

Prevalence of *BRCA1/2* mutations in sporadic breast/ovarian cancer patients and identification of a novel de novo *BRCA1* mutation in a patient diagnosed with late onset breast and ovarian cancer: implications for genetic testing

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Received: 2 February 2011 / Accepted: 20 April 2011 / Published online: 7 May 2011
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Abstract In order to adequately evaluate the clinical relevance of genetic testing in sporadic breast and ovarian cancer patients, we offered comprehensive *BRCA1/2* mutation analysis in patients without a family history for the disease. We evaluated the complete coding and splice site regions of *BRCA1/2* in 193 sporadic patients. In addition, a de novo mutation was further investigated with ultra deep sequencing and microsatellite marker analysis. In 17 patients (8.8%), a deleterious germline *BRCA1/2* mutation was identified. The highest mutation detection ratio ($3/7 = 42.9\%$) was obtained in sporadic patients diagnosed with breast and ovarian cancer after the age of 40. In 21 bilateral breast cancer patients, two mutations were identified (9.5%). Furthermore, 140 sporadic patients with unilateral breast cancer were investigated. Mutations were only identified in patients diagnosed with breast

cancer before the age of 40 ($12/128 = 9.4\%$ vs. $0/12$ with $Dx > 40$). No mutations were detected in 17 sporadic male breast cancer and 6 ovarian cancer patients. *BRCA1* c.3494_3495delTT was identified in a patient diagnosed with breast and ovarian cancer at the age of 52 and 53, respectively, and was proven to have occurred de novo at the paternal allele. Our study shows that the mutation detection probability in specific patient subsets can be significant, therefore mutation analysis should be considered in sporadic patients. As a consequence, a family history for the disease and an early age of onset should not be used as the only criteria for mutation analysis of *BRCA1/2*. The relatively high mutation detection ratio suggests that the prevalence of *BRCA1/2* may be underestimated, especially in sporadic patients who developed breast and ovarian cancer. In addition, although rare, the possibility of a de novo occurrence in a sporadic patient should be considered.

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Keywords Breast and ovarian cancer · Sporadic cancer · De novo mutation · *BRCA1/2*

Introduction

Germline *BRCA1* (MIM 113705) [1] and *BRCA2* (MIM 600185) [2, 3] mutations confer high risks for breast and ovarian cancer and are most prevalent in patients with a family history for the disease. The incidence of mutations in high-risk families varies widely among different populations; some present a wide spectrum of different mutations, while in particular ethnic groups specific mutations show a high frequency due to a founder effect. For example, approximately 2.5% of all people of Ashkenazi Jewish descent carry one of three ancient mutations in *BRCA1* or *BRCA2* (c.68_69delAG

(185delAG) and c.5266dupC (5382insC) in *BRCA1* and c.5946delT (6174delT) in *BRCA2*) [4].

Fewer reports and data on the prevalence of *BRCA1* and *BRCA2* germline mutations in sporadic breast and ovarian cancer patients are available, since inclusion criteria for BRCA mutation testing are most often based on the family history. A negative family history can be the result of a small family size, predominance of males in the family, or incomplete penetrance [5]. For sporadic patients, genetic testing is often limited to patients with early age at onset, bilaterality or tumor characteristics like multifocality, multicentricity, and triple-negative breast tumors, as these features increase the probability to detect a germline *BRCA1* or 2 mutation.

Despite the high number of *BRCA1/2* mutations identified, de novo BRCA mutations are rare. To the best of our knowledge, to date only two de novo mutations in *BRCA1* are reported [6, 7] and five in *BRCA2* [8–12]. All these mutations were identified in patients with tumors occurring before the age of 40.

In this study, we present our data on the prevalence of *BRCA1&2* mutations in 193 sporadic Belgian breast and/or ovarian cancer patients. Furthermore, we present the identification of a de novo *BRCA1* mutation in a patient diagnosed with breast cancer and ovarian cancer in her fifties.

Materials and methods

Patient population

The patients evaluated in this study are a subgroup of all Belgian patients referred to our center for diagnostic testing for *BRCA1* and *BRCA2*. All the tested individuals provided a signed informed consent after appropriate genetic counseling. Personal and family histories of all patients were recorded, including ages of diagnosis of all cancers.

We investigated 193 breast and/or ovarian cancer patients without any family history for the disease. The majority of the study population (128 patients) are unilateral breast cancer patients who were selected because of an early age of onset (<40 years). Additionally, we analyzed 12 unilateral breast cancer patients with a later age at onset (Dx > 40). Furthermore, we investigated the *BRCA1/2* coding region in 21 bilateral breast cancer patients and six ovarian cancer patients with an age at diagnosis <50. Male breast cancer patients ($n = 17$) and patients who developed breast in combination with ovarian cancer ($n = 9$) were selected without limitations based on the age of onset.

Mutation detection in *BRCA1/2*

Genomic DNA was isolated from peripheral blood mononuclear cells of all patients. The complete coding sequence

and flanking splice site regions of *BRCA1/2* were evaluated, by a wide variety of techniques, since these patients were selected retrospectively. Analysis of the samples was performed with a combination of techniques: Protein Truncation Test (PTT) [13], Denaturing Gradient Gel Electrophoresis (DGGE) [14], High Resolution Melting Curve Analysis (HRMCA) [15, 16], or Sanger sequencing.

In addition, large intragenic rearrangements in *BRCA1* and *BRCA2* were evaluated with Multiplex Ligation Probe Amplification (MLPA).

Paternity testing

We identified a *BRCA1* mutation c.3494_3495delTT (p.Phe1165fs) in one of the patients, which was absent in both parents. Paternity was verified using Powerplex16 assay (Promega). The PowerPlex[®] 16 System allows the co-amplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin). The system contains the loci Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818. One primer specific for Penta E, D18S51, D21S11, TH01, and D3S1358 is labeled with fluorescein (FL); one primer specific for FGA, TPOX, D8S1179, vWA, and Amelogenin is labeled with carboxy-tetramethylrhodamine (TMR); and one primer specific for Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 is labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxy-fluorescein (JOE). All the 16 loci are amplified simultaneously in a single tube and analyzed on an ABI3730 (Applied Biosystems). Data are analyzed with the Genemapper v1 software. The power of exclusion of this system exceeds 0.999998 in all populations tested.

Microsatellite marker analysis

To determine if a possible de novo mutation (*BRCA1* c.3494_3495delTT) originated on the maternal or paternal allele, the region encompassing the *BRCA1* gene was investigated with microsatellite markers in the parents, sibs, and children of the proband. Twelve microsatellite markers within or flanking *BRCA1* were selected, spanning a 3 cM region. A schematic overview of the used markers and their location is shown in Fig. 1. Primer sequences for amplification of these markers were obtained from the Genome database. The forward primer was labeled with a FAM label and the amplification products were separated with capillary electrophoresis on an ABI3730 (Applied Biosystems). Plots were analyzed with the Genemapper software.

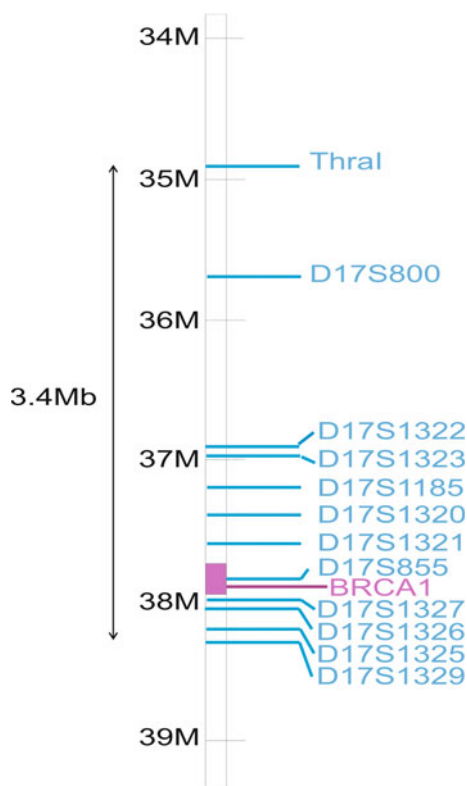


Fig. 1 Schematic overview of the location of the used microsatellite markers. Thirteen microsatellite markers within or flanking *BRCA1* were selected, spanning a 3.4 Mb region

Deep sequencing

Mosaicism in the parents of the patient with the possible de novo mutation (*BRCA1* c.3494_3495delTT (p.Phe1165fs)) was further investigated with ultra deep sequencing. The relevant amplicon was sequenced on a GS-FLX instrument (454-Roche). Data analysis was performed with the commercial available software Nextgene v2. (Softgenetics) [17].

Statistical analysis

Comparison of mutation prevalence between groups was performed with the chi-square test. For the statistical analysis of the mutation prevalence of smaller subgroups ($n < 40$), the Fisher's exact test was used.

Results and discussion

We investigated the complete coding region of *BRCA1* & 2 in 193 breast and/or ovarian cancer patients, without evidence for a family history of the disease. These patients were selected because they presented early onset or bilateral or multifocal tumors. In 17 (8.8%), a deleterious

germline *BRCA1/2* mutation was identified (an overview is shown in Table 1).

In three patients, previously unreported mutations were identified: *BRCA1* c.3494_3495delTT, *BRCA2* c.6948_6949insTT, and *BRCA1* c.2507_2508delAA. These were, therefore, considered as potentially de novo. For two of them parental DNA was available to verify this hypothesis.

For the patient with the *BRCA2* c.6948_6949insTT mutation; Sanger sequencing showed that she inherited the mutation from her father. An autosomal dominant inheritance pattern for this mutation is masked by the predominance of males in this branch of the pedigree (father has only male siblings).

BRCA1 c.3494_3495delTT was identified in a patient diagnosed with breast and ovarian cancer at the age of 52 and 53, respectively. This mutation was detected with high resolution melting curve analysis in our routine diagnostic screening [16]. Sanger sequencing revealed a truncating *BRCA1* mutation c.3494_3495delTT (p.Phe1165fs) which to the best of our knowledge, has not yet been described before (Fig. 2).

The mutation was not detectable in DNA extracted from blood lymphocytes of both parents by Sanger sequencing. Since the lower limit to detect mosaicism with Sanger sequencing is around 15% [18], we investigated possible mosaicism in lymphocytes with ultra deep sequencing on the GS-FLX instrument (454/Roche). The relevant amplicon was deep sequenced for the patient DNA, the maternal and the paternal sample. This ultradeep sequencing allows the identification of low grade mosaic variants [19]. The mutation was detected in 43% of the reads in the patient at 157-fold coverage, but was absent in the maternal and paternal sample both covered 1,840 and 2,780 fold, respectively, consistent with a de novo occurrence. A prevalence of a variant in 43% of all reads is consistent with 50% heterozygosity as heterozygous mutations can be present in 25–60% of the reads [17]. The coverage of 1,840 obtained for the maternal sample allows detection of mosaic mutations present in 5% of the blood lymphocytes with a probability of $\geq 99\%$; the higher coverage of the paternal sample (2,780 \times), results in a lower detection limit of 2% mosaicism with a 99% probability. Deeper coverage would be needed to detect variants with a frequency lower than 2% with a 99% probability.

To determine if the mutation originated on the maternal or paternal allele, the region encompassing the *BRCA1* gene was investigated with 12 microsatellite markers in the parents, sibs, and children of the proband. Fragment analysis revealed that the mutation originated on the paternal allele of the proband. Five of her sibs inherited the same paternal allele but do not carry the mutation, providing additional evidence for the de novo occurrence of this mutation (data are shown in Fig. 3).

Table 1 Overview of the different *BRCA1* and *BRCA2* mutations detected in the selected population

ID	Age at onset	Diagnosis	Mutation HGVS nomenclature (BIC nomenclature)			
32-0868	37	Breast cancer	BRCA1 c.2197_2201del5 (2316del5)	BRCA1 p.Glu733 fs	Belgian founder mutation	Parental DNA not available ^a
32-0778	31	Breast cancer	BRCA1 c.2380dupG (2478insG)	BRCA1 p.Glu787fs	Belgian founder mutation	Paternal inheritance
32-0331	27	Breast cancer	BRCA1 c.2507-2508delAA (2626-2627delAA)	BRCA1 p.Glu836fs	Previously not reported in literature	Parental DNA not available
32-0368	27	Breast cancer	BRCA1 c.2603C>G (2722C>G)	BRCA1 p.Ser868X	Previously reported in British population	Paternal inheritance
32-0452	38	Breast cancer	BRCA1 c.3607C>T (3726C>T)	BRCA1 p.Arg1203X	Belgian founder mutation	Parental DNA not available ^a
32-1047	29	Breast cancer	BRCA1 c.5075-?_5194+?del		In the Italian population a founder deletion spanning exons 18–19 is reported ^b as we did not determine the breakpoints in our patient, it is not clear if this is the same mutation	Paternal inheritance
32-0110	32	Breast cancer	BRCA1 c.212+3A>G (IVS5+3A>G)	Non coding	Belgian founder mutation	Paternal inheritance
32-1219	33	Breast cancer	BRCA1 c.212+3A>G (IVS5+3A>G)	Non coding	Belgian founder mutation	Maternal inheritance
32-0080	34	Breast cancer	BRCA1 c.212+3A>G (IVS5+3A>G)	Non coding	Belgian founder mutation	Maternal inheritance
32-1279	32	Breast cancer	BRCA2 c.4936_4939del4 (5164del4)	BRCA2 p.Glu1646fs	Previously reported in several populations	Parental DNA not available
32-0982	35	Breast cancer	BRCA2 c.6275_6277delTT (6503delTT)	BRCA2 p.Leu292fs	Belgian founder mutation	Maternal inheritance
32-0288	37	Breast cancer	BRCA2 c.6275_6277delTT (6503delTT)	BRCA2 p.Leu292fs	Belgian founder mutation	Parental DNA not available
32-0876	52 and 53	Breast and ovarian cancer	BRCA1 c.3494_3495delTT (3613-3614delTT)	BRCA1 p.Phe1165fs	Previously not reported in literature	Novel de novo mutation
32-0048	53 and 55	Breast and ovarian cancer	BRCA1 c.3661G>T (3780G>T)	BRCA1 p.Glu1221X	Belgian founder mutation	Parental DNA not available
32-0612	43 and 57	Breast and ovarian cancer	BRCA2 c.469_470delAA (697delAA)	BRCA2 p.Lys157fs	Previously reported in WECARE study ^c	Parental DNA not available
32-1077	45 and 45	Bilateral breast cancer	BRCA2 c.6948insTT (7176insTT)	BRCA2 p.Phe2355fs	Previously not reported in literature	Paternal inheritance
32-0924	36 and 45	Bilateral breast cancer	BRCA1 c.134+3A>C (IVS3+3A>C)	Non coding	Belgian founder mutation	Parental DNA not available

^a Mutation was detected in one of the patients' siblings, hence a de novo event can be ruled out

^b See ref [39]

^c See ref [40]

Our data suggest a de novo event in a testicular germ cell, although a zygotical origin cannot be excluded. This is in agreement with previous findings in de novo mutations

in *BRCA1* and *BRCA2*, in which the paternal origin could be ascertained [8, 12]. Of note, the age of the patients' father was only 29 years at the time of birth of his

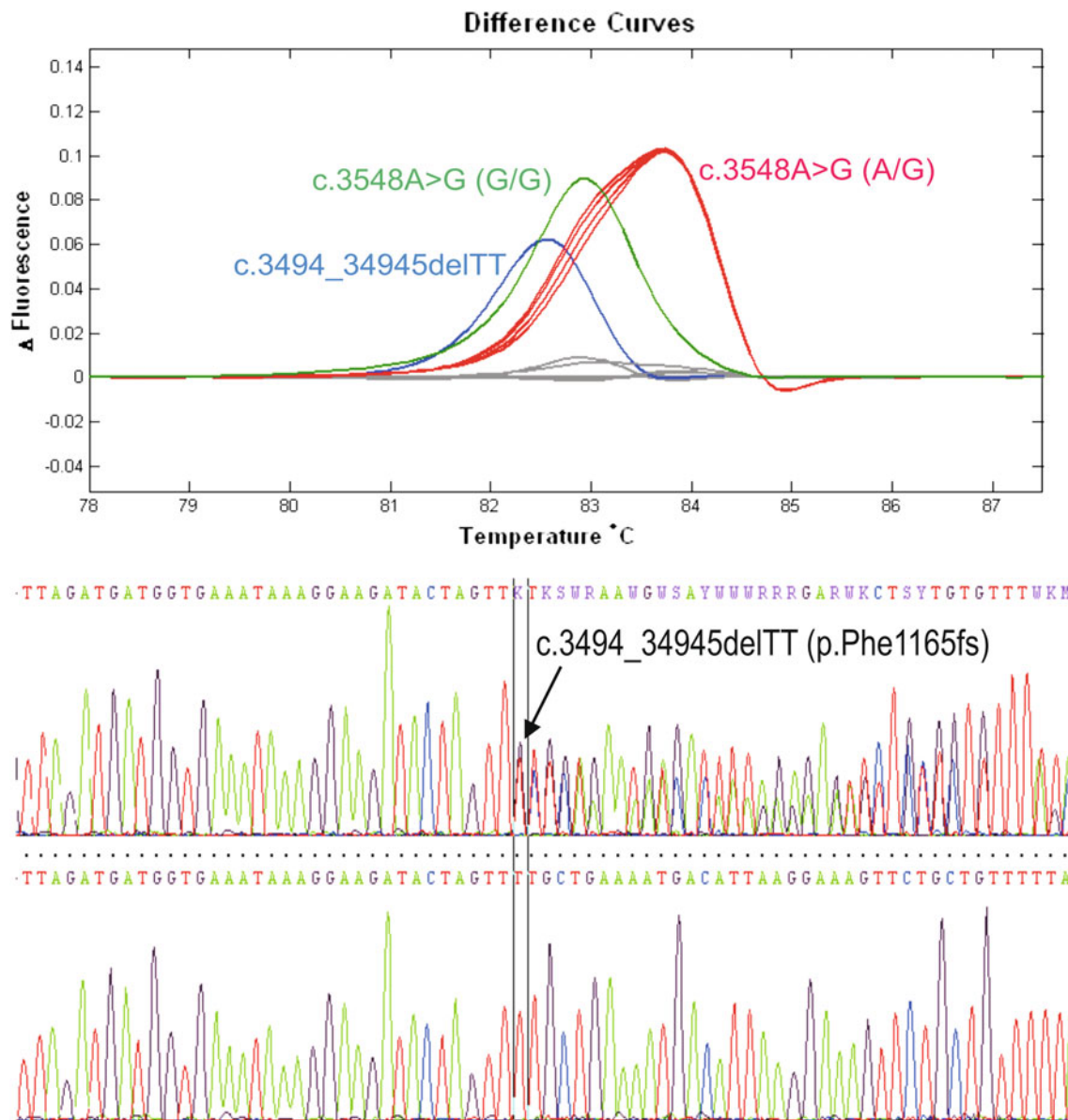


Fig. 2 Difference plot and Sanger sequencing of *BRCA1* c.3494_3495delTT (p.Phe1165fs). Aberrant melting curves by high resolution melting curve analysis for one of the amplicons in exon 11 were detected in our routine diagnostic screening. A frequent

polymorphism *BRCA1* c.3548 A>G was identified in most of the samples. Sanger sequencing revealed a truncating *BRCA1* mutation c.3494_3495delTT (p.Phe1165fs) in the sample

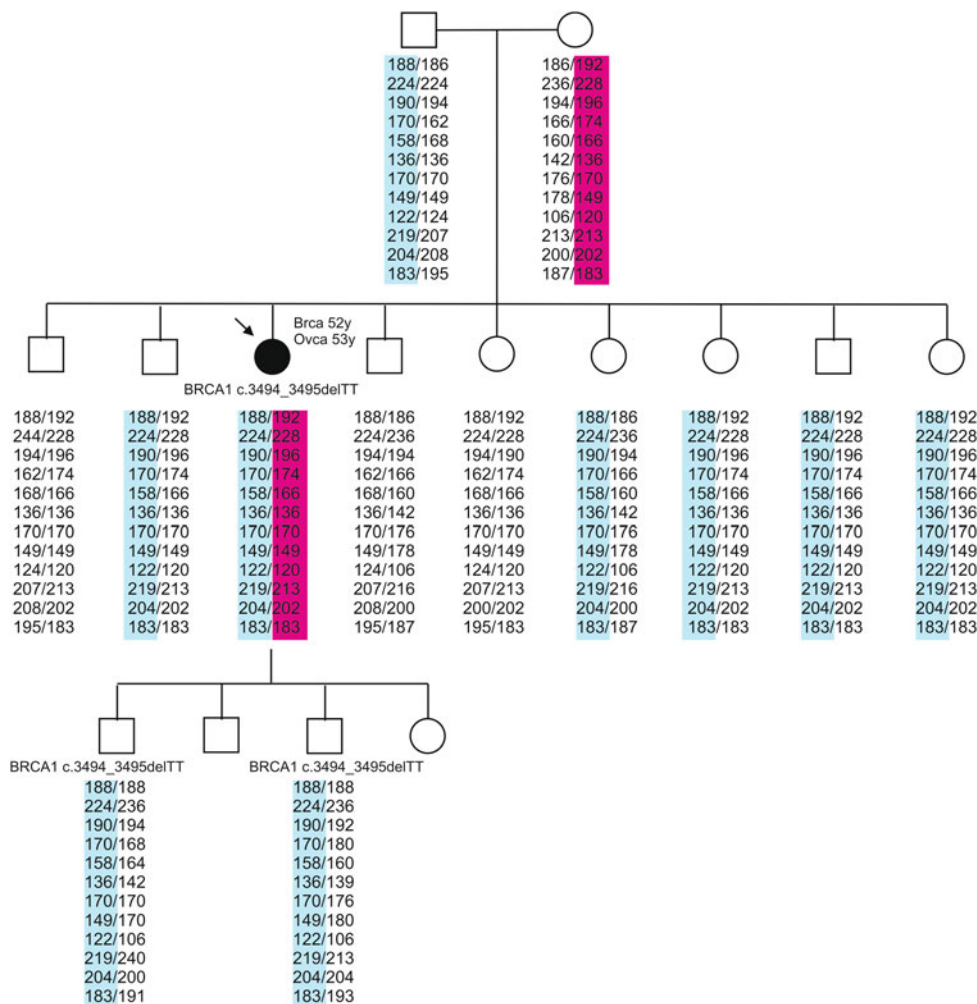
daughter. It has been suggested that the origin of new mutations may be influenced by both genomic imprinting effects and the increased number of cell divisions in spermatogenesis compared with oogenesis. A mainly paternal origin of mutations is seen in other hereditary syndromes as well, for instance in *FGFR3*, *FGFR2*, and *RET*, de novo mutations have been analyzed and all shown to be of paternal origin [20–22].

Not all disorders show such an extraordinary paternal bias, some single gene traits show only a moderate paternal bias in the male:female mutation ratio and a small paternal age effect. An example is neurofibromatosis type 1.

The *NF1* gene has one of the highest mutation rates. Many of the mutations are intragenic deletions, which are more common in larger genes. Whereas base substitutions occur primarily in males and are age-dependent, small chromosomal changes (intragenic deletions) are not age-dependent because they occur by different mechanisms [23].

This is the first de novo *BRCA1* mutation identified in a patient with age of onset for the disease after the age of 50. In all patients previously reported with de novo mutations, breast tumors occurred at young age (<40 years), which may reflect recruitment bias, since genetic testing in sporadic cases is most often limited to patients with early onset

Fig. 3 Microsatellite marker investigation of the de novo mutation *BRCA1* c.3494_3495delTT. To determine if the mutation originated on the maternal or paternal allele, the region encompassing the *BRCA1* gene was investigated with microsatellite markers in the parents, sibs, and children of the proband. Fragment analysis revealed that the mutation originated on the paternal allele of the proband. Five of her sibs inherited the same paternal allele but do not carry the mutation, providing additional evidence of the de novo occurrence of this mutation



breast or ovarian cancer. To our knowledge, only two other de novo *BRCA1* mutations have been reported to date. The prevalence of de novo *BRCA1* and *BRCA2* mutations is still unknown and making a reliable estimation is challenging. Taking into account that recurrent *BRCA1/2* mutations might appear as de novo alterations, might increase the prevalence to a higher rate than generally accepted [12]. As we could not evaluate parental DNA for all mutations detected in our sporadic patient population, the prevalence we observed might be an underestimation.

The majority of the mutations identified in this study (10/17), are well known founder mutations in our population. Although the Belgian population has a mixed ethnicity, we do see a considerable number of founder mutations in our population [24, 25]. In our overall patient population, 58 distinct mutations were detected, 17 of those represent approximately 75% of the 206 *BRCA1/2* mutations identified to date in our center. Ten of the 17 mutations detected in this study are part of this top 17. Three mutations (*BRCA1* p.Ser868X, *BRCA2* c.4936_4939del4, and *BRCA2* c.469_470delAA) are less prevalent in the

Belgian population, but are reported in literature as founder mutations in other populations (Table 1).

We detected *BRCA1/2* mutations in 189 of 1,170 (17%) unrelated index patients with a family history for breast/ovarian cancer referred to our center for genetic testing. This is statistically significantly higher than in our sporadic group (17/193 = 8.8%) ($P < 0.01$), demonstrating the relevance of inclusion criteria based on a family history. However, some subgroups of sporadic patients investigated in this study revealed a much higher frequency (see below and Table 2), indicating the importance of genetic testing for these patients.

Mutation analysis in 140 patients with unilateral breast cancer led to the identification of 12 germline mutations (8.6%). However, all the 12 mutations were detected in the subgroup of 128 patients diagnosed before the age of 40 (9.4%), of which three in the group of 55 patients diagnosed with unilateral breast cancer between the age of 35 and 40. Therefore, we have no evidence for a contribution of inherited *BRCA1/2* mutations in unilateral breast cancer patients diagnosed in their forties, however, this observation

Table 2 Overview of all patients investigated and mutations detected

	Mean age at diagnosis (range)	# Mutations detected	Mean age at diagnosis mutation carriers (range)
193 Sporadic breast cancer patients			
17 Male breast cancer patients	56 years (25–72)	0%	
176 Female breast and/or ovarian cancer patients	36 years (24–68)	8.80%	
Dx < 40			
128 Unilateral breast cancer patients	33 years (24–40)	9% (9 in <i>BRCA1</i> and 3 in <i>BRCA2</i>)	33 years (27–38)
5 Ovarian cancer patients	29 years (25–35)	0%	
12 Bilateral breast cancer patients	Dx1 = 35 years/Dx = 39 years (Dx1: 29–39/Dx2: 30–50)	8% (1 in <i>BRCA1</i>)	Dx1: 36 years/Dx2: 45 years
2 Patients with breast and ovarian cancer	Dx1 = 39 years/Dx = 46 years (Dx1: 38–40/Dx2: 43–49)	0%	
Dx > 40			
12 Unilateral breast cancer patients	44 years (41–49)	0%	
1 Ovarian cancer patient	49 years	0%	
9 Bilateral breast cancer patients	Dx1 = 46 years/Dx = 49 years (Dx1: 42–49/Dx2: 45–53)	11% (1 in <i>BRCA2</i>)	Dx1: 45 years/Dx2: 45 years
7 Patients with breast and ovarian cancer	Dx1 = 55 years/Dx = 60 years (Dx1: 43–68/Dx2: 47–72)	30% (2 in <i>BRCA1</i> and 1 in <i>BRCA2</i>)	Dx1: 49 years/Dx2: 55 years (Dx1: 43–53/Dx2: 53–57)

is based on the analysis of only 12 patients. In the subgroup of 30 patients with at least two *BRCA1/2* associated tumors (bilateral breast cancer or breast and ovarian cancer), we identified five mutations (16.7%). Interestingly, mutations were more prevalent in the patients diagnosed with their first tumor after the age of 40 (4/16 = 25%) compared to the group diagnosed before 40 (1/14 = 7.1%), however, the difference is not statistically significant ($P = 0.36$). Especially in patients with both breast and ovarian cancer ($n = 9$), a high mutation detection ratio was obtained (3/9 = 30%), even with a diagnosis at a more advanced age. Therefore, testing these patients is mandatory, independent of the age of onset. An overview of the prevalence of *BRCA1/2* mutations in the different subgroups of our study population is given in Table 2.

The average age to develop breast cancer caused by a germline mutation is calculated to be 42 years for *BRCA1* and 49 years for *BRCA2* [26]. In *BRCA1* mutation carriers, ovarian cancer rarely occurs before the age of 40 years, while 50 years seem to be a critical age for ovarian cancer in *BRCA2* mutation carriers [26, 27]. In only two of our sporadic patients diagnosed with breast cancer before the age of 40 a *BRCA2* mutation was identified (2x *BRCA2* c.6280_6281delTT), in all other cases mutations in *BRCA1* were detected. This is in agreement with the study of Peto et al. [28], who concluded that *BRCA1* mutations may be more penetrant at young ages compared to *BRCA2* mutations. Average age at diagnosis for breast cancer patients in

whom a *BRCA1* mutation was identified in our study population was 35.8 years [range: 27–53] compared to 38.4 years [range: 32–45] for *BRCA2* mutations. Young age at primary breast cancer diagnosis is associated with an increased susceptibility to bilateral breast cancer, arguably due to the remaining life span and the resulting interval at which the patient remains at risk for a second primary tumor. In total, we investigated 21 patients diagnosed with bilateral breast cancer. All of these cases were diagnosed with their first breast cancer before the age of 50, but in only two patients a pathogenic *BRCA* mutation was identified.

We investigated the complete *BRCA1/2* coding sequence in six sporadic ovarian cancer patients. Five of them were diagnosed with ovarian cancer at a young age (range 25–35 years) but no *BRCA1* or *BRCA2* mutations were detected. The estimated prevalence of *BRCA1* mutations in familial ovarian cancer patients is 5% [29]. Although genetic testing to these five patients was offered because of their very young age of onset, other genetic factors may have contributed to the tumor development.

We identified no *BRCA1/2* mutations in 17 sporadic male breast cancer patients (mean age at diagnosis: 52, range 25–72). As male breast cancer is rare, genetic testing is offered to these patients since it is believed to result from genetic susceptibility. Population and clinic based studies estimate the prevalence of *BRCA2* mutations in males from 4% up to 40% depending on the ethnic background of the

studied population [30–34]. One extreme example is the Icelandic population where 40% of all male breast cancers diagnosed carry the *BRCA2* 999del5 (*BRCA2* c.771_775del) mutation. This is mainly due to a founder effect and is very unlikely to be replicated in other populations. The absence of *BRCA2* mutations in our male sporadic breast cancer patients might be due to small sample size since we detected a *BRCA2* mutation in 14% of all males with a family history for the disease referred to our center for genetic testing.

A *BRCA1/2* mutation occurrence of ~10% is in agreement with previously reported findings in young (age at diagnosis <45 years) sporadic breast cancer patients in different populations [28, 35, 36]. However, prevalence is population dependent; for instance, the prevalence of *BRCA1/2* mutations in British sporadic patients is lower (<4%) [37].

The ratio of *BRCA1* to *BRCA2* mutations varies widely between subpopulations globally. In this study, the prevalence of *BRCA1* mutations is higher compared to *BRCA2* mutations: 70% (12/17) of the mutations detected in the sporadic patients are present in *BRCA1*, this is higher than the prevalence of *BRCA1* mutations (115/189 = 61%) in patients with a family history of breast/ovarian cancer, however, the difference is not statistically significant ($P < 0.2$). This increased prevalence of *BRCA1* mutations can be explained by the higher penetrance of *BRCA1* mutations in patients mainly selected on early age of onset, but also to founder effects, since the two most prevalent mutations (c.2380dupG and c.212+3A>G) in our population are both *BRCA1* mutations.

Several tools to predict the likelihood for a patient to carry a mutation have been developed, unfortunately for most of them risk estimation is based on a family history for the disease. Important and widely used examples are the Claus, Tyrer-Cuzick, and BRCAPRO model but these cannot be reliably used for patients without any family history for breast or ovarian cancer. Our data and other studies demonstrate that these women may have a significant risk to carry a *BRCA1* or *BRCA2* mutation. Suggestions to adopt or expand the inclusion criteria for sporadic breast cancer patients have already been made [38]. Kwon et al. [38] suggest offering genetic testing to all women with triple-negative breast cancers diagnosed before the age of 50. Our study shows that a cut-off based on the age of onset cannot be used as the sole selection criterion for genetic testing of sporadic breast/ovarian cancer patients.

Conclusion

Selection of breast cancer patients based on the family history will not allow detection of all *BRCA1/2* carriers that

may benefit from preventive interventions. However, formulating a general cost effective set of guidelines to identify all sporadic *BRCA1/2* mutation carriers is challenging. Our study proves that *BRCA1/2* mutation detection in sporadic patients is worthwhile, even with diagnoses at later age, especially in patients with two tumors. The identification of a de novo *BRCA1* mutation in a sporadic patient diagnosed with breast and ovarian cancer in her fifties, suggests that the prevalence of *BRCA1/2* de novo mutations may currently be underestimated.

Acknowledgments This project was realized with the funding of an Emmanuel van der Schueren scholarship of the Flemish foundation against cancer to Kim De Leeneer. This research was supported by grant 1.5.150.07 from the Fund for Scientific Research Flanders (FWO) to Kathleen Claes and by GOA grant BOF10/GOA/019 (Ghent University). Bruce Poppe is a senior clinical investigator from FWO.

Conflict of interest None.

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