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Abstract

Background. Invasive lobular carcinoma (ILC) of the breast has epidemiological, molecular and clinical specificities, and should likely be considered a unique entity. As for genetic susceptibility, *CDH1* germline mutations predispose exclusively to ILC. Data are however scarce regarding ILC in women with *BRCA1/2* (Hereditary Breast and Ovarian Cancer) and *TP53* (Li-Fraumeni syndrome) germline mutations. **Methods.** We included all breast cancers from female patients tested at our Institute between 1992 and 2016 (n=3469) for which pathology data were available. ILC proportion comparison according to mutational status was performed by a Chi-squared test. The impact of susceptibility genes on ILC proportion was investigated by univariate logistic regression with wild-type patients as reference. **Results and Discussion.** There were 265 (7.64%) ILC: 2/342 (0.58%) in *BRCA1* patients, 24/238 (10%) in *BRCA2* patients, 1/57 (1.75%) in *TP53* patients and 238/2832 (8.4%) in non-carriers. The majority of breast cancers in all groups were invasive ductal and ductal *in situ* carcinomas. The difference in ILC proportion was highly significant (p<0.001). Compared to wild-type patients, *BRCA1* was associated with a lower ILC proportion (OR 0.064 [95%CI 0.016;0.259], p<.0001). *BRCA2* OR was 1.222 [95%CI 0.785;1.902] (p=0.374), *TP53* OR was 0.195 [95%CI 0.027;1.412] (p=0.105). ILC are therefore underrepresented in *BRCA1* and *TP53* mutation carriers. Formal significance (p=0.05) was not reached for *TP53*, but statistical power was only 38%. Based on ILC incidence in the general population, we make the hypothesis that *BRCA1* and *TP53* do not predispose to ILC, as the few occurrences of ILC in mutation carriers could be attributed to chance and not to germline mutations. Our observations will be useful to clinical cancer geneticists managing patients with ILC, as a *BRCA1* or *TP53* mutation in these patients would be unlikely. Genetic counselling should be adapted accordingly,

Introduction

Invasive lobular carcinomas (ILC) represent about 10% of invasive breast cancers [1,2], while the remainder are mostly ductal carcinomas (IDC), also called invasive carcinomas of no special type. Recent data support the notion that ILC is a disease with epidemiological, molecular and clinical specificities, and that it should be considered a unique entity among breast cancers. For example, menopausal hormone therapy is more strongly related to the risk of ILC compared to IDC, regardless of hormone receptor status [3]. In a comprehensive molecular profile of 817 breast cancers, Ciriello *et al.* observed CDH1 and PTEN loss, AKT activation, and mutations in *TBX3* and *FOXA1* in ILC, and showed that these profiles were not seen in IDC [4]. Finally, in the BIG 1-98 study, the magnitude of benefit of adjuvant hormone therapy was greater for patients diagnosed with ILC versus IDC [5].

As for genetic susceptibility to breast cancer, there are plenty of gaps to fill with regard to ILC. While it is now accepted that germline mutations in *CDH1,* the breast and diffuse gastric cancer susceptibility gene, predispose exclusively to ILC [6,7], data are scarce regarding women with germline mutations in the other major genes, more specifically *BRCA1/2* (hereditary breast and ovarian cancer) and *TP53* (Li-Fraumeni syndrome). For example, even the largest and most comprehensive work on histopathological breast cancer features in *BRCA1/2* mutation carriers only focused on hormone receptors and grade without making any mention of type [8]. Of note, *CDH1* is the only ILC-specific susceptibility gene, along with a common, low-penetrance polymorphism at the 7q34 locus [9].

In this study, we collected data on 3469 consecutive breast cancers diagnosed in female patients. We compared 637 cancers in carriers of *BRCA1, BRCA2* and *TP53* germline mutations with 2832 cancers in non-carriers (wild-type patients) regarding ILC frequency. We show that ILC is underrepresented in carriers of *BRCA1* and *TP53* mutations. Given the rarity of ILC in these patients, we also make the hypothesis that their risk of ILC is close to, if not similar, to the general population risk. Our observations strengthen the hypothesis that even though both are invasive epithelial breast carcinomas, ILC and IDC are different diseases.

Patients and methods

We included all consecutive invasive and ductal *in situ* breast cancers diagnosed in female patients tested at our Cancer Genetics Clinic (Gustave Roussy Cancer Institute, Villejuif, France) between 1992 and 2016, and for which pathology data were available. Patients had been referred to us when cancer susceptibility was suspected, e.g. breast cancer ≤ age 40, breast cancer with one relative with breast cancer ≤ 50, or breast cancer with a relative with ovarian cancer for *BRCA1/2* , classical Li-Fraumeni criteria, Chompret criteria, or breast cancer ≤ age 30 for *TP53* [10,11]. Informed consent was obtained, and germline DNA testing was performed using different methods depending on the period, e.g. SSCP, fluorescence, Sanger and NGS sequencing, qPCR and MLPA.

Statistical analyses were carried out with the SAS software (SAS Institute Inc., Cary, NC, USA.). The significance level was set at 0.05. The comparison of ILC proportion according to mutational status was performed by a Chi-squared test. The impact of susceptibility genes on ILC proportion was investigated by univariate logistic regression with wild-type patients as reference. Results were expressed by odds ratio (OR) and 95% confidence interval.

Results

We included 3469 breast cancers. Of these, 637 (18.36%) were from mutation carriers: 342 (9.86%), 238 (6.86%) and 57 (1.64%) from *BRCA1, BRCA2*, and *TP53* patients respectively. The remaining 2832 had been diagnosed in wild-type patients. There were 265 (7.64%) ILC: 2/342 (0.58%) in *BRCA1* patients, 24/238 (10%) in *BRCA2* patients, 1/57 (1.75%) in *TP53* patients and 238/2832 (8.4%) in non-carriers (table 1). The difference in ILC proportion according to the mutational status was highly significant (p<0.001). Compared to wild-type patients, *BRCA1* was significantly associated with a lower proportion of ILC (OR 0.064 [95%CI 0.016 ; 0.259], p<.0001). *BRCA2* OR was 1.222 [95%CI 0.785 ; 1.902] (p=0.374) and TP53 OR was 0.195 [95%CI 0.027 ; 1.412] (p=0.105). As expected, the overwhelming majority of breast cancers in all groups were IDC and ductal *in situ* carcinomas: 327/342 (96%) in *BRCA1* patients, 54/57 (95%) in *TP53* patients, 204/238 (86%) in *BRCA2* patients and 2490/2832 (88%) in non-carriers, the remainder being mostly rare types such as medullary, mucinous, papillary or tubular carcinomas, there was also one case of mixed ductal-lobular cancer (in a *BRCA1* patient), and obviously the above-mentioned ILC.

Discussion

In this retrospective study of nearly 3500 breast cancers, the difference in ILC proportion according to the mutational status was highly significant. ILC was significantly underrepresented in *BRCA1* mutation carriers compared to wild-type patients with an OR of 0.064 (p<0.0001). *TP53* germline mutations were associated with an 80% reduction in the odds of ILC (OR=0.195), admittedly with a p-value of 0.105, meaning we came close but did not reach significance. That should not be a reason to dismiss our results as we only had 38% statistical power to detect a difference at the consensus 0.05 level. One hundred and twelve breast cancers in patients with *TP53* mutations out of a total sample size of 6769 would have been required in order to have 80% power, but that would be hard to achieve without multicentre collaborations considering the rarity of Li-Fraumeni. Furthermore, the OR were lower than 0.3 and therefore indicate a strong relationship with ILC, suggesting a real, meaningful difference between the two groups [12]. There were no differences for *BRCA2.*

Surprisingly, the issue of breast cancer type in patients with genetic susceptibility to the disease is hardly addressed in the literature, the most striking example being the large, multi-

consortia study by Spurdle *et al.* published in 2014 and based on 54607 breast cancer cases [8]. The authors compared hormone receptor, HER2, CK5/6 status, and grade in *BRCA1* patients, *BRCA2* patients and non-carriers, but nowhere in the article did they mention cancer type. In an earlier paper, the CIMBA consortium reported, amongst other characteristics, breast cancer type and showed that ILC was more likely to be associated with *BRCA2* compared to *BRCA1* [13]. However, this observation was apparently considered of minor importance since it did not feature in the discussion. Furthermore, there was no comparison with non-carriers. On the contrary, we did have control subjects (our non-carriers) from the same population as cases and recruited under similar conditions. There is a similar phenomenon regarding Li-Fraumeni. Two recent studies evaluated 415 French and 286 US patients, and reported 172 and 118 breast cancers, respectively [14,15]. No mention was made of tumour type. In 2012, Masciari *et al.* performed a central review of 43 breast cancers from Li-Fraumeni patients and only observed ductal histologies [16]. Like in the CIMBA study, the absence of ILC was not addressed in the discussion, neither was it in the abstract, and the emphasis was put on hormone receptor and HER2 status. There was no comparison with tumours in non-carriers either.

Somatic studies support our observations. Indeed, in large-scale molecular portraits of ILC, alterations in both *BRCA1* and *TP53* are rare in ILC, and underrepresented compared to IDC [4,17]. Only two *BRCA1* and 31 *TP53* mutations were observed in 413 ILC in the Desmedt paper vs. for example 285 and 182 in the classical ILC genes *CDH1* and *PIK3CA*. In the Ciriello paper, *TP53* alterations were present in 8% of ILC vs. 44% of IDC ($q = 1.9e^{-14}$), there were no data for *BRCA1.* These molecular portraits show that different oncogenic pathways are involved in ILC/IDC development, and that *BRCA1* and *TP53* are likely of minor importance in ILC.

The question raised by our results is whether *BRCA1* and *TP53* predispose to ILC at all. Our data do not allow for definitive conclusions, but we make the hypothesis they do not. Indeed, estimates for average annual breast cancer incidence in the general population are 118/100'000 [18]. For *BRCA1,* our patients had an average age of 51.3 at last follow up. Combining incidence and last follow up, nineteen breast cancers would have been expected, two of them (10%) ILC. This is exactly the observed number in our study. As for *TP53,* it is conceivable that one single occurrence of ILC could be due to chance, even more so considering that the only patient with ILC was diagnosed at the age of 55, while breast cancers in Li-Fraumeni are commonly seen in very young patients [14].

Our observations have direct implications for clinical cancer geneticists. Indeed, they will now be aware that a *BRCA1* or *TP53* mutation is unlikely in a patient with ILC. Genetic counselling should be adapted accordingly, i.e. no mention of a possible >40% lifetime risk of ovarian cancer as seen with *BRCA1* or of a potential risk of multiple cancers regardless of age as seen in Li-Fraumeni

syndrome [19]. Patients might experience reduced stress levels as a result, considering the well-documented physical and psychological consequences of ovarian and of Li-Fraumeni-associated cancers [20-22].

Compared to other cancers (IDC included), ILC shows unusually high levels of familial clustering, pointing towards a major contribution of genetic, inherited factors to the disease [23]. Only one clinically relevant ILC-specific susceptibility gene, *CDH1,* has been identified so far. The possibly null, at best minor, implication of *BRCA1* and *TP53* in ILC causality tells us there is a lot to discover regarding genetic susceptibility to ILC. We hope whole exome sequencing studies in ILC families will soon provide an answer. Our observations on the genetic specificities of ILC are all the more relevant today. Indeed, researchers and clinicians are finally starting to give ILC the importance it deserves, as illustrated for example by the first International ILC Symposium held last year in Pittsburgh (https://upci.upmc.edu/wcrc/ilcsymposium-info.cfm). Even though both are invasive epithelial breast carcinomas, ILC and IDC should likely be considered different diseases.

	No mutation	***BRCA1***	***BRCA2***	***TP53***	**Total**
Total breast cancers	2832	342	238	57	3469
ILC	238 (8.40%)	2 (0.58%)	24 (10%)	1 (1.75%)	265
Other histologies	2594	340	214	56	3204
OR [95%CI]	1 (ref)	0.064 [0.016;0.259]	1.222 [0.785;1.902]	0.195 [0.027;1.412]	
p-value		**<0.001**	0.374	0.105	

Table 1. Invasive lobular carcinoma (ILC) and other types according to germline mutations in major susceptibility genes. Odds Ratios (OR) represent the risk of ILC in mutation carriers vs. patients with no mutation.

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