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Targeted sequencing of *BRCA1* and *BRCA2* across a large unselected breast cancer cohort suggests that one-third of mutations are somatic

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Background: A mutation found in the *BRCA1* or *BRCA2* gene of a breast tumor could be either germline or somatically acquired. The prevalence of somatic *BRCA1/2* mutations and the ratio between somatic and germline *BRCA1/2* mutations in unselected breast cancer patients are currently unclear.

Patients and methods: Paired normal and tumor DNA was analyzed for *BRCA1/2* mutations by massively parallel sequencing in an unselected cohort of 273 breast cancer patients from south Sweden.

Results: Deleterious germline mutations in *BRCA1* ($n = 10$) or *BRCA2* ($n = 10$) were detected in 20 patients (7%). Deleterious somatic mutations in *BRCA1* ($n = 4$) or *BRCA2* ($n = 5$) were detected in 9 patients (3%). Accordingly, about 1 in 9 breast carcinomas (11%) in our cohort harbor a *BRCA1/2* mutation. For each gene, the tumor phenotypes were very similar regardless of the mutation being germline or somatically acquired, whereas the tumor phenotypes differed significantly between wild-type and mutated cases. For age at diagnosis, the patients with somatic *BRCA1/2* mutations resembled the wild-type patients (median age at diagnosis, germline *BRCA1*: 41.5 years; germline *BRCA2*: 49.5 years; somatic *BRCA1/2*: 65 years; wild-type *BRCA1/2*: 62.5 years).

Conclusions: In a population without strong germline founder mutations, the likelihood of a *BRCA1/2* mutation found in a breast carcinoma being somatic was ~1/3 and germline 2/3. This may have implications for treatment and genetic counseling.

Key words: breast cancer, mutation, somatic, germline, carrier, prevalence

introduction

The tumor suppressor genes *BRCA1* and *BRCA2* have a critical role in the repair of DNA. Inactivation of either of these genes fundamentally influences cancer risk and development [1–3]. Alleles can be inactivated by several mechanisms including germline mutation, somatic mutation, and epigenetic downregulation. The prevalence of somatic *BRCA1* and *BRCA2* mutations in breast cancer is currently unclear, as studies have either been small in size or have focused only on a selected group of patients [4–13]. Somatic mutation status is important to know since not only germline but also somatic mutations are believed to be treatment predictive for response to poly(ADP-ribose) polymerase (PARP) inhibitors and platinum agents [14]. For relapsed ovarian cancer, the PARP inhibitor olaparib has recently been approved in Europe for use in patients with *BRCA1/2* mutations—regardless of the mutations being germline or

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somatic [15, 16]. Ongoing trials will determine whether this will be the case also for breast cancer patients [17].

Importantly, the ratio between somatic and germline mutations has implications for pretest genetic counseling of breast cancer patients and for settings where only tumor specimens, not necessarily the matched germline DNA, are analyzed. Additionally, cataloguing somatic *BRCA1* and *BRCA2* alterations could aid in the interpretation of germline variants of unknown significance [18].

For the present study, we have analyzed an unselected cohort of 273 primary breast cancer patients treated in south Sweden at the Skåne University Hospital in Malmö. The aims were to determine the prevalence of germline and somatic *BRCA1/2* mutations, to determine the ratio between somatic and germline *BRCA1/2* mutations, and to describe clinicopathological and molecular characteristics of the tumors. Here, we use the term 'mutation' to describe a deleterious sequence variant, and the term 'carrier' to describe an individual with a germline mutation.

materials and methods

patient cohort and samples

Patients with preoperative diagnosis of invasive breast cancer scheduled for surgery in Malmö, Sweden, during the years 2007–2009, were asked to participate in the population-based All Breast Cancer in Malmö (ABiM) study. Approximately 80% of all breast cancer patients in Malmö during this period were included in the ABiM study (Figure 1A). For consenting patients, fresh-frozen tumor tissue was obtained for molecular analyses, and blood samples were taken before surgery and biobanked within 2 h similar as previously described [19]. Tumor DNA/RNA and buffy coat normal DNA were isolated as previously described [19, 20]. No research tissue was taken unless it was certain not to influence the quality of diagnostic procedures. As a consequence, as well due to the quantity requirements of 10 μg tumor and 3 μg normal DNA, 276 patients were analyzed of which 3 were excluded after quality control of the sequencing data. The remaining 273 patients constitute our study population. Comparisons between the study population and the patients from the ABiM cohort that were not included in the present study population are presented in supplementary Table S1, available at *Annals of Oncology* online. Compared with the ABiM patients not analyzed here, patients included in the study population differed significantly with respect to tumor size, grade, Ki-67, and St Gallen subtype, but were similar with regard to all other clinicopathological parameters (supplementary Table S1, available at *Annals of Oncology* online).

targeted sequencing and variant calling

For paired normal and tumor DNA, the coding exons plus 14 bp of each intron boundary of the *BRCA1* and *BRCA2* tumor suppressor genes were target captured (Agilent SureSelect; supplementary Table S2, available at *Annals of Oncology* online) and sequenced to a median coverage of 603× (supplementary Table S3, available at *Annals of Oncology* online) on Illumina HiSeq 2000 instruments with paired-end 101 bp reads. After alignment, identity and match between tumor and normal samples were confirmed by single nucleotide polymorphism analysis. We used VarScan [21] to call single-nucleotide variants (SNVs) and indels in *BRCA1* and *BRCA2* (see supplementary Methods, available at *Annals of Oncology* online). Variants were classified as somatic if they were present in the tumor sample only, as germline if they were present in both the tumor and the normal sample, and were removed if they were present only in the normal sample.

assessment of the deleteriousness of *BRCA1* and *BRCA2* variants

Variants located in introns (excluding splice sites) and synonymous SNVs were excluded. All variants (SNVs or indels) that resulted in a frameshift or a loss or gain of a stop codon were considered deleterious, except variants in the last exon of *BRCA2*. We considered SNVs that resulted in the change of one amino acid as deleterious if they were annotated as class 5 (pathogenic) in the Breast Cancer Information Core (BIC) [22], or pathogenic in ClinVar [23], or if Align-GVGD (http://agvgd.iarc.fr) predicted a class of C65 (deleterious).

gene expression profiling and intrinsic breast cancer subtype

Tumors were subtyped according to St Gallen criteria as well as by PAM50 gene expression subtyping (supplementary Methods, available at *Annals of Oncology* online) based on RNA sequencing [19].

survival analysis

For overall survival (OS), vital status was checked in the Swedish Census Register. For recurrence-free survival (RFS), recurrence information was obtained from the clinical cancer database INCA. Events were death of any cause for OS, and local or distant recurrence for RFS. Survival analysis was done using the Kaplan–Meier method and the log-rank test (two-tailed).

results

germline mutations in *BRCA1* and *BRCA2*

An unselected cohort of 273 breast cancer patients constituted our study population (Figure 1A and supplementary Table S1, available at *Annals of Oncology* online). The analysis of targeted sequencing data for *BRCA1/2* genes revealed germline mutations in 20 patients (7%): 10 *BRCA1* mutation carriers and 10 *BRCA2* mutation carriers (Table 1 and Figure 1B). Seventeen (85%) of the mutations were caused by substitution, deletion, or insertion of a single nucleotide. One mutation was a heterozygous deletion of exons 1–17, and the other two were deletions of two and five nucleotides resulting in a frameshift.

BRCA1/2 *carriers versus noncarriers.* Comparing the 20 *BRCA1/2* germline mutation carriers with the 253 noncarriers (supplementary Table S4, available at *Annals of Oncology* online), as expected we found that carriers were significantly younger at time of diagnosis (median age 45 versus 63 years; $P < 0.001$), that carriers more often had present or past contralateral breast cancer than noncarriers (20% versus 3%, $P < 0.01$), and that tumors of carriers were more often Nottingham grade 3 ($P = 0.04$) and basal subtype (St Gallen 30% versus 13%, $P = 0.04$; PAM50 30% versus 12%, $P = 0.03$), and less often of the St Gallen luminal A subtype (25% versus 53%, $P = 0.02$). However, the median tumor size in both groups was identical (20 mm).

germline mutations in BRCA1 *versus* BRCA2. When comparing tumors from the 10 *BRCA1* germline mutation carriers with those from the 10 *BRCA2* germline mutation carriers, we found remarkable similarities in tumor size, lymph node status, and grade (supplementary Table S5, available at *Annals of Oncology* online). The median age at diagnosis was 41.5 years for *BRCA1* carriers and 49.5 years for *BRCA2* carriers.

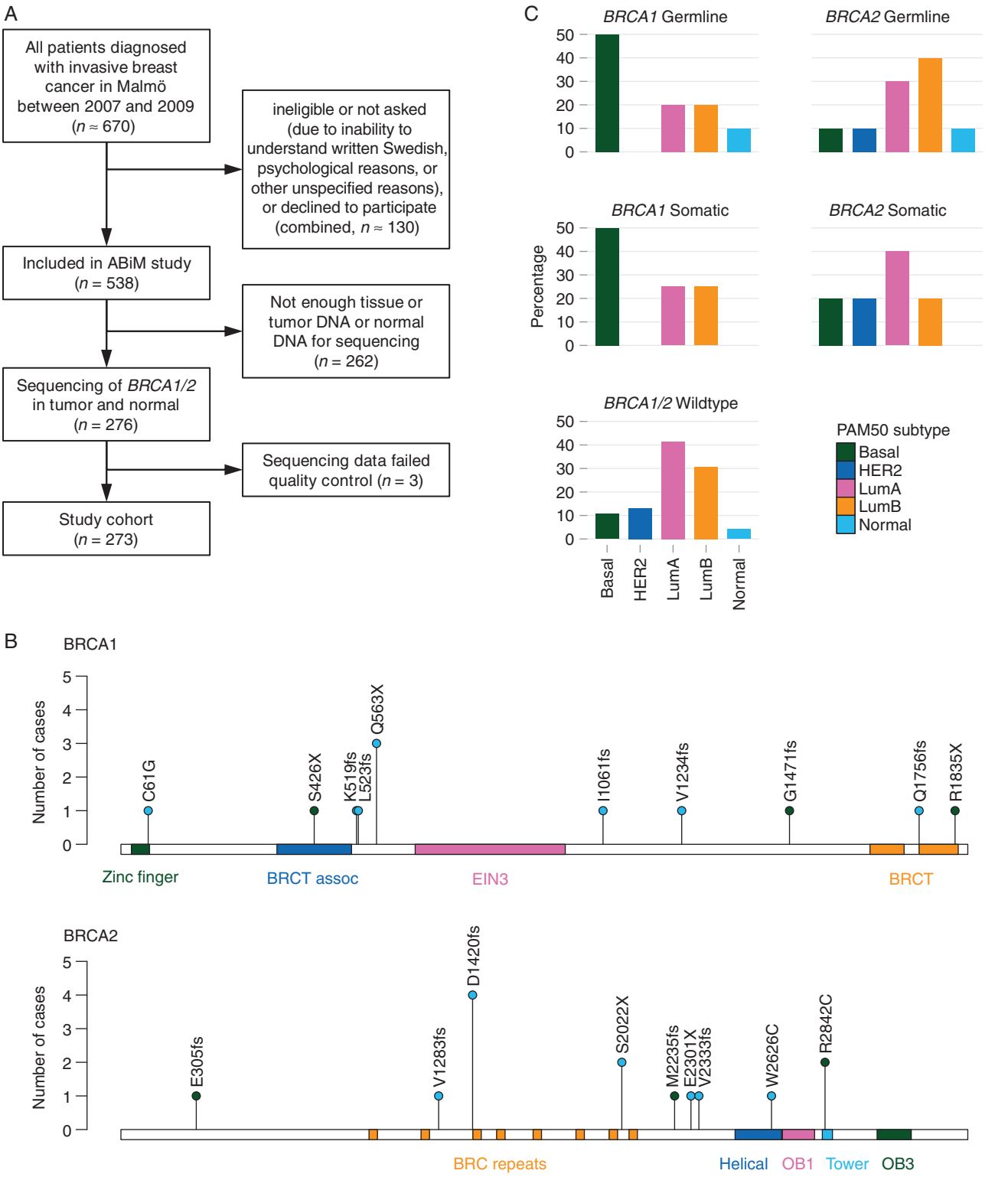

Figure 1. (A) Study flowchart. Approximately 80% of all patients diagnosed with invasive breast cancer in Malmö between 2007 and 2009 were included in the All Breast Cancer in Malmö (ABiM) study. Patients not included were either not asked, ineligible, could not be consented due to language difficulty, or declined to participate. As a result, 538 patients were included in the ABiM study during that period. With the limitation of tumor and normal DNA of sufficient sequencing quality, we were able to study *BRCA1* and *BRCA2* mutations in 273 patients. (B) *BRCA1* and *BRCA2* mutations by protein position. Single-nucleotide variants and small (≤5 bp) indels mapped to the canonical protein sequence are shown. blue, germline mutations. Dark Green, somatic mutations. Protein domains are shown as colored bars. BRCT, BRCA1 C-terminus; EIN3, ethylene insensitive 3; OB, oligonucleotide/oligosaccharide-binding. (C) Distribution of the PAM50 intrinsic subtype across mutation subgroups. *BRCA1* germline mutant tumors have a similar subtype distribution as *BRCA1* somatic mutants, whereas *BRCA2* germline and somatic mutants are similar to *BRCA1/2* wild-type tumors.

Table 1. All germline and somatic *BRCA1* and *BRCA2* mutations identified in the 273 patient cohort

Patient ID	Age	Gene	Somatic status	Mutation type[a]	Exon	cDNA change	Protein change	Evidence[b]	Tumor size (mm)	Lymph node status[c]	NHG[d]	Ki-67 (%)	St Gallen subtype
P1	<40	*BRCA1*	Germline	Nonsynonymous SNV	5	c.181T>G	p.C61G	A	11–20	Pos	3	>20	Basal
P2	50–59	*BRCA1*	Germline	Frameshift del	11	c.1556delA	p.K519fs	B, F	11–20	Neg	3	>20	Basal
P3	50–59	*BRCA1*	Germline	Frameshift del	11	c.1568delT	p.L523fs	F, N	>20	Pos	3	≤20	LumA
P4	40–49	*BRCA1*	Germline	Stop-gain SNV	11	c.1687C>T	p.Q563X	B, X	>20	Pos	3	>20	LumB HER2+
P5	50–59	*BRCA1*	Germline	Stop-gain SNV	11	c.1687C>T	p.Q563X	B, X	11–20	Neg	3	>20	Basal
P6	60–69	*BRCA1*	Germline	Stop-gain SNV	11	c.1687C>T	p.Q563X	B, X	11–20	Neg	2	≤20	LumA
P7	<40	*BRCA1*	Germline	Frameshift del	11	c.3182delT	p.I1061fs	F, N	>20	Pos	3	>20	LumB HER2−
P8	<40	*BRCA1*	Germline	Frameshift del	11	c.3700_3704del	p.V1234fs	B, F	Unknown	Neg	2	≤20	LumA
P9	40–49	*BRCA1*	Germline	Frameshift ins	20	c.5266dupC	p.Q1756fs	B, F	>20	Unknown	3	>20	LumB HER2+
P10	<40	*BRCA1*	Germline	Heterozygous del	1–17			L	11–20	Pos	3	>20	Basal
P11	≥80	*BRCA2*	Germline	Frameshift del	11	c.3846_3847del	p.V1283fs	B, F	>20	Neg	3	>20	Basal
P12	<40	*BRCA2*	Germline	Frameshift del	11	c.4258delG	p.D1420fs	B, F	11–20	Neg	3	>20	LumB HER2−
P13	40–49	*BRCA2*	Germline	Frameshift del	11	c.4258delG	p.D1420fs	B, F	>20	Pos	3	≤20	LumA
P14	40–49	*BRCA2*	Germline	Frameshift del	11	c.4258delG	p.D1420fs	B, F	11–20	Pos	2	>20	LumB HER2−
P15	60–69	*BRCA2*	Germline	Frameshift del	11	c.4258delG	p.D1420fs	B, F	11–20	Pos	3	>20	LumB HER2−
P16	50–59	*BRCA2*	Germline	Stop-gain SNV	11	c.6065C>G	p.S2022X	B, X	11–20	Pos	2	≤20	LumA
P17	≥80	*BRCA2*	Germline	Stop-gain SNV	11	c.6065C>G	p.S2022X	B, X	11–20	Pos	3	>20	LumB HER2−
P18	60–69	*BRCA2*	Germline	Stop-gain SNV	12	c.6901G>T	p.E2301X	N, X, D	11–20	Neg	3	≤20	Basal
P19	<40	*BRCA2*	Germline	Frameshift ins	13	c.6998dupT	p.V2333fs	F, N	>20	Neg	3	>20	LumB HER2−
P20	40–49	*BRCA2*	Germline	Nonsynonymous SNV	17	c.7878G>C	p.W2626C	A	>20	Pos	3	≤20	Non-lum HER2+
P21	≥80	*BRCA1*	Somatic	Stop-gain del	11	c.1277delC	p.S426X	X	>20	Pos	3	>20	LumB HER2−
P22	70–79	*BRCA1*	Somatic	Frameshift del	14	c.4412delG	p.G1471fs	F	11–20	Neg	3	>20	LumB HER2−
P23	40–49	*BRCA1*	Somatic	Splicing SNV	18	c.5074+1G>T		C, P	11–20	Pos	3	>20	Basal
P24	60–69	*BRCA1*	Somatic	Stop-gain SNV	24	c.5503C>T	p.R1835X	C, S, X	11–20	Neg	3	>20	Basal
P10	<40	*BRCA1*	Somatic	Homozygous del	1–17			L	11–20	Pos	3	>20	Basal
P25	60–69	*BRCA2*	Somatic	Frameshift del	10	c.914_915del	p.E305fs	F	11–20	Neg	3	≤20	LumA
P26	70–79	*BRCA2*	Somatic	Frameshift del	11	c.6705delG	p.M2235fs	F	>20	Unknown	3	>20	Basal
P27	60–69	*BRCA2*	Somatic	Nonsynonymous SNV	20	c.8524C>T	p.R2842C	A, S, D	>20	Neg	2	≤20	LumA
P28	60–69	*BRCA2*	Somatic	Nonsynonymous SNV	20	c.8524C>T	p.R2842C	A, S, D	>20	Pos	2	≤20	LumA
P15	60–69	*BRCA2*	Somatic	Splicing SNV	26	c.9502−1G>A		P	11–20	Unknown	3	>20	LumB HER2−
P29	60–69	*BRCA2*	Somatic	Heterozygous del	All			L	>20	Pos	3	>20	LumB HER2+

In total, 31 mutations were identified in 29 patients, with 2 patients having a germline and a somatic mutation in the same gene.

[a]SNV, single-nucleotide variant; del, deletion; ins, insertion.

[b]Evidence for deleteriousness: A, Align-GVGD; B, BIC; C, ClinVar; D, see Discussion; F, frameshift; L, loss/LOH; N, novel variant; P, affects splice donor or acceptor site; S, COSMIC; X, stop-gain/loss.

[c]Pos, positive (N1–N3); Neg, negative (N0).

[d]NHG, Nottingham histologic grade.

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somatic mutations in *BRCA1* and *BRCA2*

Somatic *BRCA1* mutations were found in four noncarrier patients and one carrier patient, and somatic *BRCA2* mutations were found in five noncarriers and one carrier (Table 1). No patient had somatic mutations in both *BRCA1* and *BRCA2*, and no patient presented with more than one somatic alteration in *BRCA1* or *BRCA2*. Combined, the overall prevalence of patients with only somatic *BRCA1/2* mutations was 3% (9/273), with *BRCA1* and *BRCA2* each contributing approximately half. The median age at diagnosis for patients with somatic *BRCA1/2* mutations was 65 years and comparable to the age of *BRCA1/2* wild-type tumor patients (median 62.5 years; supplementary Tables S4 and S6, available at *Annals of Oncology* online). Nine (82%) of the 11 mutations were caused by substitution or deletion of a single nucleotide, of which 2 affected splice donor or acceptor sites (Table 1). The other two mutations were larger deletions of several exons: one somatic homozygous deletion of *BRCA1* exons 1–17 in a carrier with a germline heterozygous deletion of these exons and one heterozygous deletion of all *BRCA2* exons. Of the 11 somatic mutations, 3 were found in previous studies and listed in the COSMIC database of somatic mutations in cancer [24].

combined germline and somatic mutations in *BRCA1* and *BRCA2*

BRCA1 *and* BRCA2 *mutations occur in equal frequency.* The prevalence of *BRCA1* mutations (regardless of germline or somatic origin) was 5% (14/273; supplementary Table S7, available at *Annals of Oncology* online), and the prevalence of *BRCA2* mutations was 5% (15/273). Combined, the prevalence of *BRCA1/2* mutations was 11% (29/273). The highest prevalence was found in patients younger than 40 years at diagnosis (46%, 6/13), all of whom were germline carriers (supplementary Table S8, available at *Annals of Oncology* online).

germline BRCA1/2 *mutations versus somatic* BRCA1/2 *mutations.* While patient age at diagnosis differed between germline and somatic *BRCA1/2* mutation tumors, the molecular characteristics of the tumors were similar. Tumor size, lymph node status, estrogen receptor status, progesterone receptor status, human epidermal growth factor receptor 2 (HER2) status, and St Gallen/PAM50 subtype had similar distribution in both groups (supplementary Table S4, available at *Annals of Oncology* online). Two of 20 germline mutation carriers had an additional somatic mutation, presumably inactivating both alleles (patients P10 and P15 in Table 1).

intrinsic subtype is associated with mutated gene rather than germline or somatic origin. We compared the PAM50 intrinsic subtypes across the five subgroups of *BRCA1* germline, *BRCA1* somatic only, *BRCA2* germline, *BRCA2* somatic only, and *BRCA1/2* wild-type tumors (Figure 1C). We found that *BRCA1*-mutated tumors (regardless of germline or somatic origin) had a significantly different intrinsic subtype distribution than wild-type tumors ($P = 0.003$), with half of the tumors being of the basal subtype. In contrast, *BRCA2* had a subtype distribution that resembled more the wild-type tumors.

molecular details. The predominant mutation type was deletion of one or several bases that resulted in a frameshift, which occurred in 12/31 mutations. We observed no difference in the distribution of the type of mutation (deletion, insertion, and SNV) between germline and somatic mutations (Table 1). Mutant allele frequencies of germline *BRCA1/2* mutations in normal samples ranged from 38% to 51% (median 47%), consistent with a heterozygous carrier. In the tumor samples, the same mutations generally had an increased mutant allele frequency consistent with loss of the wild-type allele in the tumor (34%–91%, median 68%). For all but two mutations, the mutant allele frequency was higher in the tumor than in the normal sample (supplementary Figure S1, available at *Annals of Oncology* online).

survival analysis

The median follow-up was 6.4 years (range 0.6–7.6 years). Between patients with a *BRCA1/2* mutation and wild-type patients, we found no significant difference in OS (5-year OS 86% in both groups, log-rank $P = 0.81$) or RFS (5-year RFS 84% for mutants, 92% for wild type, log-rank $P = 0.35$, supplementary Figure S2, available at *Annals of Oncology* online). However, in the subgroup of patients who did not receive adjuvant chemotherapy (147 of 273 patients), RFS was significantly inferior for patients with *BRCA1/2* mutations (5-year RFS: 75% versus 92%; $P = 0.049$, supplementary Figure S2, available at *Annals of Oncology* online). The low number of events precluded survival analysis of germline and somatic subgroups.

discussion

In the present study, we report that 3% of the tumors from our cohort of unselected breast cancer patients harbored only somatic mutations in *BRCA1* or *BRCA2* and 7% harbored germline mutations. Accordingly, the likelihood of a mutation found in a breast carcinoma being somatic was 1/3 and germline 2/3, with ratio of 1 : 2. The tumor phenotypes were found to be similar regardless of the mutation being germline or somatically acquired, but germline mutations carriers were much younger at diagnosis (median age at diagnosis, germline *BRCA1* 41.5 years; germline *BRCA2* 49.5 years; somatic *BRCA1/2* 64 years; wild-type *BRCA1/2* 62.5 years), consistent with the Knudson two-hit hypothesis [25].

Although our cohort is derived from a population-based series of breast cancer patients, we have previously shown that small- and low-grade tumors can be undersampled [19]. Therefore, and also influenced by DNA quantity requirements, patients with larger tumors and more aggressive features, such as high grade and high proliferation, were enriched in our study population. Accordingly, the prevalence of *BRCA1/2* mutations, both germline and somatic, may be overestimations. However, given that there was no inclusion bias for age at diagnosis or family history, the ratio of somatic to germline mutations should be unbiased and representative. Our finding that 2/3 of the *BRCA1/2* mutations found in the tumors were germline highlights the need for a strategy on how to deal with identified mutations when sequencing tumors without matched peripheral blood for comparison.

A similar rate of 1/3 somatic versus 2/3 germline was found in the The Cancer Genome Atlas breast cancer study, which carried out exome sequencing of tumor and normal samples of a selected breast cancer patient cohort [26]. Meric-Bernstam et al. [13] recently published a study of germline and somatic mutations in cancer patients treated at MD Anderson. In 251 breast cancer patients, 6 somatic and 21 germline *BRCA1/2* mutations were found, corresponding to a ratio of 1 : 3.5. Their cohort consisted of patients referred to a tertiary cancer center who were likely to benefit from somatic genomic testing, and most patients had metastatic or inoperable disease. Although their study is informative for that kind of setting, it is possible that such ascertainment inflates the ratio of somatic to germline *BRCA1/2* mutations.

BRCA1/2 mutations may soon prove treatment predictive for breast cancer. The Triple-Negative Breast Cancer Trial (TNT) compared carboplatin with docetaxel in metastatic triple-negative breast cancer, and found that patients with germline *BRCA1/2* mutations had a higher response rate with carboplatin [27]. Furthermore, a number of trials have shown a greater efficacy of PARP inhibitors in germline *BRCA1/2* mutation carriers than in noncarriers, and phase III trials in this subset are ongoing [17]. In ovarian cancer, both somatic and germline *BRCA1/2* mutations are now used for treatment prediction, with the approval of olaparib for relapsed *BRCA1/2*-mutated ovarian cancer, regardless of the mutation being germline or somatic [16].

A strength of our study is that we have detailed information about patients who were not included in the study population. Consequently, we can assess and interpret inclusion bias. Another strength is the comprehensive analytic method used, which is expected to detect a great majority of pathogenic mutations in *BRCA1* and *BRCA2*, including missense mutations and DNA copy number losses.

There are also limitations to our study. First, a rather small number of carriers results in imprecise point estimates. Second, the ratio between somatic and germline *BRCA1/2* mutations depends on the study population, which must be considered for the generalizability of our results. Sweden is a country with a high incidence of breast cancer similar to that of the USA and most European nations: the lifetime risk for women is 12%. The median age at diagnosis (64 years) is higher than in many other countries. Although founder mutations in *BRCA1* and *BRCA2* have been reported, carriers of the five most common mutations account for only 19% of the total number of mutation carriers (Å. Borg, personal communication). Therefore, Sweden should be viewed as a country without strong founder mutations. Third, two of the mutations we found are not yet classified as definitely pathogenic (IARC class 5). For example, although *BRCA2* c.8524C>T is not listed in BIC or ClinVar as definitely pathogenic, we consider it deleterious since it was classified pathogenic by Align-GVGD and found in one ovarian cancer sample according to COSMIC and independently in two of our tumor samples. In a homology-directed repair assay, it has intermediate function [28]. The stop-gain mutation *BRCA2* c.6901G>T is located in exon 12. The fact that a low expressed transcript isoform carrying an in-frame exon 12 deletion has been described [29, 30] adds some uncertainty to the pathogenicity of this mutation. Functional and larger population-based studies are likely to be carried out over the next years, and they are needed to validate our results.

In conclusion, in our data from a population without strong germline founder mutations, the likelihood of a *BRCA1/2* mutation found in a breast carcinoma being somatic was ∼1/3 and germline 2/3. This could have implications for treatment and genetic counseling.

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disclosure

The authors have declared no conflicts of interest.

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