALB2* (*FANCM* was not targeted), we found no likely pathogenic germline mutations. Germline mutations in these genes predicted to have medium or high impact are described in the Supplementary results section.

Discussion

Compared to FBC, MBC has been relatively poorly characterized on the molecular level. Here, we have characterized a cohort of 135 MBC, 46% of which were deemed luminal B-like tumors. This is consistent with a recent pathology review of the EORTC 10085/TBCRC/BIG/NABCG International Male Breast Cancer Program where 49.3% of 1328 MBC were luminal B-like tumors (Doebar *et al.* 2017). In our MBC series, 46% of patients received radiotherapy, 16% chemotherapy and 42% endocrine therapy. Compared to the EORTC cohort, fewer men received chemotherapy (EORTC 27.7%; 220/794 patients with available data) and endocrine therapy (EORTC 82.6%; 583/706 patients with available data). These discrepancies are likely mainly due to uncertainties in treatment decision making. Studies investigating MBCs at the molecular level and correlating their findings with survival are therefore of the utmost importance.

TP53, *CDH1*, *MAP3K1* and to a lesser extent *PIK3CA*, *TBX3*, *MAP2K4*, *RUNX1* and *PTEN* somatic mutations appear to be less frequent in MBC compared to smFBC, whereas *PBRM1*, *NSD1* and *SETD2* somatic mutations seem to be more frequent.

After correction for multiple comparisons, there were no significant differences between our study and the MBC study of Piscuoglio *et al.* Similar to their MBC study, somatic mutations in *TP53* were very rare (Piscuoglio *et al.* 2016). Their *TP53* mutation frequency was however higher (8.5%) than ours (3%), possibly due to a different cohort composition. In FBC, luminal B-like tumors show a higher *TP53* mutation frequency than luminal A-like tumors and the MBC set of Piscuoglio *et al.* was likely enriched for luminal B-like cancers (71% of their cohort) (Cancer Genome Atlas Network 2012, Piscuoglio *et al.* 2016). In addition, a study using

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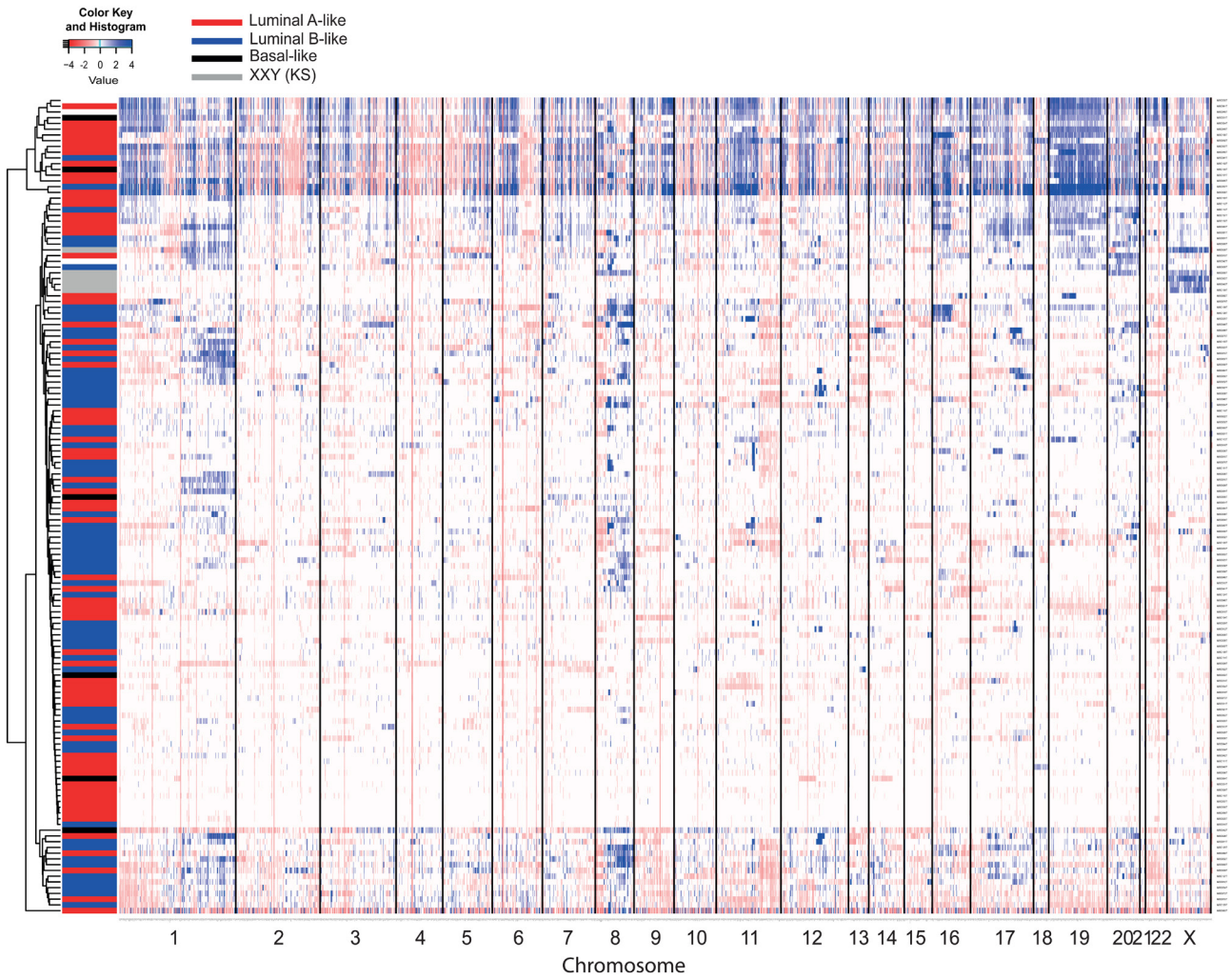

Figure 4
Heatmap of copy number alterations in the male breast cancers following unsupervised hierarchical cluster analysis. Two clusters seem to stand out: the top cluster with many events dominated by luminal A-like tumors and the bottom cluster dominated by luminal B-like cancers. Klinefelter syndrome patients (gray) show few copy number alterations.

a highly similar predecessor 'Cancer mini-genome' gene panel and approach in triple-negative FBC identified *TP53* mutations in 55% of the specimens, thereby excluding technical bias (Lips *et al.* 2015). In contrast to *TP53*, the pattern of somatic mutations found in *PIK3CA* resembled that of FBC. Its somatic mutation frequency in our MBC cohort (36%) was in between that reported by Piscuoglio *et al.* (MBC; 20%) and ER-positive FBC (n=1431; 48%). *PIK3CA* mutations were associated with more aggressive tumor characteristics and reduced survival, in line with findings in ER-positive FBC subgroups (Pereira *et al.* 2016, Ishida *et al.* 2018).

Aside from *PIK3CA*, the serine/threonine protein kinase *AKT1* was the only other mutated gene with hotspots corresponding to FBC. The majority of *AKT1* missense mutations were E17K (4/9) or L52R (2/9) substitutions. Both mutations are known to be oncogenic and there is promising clinical data in FBC patients with *AKT1* E17K mutant ER+ ductal breast cancer treated with the pan-AKT targeted inhibitor AZD5363 (level 3A evidence; oncoKB) (Davies *et al.* 2012, 2015, Addie *et al.* 2013, Hyman *et al.* 2017).

As expected from FBC, most *GATA3* mutations were likely oncogenic frameshift mutations including K358fs, H435fs, A332fs, R330fs and M294K/R (Cerami *et al.* 2012, Ellis *et al.* 2012, Gao *et al.* 2013). In contrast to Piscuoglio *et al.*, *GATA3* somatic mutations were not exclusive to luminal B-like MBC tumors (Piscuoglio *et al.* 2016). Overall, genes regulating chromatin function appear more often mutated in MBC, and mutational

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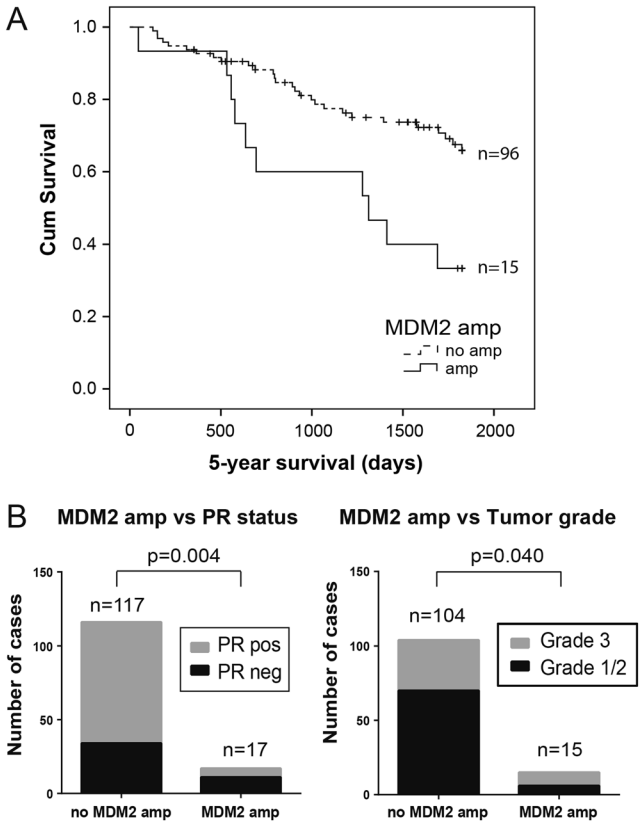

Figure 5
MDM2 amplification in male breast cancer is associated with poor 5-year overall survival, PR negativity and high tumor grade.

signatures 3 and 8, both associated with homologous recombination deficiency, are more prevalent than in ER/HER2-matched FBC. Of particular clinical importance, homologous recombination deficiency may point to a selective sensitivity to PARP (poly-ADP ribose polymerase) inhibitors (Nik-Zainal & Morganella 2017).

Like FBC, MBC is CNA driven, and despite many similarities in the CNA landscape between MBC and FBC (Curtis *et al.* 2012, Piscuoglio *et al.* 2016), only 40% of frequently amplified genes in MBC were also frequently amplified in FBC, underlining differences in biology and genetics. *MDM2* amplifications were observed in 13% of the MBC cohort while in FBC, these amplifications occur in merely 3.7% of cases. Since *MDM2* mediates ubiquitination of p53, its increased amplification frequency may be related to the decreased *TP53* mutation frequency observed in MBC, the two events being mutually exclusive in the same pathway. Of note, besides *MDM2*, many of the aberrant genes in MBC seem to be involved in the inactivation of the p53 pathway (e.g. amplification of *PAK1* and *SMYD2*, and inactivating mutations in *ARID1A*, *PBRM1* and *KMT2C*) (Huang *et al.* 2006, Wu *et al.* 2014, Woo *et al.* 2017). *MDM2* amplifications predicted more aggressive tumor behavior and correlated with protein overexpression. The high rate of MDM2 overexpression in prostate and ER-positive FBC, and the ability of MDM2 inhibitors to ubiquitinate steroid hormone receptors, has led to the evaluation of this class of drugs in combination with endocrine therapies (CRUKE/12/032). Many MDM2 small-molecule inhibitors have only recently progressed from preclinical development into early clinical trials (such as RG7112, also known as RO5045337, and AMG-232) (Burgess *et al.* 2016). Thus, MDM2 is a putative therapeutic target in MBC.

Four genes independently predicted poor OS in MBC when amplified: *PAK1* (11q13.5-q14.1), *ASH1L* (1q22), *E2F7* (12q21.2) and *TGFB2* (1q41). *PAK1* (p21 activated kinase 1) amplifications were present at a similar frequency compared to ER-positive FBC (12 vs 10%) (Ong *et al.* 2011, Curtis *et al.* 2012, Ye & Field 2012, Radu *et al.* 2014) and amplification and overexpression of *PAK1* is associated with poor outcome in luminal FBC as well. *In vitro* testing of a small-molecule inhibitor, FRAX1036, in combination with docetaxel, further supported PAK1 as a potential target in breast cancer (Ong *et al.* 2015). *PAK1* has also been implicated in tamoxifen resistance (Holm *et al.* 2006, Bostner *et al.* 2007, 2010), and in upregulating genes involved in the Fanconi anemia (FA)/BRCA pathway, paving the way for combined PAK1 and PARP inhibition in FA/BRCA-proficient cancers (Villamar Cruz *et al.* 2016). *ASH1L*, encoding a histone methyltransferase (HMT), showed a similar amplification frequency in MBC compared to ER-positive FBC (18 vs 21%). This HMT is classified as dysregulated by genetic alterations and as candidate therapeutic target in FBC (Liu *et al.* 2015). ASH1L inhibitors are currently being developed (Rogawski *et al.* 2015). Atypical E2F transcription factor 7 (*E2F7*) amplification appears to be more frequent in MBC than ER-positive FBC (13 vs 1.4%). E2F7 participates in various processes such as angiogenesis, polyploidization and DNA damage response. E2F7 overexpression leads to tamoxifen resistance in breast cancer cells (Chu *et al.* 2015) and anthracycline resistance in squamous cell carcinoma (Hazar-Rethinam *et al.* 2015). A small-molecule pan-E2F inhibitor (HLM006474) has been developed and was proven to be effective in a melanoma and lung cancer cell culture model (Ma *et al.* 2008, Kurtyka *et al.* 2014). *TGFB2* encodes a secreted ligand of the transforming growth factor-beta superfamily and its mRNA levels predict tamoxifen response in breast cancer cells (Buck *et al.* 2008). Neutralizing antibodies to TGFB2 have been shown to reverse tamoxifen resistance of human

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breast carcinomas *in vivo* (Arteaga *et al.* 1999). Several pharmacological strategies have been developed to block TGF-beta signaling (Colak & Ten Dijke 2017). Overall, 19% of the most frequently amplified genes in MBC are clinically actionable and for 26%, drug interactions have been described, suggesting many potential druggable targets.

We detected pathogenic *BRCA2* germline mutations in 11% of the paired tumor/normal subcohort (5/44). Other larger studies have described similar frequencies (8–14%) (Couch *et al.* 1996, Basham *et al.* 2002, Pritzlaff *et al.* 2017). In contrast to Silvestri *et al.* who reported a median age at diagnosis for male *BRCA2* mutation carriers of 62 years and an inverse relationship between age at diagnosis and tumor grade in 375 male *BRCA2* mutation carriers, our *BRCA2* cohort consisted of older patients with poorly differentiated tumors (Silvestri *et al.* 2016). In our study, tumors from *BRCA2* germline mutation carriers harbored a similar amount of somatic mutations and total CNAs compared to other MBCs, but appeared to have increased CNA counts on 8q and decreased counts on 8p. In light of the small *BRCA2* mutation carrier cohort reported here, results based on this subgroup should however be interpreted with caution.

KS patients tended to be younger at presentation, with generally well-differentiated tumors with few CNAs. The increased risk of developing breast cancer for these KS patients can be attributed to a direct effect of the supernumerary X chromosome(s) or the combined action of abnormal chromosome dosage and hormonal imbalances (Kawakami *et al.* 2004, Bonomi *et al.* 2017).

It should be noted that this study used FFPE as well as fresh-frozen material. It is well recognized that the quality of FFPE samples due to fixation and tissue processing is inferior to that of frozen material. DNA extracted from FFPE tissues is fragmented which can lead to sequence artifacts (Do & Dobrovic 2015). Sequence artifacts can be difficult to distinguish from true mutations, especially in the context of tumor heterogeneity and are an increasing interpretive problem in this era of massively parallel sequencing. We therefore included as much fresh-frozen material as possible. Nevertheless, in light of the rarity of MBC, we have included archived FFPE material as well. This might have led to an overestimation of mutational load (Yost *et al.* 2012).

In conclusion, this comprehensive molecular analysis of MBC suggests that, while MBCs show remarkable similarities to FBC, clear differences are also found, subscribing the amounting evidence that MBC should be seen as an entity of its own. This should also be reflected in the clinical approach that should refrain from merely imitating FBC management. As we have observed some genetic alterations to be clearly more prevalent in MBC, for example *MDM2* amplifications and homologous recombination-deficient mutational signatures, focus should be on associated relevant drugs. As such, honoring MBC for its specific molecular portrait may result in improvements in outcome that parallel those we have observed over the last years in FBC.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/ERC-19-0278.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Ethics approval and consent to participate

The Dutch national ethical guidelines (www.federa.org) state that no ethical approval is required for the use of anonymous leftover tissue, and this is also part of the standard treatment agreement (Van Diest 2002).

Availability of data and materials

All sequencing data have been deposited in EGA under accession number EGAS00001002683. All data generated or analyzed during this study are included in this published article and its Supplementary information files.

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