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Adiposity is associated with *p53* gene mutations in breast cancer

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Abstract

Purpose—Mutations in the *p53* gene are among the most frequent genetic events in human cancer and may be triggered by environmental and occupational exposures. We examined the association of clinical and pathological characteristics of breast tumors and breast cancer risk factors according to the prevalence and type of *p53* mutations.

Methods—Using tumor blocks from incident cases from a case-control study in western New York, we screened for *p53* mutations in exons 2–11 using the Affymetrix *p53* Gene Chip array and analyzed case-case comparisons using logistic regression.

Results—The *p53* mutation frequency among cases was 28.1%; 95% were point mutations (13% of which were silent) and the remainder were single base pair deletions. Sixty seven percent of all point mutations were transitions; 24% of them are G:C>A:T at CpG sites. Positive *p53* mutation status was associated with poorer differentiation (OR, 95% CI: 2.29, 1.21–4.32), higher nuclear grade (OR, 95% CI: 1.99, 1.22–3.25) and increased Ki-67 status (OR, 95% CI: 1.81, 1.10–2.98). Cases with *P53* mutations were more likely to have a combined ER-positive and PR-negative status (OR, 95% CI: 1.65, 1.01–2.71), and a combined ER-negative and PR-negative status (OR, 95% CI: 2.18, 1.47–3.23). Body mass index>30 kg/m², waist circumference>79 cm, and waist-to-

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hip ratio > 0.86 was also associated with *p53* status; obese breast cancer cases are more likely to have *p53* mutations (OR, 95% CI: 1.78, 1.19–2.68).

Conclusions—We confirmed that *p53* mutations are associated with less favorable tumor characteristics and identified an association of *p53* mutation status and adiposity.

Keywords

p53 mutation; breast cancer; adiposity

Introduction

p53 is a multifunctional tumor suppressor gene that plays a central role in deciding cellular fate after DNA damage by arresting cell cycle and allowing DNA repair or inducing apoptosis (1–3). In sporadic breast cancers, *p53* is mutated in about 25% of all tumors (4). Mutation frequencies are higher than 80% in the basal-like breast cancer subtype (5, 6) and range from 43–62% in triple negative breast cancer (7).

P53 mutations are a hallmark of many cancers, and the majority of them are point missense mutations in the coding region (8–11). More than 90% of *p53* mutations are clustered in exons 4–10 and correspond to the DNA binding domain of the *p53* protein (4). The spectrum and distribution of *p53* mutations in sporadic breast cancer is similar to other cancers, especially lung cancer, with respect to the substantial rate of G:C to T:A transversions, and similar to colon cancer with respect to the increased rate of G:C to A:T transitions, deletions and insertions (8, 9, 12).

Several studies have measured nuclear staining of *p53* as a proxy for *p53* gene alterations in breast cancer, and reported associations with poor outcomes and worse pathological characteristics, such as hormone receptor negative, HER2 positive, high grade, and high proliferative tumors (8). However, as DNA sequencing is the gold standard for detecting mutations, other studies have performed mutation screening followed by direct sequencing of *p53* exons 2–11 (8). More recent work involves next-generation sequencing for detection of *p53* mutations (13). In general, *p53* mutations are independent predictive factors of poor prognosis for breast cancer survival; however, more recent studies indicate that certain specific *p53* mutations located in the DNA binding domain are associated with even lower survival rates (14–17). Therefore the full spectrum of *p53* mutation needs to be studied more specifically with regard to clinical characteristics and outcomes.

Although there is clear evidence that *p53* mutations are critical to breast carcinogenesis, little is known about the determinants and time course of these mutations. Several studies have described the presence of *p53* mutations in ductal carcinoma *in situ* lesions, suggesting that these mutations are an early event in the carcinogenic process (18–20). Specific patterns of *p53* mutations have been linked to certain exposures in several other cancer types providing etiological clues, including aflatoxin exposure for liver cancer, smoking for lung cancer and sun exposure for skin cancer (9, 21–24). There are several studies which have investigated the relationship between *p53* mutations and exposures in breast cancer likely to

be important earlier in the carcinogenic process, although there are no consistent findings from these reports (25–29).

In the present study we characterized the prevalence and spectrum of *p53* mutations in a large sample of breast cancer tumors to study the association of *p53* mutations and markers of tumor aggressiveness, and breast cancer risk factors to determine if any particular exposures are differentially associated with particular *p53* mutations.

Materials and Methods

Study population

All subjects were part of the Western New York Exposures and Breast Cancer (WEB) study, a population-based case-control study of breast cancer conducted between 1996–2001, described in detail elsewhere (30). Analyses presented here were restricted to cases from that study. Briefly, cases were residents of Erie and Niagara Counties, 35–79 years old, with pathologically confirmed primary, incident breast cancer and no history of cancer other than non-melanoma skin cancer.

Data on demographics, breast cancer risk factors and anthropometric measures were obtained through extensive in-person interviews and self-administered questionnaires. Medical data, including clinical and pathological information on tumors were abstracted from medical records using a standard protocol. We obtained archived tissue blocks for 920 cancer cases in the WEB study, and only tissue blocks with tumor tissue in them were analyzed (n=734). A senior pathologist from Georgetown University confirmed the pathological diagnosis of breast cancer based on the tumors. All subjects provided informed consent and the study was approved by the Institutional Review Boards of the University at Buffalo, Georgetown University and all participating hospitals.

Hormone receptor status

Estrogen receptor status, progesterone receptor status, Ki-67 and HER2 status was determined by immunohistochemistry using standard protocols as previously described (31). Briefly, 5µm tissue slides were stained using a DAKO Autostainer (DAKO, Carpinteria, CA) and the DakoCytomation EnVision+ system-HRP (DAB) kit. The Allred score for staining intensity and distribution of positive-stained tumor cells was used to determine ER and PR status. A score cutoff value of 3 was chosen to establish hormone receptor status, with a score of 3 or more being considered positive. For Ki-67, in accordance with the majority of studies using Ki-67 as a categorical variable in breast cancer, we used a cutoff of 20% positive stained cells (32). HER2 protein expression was scored in a similar manner as the clinically used HercepTest™. Tumors with a score of 0–2 classified as HER2-negative and tumors with a score of 3 classified as HER2-positive. All stained slides were interpreted by a board certified pathologist.

p53 mutation detection

Mutations were detected by the Affymetrix p53 Gene Chip System (Affymetrix, Santa Clara, CA) to screen for mutations in the *p53* gene as previously described (33). Briefly,

micro-dissection of the tumor tissue from a 20µm thick slide was performed with a fine needle under the microscope, using an adjacent H&E stained slide for guidance to minimize the inclusion of normal cells. The dissected tissue was then subjected to DNA isolation using a phenol/chloroform/isoamyl alcohol extraction protocol. A multiplex PCR was carried out to amplify exons 2 to 11 of the *p53* gene and the product was fragmented, labeled, hybridized on the microarray and scanned according to the manufacturer's instructions. A commercially available wild type DNA was used as control. All positive mutation readings were subsequently confirmed by bidirectional sequencing using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the Mega BACE 1000 DNA Analysis capillary electrophoresis system (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) according to the manufacturers' instructions.

Statistical analysis

We examined differences in demographics, breast cancer risk factors and tumor characteristics according to *p53* mutation status (present/absent) using t-tests and chi-square tests as appropriate and summarized the frequencies of *p53* mutation types. We used unconditional logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) in case-case analyses to study the association of various tumor characteristics, including clinical state, histologic and nuclear grade, tumor size, lymph node involvement, combined ER/PR status, Ki-67 status and HER2 status with *p53* mutation status. We also evaluated the association of *p53* status with smoking status, drinking status, and several measures of adiposity including body mass index (BMI), waist circumference, and waist-to-hip ratio, in strata defined by menopausal status. All models were adjusted for age at diagnosis, education, and race. When analyzing BMI, we used cutoffs of 25 and 30 kg/m². We utilized clinically meaningful cutoffs for waist circumference and waist-to-hip ratio to present results by category [Janssen et al., 2002; Yusuf et al., 2004]. Statistical analyses were conducted using SAS Version 9.1 (SAS Institute, Cary, NC).

Results

We obtained tumor blocks from 920 (79%) of the 1170 cases enrolled in our study, however only 865 (94%) had sufficient tumor tissue on slides and were subjected to *p53* mutation analysis. Women for whom we obtained breast tumor tissue were slightly younger at diagnosis and had higher TNM stage disease, compared to women who either did not have blocks or insufficient tissue for analysis, but were similar in terms of tumor size, histologic and nuclear grade, and ER and PR status (34). We obtained valid results for 734 subjects, with the remainder failing during the analytical process due to insufficient or poor DNA quality. There were few differences between women with and without *p53* mutation status in our study; women with *p53* status were approximately two years younger, more likely to have metastatic disease, lymph node involvement, higher nuclear grade and higher TNM stage. There were no differences in tumor size, histologic grade, ER status, PR status, HER2 status, or Ki-67 between women with measurable versus not measurable *p53* mutation status.

Characteristics of breast cancer cases according to *p53* mutation status are shown in Table 1. In bivariate comparisons, there were no differences for most risk factors between those with and without *p53* mutations in their tumors. Breast cancer cases with *p53* positive tumors had a statistically significant larger BMI (28.5 kg/m² versus 28.0 kg/m² for *p53* negative tumors, $p<0.01$). With regard to tumor characteristics, *p53* mutation positive cases had a higher proportion of poorly differentiated tumors (65% versus 46%; $p<0.01$), and a distribution weighed toward higher nuclear grade ($p<0.01$). Cases who have *p53* positive tumors were also more likely to have ER-negative and PR-negative tumors, and more likely to be Ki-67 positive (all $p<0.02$). We examined differences with regard to molecular subtype, which represents a subgroup of our larger set. Here we observed that 26% of *p53* positive tumors were triple negative versus 15% of *p53* negative tumors, $p<0.01$.

Distributions of cases across the different types of *p53* mutations and effect on DNA sequence are detailed in Table 2. The *p53* mutation frequency among cases was 28%, of which 95% were point mutations (13% of them silent) and the remainder were single base pair deletions. Transitions represented 67% of all point mutations, with 36.5% of transitions being G:C>A:T at CpG sites. Similar to the IARC database (4), most mutations were localized in exons 5 (35%), 6 (16%), 7 (23%) and 8 (20%), with hotspots in codons 303, 338 and 350 (data not shown).

Descriptive statistics according to mutation type and mutation effect are detailed in Supplementary Table 1. Women with transition mutations had a slightly higher BMI than women whose tumors are *p53* negative (29.4 versus 28.0 kg/m²; $p=0.02$), as well as a higher frequency of poorly differentiated (67% versus 46% for *p53*-negative; $p=0.001$) and high nuclear grade tumors (60% versus 38% for *p53*-negative; $p<0.001$). Also, tumors for women with transition mutations in *p53* were more frequently ER and PR negative, and more frequently of the triple negative subtype (27% versus 15% in *p53* negative cases; $p=0.01$). Notable differences for transversion-type mutations include a higher frequency in non-white cases (16% versus 8% in *p53* negative cases; $p=0.04$), poor nuclear grade (59% versus 38% in *p53* negative cases; $p=0.01$), higher frequency of PR negative tumors (50% versus 32% in *p53* negative cases; $p=0.01$), and more staining for Ki-67 compared to breast cancer cases with no *p53* mutation (18% versus 9% in *p53* negative cases; $p=0.04$).

We observed several statistically significant differences in groups of cases according to *p53* mutation effect. Women with missense mutations had a higher BMI (29.4 kg/m² versus 28.0; $p=0.02$), larger tumors (2.1 versus 1.8 cm; $p=0.03$), poor histologic and nuclear grade (both p -values <0.001), higher frequency of ER and PR negative tumors as well as more triple negative tumors (27% versus 15% in *p53* negative cases; $p=0.002$), and higher Ki-67 index compared to women with no *p53* mutation (16% versus 9%; $p=0.03$). Other types of *p53* mutations classified by type had small sample size, therefore we are unable to draw robust conclusions for other *p53* effects (frameshift, nonsense, silent and splice).

The associations of *p53* mutations (yes/no) and of mutation type (transitions and transversions) with several tumor characteristics and various measures of adiposity are shown in Table 3. We observed that cases with poorly differentiated tumors and those with a higher nuclear grade are more likely to have *p53* mutation positive tumors; OR, 95% CI:

2.29, 1.21–4.32 and 1.99, 95% CI: 1.22–3.25, respectively. These results were similar when we stratified these analyses according to mutation type (transitions and transversions); however the estimates did not reach statistical significance for transversions. Tumors that were ER and PR negative were more likely to be *p53* positive. Similarly, the luminal A type was inversely associated with *p53* OR, 95% CI: 0.44, 0.28–0.68. Ki67 was also associated with having a *p53* mutation, OR, 95% CI: 1.81, 1.10–2.99.

We observed no difference in the associations of ER and PR status with *p53* mutations by menopausal status. ER and PR negative cases were more likely to have a *p53* positive tumor, (OR, 95% CI: 2.17, 1.09–4.30 and 2.14, 95% CI: 1.31–3.50 for pre- and postmenopausal women, respectively) (data not shown).

All three measures of adiposity were examined (BMI, waist circumference, and waist-to-hip ratio) and all three were associated with having a *p53* positive tumor; ORs ranged from 1.52, 95% CI: 1.00–2.31 for the highest category of WHR (WHR>0.86) to 1.82, 95% CI: 1.18–2.82 for waist circumference (>79 cm).

Because adiposity is associated with risk of postmenopausal breast cancer, we stratified these analyses by menopausal status. Among postmenopausal women, we found that cases with a BMI >25 kg/m² were more likely to have a *p53* mutation (OR: 2.20, 95% CI: 1.31–3.70), which was a statistically significant trend ($p=0.01$), as well waist circumference >79 cm (OR: 1.97, 95% CI: 1.09–3.58), and WHR>0.86 (OR: 1.19, 95% CI: 1.12–3.25). Our sample of pre-menopausal women showed none of these statistically significant associations (data not shown).

We observed no statistically significant associations of *p53* mutation status with the following breast cancer risk factors: age at first birth, age at menarche, number of births, family history, or previous benign breast disease (Supplementary Table 2); nor did we observe differences by menopausal status (data not shown).

Discussion

In this study, the spectrum and distribution of *p53* mutations in breast cancer cases from a large population-based case control study *p53* mutations were identified in 25.6% of tumors, similar to other reports for breast cancer and the IARC *p53* mutation database (4). We confirm that women with *p53* mutations in their breast tumors were more likely to have poorly differentiated, high grade, hormone receptor negative and highly proliferative tumors, especially in postmenopausal cases. Moreover, postmenopausal, but not premenopausal breast cancer cases with high adiposity measures were more likely to have *p53* positive breast tumors. Our work may suggest a role of *p53* in mechanisms associated with adiposity and cancer, including inflammation and insulin resistance.

Our findings are in agreement with others, as *p53* mutations have been associated with larger tumor size and higher grade, positive nodal status, and negative ER and PR status (17, 36). In spite of the fact that most studies have relied on measurement of *p53* expression rather than the more direct measurement of *p53* mutations, there is a consistent finding of an association of *p53* mutations with poor prognosis and breast cancer mortality (17, 35). When

we stratified the subjects by menopausal status, the associations of tumor characteristics that are related to poor prognosis and *p53* status were present in both pre- and post-menopausal women; however they were only statistically significant in post-menopausal cases. The lack of significance in pre-menopausal women may be due to the limited sample size in that group; our study is unable to rule out the possibility of no association in pre-menopausal women.

Among a number of breast cancer risk factors examined herein for associations with *p53* mutations, we identified an association of higher adiposity values (larger BMI, waist circumference and waist-to-hip ratio) with *p53* mutations in postmenopausal women. We observed an approximate 2-fold increased odds of having a *p53* mutation for women in the higher categories of adiposity. It is well established that BMI is associated with increased risk of breast cancer among postmenopausal women (37); in studies of premenopausal women, BMI is either not associated with risk or is negatively associated with risk (38). A Dutch study reported no significant trends in risk for increasing BMI according to *p53* status measured using immunohistochemistry (39). While our findings need to be replicated, there is mounting evidence for *p53* involvement in inflammation and insulin resistance (40).

There is evidence that genes associated with obesity are regulators of *p53* (41, 42). One of these genes is *SIRT1*, which can bind and deacetylate p53, preventing cell senescence and apoptosis following DNA damage (41). Another negative regulator of p53 is *MDM2*; the Mdm2-p53 pathway and the PI3K/Akt pathways are involved in stress response and important in cellular homeostasis, controlling proliferation, senescence, and apoptosis (43–45).

Animal models have demonstrated that excess caloric intake can lead to oxidative stress in adipose tissue and increased *p53* expression, resulting in a pro-inflammatory state (40). Mice injected with Wnt-1 mammary tumor cells with either wild-type *p53* (*p53*^{+/+}) or heterozygous *p53* (*p53*^{+/-}) that were subjected to diet-induced obesity had reduced p53 expression signaling and downstream expression of p21. (46). Other evidence comes from breast cancer cell lines where the proliferative effects of leptin may down-regulate *p53* (47). Collectively, this evidence suggests that obesity may influence p53 expression via inflammatory processes.

There are few studies of the association of *p53* and adiposity in other cancers. Figueroa *et al* studied different types of esophageal and gastric adenocarcinoma and although the groups of cases were fairly small, they reported that the association of BMI and esophageal adenocarcinoma (n=122) showed higher odds of disease in P53 positive cases compared to P53 negative cases (48). Their estimates were OR=5.32, 95% CI: 2.75–10.29 and OR=3.34, 95% CI: 1.30–8.62 for the BMI>30 kg/m² and <25kg/m² groups, respectively (48). In colon cancer, another obesity-related cancer, a study by Zhang et al of advanced colon cancer showed an association of P53 and BMI for BMI >21.4 kg/m² (49). Slattey et al analyzed whether *p53* mutation status modified the association of BMI and colon cancer; ORs for BMI>29 kg/m² are similar for the wild type and mutation. Interestingly, when they stratified mutation type further, transitions, transversions, missense *p53* mutations were all significantly associated with colon cancer however in the BMI>29 kg/m² group only (50).

These results differ from our findings; we did not find an association with transversion type mutations and breast cancer. Finally, in a study of 75 patients with bladder cancer, overweight patients had the lowest frequency of *p53* mutations; obesity was not positively related to *p53* status for tumors at that site (51). While the epidemiologic evidence is limited, studies on obesity-related cancers show some evidence of a link between adiposity and *p53* mutations that deserves further attention.

In three previous studies, cigarette smoking was associated with the prevalence and spectrum of *p53* mutations in breast cancers, measured both by immunohistochemistry and direct sequencing (36, 52). However, this was not observed in this study. In one of these studies, a higher proportion of G:C→T:A transversions in postmenopausal breast cancer cases who are smokers also was reported (36). In our study, 39% of smokers had this particular mutation (7 of 18 with the mutation), but it was not statistically increased, perhaps due to small study size.

In evaluation of the findings from this study, it is important to understand its strengths and weaknesses. The main strength of this study is the detailed examination of *p53* mutations in tumors from a large sample of breast cancer cases as well as the extensive questionnaire data and interviewer-administered measurements of anthropometry. In addition, because this is a population-based study our study is representative of the broader population of breast cancer cases. The microarray assay that we used to measure *p53* mutations is more robust compared to immunohistochemistry, and has similar or even superior performance in detecting point mutation compared to sequencing as previously showed by us and others (33, 53–55).

Our study is not without limitations. Our relatively small sample size of pre-menopausal women and small number of women with certain types of *p53* mutations (transversions) limited our ability to detect associations of *p53* mutations and other characteristics in these groups. In spite of low power to detect associations in our pre-menopausal sample, the ORs were in similar direction to those of post-menopausal cases, with respect to women with the clinical characteristics of the tumors, indicating that the associations are likely similar. The associations were less similar with respect to adiposity; the trends with increasing waist circumference and waist-to-hip ratio were not linear nor statistically significant. These differences are consistent with the lack of a positive association of BMI and premenopausal breast cancer risk. However, these findings should be interpreted cautiously because of the limitations in sample size. Other limitations to the study include participation rates and the final analytic sample representing approximately 62% of the entire sample of women who participated in the study. While 95% of study participants with breast cancer gave consent for us to access their archived tumor specimen, we were unable to obtain tumor samples for a number of participants. As expected, we were less likely to obtain tissues for those with smaller tumors. Our sample from which we obtained tumor blocks also tended to be younger. These differences necessarily limit the generalizability of the study findings.

Clinical relevance comes into play with regard to identifying risk factors for heterogeneous breast cancer tumors, and identifying biologic pathways through which the risk factors influence breast cancer development. While obesity is not recognized as a risk factor for pre-menopausal breast cancer, our study suggests that adiposity is associated with unfavorable

tumor characteristics, and thus obesity prevention may be important for women who are at risk and also for women who have already developed aggressive breast cancer, since adiposity is considered to be a negative prognostic factor for breast cancer in both pre- and postmenopausal women. Our study can be validated in other case control studies that have measured *p53* status and adiposity similarly using a case-only design; however large studies are needed with larger representation of more aggressive tumor subtypes.

To our knowledge, this study is the first to identify an association of *p53* status and adiposity in breast cancer. A causal relationship remains to be established. Replication of these findings would be important to better understand the mechanism of this association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* 1991; 51(23 Pt 1):6304–11. [PubMed: 1933891]
2. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell.* 1997; 88(3):323–31. [PubMed: 9039259]
3. Tian K, Rajendran R, Doddananjaiah M, Krstic-Demonacos M, Schwartz JM. Dynamics of DNA damage induced pathways to cancer. *PLoS One.* 2013; 8(9):e72303. [PubMed: 24023735]
4. Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P, Olivier M. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat.* 2007; 28(6):622–9. [PubMed: 17311302]
5. Dumay A, Feugeas JP, Wittmer E, Lehmann-Che J, Bertheau P, Espie M, Plassa LF, Cottu P, Marty M, Andre F, Sotiriou C, Pusztai L, de The H. Distinct tumor protein p53 mutants in breast cancer subgroups. *Int J Cancer.* 2013; 132(5):1227–31. [PubMed: 22886769]
6. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lonning PE, Borresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A.* 2001; 98(19):10869–74. [PubMed: 11553815]
7. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, Turashvili G, Ding J, Tse K, Haffari G, Bashashati A, Prentice LM, Khattra J, Burleigh A, Yap D, Bernard V, McPherson A, Shumansky K, Crisan A, Giuliany R, Heravi-Moussavi A, Rosner J, Lai D, Birol I, Varhol R, Tam A, Dhalla N, Zeng T, Ma K, Chan SK, Griffith M, Moradian A, Cheng SW, Morin GB, Watson P, Gelmon K, Chia S, Chin SF, Curtis C, Rueda OM, Pharoah PD, Damaraju S, Mackey J, Hoon K, Harkins T, Tadigotla V, Sigaroudinia M, Gascard P, Tlsty T, Costello JF, Meyer IM, Eaves CJ, Wasserman WW, Jones S, Huntsman D, Hirst M, Caldas C, Marra MA, Aparicio S. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature.* 2012; 486(7403):395–9. [PubMed: 22495314]

8. Feki A, Irminger-Finger I. Mutational spectrum of p53 mutations in primary breast and ovarian tumors. *Crit Rev Oncol Hematol*. 2004; 52(2):103–16. [PubMed: 15501075]
9. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res*. 1994; 54(18):4855–78. [PubMed: 8069852]
10. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science*. 1991; 253(5015):49–53. [PubMed: 1905840]
11. Soussi T, Legros Y, Lubin R, Ory K, Schlichtholz B. Multifactorial analysis of p53 alteration in human cancer: a review. *Int J Cancer*. 1994; 57(1):1–9. [PubMed: 8150526]
12. Biggs PJ, Warren W, Venitt S, Stratton MR. Does a genotoxic carcinogen contribute to human breast cancer? The value of mutational spectra in unravelling the aetiology of cancer. *Mutagenesis*. 1993; 8(4):275–83. [PubMed: 8377645]
13. Uji K, Naoi Y, Kagara N, Shimoda M, Shimomura A, Maruyama N, Shimazu K, Kim SJ, Noguchi S. Significance of TP53 mutations determined by next-generation “deep” sequencing in prognosis of estrogen receptor-positive breast cancer. *Cancer Lett*. 2014; 342(1):19–26. [PubMed: 23973262]
14. Baker L, Quinlan PR, Patten N, Ashfield A, Birse-Stewart-Bell LJ, McCowan C, Bourdon JC, Purdie CA, Jordan LB, Dewar JA, Wu L, Thompson AM. p53 mutation, deprivation and poor prognosis in primary breast cancer. *Br J Cancer*. 2010; 102(4):719–26. [PubMed: 20104224]
15. Lai H, Ma F, Trapido E, Meng L, Lai S. Spectrum of p53 tumor suppressor gene mutations and breast cancer survival. *Breast Cancer Res Treat*. 2004; 83(1):57–66. [PubMed: 14997055]
16. Langerod A, Zhao H, Borgan O, Nesland JM, Bukholm IR, Ikdahl T, Karesen R, Borresen-Dale AL, Jeffrey SS. TP53 mutation status and gene expression profiles are powerful prognostic markers of breast cancer. *Breast Cancer Res*. 2007; 9(3):R30. [PubMed: 17504517]
17. Olivier M, Langerod A, Carrieri P, Bergh J, Klaar S, Eyfjord J, Theillet C, Rodriguez C, Lidereau R, Bieche I, Varley J, Bignon Y, Uhrhammer N, Winqvist R, Jukkola-Vuorinen A, Niederacher D, Kato S, Ishioka C, Hainaut P, Borresen-Dale AL. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. *Clin Cancer Res*. 2006; 12(4):1157–67. [PubMed: 16489069]
18. Aubele M, Werner M, Hofler H. Genetic alterations in presumptive precursor lesions of breast carcinomas. *Anal Cell Pathol*. 2002; 24(2–3):69–76. [PubMed: 12446956]
19. Done SJ, Eskandarian S, Bull S, Redston M, Andrulis IL. p53 missense mutations in microdissected high-grade ductal carcinoma in situ of the breast. *J Natl Cancer Inst*. 2001; 93(9):700–4. [PubMed: 11333292]
20. Kang JH, Kim SJ, Noh DY, Choe KJ, Lee ES, Kang HS. The timing and characterization of p53 mutations in progression from atypical ductal hyperplasia to invasive lesions in the breast cancer. *J Mol Med (Berl)*. 2001; 79(11):648–55. [PubMed: 11715068]
21. Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ, Ponten J. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci U S A*. 1991; 88(22):10124–8. [PubMed: 1946433]
22. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature*. 1991; 350(6317):429–31. [PubMed: 1672732]
23. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature*. 1991; 350(6317):427–8. [PubMed: 1849234]
24. Hussain SP, Hollstein MH, Harris CC. p53 tumor suppressor gene: at the crossroads of molecular carcinogenesis, molecular epidemiology, and human risk assessment. *Ann N Y Acad Sci*. 2000; 919:79–85. [PubMed: 11083100]
25. Borresen-Dale AL. TP53 and breast cancer. *Hum Mutat*. 2003; 21(3):292–300. [PubMed: 12619115]
26. Freudenheim JL, Bonner M, Krishnan S, Ambrosone CB, Graham S, McCann SE, Moysich KB, Bowman E, Nemoto T, Shields PG. Diet and alcohol consumption in relation to p53 mutations in breast tumors. *Carcinogenesis*. 2004; 25(6):931–9. [PubMed: 14742318]
27. Hartmann A, Blaszyk H, Kovach JS, Sommer SS. The molecular epidemiology of p53 gene mutations in human breast cancer. *Trends Genet*. 1997; 13(1):27–33. [PubMed: 9009845]

28. Olivier M, Hainaut P. TP53 mutation patterns in breast cancers: searching for clues of environmental carcinogenesis. *Semin Cancer Biol.* 2001; 11(5):353–60. [PubMed: 11562177]
29. Starks AM, Martin DN, Dorsey TH, Boersma BJ, Wallace TA, Ambs S. Household income is associated with the p53 mutation frequency in human breast tumors. *PLoS One.* 2013; 8(3):e57361. [PubMed: 23469190]
30. Tao MH, Shields PG, Nie J, Millen A, Ambrosone CB, Edge SB, Krishnan SS, Marian C, Xie B, Winston J, Vito D, Trevisan M, Freudenheim JL. DNA hypermethylation and clinicopathological features in breast cancer: the Western New York Exposures and Breast Cancer (WEB) Study. *Breast Cancer Res Treat.* 2009; 114(3):559–68. [PubMed: 18463976]
31. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol.* 1998; 11(2):155–68. [PubMed: 9504686]
32. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. *Lancet Oncol.* 2010; 11(2):174–83. [PubMed: 20152769]
33. Tennis M, Krishnan S, Bonner M, Ambrosone CB, Vena JE, Moysich K, Swede H, McCann S, Hall P, Shields PG, Freudenheim JL. p53 Mutation analysis in breast tumors by a DNA microarray method. *Cancer Epidemiol Biomarkers Prev.* 2006; 15(1):80–5. [PubMed: 16434591]
34. Tao MH, Marian C, Shields PG, Nie J, McCann SE, Millen A, Ambrosone C, Hutson A, Edge SB, Krishnan SS, Xie B, Winston J, Vito D, Russell M, Nochajski TH, Trevisan M, Freudenheim JL. Alcohol consumption in relation to aberrant DNA methylation in breast tumors. *Alcohol.* 2011; 45(7):689–99. [PubMed: 21168302]
35. Pharoah PD, Day NE, Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. *Br J Cancer.* 1999; 80(12):1968–73. [PubMed: 10471047]
36. Conway K, Edmiston SN, Cui L, Drouin SS, Pang J, He M, Tse CK, Geradts J, Dressler L, Liu ET, Millikan R, Newman B. Prevalence and spectrum of p53 mutations associated with smoking in breast cancer. *Cancer Res.* 2002; 62(7):1987–95. [PubMed: 11929815]
37. Neuhauser ML, Aragaki AK, Prentice RL, Manson JE, Chlebowski R, Carty CL, Ochs-Balcom HM, Thomson CA, Caan BJ, Tinker LF, Peragallo Urrutia R, Knudtson J, Anderson GL. Overweight, obesity and postmenopausal invasive breast cancer risk. *JAMA Oncol.* 2015
38. Cheraghi Z, Poorolajal J, Hashem T, Esmailnasab N, Doosti Irani A. Effect of body mass index on breast cancer during premenopausal and postmenopausal periods: a meta-analysis. *PLoS One.* 2012; 7(12):e51446. [PubMed: 23236502]
39. van der Kooy K, Rookus MA, Peterse HL, van Leeuwen FE. p53 protein overexpression in relation to risk factors for breast cancer. *Am J Epidemiol.* 1996; 144(10):924–33. [PubMed: 8916503]
40. Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T, Nojima A, Nabetani A, Oike Y, Matsubara H, Ishikawa F, Komuro I. A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat Med.* 2009; 15(9):1082–7. [PubMed: 19718037]
41. Kim EJ, Kho JH, Kang MR, Um SJ. Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity. *Mol Cell.* 2007; 28(2):277–90. [PubMed: 17964266]
42. Schug TT, Li X. Sirtuin 1 in lipid metabolism and obesity. *Ann Med.* 2011; 43(3):198–211. [PubMed: 21345154]
43. Deisenroth C, Zhang Y. The Ribosomal Protein-Mdm2-p53 Pathway and Energy Metabolism: Bridging the Gap between Feast and Famine. *Genes Cancer.* 2011; 2(4):392–403. [PubMed: 21779508]
44. Levine AJ, Feng Z, Mak TW, You H, Jin S. Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. *Genes Dev.* 2006; 20(3):267–75. [PubMed: 16452501]
45. Huang XF, Chen JZ. Obesity, the PI3K/Akt signal pathway and colon cancer. *Obes Rev.* 2009; 10(6):610–6. [PubMed: 19527447]
46. Ford NA, Dunlap SM, Wheatley KE, Hursting SD. Obesity, independent of p53 gene dosage, promotes mammary tumor progression and upregulates the p53 regulator microRNA-504. *PLoS One.* 2013; 8(6):e68089. [PubMed: 23840816]
47. Chen C, Chang YC, Liu CL, Chang KJ, Guo IC. Leptin-induced growth of human ZR-75-1 breast cancer cells is associated with up-regulation of cyclin D1 and c-Myc and down-regulation of tumor

- suppressor p53 and p21WAF1/CIP1. *Breast Cancer Res Treat.* 2006; 98(2):121–32. [PubMed: 16752079]
48. Figueroa JD, Terry MB, Gammon MD, Vaughan TL, Risch HA, Zhang FF, Kleiner DE, Bennett WP, Howe CL, Dubrow R, Mayne ST, Fraumeni JF Jr, Chow WH. Cigarette smoking, body mass index, gastro-esophageal reflux disease, and non-steroidal anti-inflammatory drug use and risk of subtypes of esophageal and gastric cancers by P53 overexpression. *Cancer Causes Control.* 2009; 20(3):361–8. [PubMed: 18989634]
49. Zhang ZF, Zeng ZS, Sarkis AS, Klimstra DS, Charytonowicz E, Pollack D, Vena J, Guillem J, Marshall JR, Cordon-Cardo C, et al. Family history of cancer, body weight, and p53 nuclear overexpression in Duke's C colorectal cancer. *Br J Cancer.* 1995; 71(4):888–93. [PubMed: 7710960]
50. Slattery ML, Curtin K, Ma K, Edwards S, Schaffer D, Anderson K, Samowitz W. Diet activity, and lifestyle associations with p53 mutations in colon tumors. *Cancer Epidemiol Biomarkers Prev.* 2002; 11(6):541–8. [PubMed: 12050095]
51. Ecke TH, Schlechte HH, Gunia S, Lenk SV, Loening SA. Body mass index (BMI) and mutations of tumor suppressor gene p53 (TP53) in patients with urinary bladder cancer. *Urol Oncol.* 2008; 26(5):470–3. [PubMed: 18367114]
52. Gammon MD, Hibshoosh H, Terry MB, Bose S, Schoenberg JB, Brinton LA, Bernstein JL, Thompson WD. Cigarette smoking and other risk factors in relation to p53 expression in breast cancer among young women. *Cancer Epidemiol Biomarkers Prev.* 1999; 8(3):255–63. [PubMed: 10090304]
53. Chiaretti S, Tavoraro S, Marinelli M, Messina M, Del Giudice I, Mauro FR, Santangelo S, Picocchi A, Peragine N, Truong S, Patten N, Ghia EM, Torrente I, De Propriis MS, Nanni M, Lawrence J, Guarini A, Foa R. Evaluation of TP53 mutations with the AmpliChip p53 research test in chronic lymphocytic leukemia: correlation with clinical outcome and gene expression profiling. *Genes Chromosomes Cancer.* 2011; 50(4):263–74. [PubMed: 21319261]
54. Lumachi F, Marino F, Varotto S, Basso U. Oligonucleotide probe array for p53 gene alteration analysis in DNA from formalin-fixed paraffin-embedded breast cancer tissues. *Ann N Y Acad Sci.* 2009; 1175:89–92. [PubMed: 19796081]
55. Robles AI, Harris CC. Clinical outcomes and correlates of TP53 mutations and cancer. *Cold Spring Harb Perspect Biol.* 2010; 2(3):a001016. [PubMed: 20300207]

Table 1

P53 status and participant demographics, breast cancer risk factors, and tumor characteristics, WEB Study.

Characteristic Mean (SD) or %	Breast Cancer Cases (n=733)		p-value
	P53 negative (n=528)	P53 positive (n=205)	
Age, yr	57.3 (11.3)	56.6 (11.7)	0.43
Education, yr	13.6 (2.6)	13.6 (2.3)	0.91
Age at menarche, yr	12.5 (1.6)	12.6 (1.7)	0.40
Number of births	2.3 (1.7)	2.3 (1.7)	0.67
Nulliparous, %	17.6	17.6	0.54
First-degree relative with breast cancer, %	22.0	18.4	0.30
Previous benign breast disease, %	35.8	32.3	0.38
Menopausal status, %			
Pre-menopausal	30.7	33.7	0.44
Post-menopausal	69.3	66.3	
Ever smokers, %	56.0	52.9	0.46
Lifetime pack years of smoking	19.9 (18.9)	20.6 (18.2)	0.72
Body mass index, kg/m ²	28.0 (6.2)	29.5 (7.3)	<0.01
Waist circumference (cm)	88.9±15.8 n=516 [§]	91.1±14.1 n=201 [§]	0.09
Waist to hip ratio	0.8±0.1 n=514 [§]	0.8±0.1 n=200 [§]	0.16
Tumor size, cm, n=666 [§]	1.8 (1.2)	1.9 (1.2)	0.22
Metastatic disease, %, n=692	27.8	30.2	0.53
Lymph node involvement, %, n=680	28.8	31.6	0.47
TNM stage, %, n=577			
I	53.6	45.3	0.20
II _a /II _b	39.4	46.0	
III/IV	7.0	8.7	
Histologic grade, %, n=583			
Well	13.9	8.8	<0.01
Moderate	40.4	26.3	
Poorly	45.6	65.0	
Nuclear grade, %, n=651			
I	19.5	15.8	<0.01
II	42.6	23.9	
III	37.9	60.3	
ER-positive, %, n=729	72.8	60.8	<0.01
PR-positive, %, n=721	67.7	52.2	<0.01
HER2-positive, %, n=606	9.0	12.6	0.18
Ki-67 positive, %, n=683	9.2	15.6	0.02
Molecular subtype (%) [*]	n=430 [§]	n=172 [§]	<0.01
Luminal A	76.5	61.0	

Characteristic Mean (SD) or %	Breast Cancer Cases (n=733)		p-value
	P53 negative (n=528)	P53 positive (n=205)	
Luminal B	4.7	7.6	
HER2 expressing	4.2	5.2	
Triple negative	14.7	26.2	

^sNumber of subjects with available data.

Table 2

Distribution and spectrum of *p53* mutations according to mutation type and effect of mutation on DNA sequence, WEB study

Mutations classification		N	% of total(% within group)
Mutation type	All Transitions	137	66.5
	G:C>A:T at CpG	50	(25.6)
	G:C>A:T at nonCpG	56	(28.7)
	A:T>G:C	31	(15.9)
	All Transversions	58	28.2
	G:C>T:A	18	(31.0)
	T:A>G:C	6	(3.1)
	G:C>C:G	22	(11.3)
	A:T>T:A	12	(6.2)
	All Point mutations	195	95.0
Effect on DNA sequence	Missense	147	(71.7)
	Nonsense	15	(7.3)
	Splice site	6	(2.9)
	Silent	27	(13.1)
	Frameshift Deletions	10	4.9

Table 3
Associations of *p53* mutations and clinical characteristics and adiposity: WEB study*

Characteristics	<i>p53</i> positive	Trans-itions	Trans-versions	<i>p53</i> negative	OR (95% CI)*		
					<i>p53</i> positive vs. <i>p53</i> negative	Transitions vs. <i>p53</i> negative	Transversions vs. <i>p53</i> negative
TNM stage							
I	73	50	21	223	1.00	1.00	1.00
II _a /II _b	74	50	20	164	1.37 (0.93–2.02)	1.34 (0.86–2.09)	1.32 (0.68–2.54)
III/IV	14	6	6	29	1.42 (0.71–2.87)	0.85 (0.33–2.18)	2.41 (0.87–6.66)
<i>p</i> for trend					0.11	0.56	0.11
Histologic grade							
Well	14	10	4	59	1.00	1.00	1.00
Moderate	42	25	14	171	1.04 (0.53–2.04)	0.87 (0.39–1.93)	1.16 (0.37–3.68)
Poorly	104	70	29	193	2.29 (1.21–4.32)	2.18 (1.05–4.52)	2.08 (0.69–6.27)
<i>p</i> for trend					0.001	0.001	0.07
Nuclear grade							
I	29	21	8	91	1.00	1.00	1.00
II	44	27	14	199	0.69 (0.40–1.17)	0.58 (0.31–1.08)	0.78 (0.32–1.93)
III	111	73	31	177	1.99 (1.22–3.25)	1.78 (1.02–3.11)	1.96 (0.85–4.54)
<i>p</i> for trend					0.001	0.001	0.03
Tumor size							
2 cm	119	80	32	346	1.00	1.00	1.00
>2 cm	65	43	21	136	1.39 (0.96–2.00)	1.36 (0.89–2.09)	1.66 (0.91–3.02)
Vascular/lymph invasion							
No	130	89	37	349	1.00	1.00	1.00
Yes	60	38	16	141	1.12 (0.78–1.62)	1.03 (0.67–1.58)	1.07 (0.57–2.01)
Combined ER and PR status							
ER+/PR+	92	64	26	313	1.00	1.00	1.00
ER+/PR–	31	18	12	66	1.65 (1.01–2.71)	1.37 (0.75–2.47)	2.31 (1.10–4.88)
ER–/PR+	13	10	2	39	1.11 (0.56–2.17)	1.20 (0.57–2.55)	0.62 (0.14–2.76)
ER–/PR–	64	43	16	102	2.18 (1.47–3.23)	2.14 (1.36–3.37)	1.80 (0.91–3.54)

Characteristics	<i>p53</i> positive	Trans-itions	Trans-versions	<i>p53</i> negative	OR (95% CI) [*]		
					<i>p53</i> positive vs. <i>p53</i> negative	Transitions vs. <i>p53</i> negative	Transversions vs. <i>p53</i> negative
Molecular subtype ^{***}							
Luminal A	105	71	32	329	0.44 (0.28–0.68)	0.41 (0.25–0.69)	0.61 (0.28–1.30)
Luminal B	13	8	4	20	0.89 (0.39–1.99)	0.70 (0.27–1.79)	1.38 (0.38–4.95)
HER2 Expressing	9	6	3	18	0.70 (0.29–1.70)	0.65 (0.23–1.80)	1.04 (0.26–4.20)
Triple-negative	45	31	11	63	1.00	1.00	1.00
Ki67 status ^{**}							
Negative	162	108	46	446	1.00	1.00	1.00
Positive	30	18	10	45	1.81 (1.10–2.98)	1.63 (0.90–2.94)	2.11 (0.98–4.54)
BMI, kg/m ²							
25	58	41	15	195	1.00	1.00	1.00
25–30	62	41	20	161	1.35 (0.88–2.05)	1.29 (0.79–2.09)	1.52 (0.75–3.10)
>30	85	55	23	172	1.78 (1.19–2.68)	1.71 (1.07–2.74)	1.64 (0.81–3.33)
<i>p</i> for trend					0.003	0.01	0.10
Waist circumference, cm							
79.0	45	32	12	168	1.00	1.00	1.00
79.0–91.5	68	48	18	148	1.84 (1.18–2.87)	1.85 (1.11–3.08)	1.74 (0.80–3.77)
>91.5	88	55	26	200	1.82 (1.18–2.82)	1.65 (0.995–2.74)	1.82 (0.86–3.87)
<i>p</i> for trend					0.04	0.12	0.25
Waist-to-hip ratio							
0.79	62	48	13	190	1.00	1.00	1.00
0.79–0.86	59	37	19	147	1.32 (0.86–2.03)	1.08 (0.66–1.77)	1.91 (0.89–4.08)
>0.86	79	49	24	177	1.52 (1.00–2.31)	1.25 (0.77–2.02)	1.97 (0.93–4.19)
<i>p</i> for trend					0.08	0.29	0.29

* Odds ratios and 95% confidence intervals adjusted for age, education, race, and menopausal status.

** Ki67 status: positive if >20%.

*** Luminal A: ER+ or PR+, HER2–; Luminal B: ER+ or PR+, HER2+; HER2 Expressing: ER–/PR–, HER2+; Triple-negative: ER–/PR–/HER2–.