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## Association between rs2981582 polymorphism in the FGFR2 gene and the risk of breast cancer in Mexican women

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### Abstract

**Background and Aims**—The rs2981582 single nucleotide polymorphism in the Fibroblast Growth Factor Receptor 2 gene has been consistently associated with an increased risk of breast cancer. We evaluated the effect of rs2981582 polymorphism in the *FGFR2* gene on the risk of breast cancer and its interaction with non-genetic risk factors.

**Methods**—A population based case control study was conducted in Mexico. Data from 687 cases and 907 controls were analyzed.

**Results**—The T allele of the rs2981582 polymorphism was associated with an increased risk of breast cancer (OR<sub>per allele</sub> = 1.24, 95% CI 1.06 – 1.46). There was also an interaction between this polymorphism and alcohol consumption (p = 0.043); the effect of alcohol consumption on the risk of breast cancer varied according to the allelic variants of the rs2981582 polymorphism in the *FGFR2* gene: OR = 3.97 (95% CI 2.10 – 7.49), OR = 2.01 (95% CI 1.23 – 3.29) and OR = 1.21 (95% CI 0.48 – 3.05) for genotypes CC, CT and TT, respectively.

**Conclusions**—This is the first study exploring the association between rs2981582 polymorphism in the *FGFR2* gene and breast cancer risk in Mexican women. The interaction found may be of great public health interest, since alcohol consumption is a modifiable breast cancer risk factor. Therefore, replication of this finding is of foremost importance.

## Keywords

rs2981582 single nucleotide polymorphism; FGFR2 gene; breast cancer

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## Introduction

Breast cancer is known to be a partially heritable disease.(1, 2) Rare mutations in several genes including *BRCA1* and *BRCA2* are associated with a very high risk of breast cancer, but account for a small fraction of the disease.(3, 4) Recently, genome-wide association studies (GWAS) identified a series of common polymorphisms associated with modestly increased risk of breast cancer.(5, 6) However, the utility of these SNPs as risk predictors in the clinic is limited.(7) Understanding gene-environment interactions may help to develop more detailed risk prediction from these risk factors and thus increase their clinical utility.

One of the strongest and most consistent genetic risk factors for breast cancer is an intronic single nucleotide polymorphism (SNP) in the Fibroblast Growth Factor Receptor 2 (*FGFR2*) gene, rs2981582.(6) The risk appears to be consistent in Caucasian,(8–13) Asian(14, 15) and Hispanic women living in the United States.(16) *FGFR2* rs2981582 is in linkage disequilibrium ( $r^2= 1$ ) with *FGFR2* rs1219648.(17) However, to our knowledge, there are no published studies regarding the association between this SNP and the risk of breast cancer in Mexican women. We focused on *FGFR2* rs2981582 to replicate a previously published study among Caucasian women.(10)

We tested the association between the rs2981582 polymorphism in the *FGFR2* gene and breast cancer risk in Latin American women using data from a large, population-based, case-control study of women in Mexico. We also evaluated for interactions between known environmental risk factors and *FGFR2*.

## Materials and methods

### Study population

A population based case-control study was conducted in Mexico City, Monterrey, and Veracruz from January 2004 to December 2007. Incident cases (n=1000) included women aged 35 to 69 with histopathologically confirmed breast cancer. Subjects were recruited from 12 public hospitals: the Mexican Institute of Social Security (*Instituto Mexicano del Seguro Social, IMSS*, six hospitals), the Social Security and Services Institute for State Employees (*Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado, ISSSTE*, two hospitals), and the Ministry of Health (*Secretaría de Salud, SS*, four hospitals). Age distribution of eligible women was based on age parameters selected from the Histopathological Registry of Malignant Neoplasia (2002).

Controls were frequency-matched to the cases, according to 5-year age groups, membership to a health care institution and place of residence. Eligible controls were selected using a multistage method of probabilistic sampling in Basic Geo-Statistical Areas (AGEBSs) located within the catchment area of each participating hospital. A total of 1,594 Mexican cases and controls were genotyped for this study (687 cases and 907 controls).

After an informed consent was reviewed and signed by all participants, subjects were interviewed and information was collected regarding their diet, alcohol consumption, lifestyle, reproductive history, personal and health family history, including exposure to other factors associated with breast cancer risk. Additionally, anthropometric measurements and venous blood samples were taken from each subject. A more detailed description of the study has been previously published.(18, 19)

## Genotyping

Genotyping was performed using iPLEX™ reagents and protocols for multiplex PCR, single base primer extension (SBE) and generation of mass spectra, as per the manufacturer's instructions (for complete details see iPLEX™ Application Note, Sequenom, San Diego). (20) Multiplexed assays typically contain between 10–36 SNPs. Multiplexed PCR was performed in 5- $\mu$ l reactions on 384-well plates containing 5 ng of genomic DNA. Reactions contained 0.5 U HotStarTaq™ polymerase (QIAGEN), 100 nM primers, 1.25X HotStarTaq™ buffer, 1.625 mM MgCl<sub>2</sub>, and 500  $\mu$ M dNTPs. Following enzyme activation at 94 °C for 15 min, DNA was amplified with 45 cycles of 94 °C  $\times$  20 sec, 56 °C  $\times$  30 sec, 72 °C  $\times$  1 min, followed by a 3-min extension at 72 °C. Unincorporated dNTPs were removed using shrimp alkaline phosphatase (0.3 U, Sequenom). Single-base extension was carried out by addition of SBE primers at concentrations from 0.625  $\mu$ M (low MW primers) to 1.25  $\mu$ M (high MW primers) using iPLEX™ enzyme and buffers (Sequenom, San Diego) in 9- $\mu$ l reactions. Reactions were desalted and SBE products measured using the MassARRAY® Compact system, and mass spectra analyzed using TYPER software (Sequenom, San Diego), in order to generate genotype calls and allele frequencies. All samples were genotyped without knowledge about disease status (case/control) by the laboratory personnel.

Quality control (QC) was performed on all DNA using a two-part procedure. If DNA had not been quantitated prior to arrival in the core, DNA was quantitated and normalized using Picogreen® and standard methods and normalized to a standard concentration. Quantitative QC (part 1) involved non-allelic quantitative real-time PCR using a single Taqman® probe, in order to ensure amplifiability of DNA samples. Quality control (part 2) involved genotyping using a balanced polymorphism present in most human populations in order to ensure that cross- contamination of samples has not occurred.

Genetic ancestry estimation procedure used 106 AIMs and was performed using a multiplex PCR coupled with single base extension methodology with allele calls using a Sequenom analyzer. Samples were genotyped without knowledge about case/control status. The average sample call rate was 98.7%. Duplicate pairs (59) were genotyped, and of these, two pairs were excluded from the mismatch analysis because the call rate for one of the duplicates was low (7% and 26%) compared to the high call rate of most samples in the study. There was one pair that showed 1 mismatch (the call rate for one of the samples in the pair was 77%). At closer inspection, for two of the three pairs, one of the duplicate samples had a low call rate (7% and 26%). The overall error rate without including the duplicate pairs with a call rate of 7% and 26% was 0.02%. All the AIMs were in Hardy-Weinberg

equilibrium. An extensive description of genetic ancestry estimation procedure was previously published.(19)

### Statistical analyses

We evaluated the association between rs2981582 polymorphism in the *FGFR2* gene and the risk of breast cancer and whether this polymorphism modified the relative risks of breast cancer associated with established breast cancer risk factors (i.e. genetic ancestry); breast cancer in first degree relatives (yes/no); body mass index (kg/m<sup>2</sup>); lifetime tobacco smoking of  $\geq 100$  cigarettes (yes/no); occasional intake of more than one alcohol drinks within a month over a period of one or more years (yes/no); weekly moderate-intensity physical activity (hours); daily caloric intake (kilocalories); socio-economic status (low, middle, and high); oral contraceptives use once during lifetime (yes/no); age at menarche (years); parity (number of live-born offspring); age at first live child (years); complete lactation (months); personal history of benign breast disease (yes/no); menopausal status (pre-menopausal/post-menopausal); self-report of diabetes mellitus (yes/no). Genetic exposure was evaluated as a quantitative variable according to the number of additional minor alleles found (0, 1, or 2 for genotypes CC, CT, and TT, respectively). Summary statistics were used to compare cases and controls. The Hardy-Weinberg equilibrium of genotype frequencies was assessed in the control group using a chi-squared test.

Genetic ancestry procedure was based on a three populations model that included European, Native and African ancestry. We used a maximum likelihood approach (Structure software 2.3.3, University of Chicago) to estimate each participant's genetic ancestry. Socioeconomic status index was defined by belongings as previously published.(18)

To determine statistical association between the exposure variables and breast cancer risk, and their interactions, odds ratios (OR) and 95% confidence intervals (CI) were estimated by means of conditional logistic regression multiple models.(21) The following statistical models were constructed: one to estimate the association between the rs2981582 polymorphism and the mentioned factors with the risk of breast cancer and one to evaluate interaction between each established breast cancer risk factor and the rs2981582 polymorphism.

We examined whether *FGFR2* rs2981582 modified the association of known environmental exposures and breast cancer risk by categorizing women according to exposure-genotype and use of dummy variable for each category. We examined interaction terms in log-multiplicative genetic models.

Menopausal status was defined as follows: Pre-menopausal women were those having had their last period within the previous 12 months and those with a history of surgical menopause prior to age 48. Post-menopausal women were those having reached natural menopause ( $\geq 12$  months from date of last period), and women with surgical menopause aged 48 or above. The cut-off age (48 years of age) corresponds to Mexican women's median age at menopause.(22) The alcohol consumption variable used for the statistical analysis was occasional intake of more than one alcohol drinks within a month over a period of one or more years (yes/no). This variable was chosen since it reflects: a) the chronic

exposure suggested as necessary for carcinogenesis induced by certain compounds such as arsenic, and benzene, according to the International Agency for Research on Cancer;(23, 24) and b) the high prevalence of alcohol consumption among Mexican women, and the early age at starting its consumption (18 years).(25) Considering the previous aspects, we worked under the assumption that having consumed alcohol in the past (occasional intake of more than one alcohol drinks within a month over a period of one or more years), consumption has persisted during the lifetime of a woman.

## Results

The genotype frequencies of the rs2981582 polymorphism in controls were consistent with the expectation under Hardy-Weinberg equilibrium ( $p=0.361$ ). Table 1 shows the study population characteristics for selected variables. Allele T frequency was 43.6% in cases, and 38.2% in the control group. The prevalence of breast cancer in first degree relatives was higher among cases (7.3%) than controls (3.9%). Cases also reported higher daily caloric intake (2170.8 kcal vs. 1832.1 kcal), alcohol consumption (19.7% vs. 10.0%), tobacco smoking 100 cigarettes during lifetime (26.9% vs. 20.5%), high socio-economic status (44.7% vs. 33.0%), self-report of Diabetes Mellitus (20.8% vs. 15.7%), personal history of benign breast disease (14.9% vs. 7.0%), and lower weekly moderate-intensity physical activity (9.7 hrs. vs. 15.3 hrs.). Cases were older than controls ( $52.1 \pm 9.8$  vs.  $51.1 \pm 9.2$ ) and other reproductive factors, such as parity and lactation, were greater among controls than in cases. In unadjusted analyses, European ancestry was higher among cases than controls (0.36 vs. 0.32).

Table 2 shows that the rs2981582 polymorphism in the *FGFR2* gene was statistically significantly associated with the risk of breast cancer (OR<sub>per allele</sub> 1.24, 95% CI 1.06 – 1.46). When rs2981582 was analyzed as a categorical variable, where the reference category corresponded to the wild genotype (CC), the estimated OR was 1.08 (95% CI 0.84 – 1.39), and 1.63 (95% CI 1.17 – 2.27) for genotypes CT and TT, respectively (Table 2).

Other variables significantly associated with breast cancer in multiple models included breast cancer in first degree relatives (OR= 1.80; 95% CI 1.08 – 2.99), daily caloric intake (OR= 1.07 per 100 Kcal; 95% CI 1.05 – 1.09), weekly moderate-intensity physical activity (hours) (OR=0.975; 95% CI 0.966 – 0.983), body mass index (OR= 0.96 per 1 kg/m<sup>2</sup>; 95% CI 0.94 – 0.99), occasional intake of more than one drinks within a month during one year or more (OR= 2.15; 95% CI 1.53 – 3.03), increased parity (OR= 0.84 per each child; 95% CI 0.78 – 0.90), self-reported Diabetes Mellitus (OR= 1.85; 95% CI 1.36 – 2.52) and personal history of benign breast disease (OR= 2.63; 95% CI 1.77 – 3.90). Compared with women with 0–25% European ancestry, the risk was increased for women with >50 to 75% (OR= 1.50; 95% CI 1.05 – 2.14) and >75 to 100% European ancestry (OR= 3.45; 95% CI 1.11 – 10.71).

In the interactions analysis (Table 3), there was a statically significant interaction between the studied polymorphism and alcohol consumption ( $p=0.043$ ). In the multiple model, the effect of alcohol consumption on the risk of breast cancer was 3.97 (95% CI 2.10 – 7.49) for those homozygous to the major allele (CC), 2.01 (95% CI 1.23 – 3.29) for those

heterozygous (CT), and 1.21 (95% CI 0.48 – 3.05) for those homozygous to the minor allele (TT). In the cases, alcohol consumption prevalence, stratified by the polymorphism genotype was 19.31%, 20.39% and 18.62% for genotypes CC, CT and TT, respectively; while in the control group the prevalence was: 7.37%, 11.08% and 13.67%, respectively. No other interactions were significant.

## Discussion

We found that the rs2981582 polymorphism in *FGFR2* gene was associated with the risk of breast cancer and that its allelic variants modified the effect of alcohol consumption on the risk of breast cancer. No other statistically significant interactions were found.

The association of rs2981582 with breast cancer risk was previously described among Hispanic women living in the United States.(16) The gene-environment interaction found in our study was previously evaluated in other populations: Japanese women,(14) United Kingdom residents,(26) Caucasian women from the National Cancer Institute's Breast and Prostate Cancer Cohort Consortium (BPC3)(12) and European-American women living in the United States.(13)

In the first three studies,(12, 14, 26) current alcohol consumption was used for the analyses while in the case-control study conducted by Marian C et al. in the United States,(13) current/former alcohol consumption was used and no interaction was found. This could be explained because in our study, occasional intake of more than one alcohol drink within a month over a period of one or more years (yes/no) was used. This variable could reflect chronic exposure to alcohol consumption and chronic exposure to certain compounds such as arsenic, and benzene has been suggested as necessary for carcinogenesis;(23, 24) Mexican women start consuming alcohol at an early age (18 years).(25) In the Japanese study, the sample size was similar to ours, the allele T frequency was lower in Japanese than in Mexican women, as observed in cases (30.3% and 43.2%, respectively), and controls (25.9% and 38.4%, respectively). Differences in prevalence of allele T could also explain the interaction term discrepancy between both studies. In addition, none of these studies, (12–14,26) have included Latin American or Mexican women.

Our findings suggest that the effect of alcohol on the risk of breast cancer is higher in allele C homozygous women and decreases when the number of T alleles increase. This was also observed for the interaction between rs2420946 polymorphism in *FGFR2* and family history of breast cancer,(14) and between rs3050817 polymorphism in *FGFR2* and hormonal replacement therapy.(27)

We found no statistically significant interaction between family history of breast cancer and the rs2981582 polymorphism ( $p=0.160$ ). However, the ORs in our study (2.87, 1.56 and 0.98 for CC, CT and TT respectively) were similar to those in the Japanese study (2.72, 1.37 and 0.30 for CC, CT and TT, respectively).(14)

Neither the carcinogenetic mechanism related to the rs2981582 *FGFR2* gene polymorphism nor alcohol has been described yet.(28, 29) Regarding the *FGFR2* gene polymorphisms, there is *in vitro* evidence that the mechanism is more related to an anti-apoptotic than to a

mitogenic effect.(30, 31) This inhibitory effect in the apoptosis could be mediated, in part, by down- regulation of the Forkhead O transcription factors (FOXO) synthesis, one of the greatest subgroups in the Forkhead family.(32) FOXO proteins are implicated in apoptosis induction,(33) DNA damage repair,(34) reactive oxygen species (ROS) detoxification,(35) and a down- regulation of the transcriptional activity of estrogen receptors (ER)  $\alpha$  and  $\beta$ .(36) The Phosphoinositide 3-kinase (PI3K), an intracellular signaling system activated by the *FGFR2* receptor activation,(37) down-regulates the FOXO factors transcription.(38

In breast and gastric cancer, SNPs in the *FGFR2* gene are associated with the amplification of the gene and with the over-expression of the receptor codified by this gene.(39) Considering that this event could result in an aberrant expression of the receptor,(28) and of the PI3K, and subsequently in a down-regulation of the FOXO synthesis, an interaction is biologically plausible between *FGFR2* SNPs and alcohol consumption in the risk of breast cancer. This could be explained, among other mechanisms, by the reduction of ROS detoxification, less DNA repair due to adducts accumulation and loss of the down-regulation of the ER transcriptional activity. This interaction is supported by a recent study, which suggests the influence of *FGFR2* SNPs on mRNA expression levels.(40

Alcohol consumption is a well-documented modifiable risk factor associated with the risk of breast cancer.(41, 42) According to the World Health Organization, there are about 2 billion people worldwide who consume alcohol.(43) In Mexico, the National Surveys on Addictions have shown an increase in the number of adult women consuming alcohol, particularly in those ingesting 4 or more drinks per occasion (2.6% in year 1998; 3.7% in 2002). Likewise, the prevalence of alcohol consumption in adolescent women increased from 18% during 1998 to 25% in 2002.(44,45)

Due to the inherent limitations of retrospective case-control studies, our findings should be considered with caution. However, our cases were incident and our controls population-based, which increase the validity of our findings.

We found that tobacco smoking had no effect on breast cancer risk once alcohol drinking was included in the model. Therefore, despite the reported oxidative stress secondary to tobacco smoking, its association with breast cancer risk in the present study seems to be fully accounted for by its correlation with alcohol consumption (50% of subjects that reported alcohol consumption smoked).

In our study oral contraceptives use was not associated statistically significantly with breast cancer risk and was not included in the final analysis in benefit of a parsimonious regression model. Age at first life birth (years) was higher among cases ( $20.8 \pm 8.4$ ) than in controls ( $20.2 \pm 6.5$ ). It was not included in the logistic regression models since it was found highly correlated with parity ( $p < 0.001$ ).

A quantitative tobacco exposure variable was tested (pack-year, defined as the number of cigarettes smoked per day divided by 20 and multiplied by the number of years that the participant smoked). However, it was not included in the final model because we found low pack-year consumption and the difference was not statically significant among the study groups.



Socio-economic status was associated with native ( $r = -0.25$ ,  $p < 0.001$ ) and European ancestry ( $r = 0.25$ ,  $p < 0.001$ ). In the multiple model, both ancestry and socio-economic status were statistically significant, suggesting that despite the relative overlap between the two variables there might still independently account for other unknown, environmental and lifestyle factors that are associated with breast cancer risk.(46

To our knowledge this is the first study that has replicated the association between rs2981582 polymorphism in the FGFR2 gene and the risk of breast cancer in Mexican women, however its interaction with alcohol consumption needs further research in order to better understand the association between these two variables and its possible interaction with the risk of breast cancer.

## References

1. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med.* 2000; 343:78–85. [PubMed: 10891514]
2. Peto J, Mack TM. High constant incidence in twins and other relatives of women with breast cancer. *Nat Genet.* 2000; 26:411–414. [PubMed: 11101836]
3. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J Clin Oncol.* 2007; 25:1329–1333. [PubMed: 17416853]
4. Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P, Olivier M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. *Oncogene.* 2007; 26:2157–2165. [PubMed: 17401424]
5. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature.* 2007; 447:1087–1093. [PubMed: 17529967]
6. Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat Genet.* 2007; 39:870–874. [PubMed: 17529973]
7. Wacholder S, Hartge P, Prentice R, et al. Performance of common genetic variants in breast-cancer risk models. *N Engl J Med.* 2010; 362:986–993. [PubMed: 20237344]
8. Boyarskikh UA, Zarubina NA, Biltueva JA, et al. Association of FGFR2 gene polymorphisms with the risk of breast cancer in population of West Siberia. *Eur J Hum Genet.* 2009; 17:1688–1691. [PubMed: 19536173]
9. Hemminki K, Muller-Myhsok B, Lichtner P, et al. Low-risk variants FGFR2, TNRC9 and LSP1 in German familial breast cancer patients. *Int J Cancer.* 2010; 126:2858–2862. [PubMed: 19856316]
10. Huijts PE, Vreeswijk MP, Kroeze-Jansema KH, et al. Clinical correlates of low-risk variants in FGFR2, TNRC9, MAP3K1, LSP1 and 8q24 in a Dutch cohort of incident breast cancer cases. *Breast Cancer Res.* 2007; 9:R78. [PubMed: 17997823]
11. Rebbeck TR, DeMichele A, Tran TV, et al. Hormone-dependent effects of FGFR2 and MAP3K1 in breast cancer susceptibility in a population-based sample of post-menopausal African-American and European-American women. *Carcinogenesis.* 2009; 30:269–274. [PubMed: 19028704]
12. Campa D, Kaaks R, Le Marchand L, et al. Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. *J Natl Cancer Inst.* 2011; 103:1252–1263. [PubMed: 21791674]
13. Marian C, Ochs-Balcom HM, Nie J, et al. FGFR2 intronic SNPs and breast cancer risk: associations with tumor characteristics and interactions with exogenous exposures and other known breast cancer risk factors. *Int J Cancer.* 2011; 129:702–712. [PubMed: 20853316]
14. Kawase T, Matsuo K, Suzuki T, et al. FGFR2 intronic polymorphisms interact with reproductive risk factors of breast cancer: results of a case control study in Japan. *Int J Cancer.* 2009; 125:1946–1952. [PubMed: 19582883]

15. Liang J, Chen P, Hu Z, et al. Genetic variants in fibroblast growth factor receptor 2 (FGFR2) contribute to susceptibility of breast cancer in Chinese women. *Carcinogenesis*. 2008; 29:2341–2346. [PubMed: 18845558]
16. Slattery ML, Baumgartner KB, Giuliano AR, Byers T, Herrick JS, Wolff RK. Replication of five GWAS-identified loci and breast cancer risk among Hispanic and non-Hispanic white women living in the Southwestern United States. *Breast Cancer Res Treat*. 2011; 129:531–539. [PubMed: 21475998]
17. The International HapMap Project. *Nature*. 2003; 426:789–796. [PubMed: 14685227]
18. Angeles-Llerenas A, Ortega-Olvera C, Perez-Rodriguez E, et al. Moderate physical activity and breast cancer risk: the effect of menopausal status. *Cancer Causes Control*. 2010; 21:577–586. [PubMed: 20084545]
19. Fejerman L, Romieu I, John EM, et al. European ancestry is positively associated with breast cancer risk in Mexican women. *Cancer Epidemiol Biomarkers Prev*. 2010; 19:1074–1082. [PubMed: 20332279]
20. Lu B, Viscidi RP, Lee JH, et al. Human papillomavirus (HPV) 6, 11, 16, and 18 seroprevalence is associated with sexual practice and age: results from the multinational HPV Infection in Men Study (HIM Study). *Cancer Epidemiol Biomarkers Prev*. 2011; 20:990–1002. [PubMed: 21378268]
21. Hosmer DLS. *Model-Building Strategies and Methods for Logistic Regression in: Applied Logistic Regression*: Wiley. 2000
22. S B-M. La edad de la menopausia en Mexico. *Rev Endocrinol Nutr*. 2006; 14:133–136.
23. Dantzig PI. Breast cancer, dermatofibromas and arsenic. *Indian J Dermatol*. 2009; 54:23–25. [PubMed: 20049264]
24. Zheng T, Holford TR, Mayne ST, et al. Environmental exposure to hexachlorobenzene (HCB) and risk of female breast cancer in Connecticut. *Cancer Epidemiol Biomarkers Prev*. 1999; 8:407–411. [PubMed: 10350435]
25. Herrera-Vazquez M, Wagner FA, Velasco-Mondragon E, Borges G, Lazcano-Ponce E. [Onset of alcohol and tobacco use and transition to other drug use among students from Morelos, Mexico]. *Salud Publica Mex*. 2004; 46:132–140. [PubMed: 15176575]
26. Travis RC, Reeves GK, Green J, et al. Gene-environment interactions in 7610 women with breast cancer: prospective evidence from the Million Women Study. *Lancet*. 2010; 375:2143–2151. [PubMed: 20605201]
27. Prentice RL, Huang Y, Hinds DA, et al. Variation in the FGFR2 gene and the effects of postmenopausal hormone therapy on invasive breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2009; 18:3079–3085. [PubMed: 19861516]
28. Katoh M. Cancer genomics and genetics of FGFR2 (Review). *Int J Oncol*. 2008; 33:233–237. [PubMed: 18636142]
29. Poschl G, Seitz HK. Alcohol and cancer. *Alcohol Alcohol*. 2004; 39:155–165. [PubMed: 15082451]
30. Hishikawa Y, Tamaru N, Ejima K, Hayashi T, Koji T. Expression of keratinocyte growth factor and its receptor in human breast cancer: its inhibitory role in the induction of apoptosis possibly through the overexpression of Bcl-2. *Arch Histol Cytol*. 2004; 67:455–464. [PubMed: 15781986]
31. Tamaru N, Hishikawa Y, Ejima K, et al. Estrogen receptor-associated expression of keratinocyte growth factor and its possible role in the inhibition of apoptosis in human breast cancer. *Lab Invest*. 2004; 84:1460–1471. [PubMed: 15311216]
32. Yang JY, Hung MC. A new fork for clinical application: targeting forkhead transcription factors in cancer. *Clin Cancer Res*. 2009; 15:752–757. [PubMed: 19188143]
33. Tran H, Brunet A, Griffith EC, Greenberg ME. The many forks in FOXO's road. *Sci STKE*. 2003; 2003:RE5. [PubMed: 12621150]
34. Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene*. 2005; 24:7410–7425. [PubMed: 16288288]
35. Storz P, Doppler H, Toker A. Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. *Mol Cell Biol*. 2005; 25:8520–8530. [PubMed: 16166634]

36. Zou Y, Tsai WB, Cheng CJ, et al. Forkhead box transcription factor FOXO3a suppresses estrogen-dependent breast cancer cell proliferation and tumorigenesis. *Breast Cancer Res.* 2008; 10:R21. [PubMed: 18312651]
37. Katoh M. FGF signaling network in the gastrointestinal tract (review). *Int J Oncol.* 2006; 29:163–168. [PubMed: 16773196]
38. Kharas MG, Deane JA, Wong S, et al. Phosphoinositide 3-kinase signaling is essential for ABL oncogene-mediated transformation of B-lineage cells. *Blood.* 2004; 103:4268–4275. [PubMed: 14976048]
39. Adnane J, Gaudray P, Dionne CA, et al. BEK and FLG two receptors to members of the FGF family, are amplified in subsets of human breast cancers. *Oncogene.* 1991; 6:659–663. [PubMed: 1851551]
40. Huijts PE, van Dongen M, de Goeij MC, et al. Allele-specific regulation of FGFR2 expression is cell type-dependent and may increase breast cancer risk through a paracrine stimulus involving FGF10. *Breast Cancer Res.* 2011; 13:R72. [PubMed: 21767389]
41. Beasley JM, Coronado GD, Livaudais J, et al. Alcohol and risk of breast cancer in Mexican women. *Cancer Causes Control.* 2010; 21:863–870. [PubMed: 20155314]
42. Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. *Jama.* 2001; 286:2143–2151. [PubMed: 11694156]
43. Organization WH. *Global Status Report on Alcohol and Health 2011.* 2011.
44. Secretaría de Salud IMdP, Dirección General de Epidemiología, Consejo Nacional Contra las Adicciones. *Tercera Encuesta Nacional de Adicciones, Mexico.* 1999
45. Secretaría de Salud IMdP, Dirección General de Epidemiología, Consejo Nacional Contra las Adicciones. *Cuarta Encuesta Nacional de Adicciones, Mexico.* 2003
46. Ward E, Jemal A, Cokkinides V, et al. Cancer disparities by race/ethnicity and socioeconomic status. *CA Cancer J Clin.* 2004; 54:78–93. [PubMed: 15061598]

**Table 1**Characteristics of Mexican women by case control status, Mexico, 2004–2007<sup>a</sup>

	Cases n= 687 (%)	Controls n= 907 (%)	p value <sup>b</sup>
Age (years) <sup>c</sup>	52.1 (9.8)	51.1 (9.2)	0.053
Breast cancer in first degree relatives			
No	92.7	96.1	0.003
Yes	7.3	3.9	
Daily caloric intake (kcal) <sup>c</sup>	2170.8 (852.6)	1832.1 (708.0)	< 0.001
Daily caloric intake (kcal)			
< 1596.0	24.8	41.4	< 0.001
1596.0 – 2192.9	32.8	33.6	
> 2192.9	42.4	25.0	
Occasional intake of > 1 alcohol drinks within a month over a period of 1 years <sup>d</sup>			
No	80.3	90.0	< 0.001
Yes	19.7	10.0	
Weekly moderate-intensity physical activity (hours) <sup>c</sup>	9.7 (12.3)	15.3 (16.2)	< 0.001
Body Mass Index (kg/m <sup>2</sup> ) <sup>c</sup>	29.4 (5.6)	30.6 (5.4)	< 0.001
Tobacco smoking, once during life			
No	73.1	79.5	0.003
Yes	26.9	20.5	
Tobacco smoking 100 cigarettes during lifetime <sup>e</sup>			
No	73.1	79.5	0.003
Yes	26.9	20.5	
Socio-economic status			
Low	30.8	35.0	< 0.001
Middle	24.5	32.0	
High	44.7	33.0	
Menopausal status			
Premenopause	42.9	45.0	0.416
Postmenopause	57.1	55.0	
<b>Oral contraceptives use, once during lifetime</b>			
<b>No</b>	<b>52.8</b>	<b>54.2</b>	<b>0.577</b>
<b>Yes</b>	<b>47.2</b>	<b>45.8</b>	
Age at menarche (years) <sup>c</sup>	12.8 (1.7)	12.9 (1.6)	0.169
<b>Age at first live birth (years)<sup>c</sup></b>	<b>20.8 (8.4)</b>	<b>20.2 (6.5)</b>	<b>0.001</b>
Parity <sup>c</sup>	3.0 (2.2)	3.8 (2.5)	< 0.001
Lactation (months) <sup>c</sup>	21.6 (28.3)	31.0 (35.9)	< 0.001
Self-report of diabetes mellitus			

	Cases n= 687 (%)	Controls n= 907 (%)	p value <sup>b</sup>
No	79.2	84.3	0.008
Yes	20.8	15.7	
Self-report of arterial hypertension			
No	62.1	67.0	0.075
Yes	35.1	31.3	
Personal history of benign breast cancer			
No	85.1	93.0	< 0.001
Yes	14.9	7.0	
Ancestry <sup>c</sup>			
Native	0.60 (0.21)	0.64 (0.20)	< 0.001
European	0.36 (0.20)	0.32 (0.18)	< 0.001
African	0.04 (0.06)	0.04 (0.05)	0.248
rs2981582 (C>T)			
CC	33.9	38.9	0.007
CT	45.0	45.8	
TT	21.1	15.3	
Allelic frequencies			
C	56.4	61.8	0.002
T	43.6	38.2	

Due to missing values, percentages may not total 100%.

<sup>a</sup>Relative frequency is shown (%) unless otherwise specified.

<sup>b</sup>P-value.

<sup>c</sup>Arithmetic mean (standard deviation).

<sup>d</sup>Among women reporting having consumed alcohol sometime during their lifetime.

<sup>e</sup>Among women reporting having smoked tobacco sometime during their lifetime.

Percentages do not reach 100% due to missing values

**Table 2**  
Bivariate and multiple analyses between selected variables and breast cancer risk, Mexico, 2004–2007

	Bivariate analysis			Multiple analysis		
	OR <sup>a</sup>	95% CI	p value	OR <sup>b</sup>	95% CI	p value
Breast cancer in first degree relatives						
No	1.00					
Yes	2.02	1.28 – 3.18	0.003	1.80	1.08 – 2.99	0.024
Daily caloric intake (per 100 kcal)	1.07	1.05 – 1.09	< 0.001	1.07	1.05 – 1.09	< 0.001
Weekly moderate-intensity physical activity (hours)	0.976	0.968 – 0.984	< 0.001	0.975	0.966 – 0.983	< 0.001
Body Mass Index (kg/m <sup>2</sup> )	0.96	0.94 – 0.98	< 0.001	0.96	0.94 – 0.99	0.001
Occasional intake of > 1 alcohol drinks within a month over a period of 1 years						
No	1.00			1.00		
Yes	2.25	1.66 – 3.04	< 0.001	2.15	1.53 – 3.03	< 0.001
Tobacco smoking during lifetime <sup>c</sup>						
100 cigarettes						
No	1.00			1.00		
Yes	1.41	1.11 – 1.79	0.006	1.01	0.76 – 1.33	0.967
Socio-economic status						
Low	1.00			1.00		
Middle	0.82	0.62 – 1.08	0.160	0.69	0.51 – 0.94	0.020
High	1.44	1.10 – 1.88	0.009	0.91	0.67 – 1.24	0.540
Menopausal status						
Premenopause	1.00			1.00		
Postmenopause	0.91	0.65 – 1.27	0.576	0.89	0.62 – 1.28	0.531
Age at menarche (years)	0.95	0.89 – 1.01	0.109	0.95	0.88 – 1.02	0.138
Parity	0.81	0.77 – 0.85	< 0.001	0.84	0.78 – 0.90	< 0.001
Lactation (months)	0.988	0.985 – 0.992	< 0.001	0.998	0.993 – 1.003	0.351
Self-report of diabetes mellitus						
No	1.00			1.00		
Yes	1.38	1.05 – 1.81	0.021	1.85	1.36 – 2.52	< 0.001
Personal history of benign breast						

	Bivariate analysis			Multiple analysis		
	OR <sup>a</sup>	95% CI	p value	OR <sup>b</sup>	95% CI	p value
disease						
No	1.00			1.00		
Yes	2.75	1.94 – 3.92	< 0.001	2.63	1.77 – 3.90	< 0.001
European ancestry (%)						
0 – 25	1.00			1.00		
>25 – 50	1.18	0.93 – 1.49	0.164	1.18	0.91 – 1.54	0.207
>50 – 75	1.72	1.25 – 2.37	0.001	1.50	1.05 – 2.14	0.026
>75 – 100	3.65	1.37 – 9.73	0.010	3.45	1.11 – 10.71	0.032
rs2981582 (C>T) <sup>c</sup>	1.28	1.10 – 1.47	0.001	1.24	1.06 – 1.46	0.008
rs2981582						
CC	1.00			1.00		
CT	1.17	0.93 – 1.47	0.183	1.08	0.84 – 1.39	0.544
TT	1.69	1.25 – 2.27	0.001	1.63	1.17 – 2.27	0.004

<sup>a</sup> ORs adjusted by design for place of residence, health service institution membership, and 5 years age interval.

<sup>b</sup> ORs adjusted by design for place of residence, health service institution membership, and 5 years age interval, and by the variables presented in the table.

<sup>c</sup> ORs for each additional T allele.

<sup>a,b,c</sup> Conditional logistic models were used.

**Table 3**  
Interaction between FGFR2 polymorphism (rs2981582) and environmental factors

	rs2981582 [No. of cases/controls, ORs (95% CIs)] <sup>a</sup>					P interaction
	CC	CT	TT	TT	TT	
Breast cancer in first degree relatives						
No	216/343, 1.00	284/397, 1.00	137/132, 1.00			
Yes	17/10, 2.87 (1.10 – 7.47)	25/18, 1.56 (0.77 – 3.18)	8/7, 0.98 (0.25 – 3.74)			0.160
Age at menarche (years)						
15	107/153, 1.00	132/187, 1.00	65/53, 1.00			
13 - 14	94/137, 1.44 (0.79 – 2.62)	137/164, 1.63 (0.96 – 2.77)	56/66, 0.41 (0.15 – 1.14)			
12	32/63, 1.68 (0.92 – 3.06)	40/64, 1.27 (0.75 – 2.17)	24/20, 0.44 (0.15 – 1.29)			0.180
Parity						
3	138/244, 1.00	168/271, 1.00	72/94, 1.00			
1 - 2	69/88, 1.29 (0.79 – 2.12)	110/120, 1.33 (0.88 – 2.02)	54/41, 1.06 (0.50 – 2.27)			
0	26/21, 1.78 (0.83 – 3.80)	31/24, 1.91 (0.94 – 3.87)	19/4, 7.52 (0.86 – 65.58)			0.558
Breastfeeding						
Ever	185/296, 1.00	239/346, 1.00	108/123, 1.00			
Never	48/57, 0.82 (0.47 – 1.42)	70/69, 1.12 (0.70 – 1.77)	37/16, 1.50 (0.53 – 4.25)			0.187
Menopausal status						
Premenopausia	100/155, 1.00	135/191, 1.00	60/62, 1.00			
Postmenopausia	133/198, 0.67 (0.36 – 1.25)	174/224, 1.19 (0.69 – 2.05)	85/77, 0.61 (0.20 – 1.81)			0.298
Occasional intake of > 1 alcohol drinks within a month over a period of 1 years <sup>b</sup>						
No	188/327, 1.00	246/369, 1.00	118/120, 1.00			
Yes	45/26, 3.97 (2.10 – 7.49)	63/46, 2.01 (1.23 – 3.29)	27/19, 1.21 (0.48 – 3.05)			0.043
Tobacco smoking 100 cigarettes during lifetime						
No	176/282, 1.00	224/332, 1.00	102/107, 1.00			
Yes	57/71, 0.71 (0.43 – 1.17)	85/83, 1.18 (0.78 – 1.79)	43/32, 0.75 (0.33 – 1.70)			0.632
Self-report of diabetes mellitus						
No	185/306, 1.00	247/342, 1.00	112/117, 1.00			



	rs2981582 [No. of cases/controls, ORs (95% CIs)] <sup>a</sup>				
	CC	CT	TT		P interaction
Yes	48/47, 1.70 (0.98 – 2.94)	62/73, 1.43 (0.89 – 2.29)	33/22, 3.05 (1.12 – 8.25)		0.824

<sup>a</sup> ORs adjusted by design variables: place of residence, health service institution membership, and 5 year age interval and also by European ancestry, weekly moderate-intensity physical activity, daily caloric intake, BMI, socio-economic status and by variables included in the table. A conditional logistic model was used.

<sup>b</sup> Among women reporting having consumed alcohol sometime during their lifetime.