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Original Research

Identification of single nucleotide polymorphisms of the PI3K-AKT-mTOR pathway as a risk factor of central nervous system metastasis in metastatic breast cancer

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KEYWORDS
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Abstract ***Introduction:*** The PI3K-AKT-mTOR pathway may be involved in the development of central nervous system (CNS) metastasis from breast cancer. Accordingly, herein we explored whether single nucleotide polymorphisms (SNPs) of this pathway are associated with altered risk of CNS metastasis formation in metastatic breast cancer patients.
Methods: The GENEOM study (NCT00959556) included blood sample collection from breast cancer patients treated in the neoadjuvant, adjuvant or metastatic setting. We identified

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Prediction;
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patients with CNS metastases for comparison with patients without CNS metastasis, defined as either absence of neurological symptoms or normal brain magnetic resonance imaging (MRI) before death or during 5-year follow-up. Eighty-eight SNPs of phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian (or mechanistic) target of rapamycin (mTOR) pathway genes were selected for analysis: AKT1 (17 SNPs), AKT2 (4), FGFR1 (2), mTOR (7), PDK1 (4), PI3KR1 (11), PI3KCA (20), PTEN (17), RPS6KB1 (6).

Results: Of 342 patients with metastases, 207 fulfilled the inclusion criteria: One-hundred-and-seven patients remained free of CNS metastases at last follow-up or date of death whereas 100 patients developed CNS metastases. Among clinical parameters, hormonal and human epidermal growth factor receptor-2 (HER2) status as well as vascular tumour emboli was associated with risk of CNS metastasis. Only PI3KR1-rs706716 was associated with CNS metastasis in univariate analysis after Bonferroni correction ($p < 0.00085$). Multivariate analysis showed associations between AKT1-rs3803304, AKT2-rs3730050, PDK1-rs11686903 and PI3KR1-rs706716 and CNS metastasis .

Conclusion: PI3KR1-rs706716 may be associated with CNS metastasis in metastatic breast cancer patients and could be included in a predictive composite score to detect early CNS metastasis irrespective of breast cancer subtype.

1. Introduction

Breast cancer represents the most common cancer in women. Central nervous system (CNS) metastases occur in up to 10% of patients [1] and herald poor outcome: survival varies from 2.7 to 26.8 months with solid brain metastases, by breast cancer subtype [2] and is 4 months with leptomeningeal metastases [3–6]. Treatment of CNS metastasis aims not only for prolonging survival, but also at prevention or delay of neurological deterioration [7].

The identification of patients at risk could help to increase the efficacy of treatment of CNS metastasis. While cerebrospinal imaging is not part of standard follow-up in patients without neurological signs, the identification of subgroups of patients at risk could allow the implementation of more intensive follow-up and early intervention strategies.

Brain metastases risk is increased in triple-negative breast cancers (TNBCs) and human epidermal growth factor receptor 2 (HER2)-positive tumours [1,8–14]. Risk factors for leptomeningeal metastases include opening of the cerebral ventricular system during surgery for solid brain metastases and resection of cerebellar metastases [15,16] and breast cancer patients specifically lobular subtype and TNBCs [4–6,17].

Genetic variations could also help to define populations at risk. Single nucleotide polymorphisms (SNPs) represent the most frequent type of variations of the human genome [18]: they represent a single nucleotide variation at a specific position in the genome present at a frequency of 1–50% in the general population that is maintained through heredity. While not causing disease, SNPs can modify protein structure and function and thereby influence susceptibility to disease, including cancer [18].

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian (or mechanistic) target of rapamycin (mTOR) [PI3K-AKT-mTOR] pathway controls cell cycle, survival, differentiation, proliferation, motility, metabolism and genomic stability and may be the most frequently activated pathway in human cancer [19–21]. Moreover, it also regulates the behaviour of normal cells and contributes to host cell–tumour cell interactions, e.g. during angiogenesis and inflammation [21–27]. PI3K-AKT-mTOR pathway genetic lesions are frequent in breast cancer and may mediate resistance to HER2-targeted agents and hormonal agents [28]. Activation of the PI3K pathway has specifically been observed in brain metastases from breast cancer, regardless of subtype as defined by hormone receptor or HER2 status [29,30], potentially mediated by the loss of PTEN expression as demonstrated in paired primary tumour and brain metastasis samples [31]. In fact, the loss of phosphatase and tensin homologue deleted on chromosome ten (PTEN), a tumour suppressant, may directly promote brain invasiveness of metastatic breast cancer cells [27]. Herein, we sought to identify SNPs of the PI3K-AKT-mTOR pathway associated with increased risk of CNS metastasis formation in patients with metastatic breast cancer.

2. Materials and methods

2.1. Patients

We conducted a secondary analysis in a subpopulation of patients from the GENEOM study (NCT00959556) that aimed at identifying constitutional genetic variants predictive of response to chemotherapy and hormone therapy in adult patients with histologically confirmed breast cancer and included 914 women between November 2007 and January 2012. Our aim was to identify biomarkers of CNS metastasis risk among

patients who developed metastases, at diagnosis of breast cancer or during follow-up (n = 342) (Supplementary Note 1). Among patients fulfilling the inclusion criteria (n = 207), CNS metastases were diagnosed at initial diagnosis of breast cancer or during follow-up, on brain magnetic resonance imaging (MRI) (n = 87, 87%) or cranial computed tomography (CT) (n = 13, 13%). Histological confirmation was obtained in 10 brain metastasis patients. Leptomeningeal metastasis was defined by the presence of tumour cells in the cerebrospinal fluid (CSF) (n = 25) or by characteristic MRI findings in a patient presenting with clinical signs suggestive of leptomeningeal metastases in the absence of positive CSF cytology (n = 15). In nine patients, the diagnosis was based on clinical evaluation and brain imaging only. Patients with extra-CNS metastasis only were defined by the absence of neurological symptoms or signs or normal brain MRI before death or during at least 5 years of follow-up after the diagnosis of the first metastasis. Patients with neurological symptoms of unclear origin, unclear cause of death or follow-up below 5 years after the diagnosis of the first metastasis were excluded.

2.2. SNP selection

SNPs of the PI3K-AKT-mTOR pathway were selected based on a systematic literature search. Eligible SNPs had to have a minor allele frequency $\geq$0.05 in a European population, based on a 1000 genomes database (https://phase3browser.1000genomes.org/index.html). Eighty-eight SNPs of the PI3K-AKT-mTOR pathway were finally considered for the genomic analysis. Two were excluded (AKT1-rs3803304 and AKT1-rs2498786) due to a discordance between frequencies observed in our population and the database. A total of 86 SNPs was finally analysed (Supplementary Table 1). Details on genomic analyses and SNP studies are provided in Supplementary Note 2.

2.3. Statistical analysis

Overall survival (OS) was defined as time interval from diagnosis until death from any cause using the Kaplan–Meier method. Patients alive were censored at last follow-up. Cumulative incidence of CNS metastases was estimated using a competing risk approach considering the time interval from diagnosis of first metastasis to the date of diagnosis of CNS metastases, with death without CNS metastases considered as a competing event; patients alive without CNS metastases were censored at the date of last follow-up. Associations between CNS metastases and clinical parameters and genomic parameters were evaluated using sub-distribution hazard ratios estimated in Fine and Gray regression models. The first step consisted in the analysis of clinical factors. A multivariate competing risk regression model was performed for parameters significantly associated with CNS metastasis in univariate analysis ($p < 0.05$).

A similar type of modelling was used for the second step of the analysis, evaluating the association of genomic parameters with the risk of CNS metastases. An SNP was considered as evaluable after verification of comparison for genotypic frequencies according to the Hardy–Weinberg equilibrium. SNPs were excluded if the minor allele frequency was below 1%, or if genotyping analyses were performed in less than 90% of the patients of the cohort. Patients with less than 90% of SNPs analysed were excluded. For each SNP, the analysis was performed considering a dominant model (dominant genotype versus others), recessive model (recessive genotype versus others) or log-additive model (three ordered genotypic classes). The significance level was set to $p < 0.05$, and a Bonferroni correction for multiple testing was also determined according to the number of independent SNPs evaluated (threshold = 0.05/number of independent SNPs with $r^2 < 0.8$). SNPs significantly associated with CNS metastasis in univariate analysis (using level $p < 0.05$) were included in a multivariate competitive risks regression model including a step by step selection procedure of variables. Akaike information criterion (AIC) was applied for the selection of the appropriate SNP coding when several modellings were associated with CNS metastasis. A composite score was also computed from the estimated regression coefficients of the multivariate model including all SNPs significant at a 5%-significant level. Last, the association of this genomic score with the risk of CNS metastases was evaluated in multivariate analysis adjusted for clinical parameters significantly linked to CNS metastasis. Confidence intervals were re-estimated using a bootstrap approach with 1000 samplings. Harrell C discrimination index was computed. Statistical analyses were performed using Stata software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP) and SNPassoc package of R software v3.3.1.

3. Results

3.1. Clinical patient characteristics

Among the 914 patients enrolled in GENEOM trial, 342 patients had metastatic disease, 119 at diagnosis of breast cancer and 223 during follow-up. Among these 342 patients, 135 patients were excluded, leading to a study population of 207 patients (Fig. 1). The median follow-up of this cohort is 9.1 years (range 4.8–10.5) after the diagnosis of first metastasis. Overall, 107 patients remained free of CNS metastases at last follow-up or date of death whereas 100 patients developed CNS metastases. Median time interval between first diagnosis

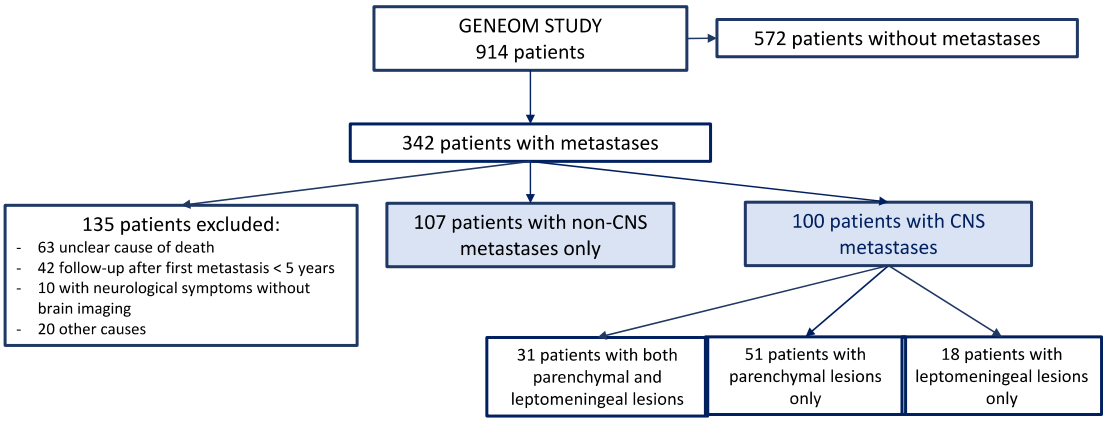

Fig. 1. **CONSORT diagram.** Patients with CNS metastases include patients with parenchymal brain or leptomeningeal metastasis, patients without CNS metastases are defined as patients with extracerebral metastases without any neurological symptoms or signs, with normal brain MRI before death or during 5-years follow-up after diagnosis of first metastasis.

of metastasis and first diagnosis of brain metastasis was 1.4 years (0–8.4); it was 2.1 years between first diagnosis of metastasis and first diagnosis of leptomeningeal metastasis. CNS metastasis was the first metastatic site in eight patients (8%). Treatment of brain metastases included surgery in 10, stereotactic radiotherapy in 14 and whole brain radiotherapy in 51 and pharmacotherapy in 53 patients. Treatment of leptomeningeal disease included intra-cerebrospinal fluid pharmacotherapy in 28, focal radiotherapy in 5 and whole brain radiotherapy in 1, and systemic pharmacotherapy in 29 patients. At last follow-up, 189 patients had died. Median overall survival was 5.3 years (95% CI 4.5–6.1) since breast cancer diagnosis and 2.8 years (95% CI 2.3–3.3) since first diagnosis of metastasis. Median overall survival after diagnosis of CNS metastasis was 4.7 months (95% CI: 3.6–6.4) for parenchymal brain metastases patients and 4 months (95% CI: 2.3–4.9) for leptomeningeal metastases patients (Fig. 2A–C).

Patient characteristics and association of clinical characteristics with risk of CNS metastases are summarised in Table 1. Median age at breast cancer diagnosis was 50 years (range, 22–79); age was not associated with occurrence of CNS metastasis. A lower time interval between breast cancer diagnosis and diagnosis of first metastasis was associated with an increased risk of CNS metastases whereas histology and Scarff Bloom Richardson scores were not. Cumulative incidence of CNS metastases was higher in patients with hormone receptor-positive and HER2-positive or triple-negative tumours, as well as in patients with peritumoural emboli on univariate and multivariate analysis (Fig. 2D and E). Initial treatment was not associated to the occurrence of CNS metastases (Supplementary Table 2).

3.2. SNP analyses

Of 86 SNPs, three were excluded from analysis: RPS56KB1-rs1292033 for not respecting the Hardy Weinberg equilibrium, and PI3KCA-rs17849071 and PI3KCA-rs7641889 because 10.1% of patients could not be genotyped for these two SNPs. No SNP had a minority allele frequency below 1%. The genotype of all patients was analysed for more than 90% of SNPs, and thus all patients were included in this analysis. Thus 83 SNPs were finally evaluated (Supplementary Table 1). Univariate analysis showed a significant association at a 5%-alpha level between seven 7 of 83 SNPs and the occurrence of CNS metastasis: AKT1-rs3803304, AKT2-rs3730050, AKT2-rs8100018, PDK1-rs11686903, PDK1-rs11904366, PI3KR1-rs251408 and PI3KR1-rs706716 (Table 2). After Bonferroni correction (p-value < 0.00085), only PI3KR1-rs706716 remained significantly associated with CNS metastasis. For the multivariate analysis including SNPs associated with occurrence of CNS metastasis in univariate analysis at the 5%-alpha level, we excluded AKT2-rs8100018 since it had an identical distribution as AKT2-rs370050 in our population. Multivariate analysis confirmed an association between AKT1-rs3803304 CC, AKT2-rs3730050 AA, PDK1-rs11686903 TT and PI3KR1-rs706716 TT and the occurrence of CNS metastases (Table 2, Fig. 3) with similar strength of association. When these 4 SNP were combined into a score, three prognostic groups could be identified: five-year cumulative incidence of CNS metastasis rate were 34.8%, 68.9% and 85.7% for patients with a score of 0 (no risk genotype), 1 (1 risk genotype) and 2 (2 risk genotypes). The score was significantly associated with the occurrence of CNS metastasis, with and without adjustment for the significant clinical parameters, hormone receptor status and HER2 status and peritumoural emboli (Table 3). Confidence intervals of subdistribution hazard ratios were re-estimated using a bootstrap approach and were close to the results previously obtained. The C index of Harrell, evaluating the discriminant capacity of the score was 0.607 (95% CI: 0.557–0.657). The interaction

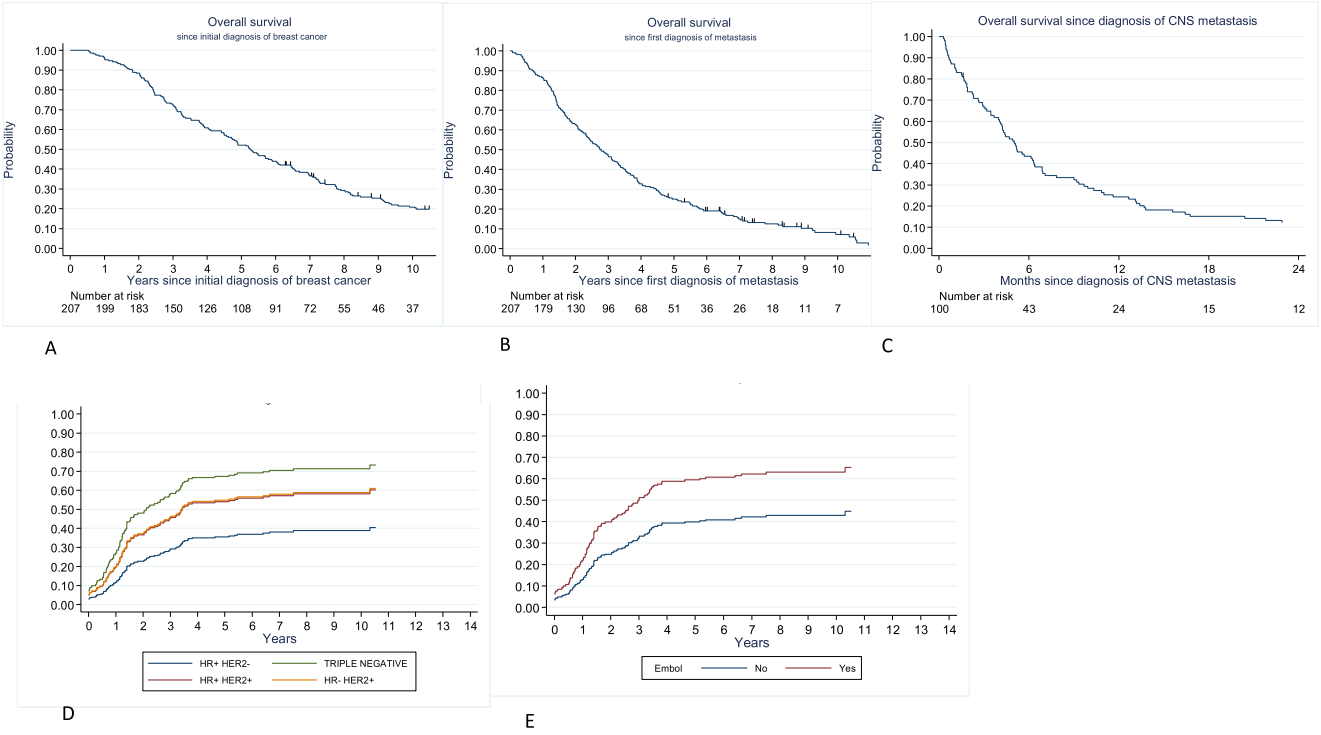

Fig. 2. **Clinical correlates of CNS metastasis and outcome.** A, B, C. OS from breast cancer diagnosis (A), first diagnosis of metastasis (B) and CNS metastasis diagnosis (C). D.E. Cumulative incidence of CNS metastasis after the diagnosis of first metastasis by hormonal and HER2 status (D) or absence or presence of tumoural vascular emboli (E).

test between the score and clinical parameters was not significant for both hormone receptors status and HER2 status, and peritumoural emboli (Supplementary Table 3).

4. Discussion

CNS metastases are a frequent and life-threatening complication of metastatic breast cancer that not only limits survival, but induces morbidity and greatly impairs quality of life. The established risk factors of CNS metastasis such as HER2-positive and triple-negative tumour status [1,8,9–13] were confirmed in the present cohort. Interestingly, we observed that peritumoural emboli were also associated with increased risk of CNS metastasis (Table 1, Fig. 2E). We sought to explore variations of PI3K-AKT-mTOR pathway genes for association with increased risk of CNS metastasis in metastatic breast cancer. AKT1-rs3803304, AKT2-rs3730050, AKT2-rs8100018, PDK1-rs11686903, PDK1-rs11904366, PI3KR1-rs251408 and PI3KR1-rs706716 were associated with risk of CNS metastasis in univariate analysis at a 5% alpha level, however, after Bonferroni correction, only PI3KR1-rs706716 remained significantly associated (Table 2). Multivariate analysis confirmed associations between AKT1-rs3803304, AKT2-rs3730050, PDK1-rs11686903 and PI3KR1-rs706716, and the risk of CNS metastasis. The risk was at least double for patients with AKT1-rs3803304 (CC), AKT2-rs3730050 (AA), PDK1-rs11686903 (TT) and PI3KR1-rs706716 (TT). The combination of SNP into a score enhanced the predictive power (Table 3).

None of these SNPs has previously been reported in studies on CNS metastases or breast cancer. PI3KR1-rs706716 and PDK1-rs11686903 have not been associated with other diseases. AKT1-rs3803304 was associated with a lower risk of death in 45 patients with recurrent or initially metastatic head and neck squamous cell carcinoma [32] and a better response to treatment was associated with this SNP in 45 oesophagealcancer patients with adenocarcinoma or squamous cell carcinoma who had undergone chemoradiotherapy and surgery [33]. AKT2-rs3730050 was associated with shorter survival in 319 patients with muscle-invasive and metastatic bladder cancer [34].

An analysis of 16 SNPs of five genes of the PI3K-AKT-mTOR pathway (PIK3CA, PTEN, AKT1, AKT2, FRAP1) and occurrence of brain metastases in 317 non-small cell lung cancer patients showed that AKT1-rs2498804, AKT1-rs2494732 and PIK3CA-rs2699887 increased the risk of brain metastases at 24-months follow-up [35]. None of these SNPs were identified as at risk of CNS metastases here.

How precisely SNPs in a given pathway modulate course of disease in cancer patients remains to be elucidated. PI3KCA mutations are the most common mutations of the PI3K pathway in breast cancer,

Table 1
Patient characteristics and association with risk of CNS metastases.

	N (%)	5-year cumulative incidence of CNS metastases (95% CI)	Univariate analysis (clinical factors)		Multivariate analysis (clinical factors)	
			SHR (95% CI)	p-value	SHR (95% CI)	p-value
Age at breast cancer diagnosis (/year)		–	0.992 (0.976–1.010)	0.39	n.a.	n.a.
Time between breast cancer diagnosis and first diagnosis of metastasis (/month)		–	0.994 (0.991–0.998)	**0.007**	0.995 (0.989–1.000)	0.051
Histology						
• Infiltrative ductal carcinoma	155 (75.2%)	45.1% (37.2–52.8)	1			
• Infiltrative lobular carcinoma	19 (9.2%)	21.1% (6.6–41.0)	0.36 (0.13–1.001)	0.06	n.a.	n.a.
• Other	32 (15.5%)	53.1% (34.7–68.5)	1.44 (0.95–2.20)	0.09	n.a.	n.a.
SBR grade						
• I	14 (6.8%)	28.6% (8.8–52.4)	1			
• II	109 (52.7%)	43.1% (33.7–52.2)	1.93 (0.70–5.37)	0.21	n.a.	n.a.
• III	49 (23.7%)	59.2% (44.2–71.4)	2.70 (0.95–7.68)	0.06	n.a.	n.a.
• Other (non-gradable/not available)	35 (16.9%)	30.4% (13.5–49.3)	1.43 (0.44–4.62)	0.55	n.a.	n.a.
HR and HER2 status						
• Hormone receptor-positive, HER2-negative	108 (56.5%)	35.2% (26.3–44.2)	1		1	
• Hormone receptor-positive, HER2-positive	33 (17.3%)	51.5% (33.5–66.9)	1.77 (1.06–2.96)	**0.028**	1.70 (1.03–2.79)	**0.037**
• Hormonal receptor-negative, HER2-positive	24 (12.6%)	58.3% (36.5–75.0)	1.81 (0.98–3.33)	0.057	1.32 (0.63–2.75)	0.45
• Triple-negative	26 (13.6%)	69.2% (47.8–83.3)	2.54 (1.43–4.51)	**0.001**	2.10 (1.16–3.79)	**0.014**
Peritumoural vascular emboli						
• No	127 (69.8%)	40.9% (32.4–49.3)	1		1	
• Yes	55 (30.2%)	58.2% (44.1–69.9)	1.78 (1.18–2.68)	**0.006**	1.83 (1.20–2.77)	**0.005**
Presence of metastatic sites:						
• at diagnosis or within 3 months						
• after breast cancer diagnosis	71 (34.3%)	40.9% (29.4–51.9)	1			
• >3 months after breast cancer diagnosis	136 (65.7%)	45.6% (37.1–53.7)	1.28 (0.83–1.96)	0.26	n.a.	n.a.
Metastatic sites at first diagnosis of metastases:						
Bone metastasis						
• No	91 (44.0%)	48.5% (37.8–58.1)	1			
• Yes	116 (56.0%)	40.5% (31.6–49.3)	0.75 (0.51–1.11)	0.15	n.a.	n.a.
Lung metastasis						
• No	141 (68.1%)	41.1% (33.0–49.1)	1			
• Yes	66 (31.9%)	50.0% (37.5–61.3)	1.30 (0.87–1.95)	0.20	n.a.	n.a.
Pleura metastasis						
• No	190 (91.8%)	43.7% (36.6–50.6)	1			
• Yes	17 (8.2%)	47.1% (23.0–68.0)	1.08 (0.49–2.39)	0.85	n.a.	n.a.
Mediastinum metastasis						
• No	156 (75.4%)	43.0% (35.1–50.5)	1			
• Yes	51 (24.6%)	47.1% (33.0–59.9)	1.13 (0.72–1.78)	0.58	n.a.	n.a.
Liver metastasis						
• No	121 (58.5%)	41.3% (32.5–49.9)	1			
• Yes	86 (41.5%)	47.7% (36.8–57.7)	1.14 (0.77–1.70)	0.51	n.a.	n.a.
Peritoneum metastasis						
• No	199 (96.1%)	44.7% (37.7–51.5)	1			
• Yes	8 (3.9%)	25.0% (3.7–55.8)	0.50 (0.10–2.18)	0.33	n.a.	n.a.
Cutaneous metastasis						
• No	178 (86.0%)	42.7% (35.4–49.8)	1			
• Yes	29 (14.0%)	51.7% (32.5–67.9)	1.27 (0.74–2.18)	0.38	n.a.	n.a.
Loco-regional metastasis						
• No	117 (56.5%)	38.5% (29.7–47.2)	1			
• Yes	90 (43.5%)	51.1% (40.4–60.9)	1.35 (0.92–2.00)	0.13	n.a.	n.a.
Other metastasis						
• No	185 (89.4%)	42.2% (35.0–49.2)	1			
• Yes	22 (10.6%)	59.1% (36.1–76.2)	1.72 (0.88–3.33)	0.11	n.a.	n.a.

CNS: central nervous system; SBR: Scarff Bloom Richardson.
SHR: subdistribution hazard ratio estimated in Fine and Gray model.
Multivariate analysis included: HR and HER2 status, peritumoural vascular emboli, time between breast cancer diagnosis and first diagnosis of metastasis.
n.a.: not applicable because not included in multivariate regression model ($p > 0.05$ in univariate analysis).

Table 2
Association between SNP and CNS metastases in univariate analysis, after Bonferroni correction, and in multivariate analysis, for the 7 SNP with p-values < 0.05 on univariate analysis.

SNP	N	5-year cumulative incidence of CNS metastases (95% CI)	Univariate analysis (SNP)				Multivariate analysis (SNP)		
			SHR (95% CI)	p-value	AIC	Significance After Bonferroni correction (0.00085)	Regression coefficient	SHR (95% CI)	p-value
AKT1 – RS3803304									
CG-GG	198	42.9% (36.0–49.7)	1					**1**	
CC (recessive)	9	66.7% (28.2–87.8)	2.17 (1.06–4.42)	0.033	1003.0	NS	**1.00**	**2.72 (1.30–5.68)**	**0.008**
AKT2 – RS3730050									
AG-GG	189	41.8% (34.7–48.7)	1					**1**	
AA (recessive)	18	66.7% (40.4–83.4)	2.07 (1.06–4.02)	0.033	1001.5	NS	**0.73**	**2.06 (1.03–4.14)**	**0.041**
AKT2 – RS8100018									
CG-GG	189	41.8% (34.7–48.7)	1						
CC (recessive)	18	66.7% (40.4–83.4)	2.07 (1.06–4.02)	0.033	1001.5	NS	ND		
PDK1 – RS11686903									
CC-CT	184	40.2% (33.1–47.2)	1					**1**	
TT (recessive)	23	73.9% (50.9–87.3)	2.35 (1.37–4.02)	0.002	997.7	NS	**0.87**	**2.38 (1.40–4.05)**	**0.001**
Log-additive:			1.57 (1.13–2.18)	0.007	998.3	NS			
CC	75	36.0% (25.3–46.8)							
CT	109	43.1% (33.7–52.2)							
TT	23	73.9% (50.9–87.3)							
PDK1 – RS11904366									
GT-TT	68	32.4% (21.7–43.5)	1						
GG (dominant)	139	49.6% (41.1–57.6)	1.67 (1.06–2.63)	0.028	1000.8	NS	NS*		
Log-additive:			1.57 (1.04–2.39)	0.033	1000.9	NS			
GG	139	49.6% (41.1–57.6)							
GT	62	33.9% (22.5-45.6)							
TT	6	16.7% (0.8–51.7)							
PI3KR1 – RS251408									
AA-AG	173	40.5% (33.1–47.7)	1						
GG (recessive)	34	61.8% (43.4–75.7)	1.62 (1.03–2.54)	0.035	1002.5	NS	NS*		
PI3KR1 – RS706716									
CC-CT	198	41.9% (35.0–48.7)	1					**1**	
TT (recessive)	9	88.9% (43.3–98.4)	3.16 (1.71–5.87)	0.0003	999.0	S	**0.88**	**2.42 (1.12–3.25)**	**0.025**

SHR: subdistribution hazard ratio estimated in Fine and Gray model.
Univariate analysis: NS: non-significant, S: significant after Bonferroni correction for multiple testing.
The backward stepwise multivariate regression model included: AKT1 – RS3803304, AKT2 – RS3730050, PDK1-RS11686903 (as recessive), PDK1-RS11904366 (as dominant), PI3KR1-RS251408, and PI3KR1-RS706716. PDK1-RS11686903 and PDK1-RS11904366 were included as recessive and dominant because of a lower AIC obtained in univariate analysis than with log-additive model.
ND: not done: AKT2 – RS8100018 was not included in multivariate analysis because of its identical distribution with AKT2 – RS3730050 in our population.
NS*: SNPs that were not significant and removed from backward stepwise multivariate regression model (p-value = 0.15 for PDK1 – RS11904366 and p-value = 0.38 for PI3KR1 – RS251408).

depending on the subtype of cancer, with the lowest rate in triple-negative breast cancer. The prognostic role of PI3KCA mutations remains controversial, but they tend to be associated with more favourable outcomes [28]. Other alterations of the PI3K-AKT-mTOR pathway in breast cancer include PI3KR1 mutations (3%), decreased PI3KR1 expression (62%), AKT1 mutations (3%) and overexpression (25.3%), PTEN mutations (2–12.5%), and PTEN loss (28%) [20,28,36]. An alteration of at least one parameter of PI3K pathway has been reported in 72% of tumours [36]. Among 52 breast cancer brain metastases and 12 matched primary breast cancers, expression of p-AKT and p-S6, and lack of PTEN expression were found in 75%, 69% and 25% of brain metastases. Concordance rates between primary tumour and brain metastases were 67% for p-AKT expression, 58% for p-S6 expression and 83% for PTEN [30]. Given this high prevalence of pathway mutations, it is conceivable that SNPs modulate tumour cell-intrinsic behaviour. Yet, it cannot be excluded that the SNPs also determine how the host's microenvironment interacts with metastatic tumour cells.

Limitations of this study include the lack of an appropriate validation cohort and the lack of systematic prospective patient assessment for CNS metastasis. A separate analysis for patients with parenchymal brain metastases as opposed to leptomeningeal metastases would have been interesting, but was not feasible for lack of statistical power. Still, our results support the notion that the identification of risk factors for CNS

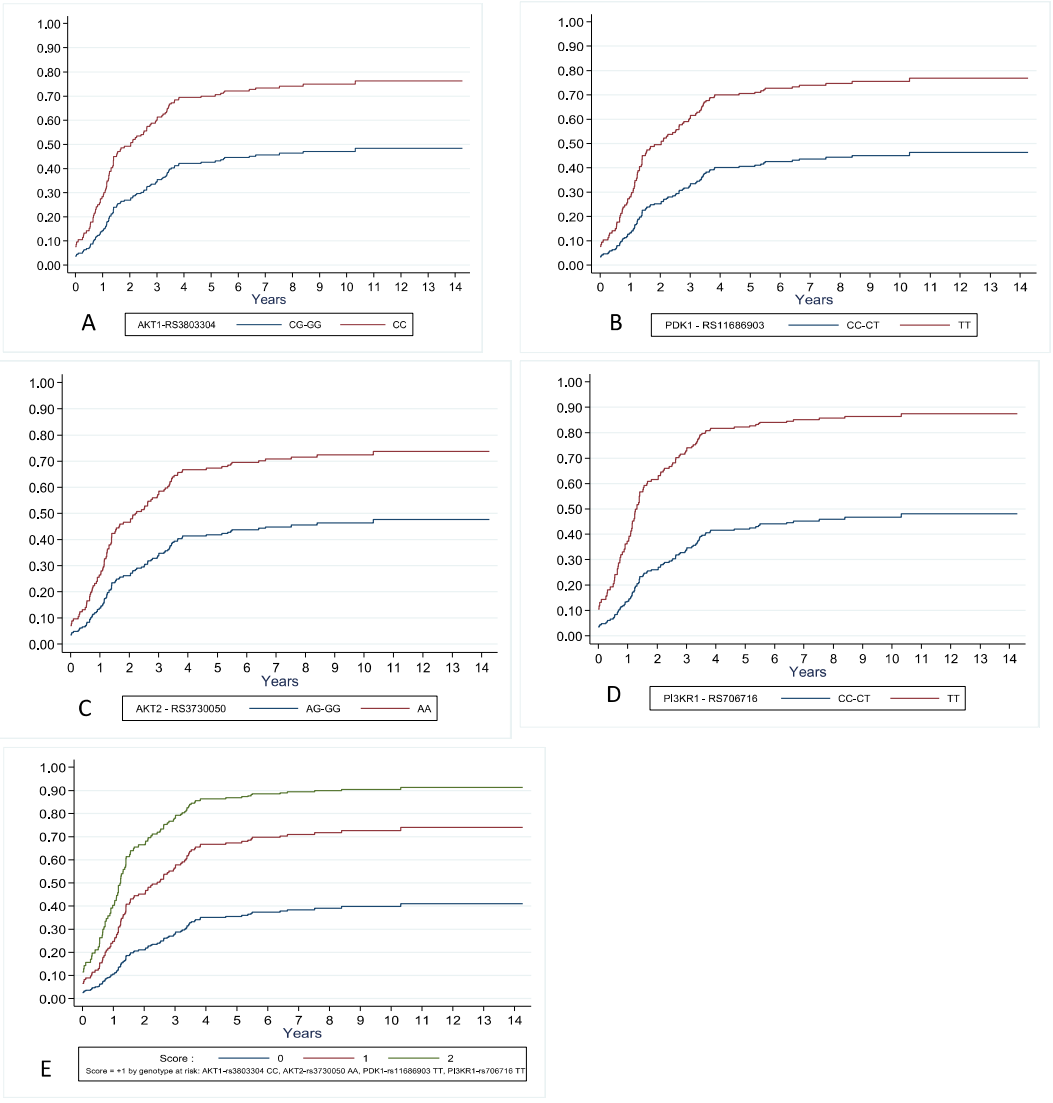

Fig. 3. **SNP linked to CNS metastasis.** Cumulative incidence of CNS metastases after diagnosis of first metastasis for SNP significantly associated with CNS metastases on multivariate analysis. A: AKT1-RS3803304, B: PDK1-RS11686903, C: AKT2-RS370050, D: PI3KR1-RS706716, E: score.

Table 3
Combination of SNP and score.

	Number of patients	5-year cumulative incidence of CNS metastases (95% CI)	Original hazard ratio				Bootstrap analysis			
			SHR (95% CI)	p-value	SHR (95% CI) adjusted for clinical parameters[a]	p-value	SHR (95% CI)	p-value	SHR (95% CI) adjusted for clinical parameters[a]	p-value
SCORE 0	155	34.8% (27.4–42.3)	1		1		1		1	
SCORE 1	45	68.9% (53.2–80.3)	2.55 (1.65–3.94)	<0.001	2.07 (1.30–3.30)	0.002	2.55 (1.60–4.07)	<0.001	2.07 (1.26–3.42)	0.004
SCORE 2	7	85.7% (33.4–97.9)	4.62 (1.82–11.7)	0.001	3.82 (1.39–10.5)	0.009	4.62 (1.53–13.9)	0.007	3.82 (0.40–36.9)	0.25
SCORE (continuous value)			2.32 (1.64–3.30)	<0.001	2.01 (1.37–2.95)	<0.001	2.32 (1.61–3.36)	<0.001	2.01 (1.34–3.03)	0.001

SHR: Subdistribution hazard ratio estimated in Fine and Gray model.
[a] Adjustment for oestrogen and progesterone receptors, HER2 status, peritumoural emboli.

metastasis may enable screening and earlier detection when numerous therapeutic options are still available, with the consequences of limiting neurological impairment and preserving quality of life longer. Such risk profiles may also facilitate the development of new strategies of prevention in populations at risk.

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Conflict of interest statement

ELR has received research grants from Mundipharma, Amgen and honoraria for lectures from Mundipharma and Novartis. MP has received research support from Böhringer-Ingelheim, GlaxoSmithKline, Merck Sharp & Dome and Roche and honoraria for lectures, consultation or advisory board participation from Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, Astra Zeneca and AbbVie. MW has received research grants from Acceleron, Actelion, Bayer, Isarna, Merck, Sharp & Dohme, Merck (EMD, Darmstadt), Novocure, OGD2, Piqur and Roche and honoraria for lectures or advisory board participation or consulting from Abbvie, BMS, Celldex, Immunocellular Therapeutics, Magforce, Merck, Sharp & Dohme, Merck (EMD, Darmstadt), Novocure, Pfizer, Roche, Teva and Tocagen. The remaining authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejca.2017.10.006.

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