Increased Risk of Cutaneous Melanoma Associated with \textit{p53} Arg72Pro Polymorphism

Peiliang Geng, Yunmei Liao, Zhihua Ruan, Houjie Liang*

Department of Oncology and Southwest Cancer Center, Southwest Hospital, Third Military Medical University, China

* lianghoujie@sina.com

Abstract

Objective

The objective of this study was to test the hypothesis that \textit{p53} Arg72Pro polymorphism may contribute to an increased risk of cutaneous melanoma (CM).

Methods

By searching the databases of PubMed, EMBASE, and Web of Science, a total of 8 eligible case-control studies with 1,957 CM cases and 2,887 controls were included in this meta-analysis. Stata software was used to analyze all the statistical data.

Results

The pooled data by a fixed-effects model suggested an increased risk of CM associated with \textit{p53} Arg72Pro polymorphism under the genetic model of Arg/Pro vs. Pro/Pro without heterogeneity (OR_{Arg/Pro vs. Pro/Pro} = 1.76, 95% CI = 1.55-1.99, \textit{P}_{\text{heterogeneity}} = 0.075). A similar trend was seen in subgroups of hospital-based studies and population-based studies.

Conclusion

Our meta-analysis based on all studies shows that the \textit{p53} Arg72Pro polymorphism may increase individual susceptibility to CM, particularly in Caucasians and could serve as a biomarker to predict the population at high risk of CM.

Introduction

Cutaneous melanoma (CM) representing one of the most malignant skin cancers has caused a large number of skin cancer-related deaths, approximately 8,700 deaths in 2010 [1]. The incidence rate steadily rises, with an average increase by 3–7% throughout the past decades among European populations [2]. It has generally been accepted that exposure to ultraviolet (UV) radiation is a main cause of skin cancer [3]. More importantly, epidemiological evidence has documented genetic variations in cancer-related genes are major contributing factors for this
malignancy [4,5]. Despite the previous efforts, the pathogenesis of this disease remains unclear.

The tumor suppressor p53 involved in a variety of biological activities, such as UV-induced DNA damage, regulates numerous downstream genes to induce cell-cycle arrest, DNA repair or apoptosis [6,7]. p53 is a most frequently mutated gene that has been described in various cancers [8,9] and the single nucleotide polymorphisms (SNPs) at this locus should account, at least in part, for the occurrence of these cancers [10]. Among these, the extensively studied SNP has been the one at codon 72, which has a substitution of Arg to Pro.

Previous reports suggest that p53 plays a pivotal role in the defense against DNA damage and breakage arising from UV exposure [11,12]. Due to the key role of p53 in the development of skin cancer, an increasing number of investigators have directed their attention to the effects of p53 Arg72Pro polymorphism on CM risk [13–20]. But there is considerable discrepancy in the findings as a result of the small-sampled studies. Therefore, in order to obtain an estimation with more statistical power, we conducted a meta-analysis to test the hypothesis that p53 Arg72Pro polymorphism may contribute to an increased risk of CM.

Methods and Materials

Literature search

We searched the databases of PubMed, EMBASE, and Web of Science for case-control studies on the association between p53 Arg72Pro polymorphism and CM risk by using the following search terms: “p53 codon 72” or “p53 Arg72Pro”, “polymorphism” or “variants”, and “melanoma” or “cutaneous melanoma”. There was no language restriction. References of the original articles and systematic reviews were manually screened for additional usable data.

Inclusion and exclusion criteria

The inclusion criteria included: (1) the association of p53 Arg72Pro polymorphism and CM risk must be examined, (2) designed as case-control study, (3) detailed genotype frequency in cases and controls to estimate odds ratios (ORs) along with 95% confidence intervals (CIs), and (4) there was no departure from Hardy-Weinberg equilibrium (HWE) in genotype distribution of the control group. Abstracts, editorials, and review articles were not considered in the final analysis.

Data extraction

On the basis of a consensus on all items, two reviewers independently collected the following characteristics from each study: first author’s name, publication year, study country, ethnicity, total numbers of genotyped cases and controls, genotype counts of p53 Arg72Pro polymorphism in cases and controls, and genotyping assays. Disagreement was resolved by discussion between the two reviewers or consulting the third reviewer.

Statistical analysis

In this meta-analysis, association of p53 Arg72Pro polymorphism and CM risk was assessed by pooled ORs with 95% CIs under five genetic comparisons (Arg/Arg vs. Pro/Pro, Arg/Arg + Arg/Pro vs. Pro/Pro, Arg/Arg vs. Arg/Pro + Pro/Pro, allele Arg vs. allele Pro, and Arg/Pro vs. Pro/Pro). HWE of the control groups was determined by the χ² test. Between-study heterogeneity was estimated by chi-square based Q test [21] and P < 0.05 indicated significant heterogeneity. I² index was also used to quantify the heterogeneity and we considered the value >50% as statistically significant. When no heterogeneity was observed across studies, the
fixed-effects model was used [22] to calculate the summary ORs for the combined studies and the random-effects model [23] was performed if there the results were heterogeneous.

In addition, sensitivity analyses were applied to detect the individual influence from the single studies on the pooled ORs. Publication bias was tested by performing Begg’s funnel plots and Egger’s test [24]. All statistical data were analyzed using Stata software (version 12.0, Stata Corp LP, College Station, TX, USA). All tests were two-sided with a significant level of 0.05.

Results

Characteristics of the studies

As graphically depicted in Fig. 1, the literature search identified 32 potentially relevant articles in all. Of these, 21 papers were excluded by reading the titles and abstracts. After examining the full-texts, we further removed 3 articles due to case-only design [25], insufficient data to calculate the combined ORs [26] and comment letter [27]. At last, a total of 8 case-control studies with 1,957 CM cases and 2,887 controls were pooled in the meta-analysis. Major characteristics of the eligible studies are listed in Table 1. All of the included studies employed Caucasian populations. There were 6 hospital-based studies [13–15,17,19,20] and two population-based studies [16,18]. The numbers of subjects recruited in each of the single studies varied substantially, from the smallest of 239 to the largest of 1,643. No deviation from HWE was seen in the control groups.

Meta-analysis results

Since the test for heterogeneity did not suggest substantial heterogeneity across studies, the fixed-effects model was performed to pool the ORs, as shown in Table 2. The combined results showed no statistically significant links between the p53 Arg72Pro polymorphism and CM susceptibility under all genetic models with the exception of Arg/Pro vs. Pro/Pro (OR_{Arg/Pro vs. Pro/Pro} = 1.76, 95% CI = 1.55–1.99, P_{heterogeneity} = 0.075, Fig. 2). When stratifying the populations by source of controls, we observed significantly increased risk in hospital-based studies (OR_{Arg/Pro vs. Pro/Pro} = 1.71,
95% CI = 1.48–1.98, \( P_{\text{heterogeneity}} = 0.058 \) as well as population-based studies (OR_{Arg/Pro vs. Pro/Pro} = 1.88, 95% CI = 1.49–2.39, \( P_{\text{heterogeneity}} = 0.186 \)).

Sensitivity analyses

We conducted leave-one-out sensitivity analyses with an aim to determine the effects of the independent studies on the overall results. The analysis did not indicate any significant alteration when the single studies was excluded from the pooling data. Thus our results are stable and credible.

Publication bias

The results of Begg’s funnel plots and Egger’s test showed that publication bias may not have a significant effect on the findings of our meta-analysis for the association of \( p53 \) Arg72Pro polymorphism and CM susceptibility (Arg/Arg vs. Pro/Pro: \( P = 0.174 \) for Begg’s test; \( P = 0.134 \) for Egger’s test, Fig. 3).

Table 1. Characteristics of studies included in the meta-analysis.

| First author | Year | Country   | Ethnicity | Study design | Genotyping method | HWE   | Cases | Controls |
|--------------|------|-----------|-----------|--------------|------------------|-------|-------|----------|
| Bastiaes     | 2001 | Netherland| Caucasian | HB           | PCR              | 0.180 | 120   | 157      |
| Shen         | 2003 | USA       | Caucasian | HB           | PCR-RFLP         | 0.793 | 289   | 308      |
| Gwosdz       | 2006 | Germany   | Caucasian | HB           | RT-PCR           | 0.419 | 49    | 193      |
| Han          | 2006 | USA       | Caucasian | PB           | TaqMan           | 0.864 | 201   | 816      |
| Stefanaki    | 2007 | Greece    | Caucasian | HB           | AS-PCR           | 0.058 | 107   | 145      |
| Li           | 2008 | USA       | Caucasian | HB           | ND               | 0.184 | 805   | 838      |
| Capasso      | 2010 | Italy     | Caucasian | PB           | PCR              | 0.599 | 240   | 284      |
| Oliveira     | 2013 | Brazil    | Caucasian | HB           | PCR              | 0.757 | 146   | 146      |

Abbreviations: PCR: polymerase chain reaction; PCR-RFLP: PCR-restriction fragment length polymorphism; RT-PCR: real-time PCR; AS-PCR: allele-specific-PCR; TaqMan: TaqMan SNP; ND: not defined; HB: hospital-based; PB: population-based; HWE: Hardy-Weinberg equilibrium.

doi:10.1371/journal.pone.0118112.t001

Table 2. Meta-analysis for the association between the \( p53 \) Arg72Pro polymorphism and CM risk.

| Subgroup (cases/controls) | Arg/Arg vs. Pro/Pro | Arg/Arg + Arg/Pro vs. Pro/Pro | Arg/Arg vs. Arg/Pro + Pro/Pro | Allele Arg vs. Allele Pro | Arg/Pro vs. Pro/Pro |
|---------------------------|---------------------|--------------------------------|--------------------------------|--------------------------|---------------------|
|                           | OR (95% CI)         | \( P_h \)                      | OR (95% CI)                     | \( P_h \)                | OR (95% CI)         | \( P_h \) |
| Fixed-effects             |                     |                                |                                |                          |                     |          |
| Ethnicity                 |                     |                                |                                |                          |                     |          |
| Caucasian (1957/2887)     | 1.00 (0.89, 1.12)   | 0.990                          | 1.06 (0.96, 1.18)              | 0.999                    | 1.76 (1.55, 1.99)   | 0.075    |
| Control source            |                     |                                |                                |                          |                     |          |
| Hospital (1516/1787)      | 1.02 (0.89, 1.16)   | 0.970                          | 1.01 (0.91, 1.11)              | 0.997                    | 1.10 (0.98, 1.12)   | 0.997    |
| Population (441/1100)     | 0.95 (0.76, 1.18)   | 0.925                          | 0.97 (0.82, 1.14)              | 0.869                    | 0.96 (0.79, 1.16)   | 0.969    |
| Total (1957/2887)         | 1.00 (0.89, 1.12)   | 0.990                          | 1.06 (0.96, 1.18)              | 0.999                    | 1.02 (0.96, 1.12)   | 0.765    |

Abbreviations: \( P_h \): p value of heterogeneity test; CI: confidence interval; OR, odds ratio.

doi:10.1371/journal.pone.0118112.t002
Discussion

In this meta-analysis consisting of 1,957 CM cases and 2,887 controls from 8 eligible case-control studies, we demonstrated that p53 Arg72Pro polymorphism may contribute to an increased risk of CM. This finding was further confirmed in subgroup analysis by ethnicity, revealing that Caucasians with the Arg/Pro genotype had 1.76-fold higher risk to develop CM compared to the Pro/Pro genotype carriers. In addition, both the studies based on hospital-based controls and those on population-based controls were found to be significantly associated with CM risk. To our knowledge, this is the first study examining the association between p53 Arg72Pro polymorphism and CM risk in Caucasians. Consistent with our initial hypothesis, our results showed that this polymorphism may have effects on the development of CM.

The p53 tumor suppressor gene that encodes a DNA-binding protein plays an important role in tumor suppression and cell cycle arrest. Loss of tumor suppression function and cell cycle control resulting from mutations and deletions of the p53 gene induces a wide range of human malignancies, including CM [28,29]. Several case-control studies have been carried out in an attempt to examine the association between p53 Arg72Pro polymorphism and CM risk. For example, Bastiaens et al. found no significant association for p53 Arg72Pro polymorphism
and CM risk in a case-control study with 120 cases and 157 controls [13]. Conversely, in another larger case-control study (805 CM patients and 838 healthy controls), Li et al. demonstrated that p53 Arg72Pro polymorphism contributed to the risk of CM [18]. A plausible explanation for the discrepancy is that the number of subjects between the two studies differs substantially, and it is the small sample that are usually underpowered to derive a precise estimation, leading to biased results as a consequence.

Recently, a meta-analysis investigating the association of p53 Arg72Pro polymorphism with skin cancer has been published [30]. In this analysis, the authors found the polymorphism of interest was not significantly associated with CM. Neither did the stratified analyses according to ethnicity detect a significant association in any subgroup, a finding that varies substantially from that indicated in our meta-analysis. Although both of the meta-analyses involves Caucasian populations only, our analysis includes three more publications contributing to additional 1,413 unique subjects, which enlarges our study substantially and hence enhances the credibility of our results consequently.

We identified a notably increased risk of CM in carriers of the Arg/Pro heterozygote, a finding that has some biological plausibilities. p53 is a signaling pathway fundamental in tumor growth suppression by promoting cellular proliferation and inducing cell death. Dumont et al. established a linkage of apoptotic potential with a common polymorphism that influences amino acid position72 at p53 locus; the Arg allele has been shown to have greater ability to induce apoptosis most likely due to the close affinity with mitochondria [31]. In addition, the p53 in conjunction with proopiomelanocortin gene within keratinocytes in defence against UV radiation stimulates melanogenesis, a potent determinant of skin color. The functional p53 polymorphism modulates proopiomelanocortin activity at the allelic level and thereby confers susceptibility to the development of skin cancer [32]. These data make us infer that the
presence of p53 Arg72Pro genotypes or alleles may possibly affect the function of p53 and ultimately modifies the risk of skin cancer, providing supportive evidence for an association between the p53 Arg72Pro and CM. Some limitations in our meta-analysis need to be addressed. To begin with, this meta-analysis is based on Caucasians only and reveals significantly increased risk of CM ascribed to the p53 Arg72Pro polymorphism. However, we can not exclude the possibility that the contribution of the polymorphism to the risk of CM differs due to different ethnic origins, and it may not represent a risk factor for other ethnicities. Furthermore, since only English-language and published articles are included in our study, selection bias may have occurred. Finally, there are no uniform criteria defined for the selection of control subjects in each of the studies included, some used population-based controls and the others selected hospital-based controls, so potential selection bias might exist in this study.

In summary, our meta-analysis provided some evidence that the Arg/Pro genotype of p53 Arg72Pro polymorphism was likely to confer susceptibility to CM. Future larger studies with the consideration of more ethnic groups and representative control groups are necessary to further identify our findings.

Supporting Information
S1 PRISMA Checklist. (DOC)

Author Contributions
Conceived and designed the experiments: PLG HJL. Performed the experiments: PLG YML. Analyzed the data: PLG ZHR HJL. Wrote the paper: PLG HJL.

References
1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010; 60: 277–300. doi: 10.3322/caac.20073 PMID: 20610543
2. Bray F, Sankila R, Ferlay J, Parkin DM. Estimates of cancer incidence and mortality in Europe in 1995. Eur J Cancer. 2002; 38: 99–166. PMID: 11750846
3. de Gruijl FR. Photocarcinogenesis: UVA vs. UVB radiation. Skin Pharmacol Appl Skin Physiol. 2002; 15: 316–320. PMID: 12239425
4. Ortonne JP. Photobiology and genetics of malignant melanoma. Br J Dermatol. 2002; 146 Suppl 61: 11–16. PMID: 11966726
5. Goldstein AM, Tucker MA. Genetic epidemiology of cutaneous melanoma: a global perspective. Arch Dermatol. 2001; 137: 1493–1496. PMID: 11708953
6. Kaelin WG Jr. The p53 gene family. Oncogene. 1999; 18: 7701–7705. PMID: 10618710
7. Robles AI, Harris CC. p53-mediated apoptosis and genomic instability diseases. Acta Oncol. 2001; 40: 696–701. PMID: 11765063
8. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat. 2002; 19: 607–614. PMID: 12007217
9. Hussain SP, Hollstein MH, Harris CC. p53 tumor suppressor gene: at the crossroads of molecular carcinogenesis, molecular epidemiology, and human risk assessment. Ann N Y Acad Sci. 2000; 919: 79–85. PMID: 11083100
10. Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer. 2009; 9: 95–107. doi: 10.1038/nrc2584 PMID: 19165225
11. Benjamin CL, Ananthawaswamy HN. p53 and the pathogenesis of skin cancer. Toxicol Appl Pharmacol. 2007; 224: 241–248. PMID: 17270229
12. Benjamin CL, Melnikova VO, Ananthawaswamy HN. P53 protein and pathogenesis of melanoma and nonmelanoma skin cancer. Adv Exp Med Biol. 2008; 624: 265–282. doi: 10.1007/978-0-387-77574-6_21 PMID: 18348463
13. Bastiaens MT, Struyk L, Tjong AHSP, Gruis N, ter Huurne J, Westendorp RG, et al. Cutaneous squamous cell carcinoma and p53 codon 72 polymorphism: a need for screening? Mol Carcinog. 2001; 30: 56–61. PMID: 11255264

14. Shen H, Liu Z, Strom SS, Spitz MR, Lee JE, Gershenwald JE, et al. p53 codon 72 Arg homozygotes are associated with an increased risk of cutaneous melanoma. J Invest Dermatol. 2003; 121: 1510–1514. PMID: 14675203

15. Gwosdz C, Scheckenbach K, Lieven O, Reifenberger J, Knopf A, Bier H, et al. Comprehensive analysis of the p53 status in mucosal and cutaneous melanomas. Int J Cancer. 2006; 118: 357–362. PMID: 16094622

16. Han J, Cox DG, Colditz GA, Hunter DJ. The p53 codon 72 polymorphism, sunburns, and risk of skin cancer in US Caucasian women. Mol Carcinog. 2006; 45: 694–700. PMID: 16739124

17. Stefanaki I, Stratigos AJ, Dimisianos G, Nikolaou V, Papadopoulos O, Polydorou D, et al. p53 codon 72 Pro homozygosity increases the risk of cutaneous melanoma in individuals with dark skin complexion and among noncarriers of melanocortin 1 receptor red hair variants. Br J Dermatol. 2007; 156: 357–362. PMID: 17223878

18. Li C, Chen K, Liu Z, Wang LE, Gershenwald JE, Lee JE, et al. Polymorphisms of TP53 Arg72Pro, but not p73 G4C14>A4TA4 and p21 Ser31Arg, contribute to risk of cutaneous melanoma. J Invest Dermatol. 2008; 128: 1584–1588. PMID: 18049450

19. Capasso M, Ayala F, Avvisati RA, Russo R, Gamble A, Mozzillo N, et al. MDM2 SNP309 and p53 Arg72Pro in cutaneous melanoma: association between SNP309 GG genotype and tumor Breslow thickness. J Hum Genet. 2010; 55: 518–524. doi: 10.1038/jhg.2010.62 PMID: 20535124

20. Oliveira C, Rinck-Junior JA, Lourenco GJ, Moraes AM, Lima CS. Assessment of the XPC (A2920C), XPF (T30028C), TP53 (Arg72Pro) and GSTP1 (Ile105Val) polymorphisms in the risk of cutaneous melanoma. J Cancer Res Clin Oncol. 2013; 139: 1199–1206. doi:10.1007/s00432-013-1430-4 PMID: 23568549

21. Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med. 1997; 127: 820–826. PMID: 9382404

22. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959; 22: 719–748. PMID: 13655060

23. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7: 177–188. PMID: 3802833

24. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997; 315: 629–634. PMID: 9310563

25. Cotogno LA, Chou JF, Roy P, Mitra N, Busam K, Halpern AC, et al. Investigation of the effect of MDM2 SNP309 and TP53 Arg72Pro polymorphisms on the age of onset of cutaneous melanoma. J Invest Dermatol. 2012; 132: 1471–1478. doi: 10.1038/jid.2012.15 PMID: 22336942

26. Spatz A, Giglia-Mari G, Benhamou S, Sarasin A. Association between DNA repair-deficiency and high level of p53 mutations in melanoma of Xeroderma pigmentosum. Cancer Res. 2001; 61: 2480–2486. PMID: 11289118

27. Zou YF, Wang F, Feng XL. TP53 Arg72Pro polymorphism may have little involvement in the pathogenesis of skin cancer in Caucasians. J Invest Dermatol. 2011; 131: 781–782. doi: 10.1038/jid.2010.367 PMID: 21191409

28. Jiang W, Ananthaswamy HN, Muller HK, Kripke ML. p53 protects against skin cancer induction by UV-B radiation. Oncogene. 1999; 18: 4247–4253. PMID: 10435637

29. Halachmi S, Gilchrest BA. Update on genetic events in the pathogenesis of melanoma. Curr Opin Oncol. 2001; 13: 129–136. PMID: 11224711

30. Jiang DK, Wang WZ, Ren WH, Yao L, Peng B, Yu L. TP53 Arg72Pro polymorphism and skin cancer risk: a meta-analysis. J Invest Dermatol. 2011; 131: 220–228. doi: 10.1038/jid.2010.270 PMID: 20861852

31. Dumont P, Leu JL, Della Pietra AC 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet. 2003; 33: 357–365. PMID: 12567188

32. Thurov HS, Haack R, Hartwig FP, Oliveira IO, Delagostin OA, Gigante DP, et al. TP53 gene polymorphism: importance to cancer, ethnicity and birth weight in a Brazilian cohort. J Biosci. 2011; 36: 823–831. PMID: 22116280