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Ethnic variation in the *HER-2* codon 655 genetic polymorphism previously associated with breast cancer

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Abstract *HER-2*, a protooncogene located on chromosome 17q21, encodes a transmembrane glycoprotein (p185) with tyrosine kinase activity. Alterations of the *HER-2* gene have been implicated in the carcinogenesis and prognosis of breast cancer and other solid tumors. It is also a cancer-therapeutic target for antibody-based therapy against the *HER-2* protein. A single-nucleotide polymorphism (SNP) at codon 655, resulting in a G-to-A transition (Ile655Val) in the transmembrane domain-coding region of this gene has been associated with an increased risk of breast cancer, particularly among younger women. To understand the importance of this finding throughout the world, we evaluated this polymorphism in Ghanaian, Kenyan, Sudanese, Caucasian, African–American, Saudi, and Filipino subjects using a polymerase chain reaction-restriction fragment length polymorphism assay. The frequency of the Val allele, which is associated with increased breast cancer risk, was highly variable between populations (0%–24%). Continental African populations had a lower frequency of the Val allele than did Saudi, Chinese, Filipino, Caucasian, and African–American subjects. The data suggest that this SNP has variable frequency in different ethnic groups. The findings in this study correspond with the lower incidence and lower risk of breast cancer in African women compared with Caucasian and African–American women.

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Introduction

Altered expression of the protooncogene human epidermal growth factor receptor 2 (*HER-2/neu* [*c-erb*B-2]) has been implicated in the carcinogenesis and prognosis of breast, ovarian, gastric, and prostate cancer, and in other solid tumors such as osteosarcomas and rhabdomyosarcomas (Dowsett et al. 2000; Hengster et al. 1999; Nakajima et al. 1999; Morote et al. 1999; Gorlick et al. 1999; Ricci et al. 2000). The *HER-2/neu* (*c-erb*B-2) protooncogene encodes a 185-kDa transmembrane tyrosine kinase receptor, a member of the type 1 tyrosine kinase receptor family, with extensive homology to epidermal growth factor receptor (Coussens et al. 1985). *HER-2* has been localized to chromosome 17q21.1 (Mulleris et al. 1997).

Recent studies indicate that *HER-2* may also be involved in determining the chemosensitivity of human cancers, especially breast and ovarian cancer (Mehta et al. 1998; Pegram et al. 1997). It has been implicated in the development of resistance to the antiestrogen tamoxifen in both advanced disease and in an adjuvant setting (De Placido et al. 1998). Overexpression of *HER-2* confers taxol resistance in breast cancers because it inhibits taxol-induced apoptosis (Yu et al. 1998). *HER-2* has been widely studied in breast cancer. The alterations of this gene are found in more than 20%–30% of human breast cancers, indicating that this gene may have an important role in this common malignancy.

Because several studies have suggested that breast cancer patients with *HER-2* overexpression exhibit a reduced response to conventional treatments, new therapeutic approaches, targeting cells that overexpress *HER-2*, have been developed (Slamon et al. 1987; Allred et al. 1992; Carter et al. 1992). Trastuzumab (Herceptin), a humanized monoclonal antibody, appears to block growth signals transmitted by *HER-2* to the nucleus and enhances

response to chemotherapeutic agents (Pietras et al. 1998; Slamon et al. 2001).

Sequence analysis of the human *HER-2* gene identified a single-nucleotide polymorphism (SNP) at codon 655, resulting in a G-to-A transition (Ile655Val) in the transmembrane domain-coding region of this gene (Papewalis et al. 1991). A population-based, case control study of this polymorphism was recently reported and the Val allele was found to be associated with an increased risk of breast cancer, particularly among younger women (Xie et al. 2000). However, there is little information on the population distribution of this SNP. This is an important issue in the context of significant ethnic differences in the incidence of breast cancer and other solid tumors (Parkin et al. 1997).

Subjects and methods

The *HER-2* polymorphism was evaluated in 200 Ghanaian, 257 Caucasian, 90 African–American, 114 Kenyan, 73 Filipino, 101 Saudi, and 52 Sudanese healthy blood donors after written informed consent and appropriate ethical committee approval. All female patients included in the study were premenopausal (average age 25.3 years). Results from some of the Ghanaian, Caucasian, and African–American subjects have been previously published in brief (Ameyaw et al. 2000). Genotyping of the G-to-A polymorphism at codon 655 of the *HER-2* gene was done using the previously described polymerase chain reaction-restriction fragment length polymorphism assay with minor modifications (Papewalis et al. 1991).

Restriction fragments were 135 bp and 13 bp for the valine allele and 101 bp, 34 bp, and 13 bp for the isoleucine allele. Comparison was made between allele frequencies and genotype frequencies for the Caucasian, Filipino, Sudanese, Saudi, African–American, and African populations as well as for the Chinese control population described by Xie et al. (2000), using the chi-square test. Hardy-Weinberg analysis was done using CLUMP software (Sham and Curtis 1995). The 95% confidence interval was calculated for all allele frequencies.

Results

The Val allele was not detected among the 400 alleles evaluated in the Ghanaian population and accounted for 1% of the Kenyan alleles and 9% of both the Sudanese and Filipino alleles (Table 1). In contrast, the Val allele was found in 20% of Caucasian, 24% of African–American, and 11% of Chinese and Saudi subjects (Table 1). The homozygous Val/Val genotype, previously associated with an increased breast cancer risk, was more frequently observed in Caucasian (5.4%) and African–American subjects (5.4%) than in Saudi (2.0%) and Chinese subjects (0.3%) ($P = 0.01$) (Table 1). This genotype was not found in any of the three continental African populations (Ghanaian, Kenyan, Sudanese). The *HER-2* allele frequency was not significantly different between African–American and Caucasian subjects ($P = 0.49$). The observed genotype frequency was not significantly different from that expected from the Hardy-Weinberg equilibrium in any population.

Discussion

In women, breast cancer is the third most common form of cancer globally and the second leading cause of cancer death among women in many populations. There are wide geographic variations in the incidence of breast cancer (Parkin et al. 1997). The reasons for the racial/ethnic variations are unclear.

Xie et al. (2000) found that women who had the Ile/Val or Val/Val genotype of the Ile655Val polymorphism in the *HER-2* gene had an elevated risk of breast cancer (odds ratio 1.4). The risk was greatest for women who were homozygous for the Val allele (odds ratio 14.1), and the association was more pronounced in women less than 45 years of age (odds ratio 1.7). The lower frequency of the Val allele in continental Africans corresponds with the lower incidence and lower risk of breast cancer in African women compared with Caucasian and African–American women (Parkin et al. 1997).

Table 1. Interethnic differences in the genotype and allele frequencies of the *HER-2* G-to-A polymorphism at codon 655

Population	*n*	Ile/Ile	Val/Ile	Val/Val	Ile (95% CI)	Val (95% CI)
Chinese (Xie et al. 2000)	359	0.780	0.217	0.003	0.89 (0.87–0.91)	0.11 (0.09–0.13)
Filipino	73	0.837	0.150	0.013	0.91 (0.84–0.98)	0.09 (0.02–0.16)
Saudi	101	0.802	0.178	0.002	0.89 (0.81–0.94)	0.11 (0.06–0.19)
Sudanese	52	0.827	0.173	0	0.91 (0.83–0.99)	0.09 (0.01–0.16)
Kenyan	114	0.974	0.260	0	0.99 (0.97–1.00)	0.01 (0–0.03)
Ghanaian (Ameyaw et al. 2000)	200	1.000	0	0	1.00 (0.99–1.00)	0 (0–0.01)
African–American (Ameyaw et al 2000)	90	0.567	0.389	0.044	0.76 (0.70–0.82)	0.24 (0.18–0.30)
Caucasian (Ameyaw et al. 2000)	257	0.654	0.292	0.054	0.80 (0.77–0.83)	0.20 (0.17–0.24)

n, Number of subjects

Age-standardized (world standard) incidence rates for female breast cancer are highest among white women in the United States (118.2 per 100,000) and lowest among women in The Gambia (3.4 per 100,000) (Parkin et al. 1997). Across the African continent, other available data for various countries are 10.2, 10.9, 16.4, and 18.6 per 100,000 for Mali, Guinea, Uganda, and Zimbabwe, respectively (Chokunonga et al. 2000). The incidence rate for African–Americans is 95.4 per 100,000 (Parkin et al. 1997). Breast cancer incidence has historically been four to seven times higher in the United States than in Asia (Ursin et al. 1999). The findings in this study correspond with the lower incidence and lower risk of breast cancer in African women compared with Caucasian and African–American women.

The Ile allele, which is present at high frequencies in all three main racial groups (African, Caucasian, and Asian), appears to be the ancestral allele, being present before the expansion of humans out of Africa over 100,000 years ago (Cavalli-Sforza et al. 1996). The Val allele was probably acquired more recently as a result of selective pressures that are not yet determinedn. Epigenetic factors such as diet and environmental influences may be maintaining the high frequency of the Val allele in the African–American population compared with the continental African population.

Several studies indicate that the genes that encode growth factor receptors can be activated through mutations in their coding sequences (Bargmann et al. 1986). Functional studies of this SNP are therefore required to assess its impact on the expression of *HER-2* in tissues. The evaluation of this SNP may be helpful in the identification of individuals at risk of breast cancer who may benefit from cancer screening and chemoprevention. This SNP needs to be evaluated in the context of the selection of therapy for tumors that overexpress *HER-2*.

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