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Joint Effects of Germ-Line p53 Mutation, *MDM2* SNP309, and Gender on Cancer Risk in Family Studies of Li-Fraumeni Syndrome

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Abstract

Li-Fraumeni syndrome (LFS) is a rare familial cancer syndrome characterized by early cancer onset, diverse tumor types, and multiple primary tumors. Germ-line p53 mutations have been identified in most LFS families. A high-frequency genetic variant of single-nucleotide polymorphism (SNP) 309 in the *MDM2* gene was recently confirmed to be a modifier of cancer risk in several case-control studies: substantially earlier cancer onset was observed in SNP309 G-allele carriers than in wild-type individuals by 7–16 years. However, risk analyses in family studies that jointly account for measured hereditary p53 mutations and SNP309 have not been evaluated. Here, we extended the statistical method that we recently developed to determine the combined effects of measured p53 mutations, SNP309, and gender and their interactions simultaneously. The method is structured for age-specific risk models based on Cox proportional hazards regression for censored age-of-onset traits. We analyzed the cancer incidence in 19 extended pedigrees with multiple germ-line p53 mutations ascertained through clinical LFS phenotype. The dataset consisted of 463 individuals with 129 p53 mutation carriers. Our analyses showed that the p53 germ-line mutation and its interaction with gender were strongly associated with familial cancer incidence and that the association between SNP309 and increased cancer risk was modest. In contrast with several outcomes in case-control studies, the interaction between SNP309 and p53 mutation was not statistically significant. The causal role of SNP309 in family studies was consistent with a previous finding that SNP309 G-alleles are associated with accelerated tumor formation in patients with sporadic and hereditary cancers.

Introduction

Li-Fraumeni syndrome (LFS) is one of the most devastating familial cancer syndromes; its major hallmarks include early cancer onset, a wide diversity of tumor types, and a high

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frequency of multiple primary tumors (1;2). LFS was initially characterized by aggregations of various tumor types within families, including soft-tissue sarcomas (STS), osteosarcomas (OST), female breast cancer, brain tumors, leukemias, and adrenocortical carcinomas (3–7). However, substantially increased risks for many other common cancers have been observed in subsequent studies, such as prostate and lung cancers (5;8). Mutations in p53 are one of the most common tumor-specific genetic alternations in human neoplasms, accounting for > 50% of various human cancers. Germ-line p53 mutations have been identified in most families with LFS (9–11). Recently, a high-frequency genetic variant of single-nucleotide polymorphism (SNP) 309 in the MDM2 gene, a direct negative regulator of p53, was found to be a genetic risk modifier of cancer incidence in p53 mutation carriers (12). The authors of this study proposed a model that the G-allele of SNP309 is associated with high levels of MDM2 RNA and protein and attenuates the p53 stress response pathway, resulting in accelerated tumor formation in both sporadic and hereditary cancers.

Several independent reports in case-control studies confirmed that MDM2 SNP309 is associated with increased cancer risk; these studies also revealed a significantly earlier mean age of cancer onset in SNP309 G-allele carriers than in wild-type (T/T) individuals by 7–16 years in p53 mutation carriers (13;14). However, risk analyses that jointly account for measured hereditary germ-line p53 mutations and MDM2 SNP309 genotypes have not been evaluated in family studies. Recently, we developed a robust and reliable statistical method to evaluate cancer risk attributable to a measured hereditary susceptibility gene in family studies (15). The method is structured for age-specific risk models based on the Cox proportional hazards regression (16) and is an extension of usual complex joint segregation and linkage analysis models for censored age-of-onset traits. It can be used to assess and determine the combined effects of individual risk factors and their interactions simultaneously and provides 95% confidence intervals (95% CIs) for the risk estimates.

In this report, we extended our developed statistical method and performed cancer risk analyses in family studies relevant to hereditary p53 germ-line mutations and MDM2 SNP309. We analyzed the cancer incidence in 19 extended pedigrees with multiple germ-line p53 mutations, ascertained through clinical LFS phenotype, and included all invasive cancers as a single combined phenotype. The final dataset consisted of 463 individuals with 129 germ-line p53 mutation carriers. Because of recent observations that female p53 mutation carriers have a higher cancer risk than males (5;17;18), we assessed and determined the combined effects of germ-line p53 mutations, MDM2 SNP309, gender, and their interactions on cancer risk simultaneously. Our analyses showed that p53 germ-line mutations and their interaction with gender were strongly associated with familial cancer incidence and that the association between MDM2 SNP309 and increased cancer risk was modest. In contrast with outcomes in case-control studies (13;14), the interaction between MDM2 and p53 mutations was not statistically significant in this family study.

Our analyses showed that SNP309 G-allele carriers had an overall 1.6-fold higher relative risk (RR) of developing cancer than did wild-type individuals (95% CI, 0.9–2.7). Men with p53 mutations had a 19-fold higher RR than did those without (95% CI, 8–41) and women with p53 mutations had a 44-fold higher RR than did those without (95% CI, 19–104). Men with both p53 mutations and SNP309 G-alleles had a 30-fold higher RR of developing

cancer than did those with neither (95% CI, 11–84). Women with both p53 mutations and SNP309 G-alleles had a 70-fold higher RR than did those with neither (95% CI, 22–219) and a 2.4-fold higher RR than did men with both (95% CI, 1.3–4.4). Our results are consistent with the finding of the original study that heightened MDM2 levels due to the SNP309 G-allele were associated with accelerated tumor formation in both hereditary and sporadic cancers (12), but that the effect of MDM2 SNP309 on cancer risk in our family study was not as strong as that reported in case-control studies.

Materials and Methods

Study population

The study population consisted of 19 extended pedigrees with multiple germ-line p53 mutations, ascertained through clinical LFS phenotypes. Of these kindreds, 11 were ascertained through systematic studies of sequential childhood STS (6 families) and OST (5 families) patients, 2 families were through second malignant neoplasm (SMN), 5 families were through medical genetic counseling (MGC), and 1 family was through adrenal neoplasm (ACC). We included their extended relatives, trimmed to include grandparents, aunts and uncles, parents, full siblings and offspring of all probands.

For STS and OST cohorts, 6 STS patients had been diagnosed before 16 years of age, survived > 3 years after diagnosis, and treated at The University of Texas M. D. Anderson Cancer Center (Houston, Texas, U.S.A.) from 1944 to 1975, and 5 OST patients had been diagnosed at M. D. Anderson from 1944 to 1982. The remaining study series (SMN, MGC, and ACC) consisted of patients referred to M. D. Anderson for research or clinical studies with a personal or family history of cancers of the types found in LFS. Genotyping included probands and adult relatives at risk of carrying a p53 germ-line mutation, without regard to affection status. Because extension through mutation status was not performed with respect to the phenotype, this approach to extending the family should not introduce an ascertainment bias during the segregation analyses.

We included all invasive cancers, excluding non-melanoma skin cancers and *in situ* carcinoma, as a single combined phenotype. These disease criteria are broader than the classic LFS component tumors, but are based on observations of diverse cancer types occurring in excess in p53 germ-line mutation carriers (5;6;8). Medical records or death certificates were used to confirm all cancers included in the analysis. Individuals were considered at risk from their date of birth to their date of cancer diagnosis, death, loss to follow-up, study termination (December 31, 2001, was the study termination date for medical record and death certificate documentation), or age 75 years, whichever came first. The evaluation of cancer incidence was truncated at age 75 years because of the limited reliability of cancer rates at older ages. The data collection methods, overall cancer incidence, germ-line p53 mutation identification, and frequencies of site-specific cancers have been described elsewhere (5;7;8;19;20)

The final dataset for these 19 extended kindreds with multiple germ-line p53 mutations consisted of 463 individuals with 76 men and 77 women affected with cancers. One hundred twenty-nine were germ-line p53 mutation carriers, 169 were wild-type, and 165 were at risk

for being a p53 mutation carrier but had unknown genotypes; 105 were MDM2 SNP309 G-allele carriers and 89 were MDM2 SNP309 wild-type (T/T) individuals. All probands are carriers of p53 germ-line mutations. The overall mean age of first cancer onset of probands was 13.7 years of age; it was 12.7 for males and 14.5 for females. Across the families, the frequency distributions of age of first cancer onset for affected relatives by p53 mutation, MDM2 SNP309 G-allele genotype and gender are shown in Table 1. Similarly, the frequency distributions of age at last examination for unaffected relatives by MDM2 SNP309 genotype and gender are shown in Table 2. We also present the frequency distributions of MDM2 SNP309 genotypes by affection and germ-line p53 mutation status in Table 3.

Joint segregation and linkage analysis models

Segregation analysis is a statistical method that is frequently used to evaluate and compare various modes of inheritance for a trait and test for evidence of age modification of genetic relative risks in association with epidemiological and environmental factors. It is often performed in statistical analyses of family studies ascertained through affected individuals. Most segregation analysis programs assume that a putative major gene is segregated within a family and allow the measured covariates, adjusted for epidemiological and environmental risk factors, to be tested for significance and accounted for in models. Adding a linked marker to the putative major gene could provide additional characterizations of the putative major gene in joint segregation and linkage analyses, resulting in greater statistical power than segregation analyses alone. Joint segregation and linkage analyses have been shown to have greater power for detecting gene-environment interactions than usual segregation analyses (21).

Recently, we developed a statistical method that proposed to use the measured susceptibility genotype (instead of the ordinary genetic allele marker) as the linked marker to the putative major gene in joint segregation and linkage analyses to estimate the cancer risk attributable to a measured susceptibility gene in family studies. We further proposed to test the significance of linkage disequilibrium (LD) between the putative gene and measured susceptibility gene and to account for LD in the model (15). Gauderman and Faucett (1997) developed the complex joint segregation and linkage analysis model based on Cox proportional hazards regression and the program package of Genetic Analysis Package (G.A.P.) for the model (21;22). Our method was based on the extension of the Cox model and program GAP that they developed.

On the basis of Cox proportional hazards regression, the age-specific risk model that accounts for variability in age of onset for right-censored traits is expressed as a function of a vector of measured covariates (z), a covariate (G) for a putative major gene, and their interactions ($G \times z$):

$$\lambda(t|z, g, \Omega) = \lambda_0(t) \exp(\beta^T z + \gamma G + \eta^T G \times z). \quad [1]$$

Let g denote the diallelic genotype at the putative major gene that has high-risk allele A with frequency q_A and normal allele a ; G is a covariate that depends on this genotype and

assumed inheritance mode. Under the assumption of dominant inheritance, G is coded as 1 for genotype $g = AA$ or Aa and 0 for $g = aa$. The letter d is a disease status indicator, and t represents the age of onset for diseased subjects ($d = 1$) or the last known disease-free age for unaffected subjects ($d = 0$). β , γ , and η are regression coefficients to be estimated; η measures the degree of departure from a purely multiplicative hazards model. The function $\lambda_0(t)$ describes the age-specific incidence rate for baseline groups; it is often expressed as a step function on a pre-determined set (e.g., 5 equal age intervals of 0–75: $\lambda_0(t) = \lambda_k$ for $t_{k-1} < t \leq t_k$, $k = 1, \dots, 5$, with $t_0 = 0$ and $t_k = 15 \times k$). The set of hazard model parameters is denoted by $\Omega = \{\beta, \gamma, \eta, \lambda_k\}$.

Suppressing subscripts, the penetrance function for a given individual is expressed as $f(d, t | z, g, \Omega) = \lambda(t | z, g, \Omega)^d S(t | z, g, \Omega)$, where $S(t | z, g, \Omega) = \exp\left(-\int_0^t \lambda(s | z, g, \Omega) ds\right)$ is the survival function (the probability of remaining disease-free up to time t). We let M_i denote a marker phenotype for a given individual (i) that is determined by a fully penetrant gene (m_i) with an arbitrary number of alleles and corresponding allele frequency (q_B). $P(M_i | m_i)$ is the marker-penetrance function that is assumed to be 1 for all m_i , consistent with M_i , and 0 otherwise. In joint segregation and linkage analysis models, the family-specific likelihood that is expressed as

$$L_I(\Omega, q_A, q_B, \theta) = \sum_{g, m} P(g, m | q_A, q_B, \theta) \prod_{i \in I} f(d_i, t_i | z, g, \Omega) P(M_i | m_i) \quad [2]$$

is formed by the summation over all possible combinations of joint genotypes and products of the individual-specific penetrance functions in a family. The first factor depends on q_A , the marker allele frequency q_B for founders, and the recombination fraction θ for non-founders (23). The total likelihood for all pedigrees is the product of the family-specific likelihoods that are expressed in [2]. Details of the methods and applications of this model have been described elsewhere (21;22).

Model measured p53 gene as a linked marker

In this study, we extended the method that we previously developed to jointly account for germ-line p53 mutations, MDM2 SNP309, and gender, and their interactions. We used the major susceptibility gene (the measured p53 genotype in this case) as the linked marker to the putative major gene and the covariates adjusted for other risk modifiers (the measured MDM2 SNP309 genotype and gender in this case) in joint segregation and linkage analyses.

Using the measured susceptibility gene as a linked marker to the putative major gene in joint segregation and linkage analyses, we will obtain a small estimated recombination fraction $\hat{\theta}$ when the study families have been ascertained through affected individuals with a measured susceptibility gene that is segregated within the families (15). We used the measured p53 susceptibility gene as the linked marker to the putative major gene in joint segregation and linkage analyses to evaluate the cancer risk attributable to p53 germ-line mutations in this study. With this design, the effect of measured p53 mutations on cancer risk can be assessed through the regression coefficient γ of the putative major gene in equation [1]. We can use the magnitude of LD between the measured p53 gene and putative major gene as a measure

to determine to what extent the putative major gene serves as a proxy of the measured p53 susceptibility gene in cancer risk estimation.

Recent rampant genome-wide association studies have used LD to associate the disease phenotype with genetic markers that are tightly linked to disease-susceptibility loci (24). In this application, the stronger the LD magnitude, the more the putative major gene represents the p53 susceptibility gene in association with cancer risk. Therefore, when strong LD exists between the measured p53 susceptibility gene and the putative major gene in joint segregation and linkage analyses, the regression coefficient γ of the putative major gene in equation [1] provides an effective and robust estimate of the cancer risk attributable to measured p53 mutations.

In this report, we used GAP software to test the significance of the LD between the measured p53 susceptibility gene and the putative major gene and account for LD in mathematical models. We further used the measured covariates (denoted by z in equation [1]) to account for the effects of modifiers of MDM2 SNP309 and gender on cancer risk in this application. More importantly, we are able to evaluate the major genetic component effect (the p53 germ-line mutation in this case) and the interaction effects between this main effect and other measured covariates simultaneously. The interaction effects can be estimated through the regression coefficient η of the interaction covariates $G \times z$ in equation [1]. This is an important feature in our study because gene-environment and gene-gene interactions play important roles in disease risk and because current statistical approaches have limited power for detecting such interactions (25). We used this feature to test the significance of the interaction effects between germ-line p53 mutations and MDM2 SNP309 and gender on cancer risk in this study.

Hypothesis testing

The logarithm $\ln(L)$ of the maximum likelihood of the data was computed for each model. The likelihood ratio test (LRT) was used to test a specific model against the baseline model – usually the general model – in which the transmission probability τ_μ of allele A for genotype μ is arbitrary (instead of Mendelian transmission modes) to identify the best fit to the data for the general model. The specific model serves as the null model, and the baseline model as the alternative model. The LRT is computed as follows:

$$\text{LRT} = -2 \{ \ln(L_{\text{specific}}) - \ln(L_{\text{baseline}}) \},$$

where LRT approximately follows a χ^2 distribution with degrees of freedom equal to the difference in the numbers of independent parameters estimated between these two models. The LRT is frequently used to compare the general model with several nested alternatives, such as the Mendelian dominant, additive, and recessive models and sporadic (no major gene) and environmental (no parent-to-offspring transmission) models. We also used Akaike's Information Criteria (AIC) ($\text{AIC} = -2 \ln(L) + 2 [\text{number of independent parameters estimated}]$) to compare non-nested models. The model with the lowest AIC value and fewest estimated parameters is generally considered the most parsimonious. The LRT is

also used to test the significance of covariate(s) when the model that includes additional covariate(s) is used as the baseline model.

Results

We assumed the underlying inheritance mode for the putative major gene to be dominant in the model setting of joint segregation and linkage analyses because the measured germ-line p53 gene is a dominant gene. It indicates the effect of genotype AA equal to that for genotype Aa on cancer risk (that is, $\gamma_{AA} = \gamma_{Aa}$). Using the germ-line p53 gene as a linked marker to the putative major gene in joint segregation and linkage analyses, we obtained estimates of the trait model parameters and recombination fraction $\hat{\theta}_{p53}$ between the p53 gene and putative major gene. We used a 5-step baseline proportional hazard model $\lambda_0(t)$ for the age-specific incidence rate, in which $t_0 = 0$, $t_k = 15 \times k$ for $k = 1, \dots, 5$ and $\lambda_0(t) = \lambda_k$ for $t_{k-1} < t \leq t_k$. We obtained the highest LOD-score of 22.64 at $\hat{\theta}_{p53} = 0.0$ and $\gamma_{AA} = \gamma_{Aa} = 3.29$, indicating that the p53 and putative major genes were tightly linked and that the RR for cancer was 26.84 ($= e^{3.29}$) for individuals with a p53 mutant allele.

Testing linkage disequilibrium

We tested the significance of LD between the p53 and putative major genes and accounted for LD in the model. The highest LOD-score was 34.18 at $\hat{\theta}_{p53} = 0.0$ and $\gamma_{AA} = \gamma_{Aa} = 2.84$. Letting q_B denote the frequency of high-risk allele B for the linked marker (the germ-line p53 mutant allele in this case) and q_{AB} denote the frequency of haplotype A and B, we obtained the LD measure of $D' \approx 1$ and $q_{AB} \approx q_B$, suggesting that nearly perfect LD exists between the p53 and putative major genes and that the haplotype of high-risk allele A and p53 mutant allele was transmitted intact through generations within families (26). Using the LRT to compare the 2 models shown in Table 4, the χ^2 value was 35.24 that gives a p-value of 2.91×10^{-9} with 1 degree of freedom, suggesting that the model that accounted for LD was significantly better than the one that did not account for LD. This was further evidenced by the difference in LOD-scores. An LOD-score difference of 1.5 is considered evidence of a better model; we found an LOD-score difference of 11.54 ($= 34.18 - 22.64$), which also provides strong evidence that the model that accounted for LD was a far better fit to the data (27).

Thus, our investigation revealed that germ-line p53 mutations are directly associated with cancer phenotype and play a causal role that is nearly identical to that of the putative major gene in cancer incidence in this application. The analyses for the models that did and did not account for LD are summarized in Table 4. The parameter λ represents the estimates of the baseline annual age-specific cancer incidence rate per 100,000 persons for the age groups of 0–15, 15–30, 30–45, 45–60, and 60–75 years, respectively.

Testing interactions among p53 mutations, MDM2 SNP309, and gender

Because of recent observations that MDM2 SNP309 is associated with significantly early ages of cancer onset in p53 mutation carriers in case-control studies (12–14) and that female p53 mutation carriers have a higher cancer risk than males in family studies (5;17;18), we not only investigated the main component effects but also the interaction effects on cancer

risk among germ-line p53 mutations, MDM2 SNP309, and gender simultaneously in family studies. We used the model that accounted for LD as the base model (rather than the one that ignores LD) to further evaluate the joint contributions of these risk factors to cancer incidence.

Letting the model that includes covariate MDM2 serve as the baseline model and the model with no covariates serve as the null model, the χ^2 value for the LRT with 1 degree of freedom was 1.57, which gives a p-value of 0.21. The null model is not rejected at a 0.05 nominal significance level, indicating that MDM2 SNP 309 is not significant. The null and baseline models for LRT testing are presented in the 2nd and 3rd columns of Table 5, respectively. Note that MDM2 is coded as 1 for SNP309 G-allele carriers and 0 for wild-type T/T individuals.

We tested the significance of the interaction between MDM2 and p53 mutations by letting the covariate MDM2 depend on the putative major gene (denoted by G in equation [1]) in the model. We had $\text{MDM2} \times \text{p53} \approx \text{MDM2} \times G$ because of nearly perfect LD with $D' \approx 1$ between the putative major gene and p53 gene in our previous analysis. The interaction covariate $\text{MDM2} \times \text{p53}$ was used to estimate the excess of cancer risk in MDM2 SNP309 G/G or G/T over wild-type genotypes among p53 mutation carriers. Using the LRT to compare the model with $\text{MDM2} \times \text{p53}$ (baseline model) and that with no covariates (null model), the χ^2 value with 1 degree of freedom was 0.68, indicating that the interaction covariate $\text{MDM2} \times \text{p53}$ was not significant. We also analyzed the model that included both covariates $\text{MDM2} \times \text{p53}$ and MDM2 and found that it was not superior. The results of the models with $\text{MDM2} \times \text{p53}$ alone and with $\text{MDM2} \times \text{p53}$ and MDM2 are presented in the 4th and 5th columns of Table 5, respectively.

We tested the significance of gender difference on cancer risk in germ-line p53 mutation carriers by letting the covariate Gender depend on the p53 gene (thus, the putative major gene). That is, the interaction covariate $\text{Gender} \times \text{p53}$ ($\approx \text{Gender} \times G$) was used to estimate the excess of cancer risk in female carriers over male carriers. Note that Gender is coded as 1 for females and 0 for males. Using the LRT to compare the model with covariate $\text{Gender} \times \text{p53}$ (baseline model) and that with no covariates (null model), the χ^2 value with 1 degree of freedom was 6.18, giving a p-value of 1.29×10^{-2} and rejecting the null model at a 0.05 nominal significance level. It indicates the significance of sex difference on cancer risk in p53 mutation carriers (5;17;18). The result of the model with $\text{Gender} \times \text{p53}$ is shown in the 6th column of Table 5.

We further analyzed the data by including both covariates $\text{Gender} \times \text{p53}$ and MDM2 in the model; the result is shown in the 7th column of Table 5. This model (baseline model) is significantly better than that with no covariate (null model); the χ^2 value with 2 degrees of freedom was 9.33, which gives a p-value of 9.42×10^{-3} and rejects the null model at a 0.01 nominal significance level. This finding suggests that $\text{Gender} \times \text{p53}$ and MDM2 are both associated with increased cancer risk. In comparison with model with covariate MDM2 alone (the 3rd column of Table 5), it is noteworthy that the contribution of MDM2 to cancer risk was substantially enhanced when both germ-line p53 and gender were incorporated into the model in our analyses. Because a substantial correlation of 0.20 was found between

covariates MDM2 and Gender×p53 in the maximization likelihood estimation, it is appropriate to compare the model with Gender×p53 and MDM2 to that with no covariates, rather than that with covariate Gender×p53 alone. It is further evidenced by the fact that the model with Gender×p53 and MDM2 had lower p-value (9.42×10^{-3}) than that with Gender×p53 alone (1.29×10^{-2}) in comparison with the model with no covariate (serves as null model). If we ignored this correlation and used the LRT to compare the model with Gender×p53 and MDM2 to that with Gender×p53, the χ^2 value was 3.15 with 1 degree of freedom, which gives a p-value of 7.59×10^{-2} , and MDM2 is not a significant risk modifier.

Parsimonious model

We also analyzed the models that included various covariate combinations, such as MDM2×Gender + Gender×p53, MDM2 + Gender + Gender×p53, and Gender + Gender×p53. They were not superior to that with Gender×p53 and MDM2. We also investigated the 3-way interaction among germ-line p53 mutations, MDM2, and gender. The numerical convergence in the maximization procedure was not satisfactory; the corresponding outcomes were, thus, not trustworthy (data not shown). In conclusion, the model that accounted for LD between the p53 and putative major genes and included the covariate MDM2 and interaction covariate Gender×p53 was most plausible. The AIC criterion confirmed this result, as shown in Table 5. Our analyses showed that both p53 and Gender×p53 were strongly associated with familial cancer incidence and that MDM2 had modest evidence to make a substantial contribution to incidence. The results of our investigation relative to the causal role of MDM2 in family studies were consistent with those of the original study, in which heightened MDM2 levels due to SNP309 G-allele were associated with accelerated tumor formation in both hereditary and sporadic cancers (12), but the marginal effect of MDM2 SNP309 on cancer risk was not as large as those in the case-control study.

According to the parameter estimation in our plausible model, the coefficient of covariate MDM2 was 0.47, indicating that the RR for developing cancer was $1.6 (= e^{0.47})$ for SNP309 G-allele carriers. The estimated coefficient $\gamma_{AA} = \gamma_{Aa}$ was 2.92, indicating that the RR for developing cancer was $18.5 (= e^{2.92})$ for men with germ-line p53 mutations. The covariate coefficient of Gender×p53 was 0.86, indicating that women with p53 mutations had a $43.8 (= e^{2.92+0.86})$ -fold higher RR of developing cancer than did those without mutations and a $2.4 (= e^{0.86})$ -fold higher RR than did men with mutations. Men with both p53 mutations and G-allele had a $29.7 (= e^{2.92+0.47})$ -fold higher RR of developing cancer than did those with neither. Women with both p53 mutations and G-allele had a $70.1 (= e^{2.92+0.86+0.47})$ -fold higher RR than did those with neither. We calculated the associated 95% CIs by inverting the Fisher Information Matrix, which was obtained as a part of the maximum likelihood estimation of independent variables. The 95% CIs of the RR for developing cancer in individuals with p53 mutations were (18.5, 103.5) and (8.3, 41.3) for women and men, respectively. The 95% CI of the RR in individuals with G-allele was (0.9, 2.7). The 95% CIs of the RR for developing cancer in individuals with both p53 mutations and G-allele were (22.4, 219.2) for women and (10.5, 83.9) for men. The 95% CI of the RR for the difference in women over men with p53 mutations was (1.3, 4.4).

We also calculated cancer-free survival curves by p53 mutation, SNP309 G-allele, gender, and age on the basis of the assumption of Cox proportional hazards model. These age-specific survival plots are shown in Figure 1. The estimated cancer-free survival probabilities for the highest risk group, female carriers of p53 mutations and G-allele, were 50.5%, 16.9%, and 0.4% at ages 30, 45, and 60 years, respectively. The corresponding survival rates for male carriers were 74.8%, 47.2%, and 9.0%. The estimated cancer-free survival probabilities for female p53 mutation carriers were 65.2%, 33.0%, and 2.9% at ages 30, 45, and 60 years, respectively. The corresponding survival rates for male carriers were 83.4%, 62.5%, and 22.2%. The estimated cancer-free survival probabilities for SNP309 G-allele carriers were 98.4%, 96.0%, and 87.8% at ages 30, 45, and 60 years, respectively. In contrast, the estimated cancer-free survival rates for non-carriers were 99.0%, 97.5%, and 92.2%.

Discussion

Since the original study that found a high-frequency genetic variant of SNP309 in the MDM2 gene to be a genetic risk modifier of cancer incidence in p53 mutation carriers (12), several subsequent and independent reports in case-control studies also showed that MDM2 SNP309 was associated with the attenuated p53 pathway, an enhanced early age of cancer onset, and an increased frequency of multiple primary tumors (13;14). However, there is paucity in family studies that systematically investigate the causal role of MDM2 SNP309 in association with germ-line p53 mutation in cancer incidence in the existing literature. In response, we proposed to evaluate the causal role of MDM2 SNP309 in family studies. Because we and others identified an increased cancer risk in female p53 mutation carriers (5;17;18) and because a recent study proposed a model in which female carriers of MDM2 SNP309 were diagnosed with sporadic cancer at an earlier age due to an active estrogen-signaling pathway (28), we designed to determine the combined effects of germ-line p53 mutations, SNP309, gender, and their interactions on cancer risk in family studies in this report. We extended the method that we recently developed and analyzed the cancer incidence in 19 extended pedigrees ascertained through clinical LFS phenotype in which the measured germ-line p53 mutation was segregated within families. To our knowledge, this study population represents the largest collection of family data with measured germ-line p53 mutations and MDM2 SNP309 genotypes.

Our analyses showed that the p53 germ-line mutation and its interaction with gender were strongly associated with familial cancer incidence and that modest evidence of an association between SNP309 and an increased cancer risk was found. Although the contribution of MDM2 SNP309 to cancer incidence became substantially greater when both germ-line p53 and gender were incorporated into the model, the interaction between MDM2 SNP309 and p53 mutations was not statistically significant in our analyses. The causal role of MDM2 SNP309 in our family study was consistent with the finding that heightened MDM2 levels, resulting from the SNP309 G-allele, were associated with the attenuated p53 tumor suppressor pathway and led to accelerated tumor formation in both hereditary and sporadic cancer (12); however, the marginal effect of MDM2 SNP309 on cancer risk was not as large as those estimates in case-control studies. We also evaluated a 3-way interaction among germ-line p53 mutations, MDM2 SNP309, and gender in family studies. Because the

numerical convergence in the maximization likelihood procedure was not satisfactory, we believe the corresponding outcomes were less trustworthy (data not shown). In conclusion, the germ-line p53 mutation, its interaction with gender, and MDM2 SNP309 were associated with increased cancer risk in our family study.

Germ-line p53 mutations are the major susceptibility component effect on cancer risk under study. Information on p53 genotypes was missing for 37% of the 444 relatives in these pedigrees. Inappropriate management of missing genotypes in relatives could lead to substantial power loss and distorted estimates of risk factors' effects. Modeling the measured p53 genotypes as the linked marker enables all possible genotypes to be assigned to individuals with missing genotypes at the susceptibility locus with corresponding probabilities conditional on the known genotypes of others in the family. We demonstrated that this approach would lead to robust estimates of main and interaction effects of a measured susceptibility gene in our previous publication (15).

Accurate age-specific assessment of cancer risk attributable to germ-line p53 mutation, gender, MDM2 SNP309, and their interactions in family studies would allow clinicians and investigators to target high-risk families for genetic counseling, clinical management, and personalized risk assessment in cancer prevention and control. More importantly, these studies provide a greater understanding of genetic and non-genetic variants' and their interactions' effects on cancer incidence, which could ultimately lead to better prevention and clinical treatment of related cancers in humans (29).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Table 1

Germline p53 Mutation Characteristics in 19 Kindreds

	Kindred #	Mutation by codon	Mutation effect	Gene Bank Ref Seq X54156	Coding DNA Sequence
1	MGC-2036	FS48	FRAMESHIFT	g.12066	c.142delG
2	MGC-2150	FS63	FRAMESHIFT	g.12113	c.189delTinsAGA
3	MGC-1375	SA INT 4	SPLICE	g.13053	c.376-2A>G
4	STS-170	M133T	MISSENSE	g.13077	c.398T>C
5	OST-921	W146X	NONSENSE	g.13117	c.437G>A
6	SMN-213	W146X	NONSENSE	g.13116	c.437G>A
7	STS-032	R175H	MISSENSE	g.13203	c.524G>A
8	STS-045	FS184	FRAMESHIFT	g.13231	c.552delT
9	MGC-1065	SD INT 5	SPLICE	g.13240	c.559+2T>G
10	OST-845	FS241	FRAMESHIFT	g.14048	c.721delT
11	STS-204	R248W	MISSENSE	g.14069	c.742C>T
12	OST-724	R248W	MISSENSE	g.14069	c.742C>T
13	ACC029	R248Q	MISSENSE	g.14070	c.743G>A
14	OST-864	I251L	MISSENSE	g.14078	c.751A>C
15	OST-502	R273H	MISSENSE	g.14487	c.818G>A
16	MGC-047	D281A	MISSENSE	g.14511	c.842A>C
17	STS-120	R282W	MISSENSE	g.14513	c.844C>T
18	STS-005	E298X	NONSENSE	g.14561	c.892G>T
19	SMN-207	SA INT8	SPLICE	g.14680	c.920-1G>A

* Accession X54156

Version X54156.1 GI:35213

Table 2

Germline p53 Mutation Characteristics in 19 Kindreds

Kindred #	Gene Bank Ref Seq X54156	Coding DNA Sequence
1	g.12066	c.142delG
2	g.12113	c.189delTinsAGA
3	g.13053	c.376-2A>G
4	g.13077	c.398T>C
5	g.13117	c.437G>A
6	g.13116	c.437G>A
7	g.13203	c.524G>A
8	g.13231	c.552delT
9	g.13240	c.559+2T>G
10	g.14048	c.721delT
11	g.14069	c.742C>T
12	g.14069	c.742C>T
13	g.14070	c.743G>A
14	g.14078	c.751A>C
15	g.14487	c.818G>A
16	g.14511	c.842A>C
17	g.14513	c.844C>T
18	g.14561	c.892G>T
19	g.14680	c.920-1G>A

* Accession X54156

Version X54156.1 G:35213

Table 3

Germline p53 Mutation Characteristics in 19 Kindreds

Kindred #	Gene Bank Ref Seq X54156*	Coding DNA Sequence [#]
1	g.12066	c.142delG
2	g.12113	c.189delTinsAGA
3	g.13053	c.376-2A>G
4	g.13077	c.398T>C
5	g.13117	c.437G>A
6	g.13116	c.437G>A
7	g.13203	c.524G>A
8	g.13231	c.552delT
9	g.13240	c.559+2T>G
10	g.14048	c.721delT
11	g.14069	c.742C>T
12	g.14069	c.742C>T
13	g.14070	c.743G>A
14	g.14078	c.751A>C
15	g.14487	c.818G>A
16	g.14511	c.842A>C
17	g.14513	c.844C>T
18	g.14561	c.892G>T
19	g.14680	c.920-1G>A

* Accession X54156

Version X54156.1 GI:35213

[#] Accession X02469 M60950

Version X02469.1 GI:35209

<http://www.ncbi.nlm.nih.gov/nuccore/x54156>

<http://www.ncbi.nlm.nih.gov/nuccore/x02469>

Table 4Age at last examination for unaffected relatives across families by MDM2 SNP309 genotype and gender

<u>MDM2</u> SNP309	G/G or T/G (<i>n</i> = 61)	T/T (<i>n</i> = 56)
Age group		
0–15	4	2
15–30	12	10
30–45	10	15
45–60	19	16
60–75	16	13
Sex		
Male	30	32
Female	31	24
Mean age at last contact		
Overall	45.24 (20.15)	46.27 (19.01)
Male	45.17 (20.38)	47.52 (19.32)
Female	45.31 (20.26)	44.60 (18.88)

Table 5

The frequency distributions of MDM2 SNP309 Genotypes by affection and TP53 mutation status across 19 families

Number (%)	Affected			Unaffected		
	G/G or G/T	T/T	Total	G/G or G/T	T/T	Total
TP53+	35 (45.45)	30 (38.96)	65 (84.41)	20 (17.09)	6 (5.13)	26 (22.22)
TP53–	9 (11.69)	3 (3.90)	12 (15.59)	41 (35.04)	50 (42.74)	91 (77.78)
Total	44 (67.14)	33 (42.86)	77 (100)	61 (52.13)	56 (47.87)	117 (100)