p53 Arg72Pro polymorphism, adiposity status, and cancer risk: Two case-cohorts within a Japanese prospective study

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Abstract
The tumor suppressor protein, p53, is a critical molecule involved in cancer development. However, the association between p53 Arg72Pro polymorphism and cancer risk remains unclear, possibly due to the pro-tumor potential of p53 under metabolic stress. Here, we hypothesized that the p53 Arg72Pro polymorphism plays different roles during tumorigenesis by adiposity status. We measured baseline body mass index (BMI) and p53 Arg72Pro polymorphism for two case-cohorts, which included 4264 cancers with up to 20 years of follow-up. Multivariable-adjusted hazard ratios (HRs) and confidence intervals (CIs) were estimated using weighted Cox proportional-hazards method. Without consideration of adiposity status, p53 Arg72Pro polymorphism was not associated with cancer risk. However, proline (Pro) homozygous genotype conferred an increased cancer risk for individuals with a BMI <25 kg/m² (HR [95% CI]: 1.12 [1.00–1.26] for total cancer and 1.19 [1.02–1.38] for obesity-related cancer), but not for those with a BMI ≥25 kg/m². The heterogeneous effect of p53 Arg72Pro polymorphism on cancer risk according to adiposity status was indicated (p heterogeneity: 0.07 for total cancer and 0.03 for obesity-related cancer). Furthermore, the association between overweight and cancer risk was only observed in arginine (Arg) carriers, but not in Pro homozygous carriers (p heterogeneity: 0.07 for total cancer and 0.02 for obesity-related cancer). Pro homozygous carriers were more likely to be predisposed to cancer than Arg carriers with normal-weight conditions. In addition, overweight was related to a higher cancer risk in Arg carriers than Pro homozygous carriers. Our findings may suggest the adiposity-dependent dual effects of p53 Arg72Pro polymorphism during tumorigenesis.

Keywords
adiposity, cancer, gene-environment interaction, p53, p53 Arg72Pro polymorphism

Abbreviations: Arg, arginine; BMI, body mass index; CI, confidence interval; HR, hazard ratio; JPHC, Japan Public Health Center-based Prospective; MAPK, mitogen-activated protein kinase; PHC, public health center; Pro, proline.
1 | INTRODUCTION

Cancer is a multifactorial disease that promotes tumorigenesis through different mechanisms depending on the endogenous and exogenous conditions. Obese individuals with insulin resistance may develop cancer through cell proliferation due to the overexpression of insulin, whereas individuals taking high ethanol and acetaldehyde may promote carcinogenesis through cell proliferation due to the activation of the mitogen-activated protein kinase (MAPK) signaling pathway and DNA damage. There are also cases in which individuals are less likely to develop cancer due to a high acetaldehyde enzymatic active variant of ALDH2 polymorphism. This led to a personalized medical treatment for cancer prevention and therapy based on individual cancer etiologies, which encourages the study of each carcinogenesis mechanism under specific conditions.

The tumor suppressor protein, p53, is a crucial transcription factor involved in many pivotal carcinogenesis pathways in response to various conditions. For example, in the occurrence of DNA damage, p53 induces apoptosis and cell cycle arrest. Interestingly, the p53 gene harbors a nonsynonymous p53 Arg72Pro polymorphism, which encodes either the arginine (CGC) or proline (CCC) at codon 72, and has been reported to alter the sensitivity of the p53 function. The proline (Pro) variant is known as a less potent inducer of apoptosis than the arginine (Arg) variant, suggesting that the Pro variant may increase cancer predisposition more. Although many epidemiological studies have already investigated the p53 Arg72Pro polymorphism, its association with cancer risks remains elusive.

Recently, there has been accumulating knowledge of bidirectional p53 functions for tumorigenesis in a context-dependent manner in basic science. The canonical p53 function including apoptosis regulation under DNA damage includes a protective effect against cancer, whereas another p53 function involves promoting insulin resistance under metabolic stress, which has a hazardous effect on cancer. With regard to epidemiological studies, one report on p53 Arg72Pro polymorphism is a Chinese study that included 20% overweight individuals. This study showed an increased risk of colorectal cancer for Pro carriers, suggesting that the Pro variant may increase cancer predisposition more. Although many epidemiological studies have already investigated the p53 Arg72Pro polymorphism, its association with cancer risks remains elusive.

2 | MATERIALS AND METHODS

We leveraged the Japan Public Health Center-based Prospective (JPHC) Study to construct two independent case-cohorts consisting of all incident cancers. We also included random subcohorts from two independent populations to avoid a false-positive result due to the nature of genomic study and replicate the results.

2.1 | Study population

The JPHC Study enrolled 140,420 residents across 11 public health center (PHC) areas and conducted baseline and follow-up surveys to examine lifestyle and possible factors that are related to the risk of cancer and other diseases. The participants were aged 40–59 years in the baseline year 1990 for 5 PHC areas and 40–69 years in that of 1993–1994 for the other PHC areas. For the present study, we excluded two PHC areas because of a different inclusion criteria. The remaining nine PHC areas were then included, in which we defined two populations for case-cohorts I and II, respectively. The first population consisted of 33,736 participants who responded to a questionnaire and provided blood samples for the baseline survey. Excluding the first population, the second population included 10,950 participants who responded to a questionnaire and provided blood samples for the 5-year follow-up survey. Then, we randomly selected a representative subcohort from each population, regardless of their incident cancer and disease histories. We included 13,024 participants for subcohort I from the first population and 4000 participants for subcohort II from the second population (constituting ~40% of each population). Finally, we identified all incident cancer cases for each population and then constructed case-cohort I from the first population and case-cohort II from the second population, with the more detailed descriptions provided in our previous case-cohort studies. The flow chart defining the two study populations are shown in Figures 1 and 2. The comprehensive study protocol, including this study, was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan (Approval No. 2011-044). The participants were informed of the study objective, and they responded to the questionnaire survey, which was regarded as consent. Additionally, before using their blood samples for research, all living participants were contacted by mail and given the opportunity to opt out. We excluded those who refused to participate and documented their withdrawal of consent.

2.2 | Follow-up and identification of cases

The participants were followed up from the date of response to the baseline questionnaire for the first population and the 5-year follow-up questionnaire for the second population until December 31, 2009. The median follow-up duration was 16.9 and 11.9 years for the first and second populations, respectively. During the follow-up, information on the participant’s vital and residential status was obtained from the residential registry. Furthermore, the incident cancer cases were obtained from the patient records of major local hospitals and population-based cancer registries. Additionally, we utilized death certificates and identified 7.9% of the cases in case-cohort I and 5.7% of the cases in case-cohort II. The cancer type in
each case was classified according to the International Classification of Diseases for Oncology Codes, Third Edition. The following types of cancer were defined as obesity-related based on the reports from the International Agency for Research on Cancer and the World Cancer Research Fund International: malignancy of the oral cavity (C00–C14), esophagus adenocarcinoma (C15 with histology code 8140-8384), gastric cardia (C160), colorectum (C18–C20), liver (C22), gallbladder (C23), pancreas (C25), breast (C50) in postmenopausal women, endometrium (C54), ovary (C56), prostate (C61), kidney (C64–C68), meningioma (C70), thyroid (C73), and multiple myeloma (histology code 9732). At the end of the follow-up period, 3977 and 1261 incident cancer cases were identified in the first population of 33,736 participants and second population of 10,950 participants, respectively. We then applied the same exclusion criteria (history of cancer and missing follow-up information) to the cases and subcohorts. Finally, we obtained a total of 3750 cases and 12,722 subcohort members in case-cohort I, and 1081 cases and 3894 subcohort members in case-cohort II.

2.3 | Genotyping

DNA samples from participants were extracted from the buffy coat of the peripheral white blood cells using FlexiGene DNA kits (QIAGEN) and genotyped using the HumanOmniExpressExome-8 v1.2 BeadChip, HumanOmniExpress-12 BeadChip or HumanOmni2.5-8 BeadChip arrays (Illumina Inc.). Detailed information is described in Methods S1.

2.4 | Statistical analysis

The association of the p53 Arg72Pro polymorphism with the risk of total, obesity-related, and other cancer types were examined using the weighted Cox proportional-hazards method, and the hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Overweight was defined as a BMI ≥ 25 kg/m² and the normal-weight as a BMI < 25 kg/m². In this study, BMI was calculated using self-reported height and weight. When we estimated Pearson’s correlation coefficients between self-reported data and recorded data in health check-ups, the coefficient for BMI was 0.90 in the first population and 0.89 in the second population. For the genotype model, the Arg homozygous carriers (Arg/Arg) were used as the reference and were compared with heterozygous carriers (Arg/Pro) and Pro homozygous carriers (Pro/Pro). A linear trend test was conducted by applying ordinal scores to each category of the Arg72Pro genotype, which was equal to the additive model. For the recessive model, HRs were estimated by comparing the Pro homozygous carriers with the Arg homozygous and heterozygous carriers as references. For this study, we used the recessive model as the main analysis because the p53 functions as a tetramer. The crude HRs were minimally adjusted for sex, age, and the first three principal components of genetic ancestral markers, then were stratified according to the PHC area.

3 | RESULTS

3.1 | Characteristics of the two case-cohorts

For this study, totals of 3368 cases and 10,142 subcohort members in case-cohort I and 896 cases and 3282 subcohort members in case-cohort II were included in the analyses (Figures S1 and S2). Based on the case-cohort design, 1376 and 339 cases occurred during follow-up in subcohorts I and II, respectively. The number of cases of obesity-related cancer is presented in Table S1 and the demographic features of the two case-cohorts are shown in Table 1. For the subcohort members, both cases showed higher tendencies for those who are men, older, smokers, and regular alcoholic drinkers.

3.2 | Associations of p53 Arg72Pro polymorphism and adiposity statuses with the risk of total, obesity-related, and other cancers

After confirmation that the results of each case-cohort were consistent, the combined results of the two case-cohorts, which examined the effect of p53 Arg72Pro polymorphism and adiposity status on the risk of cancer, are presented (Table 2). We found that there was no significant association between the genetic models for the p53 Arg72Pro polymorphism and the risk of total, obesity-related, and other cancers. The multivariable-adjusted HRs of p53 Arg72Pro polymorphism were very similar to the crude HRs, suggesting that additional factors did not substantially confound the effect of p53 Arg72Pro polymorphism. Of note, point estimates of the recessive model indicated that Pro homozygous carriers possessed a different degree of cancer risk compared with Arg carriers. Therefore, the recessive model (Arg/Arg vs. Pro/Pro) used in this study was a reasonable assumption to demonstrate the effect of p53 Arg72Pro...
polymorphism on the risk of cancer. Compared with those with normal weights (BMI <25 kg/m²), the crude HR of overweight people (BMI ≥25 kg/m²) was 1.06 [95% CI: 0.99–1.15] for total cancer and 1.18 [95% CI: 1.07–1.30] for obesity-related cancer. After adjustment for p53 Arg72Pro polymorphism and lifestyle factors, the corresponding values of the multivariable-adjusted HR were 1.08 [95% CI: 1.00–1.16] for total cancer and 1.19 [95% CI: 1.08–1.31] for obesity-related cancer, indicating that overweight people (BMI ≥25 kg/m²) have an increased risk of total and obesity-related cancers than those with normal weights (BMI <25 kg/m²).

### 3.3 Context-dependent associations of p53 Arg72Pro polymorphism and adiposity status with the risk of total, obesity-related, and other cancers

The associations between the p53 Arg72Pro polymorphism and cancer risk according to adiposity status are presented in Table 3. Compared with Arg carriers, Pro homozygous carriers showed an increased risk of total cancer and obesity-related cancer in normal-weight conditions (The multivariable-adjusted HRs: 1.12 [1.00–1.26] for total cancer and 1.19 [1.02–1.38] for obesity-related cancer). In contrast, the increased risk was not observed in overweight conditions, indicating the heterogeneous effect of the p53 Arg72Pro polymorphism on the risk of total and obesity-related cancers ($p_{interaction}$: 0.07 for total cancer and 0.03 for obesity-related cancer).

The association of the adiposity status and cancer risk and the dependence on the p53 Arg72Pro polymorphism are summarized in Table 4. A clear association between overweight and the risk of total and obesity-related cancers was observed for Arg carriers (HRs: 1.11 [1.02–1.20] for total cancer and 1.24 [1.12–1.38] for obesity-related cancer), but not for Pro homozygous carriers. The heterogeneous effect of overweight on total and obesity-related cancer risks, according to the p53 Arg72Pro polymorphism, was also observed ($p_{interaction}$: 0.07 for total cancer and 0.02 for obesity-related cancer).

### DISCUSSION

Here, we reported the findings of two case-cohorts with a total of 4264 overall cancer cases from the Japanese population-based study and subsequent stratification analyses of the p53 Arg72Pro polymorphism and pre-diagnosed adiposity status. To our knowledge, this study is the first to demonstrate context-dependent associations of the p53 Arg72Pro polymorphism and adiposity status with cancer risk. Pro homozygous carriers were found to have an increased risk of cancer for those with normal-weight conditions, but not in overweight participants. Additionally, positive associations between overweight and the risk of cancer were observed only in Arg carriers, and not in Pro homozygous carriers. These findings may provide novel evidence of the complex association between the p53 Arg72Pro polymorphism and adiposity status with cancer development.

Without the consideration of adiposity status, the results for the p53 Arg72Pro polymorphism and total cancer in this study were in line with the findings of the HuGE review comprised of 54 independent studies that reported no association between the p53 Arg72Pro polymorphism and the risk of cancer, with a high heterogeneity.
Among the site-specific cancers, the extensively examined types were colorectal and breast cancers for the obesity-related cancers, and lung and cervical cancers for the other (nonobesity-related) cancers. The results of the meta-analyses of these site-specific cancers were also equivocal due to high heterogeneities. Another comprehensive study that analyzed 302 studies on p53 Arg72Pro polymorphism suggested that the tumor site, allele frequencies in control, ethnicity, and assays for genotyping independently contributed to the heterogeneity. However, the observed high heterogeneities remained in the stratification analyses according to tumor site and ethnicity. Interestingly, heterogeneous effects of the Pro variant on breast cancer were observed even in European populations, which were as follows: hazardous in Swedish, null in Portuguese, and protective in Greek. Given

| Cancer type | Variables | Cases | HRa (95% CI) | HRb (95% CI) |
|-------------|-----------|-------|-------------|-------------|
| Total       | BMI category |       |             |             |
|             | Normal (BMI < 25 kg/m²) | 3004  | 1.00 (reference) | 1.00 (reference) |
|             | Overweight (BMI ≥ 25 kg/m²) | 1260  | 1.06 (0.99-1.15) | 1.08 (1.00-1.16) |
| p53 Arg72Pro polymorphism | ArgArg | 1703  | 1.00 (reference) | 1.00 (reference) |
|             | ArgPro | 1945  | 0.99 (0.92-1.07) | 0.99 (0.92-1.06) |
|             | ProPro | 616   | 1.06 (0.95-1.18) | 1.06 (0.95-1.18) |
| p for trend (additive model) | | | 0.43 | 0.49 |
|             | ArgArg + ArgPro | 3648  | 1.00 (reference) | 1.00 (reference) |
|             | ProPro | 616   | 1.06 (0.96-1.17) | 1.06 (0.96-1.16) |
| p for recessive model | | | 0.26 | 0.27 |
| Obesity-relatedc | BMI category |       |             |             |
|             | Normal (BMI < 25 kg/m²) | 1471  | 1.00 (reference) | 1.00 (reference) |
|             | Overweight (BMI ≥ 25 kg/m²) | 691   | 1.18 (1.07-1.30) | 1.19 (1.08-1.31) |
| p53 Arg72Pro polymorphism | ArgArg | 857   | 1.00 (reference) | 1.00 (reference) |
|             | ArgPro | 982   | 0.99 (0.90-1.09) | 0.99 (0.89-1.09) |
|             | ProPro | 323   | 1.09 (0.95-1.25) | 1.09 (0.95-1.25) |
| p for trend (additive model) | | | 0.41 | 0.45 |
|             | ArgArg + ArgPro | 1839  | 1.00 (reference) | 1.00 (reference) |
|             | ProPro | 323   | 1.08 (0.95-1.23) | 1.08 (0.95-1.23) |
| p for recessive model | | | 0.23 | 0.24 |
| Othersd | BMI category |       |             |             |
|             | Normal (BMI < 25 kg/m²) | 1533  | 1.00 (reference) | 1.00 (reference) |
|             | Overweight (BMI ≥ 25 kg/m²) | 569   | 0.95 (0.86-1.06) | 0.97 (0.88-1.08) |
| p53 Arg72Pro polymorphism | ArgArg | 846   | 1.00 (reference) | 1.00 (reference) |
|             | ArgPro | 963   | 1.00 (0.90-1.10) | 1.00 (0.90-1.10) |
|             | ProPro | 293   | 1.02 (0.88-1.17) | 1.01 (0.88-1.17) |
| p for trend (additive model) | | | 0.80 | 0.85 |
|             | ArgArg + ArgPro | 1809  | 1.00 (reference) | 1.00 (reference) |
|             | ProPro | 293   | 1.02 (0.89-1.17) | 1.02 (0.89-1.16) |
| p for recessive model | | | 0.76 | 0.78 |

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio.

aAdjusted for age, sex and first three principal components of genetic ancestral markers.
bAdjusted for age, sex, first three principal components of genetic ancestral markers, smoking status, alcohol intake, physical activity, diabetes, and BMI(normal, overweight) or p53 Arg72Pro polymorphism(recessive model).
cIncluded malignancy of the oral cavity, esophagus adenocarcinoma, gastric cardia, colorectum, liver, gallbladder, pancreas, breast in postmenopausal, endometrium, ovary, prostate, kidney, meningioma, thyroid, and multiple myeloma.
dIncluded all cancers except for obesity-related cancer.
TABLE 3  Associations of the p53 Arg72Pro polymorphism with the risk of total, obesity-related, and other cancers by adiposity status

| Cancer type         | p53 Arg72Pro | BMI category | ArgArg + ArgPro | ProPro | p | \( p_{\text{heterogeneity}} \) |
|---------------------|--------------|--------------|-----------------|--------|---|-----------------------------|
|                     |              | Normal\(^b\) | 2561 (1.00)     | 443    | 1.12 (1.00–1.26) | 0.048 |
| Total               |              | Overweight\(^c\) | 1087 (1.00)    | 173    | 0.92 (0.77–1.10) | 0.37  | 0.07 |
| Obesity-related\(^d\) |              | Normal\(^b\) | 1240 (1.00)     | 231    | 1.19 (1.02–1.38) | 0.02  |
|                     |              | Overweight\(^c\) | 599 (1.00)    | 92     | 0.88 (0.69–1.11) | 0.28  | 0.03 |
| Others\(^e\)       |              | Normal\(^f\) | 1321 (1.00)     | 212    | 1.05 (0.90–1.23) | 0.54  |
|                     |              | Overweight\(^d\) | 488 (1.00)    | 81     | 0.95 (0.74–1.23) | 0.72  | 0.53 |

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio.
\(^a\)Adjusted for age, sex, first three principal components of genetic ancestral markers, smoking status, alcohol intake, physical activity and diabetes.
\(^b\)Normal: body mass index (BMI) < 25kg/m\(^2\).
\(^c\)Overweight: BMI ≥ 25kg/m\(^2\).
\(^d\)Included malignancy of the oral cavity, esophagus adenocarcinoma, gastric cardia, colorectum, liver, gallbladder, pancreas, breast in postmenopausal, endometrium, ovary, prostate, kidney, meningioma, thyroid, and multiple myeloma.
\(^e\)Included all cancers except for obesity-related cancer.

TABLE 4  Associations of the adiposity status with the risk of total, obesity-related, and other cancers by p53 Arg72Pro polymorphism

| Cancer type         | p53 Arg72Pro | BMI category | Normal\(^a\) | Overweight\(^b\) | p | \( p_{\text{heterogeneity}} \) |
|---------------------|--------------|--------------|--------------|------------------|---|-----------------------------|
|                     |              | ArgArg + ArgPro | 2561 (1.00) | 1087 (1.11)     | 0.01 | 0.07 |
| Total               |              | ProPro       | 443 (1.00)  | 173 (0.91)      | 0.34 |
| Obesity-related\(^d\) |              | ArgArg + ArgPro | 1240 (1.00) | 599 (1.24)      | <0.001 |
|                     |              | ProPro       | 231 (1.00)  | 92 (0.89)       | 0.41  | 0.02 |
| Others\(^e\)       |              | ArgArg + ArgPro | 1321 (1.00) | 488 (0.98)      | 0.77  |
|                     |              | ProPro       | 212 (1.00)  | 81 (0.92)       | 0.54  | 0.65 |

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio.
\(^a\)Normal: body mass index (BMI) < 25kg/m\(^2\).
\(^b\)Overweight: BMI ≥ 25kg/m\(^2\).
\(^c\)Adjusted for age, sex, first three principal components of genetic ancestral markers, smoking status, alcohol intake, physical activity and diabetes.
\(^d\)Included malignancy of the oral cavity, esophagus adenocarcinoma, gastric cardia, colorectum, liver, gallbladder, pancreas, breast in postmenopausal, endometrium, ovary, prostate, kidney, meningioma, thyroid, and multiple myeloma.
\(^e\)Included all cancers except for obesity-related cancer.

that our study was conducted in a relatively homogenous Japanese population, factors other than ethnicity that were not included here may have masked our results.

Key molecules, such as p53\(^{12}\) and TGF-\(\beta\),\(^{31}\) that regulate extensive biological functions have been reported to play bidirectional roles during tumorigenesis in a context-dependent manner. Depending on specific conditions, these molecules can both inhibit and promote tumorigenesis. For example, p53 induces cell growth arrest or cell death, which can inhibit cancer under DNA stress.\(^7\) Under metabolic stress, p53 causes a pro-inflammatory response and insulin resistance\(^{13}\) that may promote obesity-related cancers. However, only a few studies have focused on the individual's adiposity status, which may cause heterogeneity in the association between the p53 Arg72Pro polymorphism and cancer risk. The results of the stratification analyses according to adiposity status indicated that Pro homozygous carriers have increased risks of total cancer and obesity-related cancer for participants with BMI < 25kg/m\(^2\), but such risks were not observed for those with BMI ≥ 25kg/m\(^2\). Furthermore, between normal-weight and overweight participants, a statistically significant heterogeneity was observed in the association of the p53 Arg72Pro polymorphism with the risk of obesity-related cancer. Laboratory studies also demonstrated that the Pro variant seemed to exert bidirectional effects on tumorigenesis including pro-tumor effects under DNA stress and antitumor effects under metabolic stress, because of its lower sensitivity to several p53 functions than the Arg variant.\(^{10,32}\) Overall, these findings on the heterogeneity according to adiposity status may suggest the following: for normal-weight
individuals with exposure to only DNA stress attributed to their daily environments, the Pro variant may potentially exhibit a pro-tumor effect and confer the increased risk of cancer; for overweight people exposed to both DNA and metabolic stress, the same Pro variant may exert both pro-tumor and antitumor effects and appears not to confer the risk. These results indicate that the polymorphisms of key molecules with extensive functions require context-dependent analyses based on their specific molecular mechanisms.

Evidence of the different sensitivities of the p53 Arg72pro polymorphism under metabolic stress has been reported in both human and animal studies. A cross-sectional study of participants with type 2 diabetes who were mostly overweight reported that Pro homozygous carriers were less likely to develop insulin resistance than Arg carriers. In an experiment in which mice were fed with a high-fat diet, mice with the Pro variant were less predisposed to insulin resistance and pro-inflammatory signature than those with the Arg variant. Therefore, the Pro variant is less likely to lead to the development of obesity-related cancer due to a lower sensitivity to insulin resistance and inflammation under metabolic stress. With or without metabolic stress, apoptosis is induced more by the Arg variant than by the Pro variant because of the difference in the ability for mitochondrial localization, which may explain why the Pro variant conferred an increased risk of cancer in normal-weight individuals with DNA stress through their life. Under the circumstances in which people could easily know the existence of p53 Arg72Pro polymorphism, the Pro homozygous carriers with normal weight would take more caution against the environmental exposure to known carcinogens and be willing to undergo cancer screening to reduce their risk of cancer-related death. Collectively, our findings highlight the complex mechanisms involved in the development of cancer that is dependent on specific conditions for different individual cases, which suggests the importance of personalized medicine.

The strength of this study is its large sample size, which included 4264 cases compared with previous studies. In addition, pre-diagnostic information on the adiposity status and other covariates obtained from a prospective cohort study is less likely to cause recall bias and reverse causality. This study also has several limitations. First, given its observational nature, there is a possibility of bias from unmeasured or unknown confounding factors. Although a major bias that affects the association between polymorphism and cancer risk is not confounding but population stratification, our study population only included Japanese individuals, and we adjusted for the first three principal components of the genetic ancestral markers. Second, we only examined the interaction between the p53 Arg72Pro polymorphism and adiposity status based on our study hypothesis. Therefore, we did not exclude the existence of other condition-dependent functions, gene–gene interactions, and other gene–environment relationships. Moreover, we only classified the total cancer into obesity-related and other cancers, instead of site-specific cancers, in the stratification analysis. Further studies according to various conditions and site-specific cancers are warranted to uncover the complex roles and different responses of the p53 Arg72Pro polymorphism in cancer development.

In summary, the current study of two case-cohorts within the Japanese population-based prospective study revealed that Pro homozygous carriers were more predisposed to cancer than Arg carriers for individuals with normal-weight conditions. In addition, overweight was related to a higher cancer risk in Arg carriers than in Pro homozygous carriers. Our findings suggest that the adiposity-dependent dual effects of the p53 Arg72Pro polymorphism during tumorigenesis might provide a deeper understanding of the complex biological functions of p53.

AUTHOR CONTRIBUTIONS
Concept and design: Shiori Nakano, Taiki Yamaji, Motoki Iwasaki. Statistical analysis: Shiori Nakano, Taiki Yamaji. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Shiori Nakano drafted the first manuscript with Taiki Yamaji’s support. Critical revision of the manuscript for important intellectual content: All authors. Administrative, technical, or material support: Taiki Yamaji, Sawada, Inoue, Shoichiro Tsugane, and Motoki Iwasaki. Obtained funding: Shoichiro Tsugane, Motoki Iwasaki. Supervision: Shoichiro Tsugane, Motoki Iwasaki.

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DISCLOSURE
All authors declare no conflict of interest for this article. Norie Sawada, Manami Inoue, and Motoki Iwasaki are Associate Editors of Cancer Science.
ETHICAL APPROVAL

Approval of the research protocol by an Institutional Reviewer Board: The comprehensive study protocol, including this study, was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan (Approval No. 2011-044).

Informed Consent: Before initiating this study, all living participants who had provided blood were contacted by mail and given the opportunity to opt out of participation. Additionally, information on the study was posted on the website of our center to provide participants with the opportunity to opt out at any time. Respondents who refused to participate were excluded from this study and their withdrawal of consent was documented.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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