Letters to the Editor

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DNA Sequence Variants of *p53:* Cancer and Aging

To the Editor:

p53 has a critical role in cell-cycle control. As such, it has been identified as an important target in human carcinogenesis. However, since human *p53* was cloned, ⩾10 DNA-sequence polymorphisms have been identified (Matlashewski et al. 1987; Weston and Godbold 1997). The codon 72 polymorphism (arginine/proline: G/C), the first to be described, has been the subject of ⩾31 epidemiological case-control studies that have explored a potential association with cancer (Olschwang et al. 1991; Weston et al. 1992, 1994, 1997; Zhang et al. 1992; Kawajiri et al. 1993; Birgander et al. 1995, 1996*b;* Jin et al. 1995; Själander et al. 1995*a,* 1996*a;* Wu et al. 1995; Murata et al. 1996; To-Figueras et al. 1996; Golovleva et al. 1997; Weston and Godbold 1997; Yung et al. 1997; Hayes et al. 1998; Helland et al. 1998; Hildesheim et al. 1998; Josefsson et al. 1998; Lanham et al. 1998; Minaguchi et al. 1998; Rosenthal et al. 1998; Storey et al. 1998; Tagawa et al. 1998; Wang-Gohrke et al. 1998). Although they err on the side of caution by citing Weston and Godbold (1997), Bonafè et al. (1999, p. 293), referring to the codon 72 polymorphism, state that "overall, the available data in the literature suggest that p53 variants may be considered as risk factors for some of the major neoplastic diseases in humans, such as lung, colorectal, breast and cervical cancer and are expected to affect survival." With respect to codon 72, we contend that this is probably not the case. In the available data, lung cancer is the subject of eight studies (Weston et al. 1992, 1994; Kawajiri et al. 1993; Birgander et al. 1995; Jin et al. 1995; Murata et al. 1996; To-Figueras et al. 1996; Tagawa et al. 1998). Three studies claim a statistically significant association: in one study, a subset analysis suggested a relationship in lung cancer cases diagnosed at age <53 years (Jin et al. 1995); in the other two, the allelic frequencies were almost identical (and the difference was not significant) between cases and controls (Kawajiri et al. [1993] observed a proline-allele frequency of .35 for controls and .36 for cases [n = 347 and 328, respectively]). Murata et al. (1996) observed a proline-allele frequency of .40 for controls and .35 for cases (n = 152 and 191, respectively), but associations of cancer risk were claimed on the basis of higher numbers of homozygotes (Kawajiri et al. 1993; Murata et al. 1996). One study (Kawajiri et al. 1993) implicated proline; the other (Murata et al. 1996), arginine.

Most recently, the relationship between cervical cancer, human papillomavirus (HPV) infection, and inheritance of the arginine allele has received considerable attention. The initial study, cited by Bonafè et al. (1999), showed a high degree of correlation between inheritance of the arginine allele and cervical cancer risk when HPV infection was present but not when it was absent (Storey et al. 1998). There are now eight studies of cervical cancer, but only one shows any association and seven are null (Hayes et al. 1998; Helland et al. 1998; Hildesheim et al. 1998; Josefsson et al. 1998; Lanham et al. 1998; Minaguchi et al. 1998; Rosenthal et al. 1998). There are five breast cancer studies (Kawajiri et al. 1993; Själander et al. 1996*a;* Weston et al. 1997; Helland et al. 1998; Wang-Gohrke et al. 1998); only one reached significance. Of the other published studies, a positive association has been claimed in 0/2 for nasopharyngeal cancer (Birgander et al. 1996*b;* Golovleva et al. 1997; Yung et al. 1997), 0/4 for colon cancer (Olschwang et al. 1991; Kawajiri et al. 1993; Jin et al. 1995; Själander et al. 1995*a*), 0/2 for bladder/urologic cancer (Kawajiri et al. 1993; Wu et al. 1995), 0/1 for acute myelogenous leukemia (Zhang et al. 1992), and 1/1 for stomach cancer (Kawajiri et al. 1993). Again, for stomach cancer, the allelic frequencies were not different for cases and controls, and an association of cancer risk with the arginine variant was claimed on the basis of higher numbers of arginine homozygotes in cases (Kawajiri et al. 1993; this report used the same control group noted above, and the proline-allele frequency in stomach cancer was .28 [n = 140]).

We contend, therefore, that the balance of the results of human molecular epidemiological association studies suggests that codon 72 allelism does not have an impact on human cancer risk. Specifically, at most, only 6 of 31 positive associations have been reported, and none have received consistent support in attempts to replicate them (Kawajiri et al. 1993; Jin et al. 1995; Murata et al. 1996; Själander et al. 1996*a;* Storey et al. 1998). Moreover, there is no obvious, plausible biological basis

Table 1

Simulated Relative-Risk Calculation for a Standard Population of 1,000 Persons

	No. of Cancer Deaths	No. of Survivors	Total
Proline-allele carriers	147	392	539
Arginine homozygotes	83	378	461
Total	230	770	1,000

NOTE.—On the basis of the control genotypic frequencies reported by Bonafè et al. (1999), a relative risk of 1.5, and a cancer death rate of 23% (WHO 1987; Lopez 1990), the frequency of proline-allele carriers in survivors is .509 (a reduction of 3.0%). If the relative risk is raised to 2.0, the frequency of proline-allele carriers in survivors is .491 (a reduction of 4.8%). This model addresses only a role for *p53* allelism in cancer as a cause of death. If limited to the major neoplasms indicated by Bonafè et al. (1999) (lung, colon, breast, and cervical), with a relative risk of 2.0, the frequency of proline-allele carriers in survivors is .526 (a reduction of only 1.3%). Also note that, if any association is limited to proline homozygotes, then, for a relative risk of 1.5, this fraction of the population would be expected to drop from .088 to .076 (a reduction of 1.2%). Bonafè et al. (1999), in fact, observed a drop of 2.0%, from .088 to .068.

for an association of cancer risk with the codon 72 polymorphism. The impact of proline on the tertiary structure of a protein is disruption of α-helices. A proline residue resides at the monomeric position codon 71; therefore, at codon 72 there is no obvious consequence to proline.

Results were reported for *p53,* codon 72, genotype frequencies in healthy Italian centenarians and younger controls (Bonafè et al. 1999). The expectation was of allelic winnowing in the case of a codon 72 variant associated with cancer susceptibility and subsequent survival. The frequency of proline-allele carriers was .539 in controls and .506 in centenarians, 3.3% lower (and the corresponding allelic frequency was 2.7% lower, but the difference was not significant). We conjecture that their comparison of centenarians (n = 176) with younger controls (n = 204) is too crude to detect the predicted allelic winnowing (Bonafè et al. 1999). First, the cause of death from cancer in the Italian population is ~23% (WHO 1987; Lopez 1990). Second, even though we do not agree that there is good evidence for an association between inheritance at codon 72 and cancer susceptibility, because of the molecular epidemiological data reviewed above, let us assume that the proline variant carries a relative risk of 1.5–2.0 (Kawajiri et al. 1993). On the basis of this assumption we might expect a reduction of 3.0%–4.8% in proline-allele carriers as the population ages (table 1 shows a simulated 2 × 2 table for a standard population of 1,000 persons, given a relative risk of 1.5 and a cancer death rate of 23% [WHO 1987; Lopez 1990]). In the letter by Bonafè et al. (1999), a reduction of 3.3% in the frequency of proline-allele carriers among centenarians was reported. This reduction is consistent with their hypothesis, although the authors suggest that it is not (Bonafè et al. 1999). Moreover, our corresponding projected reduction in allelic frequency is 2.8% for a relative risk of 1.5; Bonafè et al. (1999) observed a 2.7% reduction in frequency. However, to have, at a significance level of .05, 80% power to detect a 3.0%–4.8% reduction in the prevalence of proline-allele carriers, a minimum of 2,002–3,178 people would be needed, given a relative risk of 2.0; and 5,176–8,182 would be needed, given a relative risk of 1.5 (results of power calculations are given in table 2).

Notwithstanding these conclusions and observations, we believe that *p53* allelism is an important cancer-susceptibility factor but that it is more complex than that simply defined by the polymorphism at codon 72. A series of studies by a Swedish group (Beckman et al. 1994; Birgander et al. 1995, 1996*a,* 1996*b;* Själander et al. 1995*a,* 1995*b,* 1996*a,* 1996*b*) indicated that a constellation of three *p53* polymorphisms (intron 3, codon 72, and intron 6) constituted a haplotype predictive of increased breast cancer risk (odds ratio [OR] = 2.9, 95% confidence interval [CI] = 1.4–6.3 [Själander et al. 1996*a*]). Haplotypes were deduced on the basis of population frequencies of the individual polymorphisms. First, estimates of pairwise haplotype frequencies were calculated, and extended haplotype frequencies were deduced from this information. A subsequent study examined the same question but used a PCR-based method for physical determination of the haplotypes (Weston et al. 1997, 1998). The results of the latter study were consistent with those reported in the study by Själander et al. (1996*a*) (OR = 2.5, 95% CI = 1.3–4.8, in postmenopausal women) and further implicated a specific haplotype, designated "$p53^{1\text{-}2\text{-}1}$." More recently, a third study, estimating extended haplotypes of these three polymorphisms in a German population, provided results consistent with an elevated breast cancer risk associated with inheritance of $p53^{1\text{-}2\text{-}1}$ (OR = 2.0, 95% CI = 1.0–3.9 [Wang-Gohrke et al. 1998]). It is to be noted

Table 2

Power Calculations

	No. of Centenarians Required for Hypothesis Testing When Frequency of Proline-Allele Carriers Is[a]	
OR	3.0%	4.8%
1.5	4,091	2,588
2.0	1,589	1,001

[a] Percentages are derived from calculations in table 1.

that this latest report has adopted an allelic nomenclature different from that developed by Beckman et al. (1994) and adopted by others (Själander et al. 1996*a;* Weston et al. 1997, 1998).

The balance of the results of human breast cancer studies of molecular epidemiological haplotype association—specifically, three positive associations in three studies—suggests that inheritance of the $p53^{1\text{-}2\text{-}1}$ haplotype does have an impact on human breast cancer risk (Själander et al. 1996*a;* Wang-Gohrke et al. 1998; Weston et al. 1998). However, more research is needed to resolve this question. In our laboratory, we have adopted a complementary molecular epidemiological and basic-science approach. First, we are trying again to replicate the epidemiological studies of breast cancer, by using the PCR-based physical haplotype method in a population-based case-control study that has sufficient power. Second, we have derived normal human mammary cell strains with known haplotypes and plan to test their response to suspect breast carcinogens.

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YU SUN, CHANNA KESHAVA, DAN S. SHARP, AINSLEY WESTON, AND ERIN C. MCCANLIES
National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention
Morgantown, WV

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Address for correspondence and reprints: Dr. Ainsley Weston, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Road, Morgantown, WV 26505-2888. E-mail: AGW8@CDC.GOV

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