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Cancer Letters 173 (2001) 37–41

CANCER Letters

www.elsevier.com/locate/canlet

Distribution of *HER2*^{V655} genotypes in breast cancer cases and controls in the United States

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Received 2 May 2001; received in revised form 26 June 2001; accepted 27 June 2001

Abstract

The minor variant frequency of a *HER2* polymorphism (*HER2*^{V655}) has been determined for 471 United States women enrolled in a multiracial case-control study. Allelic frequencies varied significantly by race. Genotypic distributions showed no excess breast cancer risk associated with inheritance of *HER2*^{V655} either as carriers (OR = 1.2, 95% CI = 0.8–1.9), heterozygotes (OR = 1.2, 95% CI = 0.8–1.9), or homozygotes (OR = 1.4, 95% CI = 0.4–4.2). Nor was there a significant association when each racial group was considered separately. The current study suggests the *HER2*^{V655} allele is not a breast cancer risk factor for Caucasians, African–Americans, or Latinas.

Keywords: Breast neoplasms; *HER2*; Polymorphisms; African–Americans; Caucasians; Latinas

1. Introduction

Human epidermal growth factor receptor-2 (*HER2*) is a proto-oncogene encoding a transmembrane tyrosine kinase receptor (185 kDa) that may have an important role in breast cancer [1,2]. A single nucleotide polymorphism at codon 655 (A/G, isoleucine or valine) of human *HER2* was identified in 58 unrelated, healthy German people, and the allelic frequency of the minor variant (valine, *A2*, *HER2*^{V655}) was determined to be 0.32 [3]. A recent population-based (Shanghai, China) molecular epidemiologic study found a higher frequency of the valine variant among 339 breast cancer cases than among 359 controls ($F = 0.16$ and 0.11, respectively; $P \leq 0.011$) [4]. This study further indicated that younger women (age ≤45 years) and homozygotes were at even greater risk of breast cancer (OR = 1.7, 95% CI = 1.1–2.6 and OR = 14.1, 95% CI = 1.8–113.4, respectively) [4].

In light of inter-racial variation in gene frequencies and because geographically translocated populations adopt similar cancer incidence rates to the indigenous people, Ameyaw et al. [5] suggested that the association between *HER2*^{V655} and breast cancer may not be consistent across all racial groups. We have genotyped 471 women enrolled in a hospital based, New

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0304-3835/01/$ - see front matter
PII: S0304-3835(01)00671-1

York City, multiracial breast cancer case-control study (269 Caucasians, 97 African–Americans, and 105 Latinas). Whereas *HER2*^{V655} allele frequencies were found to vary significantly by race, no evidence was obtained to support the hypothesis that inheritance of *HER2*^{V655} was associated with increased risk of breast cancer.

2. Materials and methods

2.1. Human subjects

Construction of this hospital-based, New York City, breast cancer case-control study is described in detail elsewhere [6,7]. More than 1600 women presenting at the Mount Sinai Hospital and Kravis Women's Center were asked to participate in a breast cancer case-control study. Of 1101 (65%) women who agreed to participate, 531 were frequency-matched by race within 5-year age groups to form the multiracial case-control study (181 cases, and 350 controls: 175 benign breast disease and 175 non-breast disease). Informed consent was administered according to Institutional Review Board guidelines (Mount Sinai Medical Center, New York, NY, 1993), and further approval was obtained from the National Institute for Occupational Safety and Health – Human Studies Review Board (Cincinnati, OH, 1999). The study design was originally developed to identify major breast cancer risk factors (relative risks >3) in populations representative of New York City. Emphasis was also placed on premenopause versus postmenopause rather than early onset breast cancer, therefore, study participants were generally older than those reported by Xie et al. [4]. Ethnicity was self-described and menopausal status was defined previously [6]. A collection of constitutive DNA samples obtained from 123 Chinese people, hospitalized for lung cancer in Guang Dong Province, China, was used for comparison of gene frequencies with those reported by Xie et al. [4].

2.2. Genotyping for the HER2 codon 655 polymorphism

The *HER2* codon 655 polymorphism was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism. Our approach was similar to that of Xie et al. [4], but used different flanking primers. Use of the enzyme *BsmAI* reveals the same polymorphism as that previously described [4]. Constitutive, genomic DNA was amplified in a reaction mixture (50 μl) containing: *HER2* specific primers (*HER2*-F = 5′AGCCCTCTGACGTCCAT3′ and *HER2*-R = 5′-GCAGCAGTCTCCGCA3′), Amplitaq Gold™ and $MgCl_2$ (1 U and 2.5 mM, respectively; Perkin Elmer [PE], Foster City, CA), dNTPs (100 μM; Promega, Madison, WI), and Taq-polymerase reaction buffer (PE). Reactions were allowed to proceed by an initial melting (94°C, 6 min) followed by 35 cycles of denaturation (94°C, 30 s), annealing (56°C, 1 min), and extension (72°C, 1 min), with a final extension (72°C, 7 min). Amplicons (130 bp) were incubated (55°C, 1 h) in the presence of *Bsm*AI (1 U; New England Biolabs, Beverly, MA). Restriction fragments were separated on agarose gels (3% nusieve, containing 0.5 μg/ml ethidium bromide). Of 531 women who provided an adequate blood sample, successful genotyping was completed for 471 (269 Caucasians, 97 African–Americans, and 105 Latinas).

2.3. Statistical analysis

Genotype frequency differences, Hardy–Weinberg equilibrium analysis, and the association between *HER2*^{V655} and the development of breast cancer were examined using Exact methods and Chi square statistics. The Chi square test was used to compare the gene frequencies of *HER2* in the control populations of three racial groups, African–American, Latina, and Caucasian. If two of the four cells had an expected value less than 5, Fisher's exact test was used. This procedure results in exact results for a 2×2 table, but is only necessary when small expected numbers are encountered. The Chi-square test was also used to determine if the genotype frequencies reported in our populations followed Hardy–Weinberg laws. Logistic regression was used to determine odds ratios while adjusting for menopause status and race. Gene frequencies were estimated using standard methods [8–10]. Confidence intervals (CIs) for proportions were based on exact CIs for a binomial distribution.

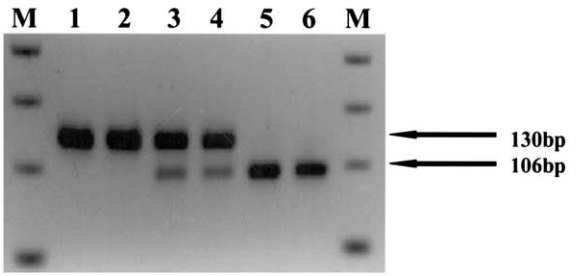

Fig. 1. Amplicons (130 bp) were generated from constitutive, genomic DNA by PCR. Restriction analysis was then performed using *BsmAI*. Three patterns were observed: isoleucine homozygotes – lanes 1 and 2; heterozygotes – lanes 3 and 4 and valine homozygotes – lanes 5 and 6; lane M shows the molecular weight marker (50 bp ladder).

3. Results

Restriction analysis for six representative samples are shown in Fig. 1 (restriction site absent homozygotes [isoleucine variant – lanes 1 and 2], restriction site present homozygotes [valine variant – lanes 5 and 6], and heterozygotes [lanes 3 and 4]). Genotypes for 471 women (269 Caucasians, 97 African–Americans, and 105 Latinas) were consistent with the patterns illustrated, and no unexpected patterns were observed (Fig. 1). Among controls, the frequency of the minor allelic variant, $HER2^{V655}$,varied by race (allele frequency of $HER2^{V655} = 0.16$, 0.05 and 0.13 for Caucasians, African–Americans, and Latinas, respectively). The differences in allele frequency were statistically significant for Caucasians versus African–Americans ($P = 0.01$), borderline for African–American versus Latinas ($P = 0.06$) and not significant for Caucasians versus Latinas ($P = 0.53$) (Table 1).

Although sample sizes were small for individual race groups in the existing breast cancer case-control study, the frequencies of $HER2^{V655}$ in each individual group were evaluated. No difference in frequency of $HER2^{V655}$ was observed for breast cancer cases compared to controls ($P = 0.70$, 1.00, and 0.32 for Caucasians, African–Americans, and Latinas, respectively; Table 1). Comparisons of the genotypic distributions for the group as a whole showed no excess breast cancer risk associated with inheritance of $HER2^{V655}$ either as carriers (OR = 1.2, 95% CI = 0.8–1.9), heterozygotes (OR = 1.2, 95% CI = 0.8–1.9), or homozygotes (OR = 1.4, 95% CI = 0.4–4.2). Nor was a significant association with $HER2^{V655}$ and breast cancer risk found when each

Table 1
Frequency of $HER2^{V655}$ in breast cancer cases and controls

Population	Genotype			F - *A2*[a] (CI[b])	HW[c]	*P*-difference
	A1/A1	*A1/A2*	*A2/A2*			
Caucasians						
Controls (180)	129	44	7	0.16 (0.12–0.20)	0.20	
Cases (89)	59	26	4	0.20 (0.14–0.26)	0.61	0.70
	OR[d] = 1.3 (0.7–2.2)					
African–Americans						
Controls (63)	57	6	0	0.05 (0.02–0.10)	0.69	
Cases (34)	32	2	0	0.03 (0.004–0.10)	0.86	1.00
	OR = 0.8 (0.1–4.2)					
Latinas						
Controls (77)	58	18	1	0.13 (0.08–0.19)	0.74	
Cases (28)	17	10	1	0.21 (0.12–0.34)	0.75	0.32
	OR = 1.8 (0.7–4.4)					

[a] F - *A2* = allelic frequency of $HER2^{V655}$, the minor allelic variant.
[b] CI = confidence interval.
[c] HW = Hardy–Weinberg statistic (*P* = value). No association was found between genotype and breast cancer risk.
[d] OR = Odds Ratios (and 95% confidence interval), and for the group as a whole OR = 1.2 (0.8–1.9). Differences in allelic frequencies were observed between control groups for Caucasians and African–Americans ($\chi^2 = 9.7$, $P = 0.01$), African–Americans and Latinas ($\chi^2 = 5.7$, $P = 0.06$), but not Caucasians and Latinas ($\chi^2 = 1.3$, $P = 0.53$).

racial group was considered separately (Table 1) or when the analysis was limited to younger women (data not shown). The current study suggests the *HER2*^{V655} allele is not a breast cancer risk factor for Caucasians, African–Americans, or Latinas. These data are in agreement with a recent report that showed no association between inheritance of *HER2*^{V655} and risk of breast cancer or ovarian cancer in a British population [11,12].

4. Discussion

Differences in *HER2*^{V655} allelic frequencies were observed between Caucasians and African–Americans ($P = 0.01$), African–American and Latinas ($P = 0.06$) but not Caucasians and Latinas ($P = 0.53$). Considering previously published reports, the difference in allelic frequencies between: United States Caucasians and German Caucasians were significant ($P = 0.001$) [3]; United States Caucasians and Chinese (0.16 versus 0.11; $P = 0.02$) [4]; German Caucasians and Chinese (0.32 versus 0.11; $P = 0.001$) [3,4]; African–Americans and Chinese (0.05 versus 0.11; $P = 0.03$) [4]; but the frequencies for Latinas and Chinese were not significantly different (0.11 versus 0.11; $P = 0.45$) [4]. There was a striking difference between the *HER2*^{V655} allelic frequency detected in New York City African–Americans compared to that reported for the African–American population studied by Ameyaw et al. [5] (0.05 versus 0.24; $P < 0.001$). The reason for this is unknown, however, regional variation is not unusual and emphasizes the need for geographically appropriate controls.

Regarding the possible association between inheritance of *HER2*^{V655} and breast cancer risk, the data from the current study are in agreement with a recent report on a British population that found no association among either breast cancer cases or ovarian cancer cases [11,12]. However, since our original study design did not specifically seek to investigate this *HER2* polymorphism, we acknowledge a lack of power and accept that a positive significant association could have been missed (e.g. type 2 error). The data are, however, valuable since, in view of the racial variation in allele frequencies for this polymorphism, design of future studies needs to weigh the allelic frequencies reported here.

We considered the *HER2*^{V655} genotypic distributions independently for all of the previously published study populations, as well as those reported here, all conformed to Hardy–Weinberg population laws (Table 1). However, Xie et al. [4] observed an under-representation of *HER2*^{V655} homozygotes ($P = 0.07$) in their Chinese control population. This factor could have contributed to the reported association with breast cancer risk [4]. We have determined the *HER2*^{V655} allelic frequency among 123 Chinese and have found it to be 0.08 (Hardy–Weinberg $\chi^2 = 0.05$, $P = 0.82$), which is not significantly different ($P = 0.18$) to that found in the control population reported by Xie et al. [4]. Clearly, further independent studies that examine this question are warranted.

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