Published in final edited form as:

Hum Genet. 2011 December ; 130(6): 789–794. doi:10.1007/s00439-011-1039-0.

Sex-specific effect of the *TP53* PIN3 polymorphism on cancer risk in a cohort study of *TP53* germline mutation carriers

Shenyang Fang,

Department of Epidemiology, The University of Texas MD

Anderson Cancer Center, Houston, TX 77030, USA

Ralf Krahe,

Department of Genetics, The University of Texas MD

Anderson Cancer Center, Houston, TX 77030, USA

Linda L. Bachinski,

Department of Genetics, The University of Texas MD

Anderson Cancer Center, Houston, TX 77030, USA

Baili Zhang,

Department of Genetics, The University of Texas MD

Anderson Cancer Center, Houston, TX 77030, USA

Christopher I. Amos, and

Department of Epidemiology, The University of Texas MD

Anderson Cancer Center, Houston, TX 77030, USA

Louise C. Strong

Department of Genetics, The University of Texas MD

Anderson Cancer Center, Houston, TX 77030, USA

Abstract

Germline mutations in the tumor suppressor gene *TP53* occur in the majority of families with Li-Fraumeni syndrome, who are at an increased risk for a wide spectrum of early onset cancers. Several genetic polymorphisms in *TP53* modify its effect on cancer risk. While some studies indicate that the *TP53* PIN3 deletion allele (D) accelerate tumor onset in carriers with *TP53* germline mutations, other studies have shown that the *TP53* PIN3 insertion allele (I) confers a significantly higher risk of developing cancer than D allele. To further determine the effects of the *TP53* PIN3 polymorphism on cancer development among *TP53* germline mutations and to evaluate if those are difference between male and female carriers, we studied a total of 152 germline mutation carriers with available DNA samples that can be used for genotyping. Our

Conflict of interest The authors declare no conflicts of interest.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-011-1039-0) contains supplementary material, which is available to authorized users.

results indicate that the *TP53* PIN3 polymorphism has a sex-specific effect on the risk of cancer in *TP53* mutation carriers, conferring cancer risk in men ($P = 0.0041$) but not women with DI or II genotypes.

Introduction

Germline mutations in the *TP53* tumor suppressor gene occur in the majority of families with Li-Fraumeni syndrome (LFS) who are at an increased risk for a wide spectrum of early onset cancers (Garber et al. 1991; Kleihues et al. 1997; Nichols et al. 2001). The polymorphic *MDM2* SNP309 G allele is strongly related to early cancer development in LFS kindreds (Bougeard et al. 2006; Marcel et al. 2009) and to accelerated tumor formation in a sex-specific fashion (Bond et al. 2006; Fang et al. 2010). *TP53* polymorphisms have been shown to modify the function of *TP53* on cancer risk, with several of them reported to increase cancer susceptibility. Some case-only studies have found that the *TP53* codon 72 R allele results in the first tumor onset at an earlier age in carriers of germline *TP53* mutations (Bougeard et al. 2006; Marcel et al. 2009) while other experimental and cohort studies have provided evidence that the PP genotype for codon 72 increases the risk of cancer (Dumont et al. 2003; Fang et al. 2010; Martin et al. 2003). Among patients genotyped for the *TP53*PIN3 polymorphism, which consists of a 16-bp duplication in intron 3, the mean age at first cancer diagnosis was found to be 19 years younger in carriers of the nonduplicated allele (A1) than in carriers of the duplicated allele (A2) (Marcel et al. 2009). Elsewhere the nonduplicated allele A1 is referred to as deletion allele (D) and the duplicated allele A2 is referred to as insertion allele (I). However, a meta-analysis of 26 population-based case-control studies showed that the insertion allele (I) conferred a significantly elevated risk of cancer development (those heterozygous for deletion/insertion and homozygous for insertion [DI + II] versus those homozygous for deletion [DD], odds ratio = 1.14 with 95% confidence interval [CI] = 1.02–1.27) (Hu et al. 2010). To further verify the relative risk of cancer in *TP53* germline mutation carriers with *TP53* PIN3 polymorphisms, we performed the present study in a cohort of families with LFS and LFS-like diseases. We also evaluated the sex-specific effect of this polymorphism on cancer susceptibility.

Patients and methods

Study population

The present study population consisted of several cohorts of families identified through patients with early onset sarcoma or multiple cancers. Details about the recruitment of these families were provided in a previous study by our group (Fang et al. 2010). A total of 213 germline mutation carriers were available for study, of whom 152 had DNA samples available for genotyping *TP53* PIN3. These 152 individuals were 47.4% male, 4.1% black, 73.0% white, and 73.7% having at least one tumor. There was no characteristic difference between genotyped and non-genotyped individuals ($P = 0.9818$ for sex and 0.1612 for race, respectively), but the non-genotyped group had higher rate of cancer development ($P = 0.0028$) (Table S1). In addition, we genotyped *TP53* PIN3 for 399 individuals with wild-type *TP53* ($N = 257$), unknown *TP53* status ($N = 133$) or genotype controls ($N = 9$). *TP53* unknown status means that these samples were not sequenced and their status could not be

inferred from the pedigree (not obligate carriers). These nine observations were excluded because they were used as a genotype quality control. The results were 100% concordant. Among 257 wild-type *TP53* mutation carriers, 43.2% were males, 12.8% black, 70.4% white, 15.5% having at least one tumor and 239 with complete follow-up information (data not shown here). Genotyping was done by fluorescent capillary electrophoresis on an Applied Biosystems 3100 Genetic Analyzer. Amplification was done by a three primer reaction using a FAM-labeled Universal primer (5' GGGACACCGCTGAT CGTTTA) as described in Bachinski et al. (2003). Forward primer (5' GACGGGACACCGCTGATCGTTTACTGAA AACACGTTCTG-GTA) and reverse primer (5' AAGGGGGACTGTAGATGGGTG) amplified fragments of 141 and 157 bp. We observed these 152 individuals for their onsets of any type of cancer and determined the effect on cancer risk of this polymorphism.

Statistical analysis

We first stratified our cancer patients with *TP53* mutations by *TP53* PIN3 genotype and tested for differences in age at cancer diagnosis among the different PIN3 genotype groups using a nonparametric Kruskal–Wallis test. The time to onset was from birth to first cancer diagnosis, for those who had cancer, and the censoring time was from birth to last contact (fixed to 31 December 2001), death, or study termination, for those who had no cancer. Among these carriers of germline *TP53* mutations, we performed a log-rank test to quantify differences in cancer risk by genotype using the Kaplan–Meier product-limit method. To adjust for correlation within families, we further modeled the relative risk of these polymorphisms in LFS patients using a weighted Cox proportional hazard model. We took into consideration the familial correlation in the model by calculating the robust variance. This model allowed us to adjust (or not adjust) for sex, race, birth year, and having *TP53* codon 72 polymorphism. To identify the haplotypes defined by the two *TP53* polymorphisms, we extracted all *TP53*-positive pedigrees in which there was one *TP53* mutation carrier and at least one family member's genotypes for both *TP53* PIN3 and *TP53* codon 72 were known. We applied a Monte Carlo-based method using the SimWalk2 software program to specify the haplotypes for these polymorphisms (Weeks et al. 1995). All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant.

Results

Of these 152 carriers with a *TP53* germline mutation, 112 (73.7%) had cancer, and their median age at cancer diagnosis or censoring was 26.9 years (mean $\pm$ standard deviation: 27.5 ± 18.3). The average age at first cancer diagnosis in those with (DD) genotypes did not differ significantly from the average age in those with (DI + II) genotypes among patients with mutant *TP53* (21.1 versus 24.0 years, $P = 0.4847$; Table 1). Furthermore, the distribution of the types of cancers (epithelial cancer, hematopoietic cancer, and sarcoma) did not differ significantly between the DD and DI + II groups using the Fisher exact test ($P = 0.300$; Table S2). We also tested for differences in age at cancer diagnosis by PIN3 polymorphisms among affected wild-type *TP53* carriers and found no significant results (51.0 vs. 39.5 years, $P = 0.2546$; Table 1).

Next, we examined the time to first cancer onset in each group. A total of 32.8% of mutation carriers with DD genotypes failed to develop cancer, whereas only 3.0% of mutation carriers with DI + II genotypes were free of cancer during the follow-up time. Among these carriers of germline *TP53* mutations, we performed a log-rank test to quantify differences in cancer risk by gender and genotype using the Kaplan–Meier product-limit method. The mean age of cancer diagnosis estimated using Kaplan–Meier method was 28 and 35 years in females and males, respectively. Long-rank test showed no difference for the mean age of tumor onset between females and males in 152 *TP53* germline mutation carriers with available DNA data ($P = 0.1160$) (Table S3) which seems non-concordant with the previous report (Fang et al. 2010) in total 213 germline mutation carriers, among which significant difference for the mean age of tumor onset between females and males was found ($P = 0.0057$). The inconsistency occurs due to non-random selection of DNA sample for genotyping. Those 152 *TP53* germline mutation carriers that were genotyped for PIN3 had lower rate of cancer patients (73.7%) than those 61 *TP53* mutation carriers that were not genotyped for PIN3 (91.8%) (Table S1). We found a significant difference in risk of cancer between the DD and DI + II genotypes ($P = 0.0154$; Fig. 1). The findings regarding the effect of *TP53* PIN3 polymorphism from the present cohort study were more reliable than those from the above case-only studies because in the cohort study we prospectively observed the effects of PIN3 polymorphisms on cancer risk. Our stratification analyses showed that male DI + II carriers had poorer disease-free survival rates than did male DD carriers ($P = 0.0358$; Fig. 2) whereas women in both genotype groups had the same survival rates over time ($P = 0.2654$; Fig. 3).

In the Kaplan–Meier method, we treated all individuals as being unrelated and did not estimate the effect size (hazard ratio) of the polymorphisms. In the further Cox proportional hazard model, while considering the correlation within families and adjusting for race and birth year, female had earlier age of tumor diagnosis than male without stratification by *TP53* PIN3 (hazard ratio [HR] = 1.43, 95% CI 1.05–1.95) or within *TP53* PIN3 DD group (HR = 1.59, 95% CI 1.08–2.34) (Table S3). A significant risk effect on onset of tumor was noted with the *TP53* PIN3 DI/II genotype in the univariate analysis (HR = 1.66, 95% CI 1.01–2.72; Table 2). Testing for the interactions between *TP53* PIN3 polymorphisms and sex revealed that the DI/II genotype conferred an increased risk among men (HR = 2.20, 95% CI 1.19–4.09) but not women (HR = 1.33, 95% CI 0.76–2.33). When we adjusted for race, birth year, and/or sex in a multivariate model, we found the same order of effect size as in the univariate model for those with a DI/II genotype in the overall study population but with higher significance. Similarly among men, HR in the multivariate model was close to that in the univariate model but had a smaller P value (Table 2 and Table S4). These results thus indicate that the *TP53* PIN3 DI/II genotype has a sex-specific risk effect against cancer development in men.

A previous study by our group showed that the *TP53* codon 72 PP homozygosity was a risk factor for cancer in *TP53* mutation carriers; this effect was significant in men but not women (Fang et al. 2010). The *TP53* PIN3 polymorphism is located just 181 bp downstream from the *TP53* codon 72 variant. To allow for linkage disequilibrium between *TP53* PIN3 and *TP53* codon 72 ($r^2 = 0.61$ and $D' = 0.90$ in our data), we included both *TP53* PIN3 and *TP53* codon 72 polymorphisms in our model and found that the effect of *TP53* PIN3 DI/II

genotypes remained significant in men when controlling for codon 72 polymorphisms (HR = 1.72, 95% CI = 1.05–2.84; Table 2 and Table S5) whereas codon 72 was not significant when controlling for PIN3 polymorphisms (HR for codon 72 PP = 1.89, 95% CI 0.85–4.21; Table S5), suggesting that the *TP53* PIN3 variant has an independent effect on cancer risk in men with *TP53* mutation.

We identified the haplotypes defined by the two *TP53* polymorphisms and evaluated their effects on cancer risk. As with the D–R haplotype (D allele at *TP53* PIN3 and R allele at codon72), the D–P haplotype had a significant risk effect on cancer risk in men carrying *TP53* mutants (HR = 1.78, 95% CI 1.18–2.70) but not women with it (HR = 1.20, 95% CI 0.57–2.59) (Table 2 and Table S6), whereas the I–P haplotype had a non-significant risk effect on cancer risk in males carrying *TP53* mutants when compared with the D–R haplotype (HR = 1.32, 95% CI 0.80–2.17; Table S6). The results of this haplotype analysis are consistent with those of our previous study in which the *TP53* codon 72 P allele increased the risk of cancer in men but not women (Fang et al. 2010).

We also estimated the effect size of the *TP53* PIN3 DI/II genotype in wild-type *TP53* carriers and found no significant results among all (HR = 0.63, 95%CI 0.28–1.42), among males (HR = 0.79, 95%CI 0.28–2.21) or among females (HR = 0.63, 95%CI 0.20–1.95) (Table S7). Our results suggest that *TP53* PIN3 polymorphism only modifies the effect of mutant *TP53* on cancer risk.

Discussion

In this study, we identified the effect of *TP53* PIN3 polymorphism on tumor onset in a cohort of *TP53* germline mutation carriers. We found that *TP53* PIN3 DI/II genotypes increased cancer risk in men but not women. Although Marcel et al. reported that *TP53* PIN3 A1(D) allele was associated with an earlier tumor onset of 19 years in mutation carriers with LFS or LFS-like syndrome (Marcel et al. 2009), we found no significant difference in age at first cancer diagnosis between *TP53* PIN3 deletion and insertion polymorphisms in affected mutation carriers. Nevertheless, when we observed all mutation carriers (cancer and non-cancer) during the follow-up period of time, we found that the log rank test results and Cox regression model agreed that the *TP53* PIN3 DI/II genotype had an increased risk effect on cancer development, which was significant in men but not women. Our findings were consistent with the meta-analysis reports by Hu et al. (2010) regarding the risk effect of the insertion allele on any type of cancer development. But we did not observe a significant effect of DI/II on breast cancer development ($P = 0.8459$) which was inconsistent with the combined analysis by Hu et al. The effect of the A2 variant in men was independent of the presence of *TP53* 72P allele. Considering the linkage disequilibrium between *TP53* PIN3 and *TP53* codon 72 polymorphisms, the D–P haplotype had a significantly higher risk effect on cancer than did the D–R haplotype in men. Dumont et al. (2003) reported that the *TP53* 72R variant was 5- to 10-times more likely to induce programmed cell death than the 72P variant, and the authors suggested that the low apoptotic potential of the 72P variant might account for increased predisposition to cancer development in carriers of the 72P variant. However, the functional basis for the risk effect of *TP53* PIN3 remains to be unclear. Gemignani et al. (2004) found that the presence of the

A2 (I) allele was associated with an elevated risk of colorectal cancer and with the reduced levels of *TP53* messenger RNA expression in lymphoblastoid cells. A possible explanation for this finding is a reduced stability of the pre-messenger RNA carrying the 16-bp insertion in intron 3 of the tumor suppressor gene *TP53*. Marcel et al. (2010) demonstrated that the formation of *G*-quadruplex structures (G4) in a GC-rich region of intron 3 that modulates the splicing of intron 2, thus resulting in differential expression of transcripts encoding distinct p53 isoforms. Our findings were inconsistent with Marcel et al. (2009) in terms of the effect of *TP53* PIN3, due to limited sample size in both studies or different study design between them. More observations are needed to better examine the relationship between *TP53* PIN3 polymorphisms and cancer risk.

In summary, our results show that *TP53* PIN3 polymorphisms have a sex-specific effect on the risk of cancer in *TP53* mutation carriers, conferring increased cancer risk in men but not women with DI/II genotypes. However, the biologic mechanisms of this effect of *TP53* PIN3 polymorphisms in men only remain to be elucidated. Our results also suggest that females generally have higher cancer risk than males in mutant *TP53* carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Bachinski LL, Udd B, Meola G, Sansone V, Bassez G, Eymard B, Thornton CA, Moxley RT, Harper PS, Rogers MT, Jurkat-Rott K, Lehmann-Horn F, Wieser T, Gamez J, Navarro C, Bottani A, Kohler A, Shriver MD, Sallinen R, Wessman M, Zhang SX, Wright FA, Krahe R (2003) Confirmation of the type 2 myotonic dystrophy (CCTG)(n) expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. *Am J Hum Genet* 73:835–848 [PubMed: 12970845]
- Bond GL, Hirshfield KM, Kirchhoff T, Alexe G, Bond EE, Robins H, Bartel F, Taubert H, Wuerl P, Hait W, Toppmeyer D, Offit K, Levine AJ (2006) MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. *Cancer Res* 66:5104–5110 [PubMed: 16707433]
- Bougeard G, Baert-Desurmont S, Tournier I, Vasseur S, Martin C, Brugieres L, Chompret A, Bressac-de Paillerets B, Stoppa-Lyonnet D, Bonaiti-Pellie C, Frebourg T (2006) Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome. *J Med Genet* 43:531–533 [PubMed: 16258005]
- Dumont P, Leu JI, Della Pietra AC3, George DL, Murphy M (2003) The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* 33:357–365 [PubMed: 12567188]
- Fang SY, Krahe R, Lozano G, Han YH, Chen W, Post SM, Zhang BL, Wilson CD, Bachinski LL, Strong LC, Amos CI (2010) Effects of MDM2, MDM4 and TP53 Codon 72 polymorphisms on cancer risk in a cohort study of carriers of TP53 germline mutations. *Plos One* 5 5(5):e10813
- Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni JF Jr, Li FP (1991) Follow-up study of twenty-four families with Li-Fraumeni syndrome. *Cancer Res* 51:6094–6097 [PubMed: 1933872]
- Gemignani F, Moreno V, Landi S, Moullan N, Chabrier A, Gutierrez-Enriquez S, Hall J, Guino E, Peinado MA, Capella G, Canzian F (2004) A TP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. *Oncogene* 23:1954–1956 [PubMed: 14647431]
- Hu Z, Li X, Qu XC, He YG, Ring BZ, Song EM, Su L (2010) Intron 3 16 bp duplication polymorphism of TP53 contributes to cancer susceptibility: a meta-analysis. *Carcinogenesis* 31:643–647 [PubMed: 20089604]

- Kleihues P, Schauble B, zur Hausen A, Esteve J, Ohgaki H (1997) Tumors associated with p53 germline mutations: a synopsis of 91 families. *Am J Pathol* 150:1–13 [PubMed: 9006316]
- Marcel V, Palmero EI, Falagan-Lotsch P, Martel-Planche G, Ashton-Prolla P, Olivier M, Brentani RR, Hainaut P, Achatz MI (2009) TP53 PIN3 and MDM2 SNP309 polymorphisms as genetic modifiers in the Li-Fraumeni syndrome: impact on age at first diagnosis. *J Med Genet* 46:766–772 [PubMed: 19542078]
- Marcel V, Thao Tran PL, Sagne C, Martel-Planche G, Vaslin L, Teulade-Fichou MP, Hall J, Mergny JL, Hainaut P, Van DE (2010) G-quadruplex structures in TP53 intron 3: role in alternative splicing and in production of p53 mRNA isoforms. *Carcinogenesis* 32(3):271–278 [PubMed: 21112961]
- Martin AM, Kanetsky PA, Amirimani B, Colligon TA, Athanasiadis G, Shih HA, Guerrero MR, Calzone K, Rebbeck TR, Weber BL (2003) Germline TP53 mutations in breast cancer families with multiple primary cancers: is TP53 a modifier of BRCA1? *J Med Genet* 40:e34 [PubMed: 12676907]
- Nichols KE, Malkin D, Garber JE, Fraumeni JF Jr, Li FP (2001) Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. *Cancer Epidemiol Biomarkers Prev* 10:83–87 [PubMed: 11219776]
- Weeks DE, Sobel E, Oconnell JR, Lange K (1995) Computer-programs for multilocus haplotyping of general pedigrees. *Am J Hum Genet* 56:1506–1507 [PubMed: 7762577]

Fig. 1.

Proportion of mutant TP53 carriers without cancer by age comparing observations with the PIN3 DD genotype and the combined DI + II genotypes. DD versus DI + II, $P = 0.0154$ (log-rank test)

Fig. 2.

Proportion of male mutant TP53 carriers without cancer by age comparing male observations with the PIN3 DD genotype and the combined DI + II genotypes. DD versus DI + II, $P = 0.0358$ (log-rank test)

Fig. 3.

Proportion of female mutant TP53 carriers without cancer by age comparing female observations with the PIN3 DD genotype and the combined DI + II genotypes. DD versus DI + II, $P = 0.2654$ (log-rank test)

Table 1

Mean age at cancer diagnosis by germline *TP53* status and PIN3 genotype

PIN3 polymorphism	Wild-type <i>TP53</i>		Mutant <i>TP53</i>		<i>P</i> value ^a
	<i>N</i> (%)	Median age, years (SD)	<i>N</i> (%)	Median age, years (SD)	
DD	29 (80.6)	51.0 (20.1)	80 (71.4)	21.1 (18.7)	0.4884
DI	7 (19.4)	39.5 (19.6)	30 (26.8)	24.0 (16.3)	
II	-	-	2 (1.8)	23.7 (8.8)	
DI + II	7 (19.4)	39.5 (19.6)	32 (28.6)	24.0 (15.9)	0.4847

SD standard deviation

^a Kruskal–Wallis test

Univariate and multivariate analyses of the *TP53* PIN3 effects on age at first cancer diagnosis in carriers of a *TP53* germline mutation

Table 2

Genotype	All ^a (N = 152)		Male ^b (N = 72)		Female ^b (N = 80)	
	Hazard ratio	P value ^d	Hazard ratio	P value ^d	Hazard ratio	P value ^d
Univariate analysis						
DD (119)	1.00	0.044	1.00	0.0123	1.00	0.3224
DI/II (33)	1.66 (1.01–2.72)		2.20 (1.19–4.09)		1.33 (0.76–2.33)	
Multivariate analysis (all = 152, male = 72, female = 80)						
DD (119)	1.00	0.0211	1.00	0.0043	1.00	0.2756
DI/II (33)	1.66 (1.08–2.54)		2.14 (1.27–3.60)		1.36 (0.78–2.36)	
Multivariate analysis including P53 codon 72 (all = 119, male = 56, female = 63)						
DD (90)	1.00	0.1840	1.00	0.0320	1.00	0.8617
DI/II (30)	1.34 (0.87–2.07)		1.72 (1.05–2.84)		1.07 (0.52–2.16)	
Haplotype analysis (all = 112, male = 56, female = 56) ^c						
D–R (172)	1.00		1.00		1.00	
I–P (25)	1.05 (0.76–1.44)	0.7806	1.32 (0.80–2.17)	0.2761	0.89 (0.58–1.36)	0.5766
I–R (5)	0.54 (0.20–1.47)	0.2303	–	–	–	–
D–P (22)	1.48 (0.89–2.45)	0.1276	1.78 (1.18–2.70)	0.0063	1.20 (0.57–2.59)	0.6423

^a Adjusted for sex, race, and birth year in multivariate and haplotype analyses

^b Adjusted for race and birth year in multivariate and haplotype analyses

^c Smaller haplotype observations due to inability to be inferred from SimWalk2 software

^d Bold values indicate $P < 0.05$