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PRECLINICAL STUDY

Prevalence and characterization of *ATM* germline mutations in Chinese *BRCA1/2*-negative breast cancer patients

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Abstract

Purpose The ataxia telangiectasia-mutated (*ATM*) gene is a moderate susceptibility gene for breast cancer. However, little is known about the breast cancer phenotypes associated with *ATM* mutation. We therefore investigated the spectrum and clinical characteristics of *ATM* germline mutations in Chinese breast cancer patients.

Methods A multi-gene panel was performed to screen for *ATM* germline mutations in 7657 *BRCA1/2*-negative breast cancer patients. All deleterious mutations were validated by independent polymerase chain reaction (PCR)-Sanger sequencing.

Results A total of 31 pathogenic mutations in the *ATM* gene across 30 carriers were identified, and the *ATM* mutation rate was 0.4% (30/7,657) in this cohort. The majority of the mutations (90.3%, 28/31) were nonsense or frameshift mutations. Of the total *ATM* mutations, 61.3% (19/31) were novel mutations and 13 recurrent mutations were found. *ATM* mutations carriers were significantly more likely to have a family history of breast and/or ovarian cancer (26.7% in carriers vs. 8.6% in non-carriers, $p < 0.001$), as well as a family history of any cancer (60.0% in carriers vs. 31.5% in non-carriers, $p = 0.001$). In addition, *ATM* mutations carriers were significantly more likely to have oestrogen receptor (ER)-positive ($p = 0.011$), progesterone receptor (PR)-positive ($p = 0.040$), and lymph node-positive breast cancer ($p = 0.034$).

Conclusions The prevalence of the *ATM* mutation is approximately 0.4% in Chinese *BRCA1/2*-negative breast cancer. *ATM* mutation carriers are significantly more likely to have a family history of cancer and to develop ER- and/or PR-positive breast cancer or lymph node-positive breast cancer.

Keywords *ATM* gene · Germline mutation · Breast cancer · Chinese population

Abbreviations

ATM	The ataxia telangiectasia-mutated gene
ANOVA	One-way analysis of variance
DSB	DNA double-strand break
ER	Oestrogen receptor
FAT	FRAP-ATM-TRRAP
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemical
PARP	Poly (ADP-ribose) polymerase
PCR	Polymerase chain reaction
PIK	PI-3 kinase
PR	Progesterone receptor
SD	Standard deviation
TNBC	Triple-negative breast cancer

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Introduction

The ataxia telangiectasia-mutated (*ATM*) gene is located on chromosome 11q22-23 and encodes a serine/threonine protein kinase. The ATM protein plays a central role in the cellular DNA damage response that is necessary to maintain genome stability. When DNA damage occurs, ATM directly phosphorylates *TP53, BRCA1*, and other proteins

involved in the DNA double-strand break (DSB) response. ATM deficiency exhibits a higher predisposition to breast cancer and other malignant diseases [1, 2], and has been demonstrated to be a moderate breast cancer susceptibility gene [3].

Together with *BRCA1, BRCA2, TP53, CHEK2*, and several other genes involved in breast cancer predisposition, *ATM* is currently tested in most gene panel assays performed for breast cancer patients [4]. In these studies, the prevalence of the *ATM* mutation ranged from 0.45 to 1.0% [5–7], and a rare mutation c.T7271G (p.V2424G) was linked with a particularly high risk [8–10]. However, germline mutations in the *ATM* gene have not been well documented in Chinese women. A recent study using a multiple gene-sequencing assay identified an *ATM* mutation rate of 0.64% among 937 Chinese breast cancer patients with high hereditary risk [11]. However, the sample size of this study was relatively small. Importantly, the relationship between the *ATM* germline mutation and clinical characteristics is still unknown, though recent study found that lower expression of *ATM* in breast cancer was associated with a higher grade in these patients [12, 13]. Therefore, the clinical relevance of the spectrum of *ATM* mutations in Chinese breast cancer patients needs to be fully elucidated.

In this study, we identified *ATM* germline mutations in 7657 *BRCA1/2*-negative breast cancer patients who were unselected for age at diagnosis or a family history of breast cancer. We further analysed the association of *ATM* germline mutations with clinical characteristics in this cohort.

Materials and methods

Study population

A total of 10,378 patients were diagnosed with breast cancer at the Breast Center of Peking University Cancer Hospital from October 2003 to May 2015. Among these patients, 8085 were sequenced on a 62-gene panel as described in our prior study [14]. After excluding 428 patients with *BRCA1/2* mutations, 7657 patients were included in our analysis. The patients' ages at diagnosis ranged from 19 to 98 years, with a mean age of 51 years (Online resource 1). The definition of a family history of breast and/or ovarian cancer, or family history of any cancer, is described in our previous study [14]. The clinical and tumour characteristics were abstracted from medical records and the family history of cancer was collected from both medical records and telephone interviews with each patient. This study was approved by the Research Ethics Committee of Peking University Cancer Hospital (No. 2011KT12), and written informed consent was obtained from all participants.

Pathology

Oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) were profiled using an immunohistochemical (IHC) assay on breast cancer tissue obtained from core-needle biopsies or surgery, as previously described [15]. A positive result for ER or PR was defined as $\geq 1\%$ of tumour cells displaying positive nuclear staining. HER2 positivity was defined as a score of 3+ or by HER-2 gene amplification using fluorescent in situ hybridization.

ATM mutation screening

Blood samples were collected from above breast cancer participants, and peripheral DNA was extracted from patient blood samples using the whole blood genome DNA isolation kit (Bioteke, Beijing, China). A 62-gene panel assay was used to screen *ATM* mutations using the HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA) as described in our previous study [14]; 2 μg of genomic DNA was used for the screening. The panel covered all coding regions and splice sites of the *ATM* gene. All pathogenic mutations were validated by polymerase chain reaction (PCR)-Sanger sequencing.

Mutation classification

Nonsense and frameshift mutations that lead to the formation of truncated proteins were classified as pathogenic mutations. Missense and splice-site mutations were classified by ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) and the American College of Medical Genetics and Genomics guidelines [16]. Previously published data and prediction software were used to support the classifications. In this study, only pathogenic or likely pathogenic mutations were included for further analysis.

Statistical analysis

The differences in age between *ATM* mutation carriers and non-carriers were described as the means ± standard deviation (SD) and were tested by one-way analysis of variance (ANOVA). Differences in clinical characteristics between *ATM* mutation carriers and non-carriers were tested using Pearson's χ^2 test and Fisher's exact test when violation existed. Two-sided p values < 0.05 were considered to be statistically significant. All analyses were carried out using SPSS 20.0 software (Chicago, IL, USA).

Results

Prevalence and the spectrum of *ATM* germline mutations

A total of 31 *ATM* germline mutations were identified in 30 of the 7657 *BRCA1/2*-negative breast cancer patients (Fig. 1; Tables 1, 2). One patient carried two *ATM* mutations, c.8929_8930insT and c.8915_8924del (Table 2). Among the 31 *ATM* mutations, 28 (90.3%) were either frameshift or nonsense mutations (9 frameshift and 19 nonsense mutations). Additionally, two missense mutations c.C6679T (p.R2227C) and c.A8711G (p.E2904G) were detected, which were previously reported as pathogenic mutations [17–19]. In total, the prevalence of pathogenic *ATM* mutations in this cohort was 0.4% (30/7,657).

Among these 31 *ATM* mutations, 13 were recurrent (p.R2486X, $n=3$; Y155X, $n=2$; p.F802fr, $n=2$; p.W1795X, $n=2$; p.Q2206X, $n=2$; p.R2227C, $n=2$; Fig. 1; Table 1), accounting for 41.9% (13/31) of all *ATM* mutations. Of the total *ATM* mutations, 61.3% (19/31) were novel and not found in databases or previous publications (Table 1). The distribution of the pathogenic mutations spanned the entire *ATM* coding sequence.

Clinicopathological characteristics

The clinicopathological characteristics of the 7657 *BRCA1/2*-negative patients are presented in Online resource 1. Among the 30 *ATM* mutation carriers, 8 (26.7%) patients had a family history of breast and/or ovarian cancer, while 18 (60.0%) had a family history of any cancer. Among the 7627 non-carriers, 653 (8.6%) had a family history of breast and/or ovarian cancer while 2406 (31.5%) had a family history of any cancer. The *ATM* mutation carriers had a significantly higher frequency of a family history of any cancer (60.0% in carriers vs. 31.5% in non-carriers, $p=0.001$), especially breast and/or ovarian cancer (26.7% in carriers vs. 8.6% in non-carriers, $p<0.001$) (Table 3). The available pedigrees are provided in Fig. 2. Additionally, the *ATM* mutation carriers were more likely to manifest as ER-positive ($p=0.011$), PR-positive ($p=0.040$), and lymph node-positive ($p=0.034$) than non-carriers (Table 3). No significant association was found between *ATM* mutations and age at diagnosis, tumour size, bilateral breast cancer, tumour grade, or HER-2 status in this cohort of 7657 patients.

Discussion

In this study, we screened *ATM* mutations in a large cohort of 7,657 unselected Chinese patients with *BRCA1/2*-negative breast cancer. To our knowledge, this is currently the largest study investigating the prevalence of *ATM* mutations and its effect on breast cancer in Asian population. In our study, 30 patients (0.4%) carried *ATM* germline mutations. *ATM* mutation carriers were more likely to have a family history of cancer and to develop ER-positive and/or PR-positive breast cancer, or lymph node-positive breast cancer.

The prevalence of the *ATM* mutation in our study was 0.4%, which is lower than that observed in Caucasian patients. Two previous large-scale gene panel studies found that the prevalence of *ATM* mutation was approximately 1% in patients with a majority of European ancestry [6, 7]. Recently, Li et al. determined the frequency of *ATM* mutation among Chinese breast cancer patients with high hereditary risk. They reported an *ATM* mutation rate of 0.77% (6/778) in *BRCA1/2*-negative patients [11]. In this study, we found that the prevalence of *ATM* mutations was 1.2%

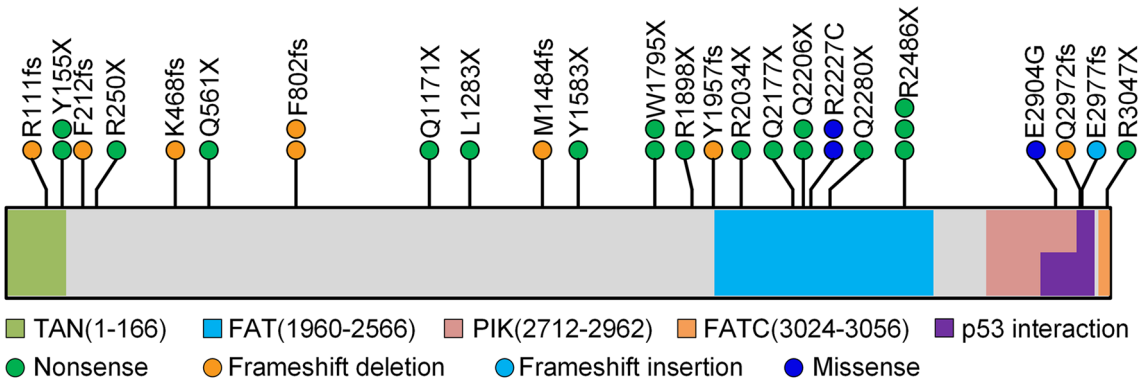

Fig. 1 The distribution of identified germline mutations in the *ATM* gene. *ATM* comprises 3056 amino acids with 4 important domains: Ter1/ATM N-terminal motif (TAN, amino acid residues 1–166), FRAP-ATM-TRRAP domain (FAT, amino acid residues 1960–2566), PI-3 kinase domain (PIK, amino acid residues 2712–2962), C-terminal FATC domain (FATC, amino acid residues 3024–3056), and a p53 interaction region (amino acid residues 2862–3012). Each solid circle presents a case with *ATM* mutation

Table 1 *ATM* germline mutation identified in this cohort

Exon	Nucleotide Change	AA Change	Effect	No. of Mutation	Reference
5	c.332_332del	p.R111fs	Frameshift deletion	1	Novel
5	c.C465G	p.Y155X	Stopgain	2	Novel
6	c.634delT	p.F212fs	Frameshift deletion	1	Novel
7	c.C748T	p.R250X	Stopgain	1	Buzin et al. [20], Keimling et al. [21] and Nakamura et al. [22]
10	c.1402_1403del	p.K468fs	Frameshift deletion	1	Buzin et al. [20], Kurian et al. [23], Micol et al. [24] and Lin et al. [25]
11	c.C1681T	p.Q561X	Stopgain	1	Novel
16	c.2404delT	p.F802fs	Frameshift deletion	2	Novel
24	c.C3511T	p.Q1171X	Stopgain	1	Jacquemin et al. [26] and Becker-Catania et al. [17]
26	c.3847delC	p.L1283X	Stopgain	1	Novel
30	c.4450_4453del	p.M1484fs	Frameshift deletion	1	Novel
31	c.C4749G	p.Y1583X	Stopgain	1	Novel
36	c.G5384A	p.W1795X	Stopgain	2	Novel
38	c.C5692T	p.R1898X	Stopgain	1	Magliozzi et al. [27]
39	c.5869_5870del	p.Y1957fs	Frameshift deletion	1	Novel
42	c.C6100T	p.R2034X	Stopgain	1	Susswein et al. [28], Telatar et al. [29] and Teraoka et al. [30]
45	c.C6529T	p.Q2177X	Stopgain	1	Novel
46	c.C6616T	p.Q2206X	Stopgain	2	Novel
46	c.C6679T	p.R2227C	Missense mutation	2	Becker-Catania et al. [17], Mitui et al. [18] and Buzin et al. [20]
47	c.C6838T	p.Q2280X	Stopgain	1	Novel
50	c.C7456T	p.R2486X	Stopgain	3	Buzin et al. [20] and Susswein et al. [28]
60	c.A8711G	p.E2904G	Missense mutation	1	Ziv et al. [19]
62	c.8915_8924del	p.Q2972fs	Frameshift deletion	1	Novel
62	c.8929_8930insT	p.E2977fs	Frameshift deletion	1	Novel
63	c.C9139T	p.R3047X	Stopgain	1	Laake et al. [31] and Barone et al. [32]

in familial breast cancer patients, which is similar to that of Li et al.

Among these 31 mutations, 90.3% mutations were either frameshift or nonsense mutations that led to a truncated protein; therefore, these mutations were classified as pathogenic mutations. Additionally, the remaining two missense mutations p.R2227C and p.E2904G were also considered as pathogenic mutations. The p.R2227C mutation was located in the FRAP-ATM-TRRAP (FAT) domain of the ATM protein, and the p.R2227C mutation leads to the ATM protein instability and eliminates phosphorylation in the S1981 self-phosphorylation site [17, 18]; these studies indicated that p.R2227C is a pathogenic mutation. Another missense mutation (p.E2904G) was located in the PI-3 kinase (PIK) domain of the ATM protein and was previously reported to be deleterious due to the mutation causing an apparent instability of the protein [19]. Approximately 61.3% of the *ATM* mutations detected in our current study have not been previously reported, suggesting that these mutations could be specific for Chinese women. The dominant negative p.V2424G mutation confers a particularly high risk but was not found in any of the Chinese breast cancer patients in our study, which indicates that the *ATM* p.V2424G mutation is absent or quite infrequent in Chinese women.

The prevalence of *ATM* mutations was significantly higher in patients with a family history of breast and/or ovarian cancer or any cancer, which is similar to that of patients with *BRCA1/2* mutation [14, 33]. Our results were consistent with previous reports that *ATM* mutations were more frequent in breast cancer with a first-degree family history [30, 34]. It is well documented that *ATM* is a moderate-penetrance breast cancer susceptibility gene, the relative risk of breast cancer in *ATM* mutation carriers was 2.2–3.3-fold [4, 7, 35–38], and the cumulative risk of breast cancer in *ATM* mutation carriers was 16–43% by age 80 years [3, 36, 39–41]. Of note, the breast cancer risk associated with *ATM* mutations varies widely and should be used with caution in clinical practice. Therefore, the family history of breast cancer should be taken into account during genetic counselling. The results from other studies also found *ATM* mutations confer an increased risk of gastric cancer, pancreatic cancer, prostate cancer, colorectal cancer, and melanoma [42, 43]. According to the 2018 National Comprehensive Cancer Network guidelines, women who are heterozygous for a

Table 2 Clinicopathological information of Breast Cancer Patients with *ATM* Mutations

Case ID	Mutation	Age	Family history	Tumour type	ER	PR	HER2
1014	c.G5384A	52	–	IDC	P	P	P
1317	c.C4749G	51	Cervical cancer, colon cancer	IDC	N	N	N
4062	c.C748T	48	Lung cancer	IDC	P	P	N
4940	c.1402_1403del	52	Breast cancer	IDC	N	N	P
5429	c.C6100T	38	–	IDC	P	N	P
5573	c.C7456T	50	Breast cancer	IDC	P	P	N
6024	c.C5692T	36	Rectal cancer	IDC	P	P	N
6298	c.C6616T	53	Breast cancer	IDC	P	P	N
7804	c.C7456T	49	Colon cancer	MC	P	P	N
6366	c.C465G	37	–	IDC	P	N	UC
7023	c.C3511T	70	Sarcoma, pancreatic cancer	IDC	P	N	N
7261	c.C1681T	25	Ovarian cancer	IDC	P	P	N
7627	c.3847delC	77	–	IDC	P	P	N
8108	c.G5384A	55	Lung cancer	IDC; DCIS	P	P	N
8200	c.C6679T	31	–	IDC; DCIS	P	P	N
8335	c.C9139T	48	–	IDC	P	P	N
9309	c.332_332del	40	–	IPC	P	P	UC
9785	c.C6616T	62	Breast cancer	ILC	P	P	N
9998	c.C6529T	57	Esophageal cancer, gastric cancer	IDC	P	P	N
10,543	c.A8711G	63	Breast cancer	IDC	P	P	N
10,732	c.4450_4453del	77	Gastric cancer, lung cancer	IDC	P	P	N
11,799	c.C7456T	48	–	IDC	P	P	P
11,773	c.8929_8930insT c.8915_8924del	30	Esophageal cancer	IDC	P	P	N
13,117	c.5869_5870del	75	Lung cancer	IDC	P	P	N
13,347	c.634delT	45	–	IDC	P	P	N
13,478	c.C465G	50	–	DCIS	NA	NA	NA
14,080	c.2404delT	43	Breast cancer, gastric cancer	IDC	P	P	N
14,262	c.2404delT	46	Breast cancer, gastric cancer	IDC	P	P	P
15,742	c.C6838T	36	–	IDC; DCIS	P	P	N
16,005	c.C6679T	37	–	IDC	P	P	N

IDC invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *DCIS* ductal carcinoma in situ, *IPC* intraductal papillary carcinoma, *MC* medullary carcinoma, *ER* oestrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor-2, *P* positive, *N* negative, *NA* not available, *UC* uncertain

pathogenic *ATM* mutation should undergo yearly mammographic screening starting by at least 40 years of age because their lifetime risk of breast cancer is likely greater than 25%. For women who also have a strong family history of breast cancer, earlier initiation of high-risk screening with both MRI and mammography should be considered [44]. This indicates that further surveillance is necessary for patients with *ATM* mutation and their close relatives.

As our understanding of the genetic heterogeneity of breast cancer grows, evidences that mutations in different genes may be associated with different breast cancer subtypes have emerged. It is known that *BRCA1* mutations are associated with triple-negative breast cancer [14, 33], whereas *TP53* mutations are associated with HER2-positive breast cancer [11]. In our study, *ATM* mutations were particularly frequent in ER-positive and/or PR-positive breast cancer, which is consistent with the findings of Renault et al. [45] who reported that *ATM*-associated breast cancers were mostly Luminal B subtype. Additionally, we found that *ATM* mutation tumours were more likely to be lymph node-positive.

ATM is the main transducer in the repair of DNA DSB, and DSB damage results in a clinical benefit from poly (ADP-ribose) polymerase (PARP) inhibitors [46]. Recent studies suggest that multiple cancer cells with lower expression of *ATM* [47–49], including breast cancer cell lines [49], were sensitive to DNA damage drug PARP1 inhibitors, and *ATM* mutant lymphoid tumour cells also benefit from PARP1 inhibitors [50]. Two recent phase II double-blind studies indicated that low *ATM* expressed metastatic gastric cancer [51] and *ATM* mutant metastatic prostate cancers patients [52] benefit from the PARP

Table 3 Association of pathologic *ATM* mutation and clinicopathological characteristics of 7,657 *BRCA1/2* non-carriers

Characteristics	No. of patients	*ATM* carriers		Non-carriers		*p* Value
		No.	%	No.	%	
Total No	7657	30		7627		
Age at diagnosis						
Mean ± SD	51.3 ± 11.6	49.4 ± 13.6		51.3 ± 11.6		0.51
Median	50	48.5		50.0		
Range	19–98	25–77		19–98		
≤ 40 years	1341	9	30.0	1332	17.5	0.07
> 40 years	6316	21	70.0	6295	82.5	
Family history of breast and/or ovarian cancer						
Positive	661	8	26.7	653	8.6	< 0.001
Negative	6996	22	73.3	6974	91.4	
Family history of any cancer						
Positive	2424	18	60.0	2406	31.5	0.001
Negative	5233	12	40.0	5221	68.5	
BBC						
Yes	196	2	6.7	194	2.5	0.18
No	7461	28	93.3	7433	97.5	
Tumour size						
≤ 2 cm	3127	12	40.0	3115	42.9	0.75
> 2 cm	4156	18	60.0	4138	57.1	
Unknown	374	0		374		
Grade						
I	618	1	4.0	617	10.6	0.28
II	4342	22	88.0	4320	74.0	
III	901	2	8.0	899	15.4	
Unknown	1796	5		1791		
ER status						
Negative	2064	2	6.9	2062	28.3	0.011
Positive	5264	27	93.1	5237	71.7	
Unknown	329	1		328		
PR status						
Negative	2557	5	17.2	2552	35.5	0.040
Positive	4655	24	82.8	4631	64.5	
Unknown	445	1		444		
HER-2 status						
Negative	5209	22	81.5	5187	74.2	0.39
Positive	1806	5	18.5	1801	25.8	
Unknown	642	3		639		
Molecular subtype						
ER+ and/or PR+, HER2−	4098	21	77.8	4077	60.0	0.16
ER+ and/or PR+, HER2+	908	4	14.8	904	13.3	
HER2+	839	1	3.7	838	12.3	
TNBC	980	1	3.7	979	14.4	
Unknown	832	3		829		
Lymph node metastasis						
Negative	5237	16	55.2	5221	72.8	0.034
Positive	1968	13	44.8	1955	27.2	
Unknown	452	1		451		
Adjuvant therapy						
C	2396	9	30.0	2387	31.3	0.80
E	1578	7	23.3	1571	20.6	
C + E	2174	10	33.3	2164	28.4	
None treatment	1509	4	13.3	1505	19.7	

Table 3 (continued)

BBC bilateral breast cancer, *ER* oestrogen receptor, *PR* progesterone receptor, *HER-2* human epidermal growth factor receptor 2, *TNBC* triple-negative breast cancer, *C* chemotherapy, *E* endocrine therapy

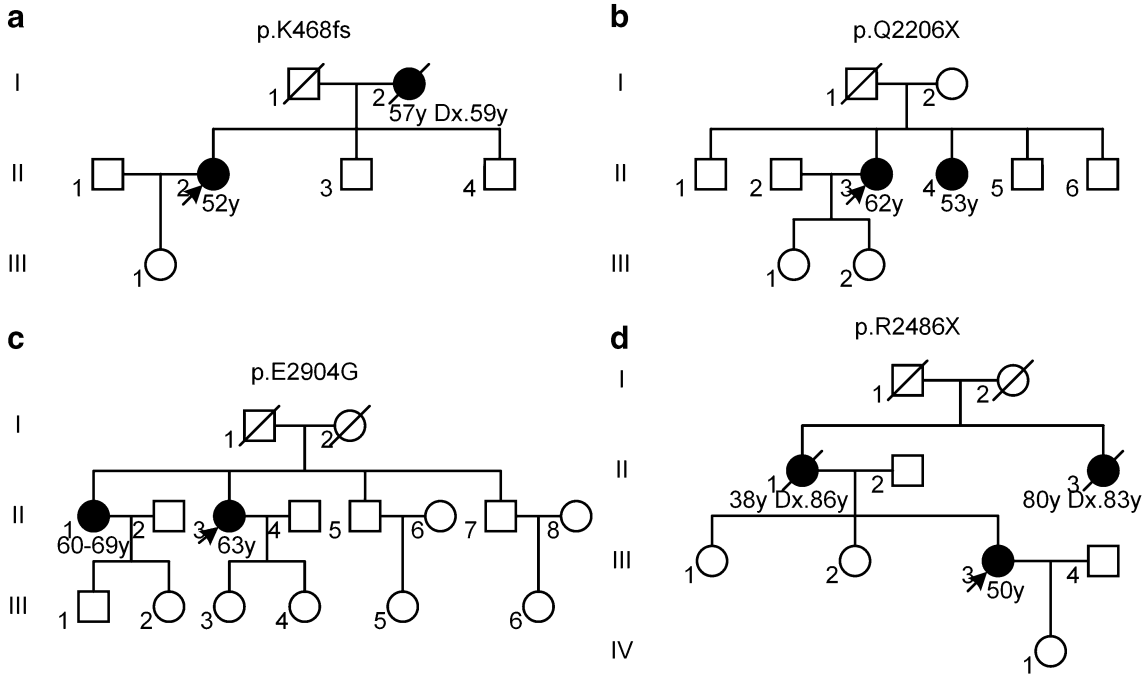

Fig. 2 Pedigrees of *ATM* germline mutations with a family history of breast cancer. Solid circles represent patients with breast cancer. Arrowheads represent probands. Slashed symbols represent deceased individuals. The age at diagnosis and the age at death (Dx.+age) are included under the symbol. **a** Pedigree of the family with proband (II-2) carrying the p.K468fr mutation of *ATM*. **b** Pedigree of the family with proband (II-3) and her sister (II-4) carrying the p.Q2206X mutation of *ATM*. **c** Pedigree of the family with proband (II-3) carrying the p.E2904G mutation of *ATM*. **d** Pedigree of the family with proband carrying the p.R2486X mutation of *ATM*

inhibitor olaparib. Therefore, *ATM* mutation carriers may be potential candidates for treatment with PARP inhibitors.

In conclusion, we found that 0.4% of *BRCA1/2*-negative breast cancer patients carry *ATM* germline mutations in this large cohort, and many mutations are specific to the Chinese population. Our study also demonstrates the characteristics of *ATM* mutations in the Chinese population. In the current study, we found *ATM* mutations were strongly associated with breast cancer patients who have a family history of breast and/or ovarian cancer. Therefore, we suggested that *ATM* should be added in genetic testing in Chinese familial breast cancer patients. And the *ATM* mutation carriers should be offered intensive surveillance and would potentially benefit from targeted therapy. Large case–control studies are needed to fully elucidate the risk and implication of *ATM* mutations in breast cancer in the Chinese population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was conducted in accordance with Helsinki Declaration, and was approved by the Research Ethics Committee of Peking University Cancer Hospital.

Informed consent Written informed consent was obtained from all participants.

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