Polymorphisms of H-ras-1 and p53 in Breast Cancer and Lung Cancer: A Meta-analysis

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Certain polymorphic variants of H-ras-1 and p53 have been investigated for an association between inheritance and cancer risk. The results of a metaanalysis, which reviews studies of H-ras-1 rare alleles and p53 codon 72 allelic variants in breast and lung cancer, are presented. The data constituted evidence for elevated risk of both breast and lung cancer with inheritance of rare H-ras-1 alleles. Calculated population attributable risks are 0.092 and 0.037 for breast and lung cancer, respectively. The frequency of the rare H-ras-1 alleles was observed to be greater in African Americans than in Caucasians, and a specific allele (A2) is common in African Americans was found only at low frequency in Caucasians. For p53 a consensus has yet to be reached. Lung cancer studies conducted in Caucasian and African-American populations have found no evidence of risk associated with the proline variant of codon 72. Two similar studies conducted in Japanese populations suggested an association between p53 genotype distribution and lung cancer risk. However, one implicates the proline allele but the other implicates the arginine allele. The frequency of the proline variant is significantly dependent on race. Frequencies have been reported for control populations of Japanese (0.347 and 0.401), Caucasian (0.295, 0.284, and 0.214), African American (0.628 and 0.527), and Mexican American (0.263). — Environ Health Perspect 105(Suppl 4):919–926 (1997)

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Introduction

Conserved nucleotide substitutions that occur during evolution are considered to be polymorphisms when the prevalence of the rare or minor allele reaches 0.01 (%) (1). Repetitive DNA sequences are composed of multiple consensus motifs. These consensus motifs may be short (di- and trinucleotides) or long (10–100 nucleotides) and are arranged in tandem array. Concatenations of these consensus motifs form minisatellite or microsatellite regions that may be tens, hundreds, or thousands of nucleotides in length. Mutational pressures that lengthen or shorten these reiterated sequences spawn multiallelic, variable (or hypervariable) tandem repeat (VTR) polymorphisms (2). These conserved DNA-polymorphisms may be silent or they may encode a structural or functional change.

Inheritance of mutations in certain critical genes predispose to various types of family cancer syndrome. These include germ line mutations in p53, BRCA-1, Rb, MCC, MSH-2, and WT-1 (3–12). The identification, isolation, and cloning of these genes has resulted in increased knowledge of inheritance of these syndromes. Overt predisposition to disease occurs when a germ line mutation in a tumor suppressor gene is inherited. Alternately, it is proposed that more subtle predisposition to common adult cancers may result from inheritance of specific genetic polymorphisms. These may alter susceptibility in conjunction with certain environmental exposures.

To test this hypothesis several molecular epidemiologic lung and breast cancer case-control studies have been performed (13–35). This report focuses on those studies that have examined possible associations between polymorphisms at the H-ras-1 and p53 gene loci and lung cancer and breast cancer predisposition.

The p53 gene is functionally characterized as a tumor suppressor gene (10). Somatic mutations (primarily nucleotide substitutions) of this gene have been detected in diverse human cancer types (36) and inheritance of germ line mutations predispose to breast, colon, and brain cancers (37,38). The role of p53 is to prevent tissue overgrowth; one mechanism operates through abrogation of cells with damaged genomes. Homeostatic controlling functions, even in the presence of already severely damaged cells that are being driven by activated protooncogenes (oncogenes) may be maintained by a fully functional p53 protein. The role of p53 in the life cycle of the cell is becoming increasingly well understood. The p53 protein has been shown to have broad functionality in cellular processes. These include cell-cycle control, DNA repair, differentiation, genomic plasticity, and apoptosis (programmed cell death) (39–41).

Ten genetic polymorphisms that have been described for p53 are cataloged in Table 1 (21,42–53). Three of the five nucleotide substitution polymorphisms are silent, conferring no change in amino acid sequence, and two cause amino acid substitutions. At codon 47 a proline to serine substitution was found in 0 of 69 Caucasians and 3 of 32 (3 of 64 alleles, 0.047) African Americans; results of in vitro experiments that used a construct of this allele containing a luciferase reporter gene indicated that this polymorphism does not interfere with growth-suppressor activity (21). At codon 72 a G to C transition, recognized by Acc II and several isoschizomers (Bsp50-1, BstUI, Bst1236I, Mmnl, and Thal), accounts for an arginine to proline amino acid substitution. The reported allelic frequencies for this polymorphism are given in Table 1 and vary with respect to race. This polymorphism has been investigated in several case-control studies of lung cancer, as well as breast, colon, stomach, and bladder cancers (13–20). Five of the ten p53 polymorphisms occur in intronic sequences. Three of these are revealed by restriction digestion in introns 1, 6, and 7 (46,48, 49,51,54). One polymorphism is a six allele VTR in intron 1 (55). In intron 3 there is a biallelic 16 bp insertion/deletion (56). The longer, minor allele (A2) is a direct repeat; and though not independently confirmed (57), it has been reported that women who
inhibit the p53 repeated motif (A2) have an excess risk of ovarian cancer [odds ratio = 8.6 (95% CI: 3.0–25.2)] (58).

The H-ras-1 gene is a protooncogene responsible for control of cell growth (proliferation) and specialization (differentiation). Almost all protooncogenes encode a protein component of the signal transduction cascade (59). This integrated multiprocess system is responsible for the smooth, orderly, and specific transmission of extracellular signals to the nucleus. Activated ras genes predominate as the family of oncogenes to be isolated from solid tumors induced by chemicals in laboratory animals. Members of the ras gene family code for proteins of molecular weight 21,000 (p21); these proteins are membrane bound, have GTPase activity, and form complexes with other proteins. The ras genes are small G-proteins (guanine nucleotide binding) that exert a powerful proliferative response through signal transduction (59).

Two VTR polymorphisms exist at the H-ras-1 gene locus. Between the pseudoexon and exon 1 there is a short (12–24) trinucleotide VTR composed of a 6 bp consensus motif ([60]; A. Weston et al., unpublished observations) (Figure 1). Approximately 1.4 kb to the 3-prime end of the structural gene there exists a longer VTR. A 28 bp consensus sequence, tandemly repeated between approximately 30 and 110 times, accounts for alleles that are approximately 900 to 3000 bp in length (61,62). More than 30 alleles have been described and their frequencies have been documented as common (0.07–0.65), intermediate (0.005–0.015) and rare (<0.005) (63). In 1985, Krontritis et al. (61) reported that individuals who inherited rare alleles were at increased risk of cancer. In the intervening years, numerous investigators have tried to replicate these findings in specific cancer types through case–control studies (22–33) and family studies (32,33), with varying success.

This report is a metaanalysis that formally summarizes the molecular epidemiologic data for the associations of both p53 and H-ras-1 polymorphisms with risk of either breast cancer or lung cancer.

### Methods

#### Study Subjects

Subjects were either lung or breast cancer cases and controls who participated in 21 reported studies (13–35). In the lung cancer studies reported by Sugimura et al. (24) and Weston et al. (25), subjects were African Americans and Caucasians from the Baltimore–Washington metropolitan area.

#### Determination of the H-ras-1 Minisatellite VTR Polymorphism

The H-ras-1 minisatellite VTR polymorphism has been determined by Southern hybridization using an H-ras-1-derived plasmid (pEC) and DNA fragment size determination. Distinction of different alleles has been achieved using a variety of

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| Location | Characterization | Frequency* | Ethnicity | References |
|----------|------------------|-------------|-----------|------------|
| Coding   |                  |             |           |            |
| Codon 21 | GAC → GAT        | —           | —         | Ahuja et al. (45) |
| Codon 36 | CCG → CCA        | 0.980       | —         | Felix et al. (52) |
| Codon 47 | CCG → TCG        | 0.953       | African American | Felley-Rosco et al. (21) |
| Codon 72 | GCC → CCC        | 0.628, 0.523 | African American | Weston et al. (13,15); Jin et al. (18) |
|          | (Arg → Pro)      | 0.705, 0.786 | Caucasian (United States/Europe) | Weston et al. (13,15); To-Figuera et al. (20) |
|          |                  | 0.653, 0.593 | Japanese | Kawajiri et al. (14); Murata et al. (19) |
|          |                  | 0.737*      | Mexican American | Jin et al. (19) |
|          |                  | 0.716*      | Swedish | Birgander et al. (17) |
|          |                  | 0.830*      | Swedish Saamis | Sjlander et al. (54) |
|          |                  | 0.968*      | United States | Carbone et al. (53) |
| Noncoding|                  |             |           |            |
| Intron 1 | VTR, six alleles | 0.02–0.46   | —         | Futreal et al. (47) |
| Intron 1 | Hae III restriction | 0.770        | Japanese | Ito et al. (51) |
| Intron 3 | 18 bp insertion  | 0.875, 0.863 | Caucasian (United States/Europe) | Runnebaum et al. (56); Lancaster et al. (57) |
| Intron 6 | Msp I/BstN1/NciI restriction (A → G) | 0.856 | Caucasian | Chumakov (49) |
|          |                  | 0.740       | Swedish | Sjlander et al. (54) |
|          |                  | 0.740       | United States | McDaniel et al. (46) |
| Intron 7 | Apal restriction  | 0.946       | Caucasian | Prosser and Condie (49) |

*Frequency of major allele. †Proline. ‡Arginine.
restriction enzymes. These include BamHI, AvaII, TaqI, PvuII, PstI, and MspI/HpaII. The restriction enzyme sites that reside closest to the H-ras-1 VTR region are MspI/HpaII. Digestion of genomic DNA with MspI/HpaII and Southern hybridization with pEC results in fragments of approximately 0.9 to 3.0 kb. This indicates that MspI (MspI/HpaII) is the restriction enzyme of choice for this analysis. Use of BamHI results in H-ras-1 fragments of approximately 6.5 to 9.0 kb. Thus, it is more difficult to discern small differences using BamHI than Msp I. Other enzyme choices have intermediate sensitivity.

Recent improvements to specific allelic identification have been made. Fifteen-centimeter format agarose gels are electrophoresed until the bromophenol blue dye-front has migrated a distance of at least 12 cm (24, 34). Each gel includes samples with known allelic composition verified through blind analysis in different laboratories (24, 34). Samples of similar migration characteristics are reanalyzed by migration in adjacent lanes (24, 25) or in the same lane (34). Concatameric DNA plasmids consisting of 30 to 50 bp increments (DNA ladders) are used for accurate fragment size determination (26). Together with the use of MspI/HpaII, these improvements yield highly consistent and accurate determination of H-ras-1 allele type.

**Determination of p53 Codon 72 Polymorphism**

The polymerase chain reaction and restriction digestion of polymerase chain reaction (PCR) amplicons are used to determine this polymorphism. Using this basic strategy, researchers have applied two methods. First, a simple restriction digestion of an amplified product has been used (14). Alternately, a 3’ mismatch primer incorporating a de novo AccII restriction site has been used to control for complete digestion and concomitant allelic misclassification (13). In the latter method the following primers were used: a) forward = 5’CCCCACACCCAGCCCCCTACGAGACCTTGGGAGCGGCG3’, b) reverse 5’TGTCATCTCTGCTCCTTCCCAGA3’, and c) reverse 5’ACACCGGCGGCCCTCGACCA3’. Primers 1 and 2 were first used to generate a 397 bp template. A heminested PCR using primers 1 and 3 was then used to generate fragments for restriction analysis. Restriction enzymes AccII (US Biochemicals, Cleveland, OH; no longer available; see “Introduction” for a listing of isoschizomers) and Bsp50I-1 (Stratagene, LaJolla, CA) were used to distinguish between the arginine (restriction site present) and proline (restriction site absent) alleles of p53. Agarose gel electrophoresis (2%), with ethidium bromide detection, was used to separate the restriction fragments and PCR products.

**Statistical Analyses**

To test whether the distribution of genotypes was the same for cases and controls within a given study, a chi-square statistic with 2 degrees of freedom was used (64). Odds ratios from multiple studies were combined to form a summary odds ratio using the Mantel–Haenszel method (65).

For case-control studies of p53 polymorphisms, the gene frequency for the proline variant was estimated separately for the cases and controls using the observed numbers of individuals for each of three genotypes (proline homozygotes, arginine homozygotes, and heterozygotes). This gene frequency in turn was used to calculate the expected numbers of individuals for the three genotypes in each group based on the Hardy–Weinberg law of equilibrium. To test whether either the cases or the controls were in equilibrium, a chi-square goodness of fit test was used (66). The Breslow–Day test was used to assess homogeneity of odds ratios across various studies (67). Population attributable risk (PAR) for inheritance of H-ras-1 rare alleles in lung cancer and breast cancer was determined according to Kuritz and Landis (68). For H-ras-1, numbers of subjects in Tables 2 to 4 represent numbers of individuals; however, chi-square tests were performed on allelic distributions and gene frequencies.

**Results**

**Lung Cancer Studies**

**Inheritance of H-ras-1 Rare Alleles.** Six case-control studies in three laboratories have examined the risk of lung cancer that can be attributed to inheritance of rare H-ras-1 alleles. Two of these studies were performed in the United Kingdom and used PvuII for allele characterization (22, 23). Both studies used the same population-based control group (n=101). Although both studies were positive (relative risks (RRs) 1.4 and 1.1), neither study reached statistical significance for risk of lung cancer with inheritance of rare H-ras-1 alleles (combined analysis: RR = 1.2, 95% CI = 0.6–2.6, p = 0.590, n = 471; Table 2). In the United States, Sugimura et al. (24) and Weston et al. (25) performed three independent studies of lung cancer risk and inheritance of rare H-ras-1 alleles [also, Weston et al., unpublished observations on 21 cases and 37 controls in an extension of

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### Table 2. H-ras-1 rare alleles and lung cancer. 

| National origin of subject (cases/controls) | Relative risk (95% CI) | p Value | Reference |
|--------------------------------------------|------------------------|---------|-----------|
| United Kingdom (n = 370/101)               | 1.2 (0.6–2.6)          | 0.590   | Heighway et al. (22, 23) |
| United States (n = 155/178)                | 1.9 (1.2–3.1)          | 0.006   | Sugimura et al. (24, 25) |
| Norway (n = 118/122)                       | 10.8 (1.4–85.4)        | 0.005   | Ryberg et al. (26) |
| All (n = 643/402)                          | 1.9 (1.3–2.8)          | 0.001   | Combined analysis |

*Represents six independent studies from three independent laboratories. Detection of racial variation in rare allele frequency between African Americans and Caucasians in the United States. Test for homogeneity, p = 0.009.

### Table 3. Evaluation of the p53 codon 72 proline variant as a risk factor for lung cancer.

| National, ethnic origin of subjects (n) | Frequency of proline variant in controls | p Value | Hardy–Weinberg | Reference |
|----------------------------------------|-----------------------------------------|---------|----------------|-----------|
| African American (95)                  | 0.628 NS                                |         | NS             | Weston et al. (13, 15) |
| Caucasian (134)                        | 0.295 NS                                |         | NS             | Weston et al. (13, 15) |
| Japanese (675)                         | 0.347 0.33 (NS)                        | 0.005   | NS             | Kawaji et al. (14) |
| Swedish (519)                          | 0.284 NS                                |         | NS             | Birgander et al. (17) |
| African American (141)                 | 0.527 NS                                |         | NS             | Jin et al. (18) |
| Mexican American (62)                  | 0.263 NS                                |         | NS             | Jin et al. (18) |
| Japanese (343)                         | 0.401 NS                                |         | NS             | Murata et al. (19) |
| Caucasian (286)                        | 0.214 NS                                |         | NS             | To-Figuera et al. (20) |

NS, not significant. Chi-square for difference in allelic frequencies between cancer cases and controls. Goodness of fit to the Hardy–Weinberg equilibrium for allelic distribution among lung cancer cases. Significant in patients diagnosed before age 53 (p < 0.05, n = 44). When analysis was performed independently for nonsmokers, the genotypic distribution was significantly different between cases and controls (p = 0.009); the nonsmoking case group was not in Hardy–Weinberg equilibrium (p = 0.018).
Table 4. H-ras-1 rare alleles and breast cancer.

| National origin of subjects (cases/controls) | Relative risk (95%CI) | p Value | Reference |
|---------------------------------------------|----------------------|---------|-----------|
| Caucasian (France, n = 104/56)             | 6.5 (3.3–12.8)       | 0.000   | Liderau et al. (27) |
| Japanese (n = 97/164)                      | 2.6 (1.3–5.2)        | 0.007   | Honda et al. (37) |
| Caucasian (Italy, n = 92/60)               | 4.0 (1.8–8.9)        | 0.000   | Saglio et al. (30) |
| Caucasian (Germany, n = 112/62)            | 1.3 (0.6–3.2)        | 0.498   | Sheng et al. (29) |
| Caucasian (Germany, n = 50/92)             | 1.8 (0.9–3.5)        | 0.075   | Corell et al. (28) |
| Icelandic (n = 56/48)                      | 1.2 (0.4–3.5)        | 0.798   | Barkardottir et al. (32) |
| Caucasian (United States, n = 23/50)       | 2.9 (0.8–11.5)       | 0.109   | Hall et al. (33) |
| United States (n = 160/202)                | 2.0 (1.1–3.7)        | 0.028   | Garrett et al. (34)* |
| United States (n = 160/203)                | 3.0 (1.5–6.1)        | 0.001   | Combined analysis |
| All (n = 694/937)                          | 2.7 (2.1–3.4)        | 0.000   | Combined analysis |
| All* (n = 580/881)                         | 2.3 (1.7–2.9)        | 0.000   | Combined analysis* |

*No evidence of linkage in 3 and 12 breast cancer families, respectively. *Detection of racial variation in rare allele frequency between African Americans and Caucasians in the United States. *Conway et al. (79) recently reported confirmation of these findings using a PCR-based allotyping assay. *All subjects in table except France, n = 104/56 (Liderau et al. [27]). *Test for homogeneity, p = 0.477.

The study reported by Sugimura et al. (24); the frequency of rare alleles was 0.21 in cases and 0.05 in controls (chi-square = 8.4, p = 0.02). All three were positive, and each used Msp I/Hpa II for allelic identification. The combined RR estimate generated from these studies was 1.9 (95% CI = 1.2–3.1, p = 0.006, for 155 cases and 178 controls) (Table 2). A sixth independent study of subjects in Norway was conducted by Byberg et al. (26). This study of 118 cases and 123 controls used Msp I/Hpa II for allelic identification and found a RR of 10.8 (95% CI = 1.4–85.4, p = 0.005) (Table 2). The combined summary statistic for all lung cancer studies of H-ras-1 rare allele inheritance indicates a RR of 1.9 (95% CI = 1.3–2.8, p = 0.001, n = 643/402; test for homogeneity, p = 0.089). From these data we calculated a lung cancer PAR for inheritance of H-ras-1 rare alleles of 0.037 (3.7%) (68).

Inheritance of Polymorphisms in p53.

For p53 most studies have examined the relationship between inheritance of the codon 72 proline allele variant and cancer risk (Table 1). Seven studies are case-control investigations of lung cancer. In these studies the allelic frequencies of the proline variant do not differ between lung cancer cases and controls (Table 3). However, the proline variant was the major allele in African Americans compared to Japanese, Caucasians, and Mexican Americans where the arginine allele was found to be most prevalent (Tables 1, 3) (13–20). Among Japanese, Kawajiri et al. (14) considered allelic distribution as it pertained to the Hardy–Weinberg equilibrium. Although the arginine/proline allelic frequencies were not different between cases and controls (arginine allele 0.653 in controls, 0.645 in cases), among controls the allelic distribution was in Hardy–Weinberg equilibrium whereas in cases it was not. In cases there was an underrepresentation of heterozygotes and an overrepresentation of homozygotes (p < 0.005, Table 3). In contrast, Murata et al. (19) examined the relationship between the codon 72 polymorphism, lung cancer, and tobacco smoking in a Japanese population but reported an association of p53 with tendency to smoke tobacco, where the arginine allele was elevated in nonsmoking cancer patients (chi-square = 13.5, p < 0.001). This association was, in turn, the driving force behind the subsequent observation that arginine homozygotes were in excess among nonsmoking lung cancer cases (chi-square = 10.9, p < 0.01) (19). Jin et al. (18) detected elevated lung cancer risk associated with inheritance of the p53 proline allele in a subset of African American lung cancer cases diagnosed prior to the age of 53 years. Overall, however, Jin et al. found no cancer risk associated with inheritance of the p53 proline allele (18). To-Figuera et al. (20) investigated this polymorphism in relation to histologic type of lung cancer in a Caucasian population in Catalonia. In this population no association of the p53, codon 72, genotype was observed (20).

Birgander et al. (17) have extended these p53 studies by considering inheritance of pairwise haplotypes of three polymorphic p53 loci. The loci of interest were the codon 72 nucleic acid base substitution, the intron three 16 bp insertion (A2/deletion (A1), and the intron 6 Msp I RFLP. Swedish Saamis were found to have the highest frequency of the codon 72 arginine variant so far described (0.830); evidence from these studies did not support the hypothesis that the codon 72 proline variant is a risk factor for lung cancer. Nor did the data suggest that the other p53 polymorphisms were independent lung cancer risk factors. However, the proportion of proline/intron three A1 to proline/intron three A2 haplotypes was found to be almost twice as frequent (1.78-fold) in lung cancer cases as in controls (p = 0.032) (17). The data suggest that the proline/intron three A1 haplotype is a lung-cancer risk factor. The same authors have also shown that this haplotype may be a risk factor for colon cancer (16).

Breast Cancer Studies

Inheritance of H-ras-1 Rare Alleles.

For breast cancer, nine studies have been reported from eight laboratories between 1986 and 1993 (Table 4). All studies indicate increased RR of breast cancer associated with inheritance of H-ras-1 rare alleles. The RR values ranged from 1.2 to 6.5; five studies were highly significant; two studies were of marginal significance, and two studies did not reach significance. Overall there was a highly significant risk of breast cancer associated with inheritance of H-ras-1 rare alleles. (RR = 2.7, 95% CI = 2.1–3.4, p = 0.000, n = 1,631; Table 4; Figure 2). The Breslow–Day test for homogeneity across these studies proved to be almost significant (p = 0.055); therefore, the data were investigated further. Studies on breast cancer used a range of restriction enzymes for allelic classification (Figure 2, legend). The enzyme likely to yield the least accurate results, BamHI, was used in the study that found the strongest association between breast cancer risk and inheritance of rare H-ras-1 alleles (27). Although the authors used Msp I/Hpa II to confirm rare alleles, when this study was omitted from the analysis, the overall combined data still yielded a statistically significant
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Figure 2. Relative risk of breast cancer associated with inheritance of H-ras-1 rare alleles calculated for nine published studies (1–7, 9, 10). Overall summary statistic of these RR values was determined (8). Five studies indicated a highly significant breast cancer risk with inheritance of H-ras-1 rare alleles. These five studies were reported as follows: Garnett et al. (34), study 4; Honda et al. (31), study 5; and Garnett et al. (34), study 7, used Msp I. Saglio et al. (30), study 9, used Ava II, and Liderau et al. (27), study 10, used BamHI. Two were of marginal significance: Corell et al. (28), study 3, used Msp I, and Hall et al. (33), study 6, used Pst I. Two studies did not reach significance: Barkardottir et al. (32), study 1, used BamHI and HinHI, and Sheng et al. (29), study 2, used Msp I. Overall a highly significant breast cancer risk with inheritance of H-ras-1 rare alleles was indicated (8, RR 2.7, 95% CI 2.1–3.4, p = 0.000, n = 1631).

relative risk (RR=2.3, 95% CI 1.7–2.9, p = 0.000, n = 1471; test for homogeneity, p = 0.477). The enzyme likely to yield the most accurate allelic classification, Msp I, was used in five studies. Analysis of the data from only these studies found a strong association between breast cancer risk and H-ras-1 rare alleles (RR = 2.1, 95% CI 1.6–2.9, p = 0.000, n = 1142; test for homogeneity, p = 0.611). Based on all of these studies a PAR for inheritance of H-ras-1 in breast cancer was calculated to be 0.092, or 9.2% (68).

Two studies examined inheritance of rare alleles in families with a high incidence of breast cancer. One study that examined three Icelandic families found no rare H-ras-1 alleles in any of 10 affected members (32). In a second study, in the United States, 12 breast cancer families were studied (33). Seven of these twelve families had members who carried rare H-ras-1 alleles. However, no evidence was found to support the hypotheses that breast cancer cosegregated with either rare or common H-ras-1 alleles (33).

Inheritance of Polymorphisms in p53

Very limited data yet exist on the potential association of p53 polymorphisms and breast cancer risk. A single study by Kawajiri et al. (14) determined the allelic frequencies of the p53 codon 72 polymorphism in breast cancer cases (n = 93) and controls (n = 347). The overall frequencies for the proline variant were 0.328 for cases and 0.347 for controls. These data do not indicate that inheritance of this p53 polymorphism is a breast cancer risk factor. Furthermore, Kawajiri et al. reported the allelic distributions for cases and controls to be similar (chi-square = 3.18, p = 0.2, degrees of freedom = 2), and both cases and control populations were in Hardy–Weinberg equilibrium (14).

Germ line mutations in the p53 gene have a role in cancer family syndromes that include breast cancer as a feature (38). However, germ line mutations in p53 have only been demonstrated in a small number of breast cancers (69). In addition, germ line p53 mutations have not been demonstrated in pedigrees that have a specific family history of breast or ovarian cancer (70, 71).

Discussion

Inheritance of H-ras-1 Rare Alleles

The data presented from molecular epidemiologic case–control studies indicate that inheritance of H-ras-1 rare alleles constitute lung cancer and breast cancer risk factors. Every study discussed (six lung cancer and nine breast cancer) indicated an elevated RR of either lung or breast cancer. Of the lung cancer studies, four of six studies, coming from two of three independent laboratories, were statistically significant. Taken together, analysis of all the data pertaining to inheritance of H-ras-1 rare alleles and lung cancer risk provided an overall relative risk of 1.9 (95% CI 1.3–2.8, p = 0.001). An even more convincing picture emerges for cancer of the breast. Nine studies from eight independent laboratories are reported. All were positive; five highly significant, two approach significance (p = 0.075 and 0.109) and only two were not significant. Taken together, analysis of all of the data pertaining to inheritance of H-ras-1 rare alleles and breast cancer risk reveals a RR of 2.7 (95% CI 2.1–3.4, p = 0.000). The significance of these findings is only diminished by the failure, so far, to demonstrate linkage of H-ras-1 rare alleles with breast cancer in family studies. However, these seemingly adverse observations may ultimately be resolved when a mechanistic basis for cancer risk and H-ras-1 rare alleles has been elucidated (see "Note Added in Proof").

In breast cancer case–control studies, based on the relative risk determinations for inheritance of H-ras-1 rare alleles, the data indicate a PAR of 0.092 (9.2%). This is consistent with the findings of Krontiris et al. (35) that considered fewer studies. The recently cloned BRCA-1 gene accounts for PAR of only 0.046 (4.6%) (72). For lung cancer the calculated PAR that we determined was 0.037 (3.7%). These findings indicate that inheritance of H-ras-1 rare alleles is an important risk factor for both breast cancer and lung cancer.

Studies of cancer risk associated with inheritance of H-ras-1 rare alleles have provided data that are both positive and negative for multiple cancer types (35). Some studies have clearly suffered from inadequate laboratory techniques leading to inaccurate allelic classification; however, controversial views have also stemmed from the lack of a plausible biologic mechanism to explain the potentially elevated cancer risk associated with inheritance of H-ras-1 rare alleles. Further laboratory investigations, at the cell and molecular level, of the association between inheritance of H-ras-1 rare alleles and increased risk of lung cancer and breast cancer seem justified from the molecular epidemiologic data presented here.

There are several explanations for the observation summarized that the inheritance of H-ras-1 rare alleles and cancer risk are associated. First, the H-ras-1 VTR may be a marker that is simply in linkage disequilibrium with another gene that is responsible for cancer risk. Second, evidence from loss-of-heterozygosity studies has been provided that indicates the presence of a tumor suppressor gene at chromosome 11p15.5 in both lung cancer (73, 74) and rhabdomyosarcoma (75). Third, the specific rare allelotypes of H-ras-1 may disrupt the controlled expression of nearby genes, including H-ras-1 itself. This could operate through transcriptional regulation. For example, four members of the rel/NF-κB family of transcription regulation factors bind to the H-ras-1 minisatellite region (76). Fourth, genetic recombinational events in regions of the genome containing repeat elements tend to be unstable; and fifth, fidelity of DNA repair may be hampered in regions containing tandem repeats (35).

Inheritance of Polymorphisms in p53

Since 1989 the importance of mutations in p53 has become central to human cancers. Mutations in the p53 tumor suppressor
gene have been recognized in many human cancer types (77). Only recently has it been questioned whether common polymorphisms in the p53 gene might have a role as a cancer risk factor. At least 10 p53 polymorphisms have been identified to date (Table 1). The codon 72 arginine/proline polymorphism has been most extensively studied, in part because it has a high prevalence. Convincing evidence that the codon 72 polymorphism has a role in cancer etiology remains to be documented. Most recently, haplotyping studies have suggested that certain p53 polymorphisms may be a marker for (that is, in linkage disequilibrium with) another chromosome 17p gene responsible for disease risk (16,17). Little evidence has been provided here to implicate p53 polymorphisms in human cancer risk (13-20). Other studies that examined molecular mechanistic questions using specific genetic constructs have also failed to indicate disease risk (21). The most recent studies that identify highly specific p53 haplotypes as cancer risk factors require further investigation (16,17). These investigations can usefully be pursued along molecular epidemiological lines as well as cell and molecular lines in the laboratory.

Although little evidence of breast and lung cancer risk is evident from current p53 polymorphism studies, reports exist to implicate p53 polymorphisms in ovarian cancer. A description has appeared in the literature that implicates the intron 3,16 bp insertion with risk of ovarian cancer (RR = 8.6, 95% CI 3.0-25.2) (57).

**Conclusions**

The published studies (22-34) that have examined an association of inheritance of the H-ras-1 VTR with breast cancer are remarkably consistent. All find a positive association and most are statistically significant (Figure 2). Fewer studies of lung cancer point to a similar trend, indicating inheritance of H-ras-1 rare alleles to be a significant risk factor.

Studies of polymorphisms in p53 are currently less well developed (13-21).

Most have focused on the codon 72 polymorphism, for which the weight of evidence appears to support the null hypothesis. However, considerable possibilities exist given the pivotal role of this gene in neoplasia and the number of polymorphisms so far reported (21,42-53). Recent studies have led to consideration of estimated haplotypes (16,17). New directions in our laboratory have focused on the absolute determination of haplotypes in diploid genomes using available molecular biologic technologies (78).

**Note Added in Proof:** For H-ras-1 an interaction between rare alleles and mutated BRCA-1 has been reported (80). In addition, two reports have been published that indicate a specific p53 haplotype, consisting of a constellation of 3 polymorphisms (intron 3, exon 4, and intron 6) and designated 1-2-1, is a breast cancer risk factor (78,81).

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