RESEARCH ARTICLE

Meta-analysis of Associations between ATM Asp1853Asn and TP53 Arg72Pro Polymorphisms and Adverse Effects of Cancer Radiotherapy

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Abstract

Background: The ataxia telangiectasia mutated (ATM) protein and p53 play key roles in sensing and repairing radiation-induced DNA double strand breaks (DSBs). Accumulating epidemiological evidence indicates that functional genetic variants in ATM and TP53 genes may have an impact on the risk of radiotherapy-induced side effects. Here we performed a meta-analysis to investigate the potential interaction between ATM Asp1853Asn and TP53 polymorphisms and risk of radiotherapy-induced adverse effects quantitatively. Materials and Methods: Relevant articles were retrieved from PubMed, ISI Web of Science and the China National Knowledge Infrastructure (CNKI) databases. Eligible studies were selected according to specific inclusion and exclusion criteria. Odds ratios (ORs) and 95% confidence intervals (CIs) were pooled to estimate the association between ATM Asp1853Asn and TP53 Arg72Pro polymorphisms and risk of radiotherapy adverse effects. All analyses were performed using the Stata software. Results: A total of twenty articles were included in the present analysis. In the overall analysis, no significant associations between ATM Asp1853Asn and TP53 Arg72Pro polymorphisms and the risk of radiotherapy adverse effects were found. We conducted subgroup analysis stratified by type of cancer, region and time of appearance of side effects subsequently. No significant association between ATM Asp1853Asn and risk of radiotherapy adverse effects was found in any subgroup analysis. For TP53 Arg72Pro, variant C allele was associated with decreased radiotherapy adverse effects risk among Asian cancer patients in the stratified analysis by region (OR=0.71, 95%CI: 0.54-0.93, p=0.012). No significant results were found in the subgroup analysis of tumor type and time of appearance of side effects. Conclusions: The TP53 Arg72Pro C allele might be a protective factor of radiotherapy-induced adverse effects among cancer patients from Asia. Further studies that take into consideration treatment-related factors and patient lifestyle including environmental exposures are warranted.

Keywords: ATM - TP53 - polymorphisms - meta-analysis - radiotherapy - adverse effects - toxicity

Asian Pac J Cancer Prev, **15 (24)**, 10675-10681

Introduction

Radiotherapy is the medical use of ionizing radiation, generally as part of cancer treatment to control or kill malignant cells. Many tumors responsive to radiation are routinely treated with curative doses of radiation therapy if they are at an early stage. During radiotherapy, normal tissues surrounding the tumor will unavoidable be irradiated, which can lead to a series of side reactions or toxicities. However, the development of normal tissue reactions in cancer patients receiving same radiotherapy treatment schedule shows considerable variation between individual patients. Researchers believe that cancer patients may have a genetic susceptibility to the development of radiation toxicities. With the development of the HapMap project and genome-wide single nucleotide polymorphism (SNP) association studies, recognition is growing that common SNPs might represent important genetic variants that correlate with clinical radiosensitivity. To date, a number of epidemiological studies have been performed to investigate the associations between SNPs and risk of radiotherapy adverse effects such as subcutaneous fibrosis, telangiectasia and radiation-induced pneumonitis. Most current studies focus on SNPs of ATM and TP53 gene.

Ataxia telangiectasia mutated (ATM) gene, is responsible for the multisystem autosomal recessive disorder ataxia-telangiectasia (A-T). This ATM gene product is a member of a novel family of large proteins that share a highly conserved carboxy-terminal region of ~300 amino acids showing highly homology to the catalytic domain of phosphoinositide 3-kinase (PI-3 kinases), which are involved in cell cycle progression, cellular responses to DNA damage and maintenance of genome stability(Uziel et al., 1996; Savitsky et al., 1997). The ATM serine/threonine kinase itself plays a crucial role as a sensor in the activation of cell cycle checkpoints in response to

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radiation-induced DNA double strand breaks (DSBs). In undamaged cells ATM acts as an inactive homodimer, but following ionizing radiation-induced DNA DSBs, it undergoes an intermolecular auto-phosphorylation on serine-1981 resulting in disassociation of the homodimer into active monomers (Kastan and Bartek, 2004; Kastan and Derheimer, 2010). The activated ATM monomer is recruited to DSB sites by the MRE11-RAD50-NBS1 (MRN) complex, and then can phosphorylate its multiple substrates, such as p53, NBS1, BRCA1 and Chk2(Kurz and Lees-Miller, 2004). The p53 transcription factor can be directly phosphorylated by ATM particularly on serine-15 or be phosphorylated on serine-20 in an ATM-Chk2-dependent way, contributing to the stabilization and accumulation of the p53 protein and its increased activity(Kastan and Lim, 2000). The key transcriptional target of p53 is the p21/WAF1 inhibitor of cyclin-dependent kinases, which inhibits the G1/S-promoting cyclinE/Cdk2 kinase activity and the capacity of cells to progress from G1 to S phase (Lavin and Kozlov, 2007). This temporarily halt of cell cycle makes adequate time for the repair of DNA damage. Genetic variants in ATM and TP53 gene can lead to the structure and function change of the proteins, as a result, aberrations of ATM and p53 proteins leads to defects in the ability of cells to sense and repair the DNA damage.

ATM Asp1853Asn (rs1801516) is a common missense variant located in exon 39 of ATM gene, and the G-to-A change leads to the substitution of asparagine for aspartic acid at amino acid position 1853. The Arg72Pro SNP (rs1042522) is located in the proline-rich domain of p53. It is known that this SNP encodes either an arginine or a proline at condon 72 in exon 4. Previously published studies have reported that these two SNPs might influence the radiosensitivity of the variant allele carriers, and the development of adverse responses to radiotherapy of prostate, breast and lung cancer (Cesaretti et al., 2005; Andreassen et al., 2006; Yang et al., 2011). However, the relationship with risk of radiotherapy adverse effects is still controversial. In an attempt to resolve these contradictory results, we performed this meta-analysis to assess whether combined evidence shows the association with ATM Asp1853Asn and TP53 Arg72Pro polymorphisms.

Materials and Methods

Search strategy

Eligible literatures published before the end of April 2013 were identified by a search of PubMed, ISI Web of Science and China National Knowledge Infrastructure (CNKI). All the relevant articles retrieved were using the following terms: "ATM", "ataxia telangiectasia mutated", "TP53", "SNP", "polymorphism" and "radiotherapy adverse effects". The last search updated was 2013 April. The references cited in the original studies or review articles concerning the relevant topic were retrieved as well for additional relevant publications.

Inclusion and exclusion criteria

The following inclusion criteria were analyzed in selecting studies for the current meta-analysis: *(i)* evaluate the association between ATM Asp1853Asn or TP53 Arg72Pro polymorphisms and radiotherapy adverse effects; *(ii)* use case-control design, consider patients developed with severe radiotherapy toxicities as cases and minimal radiotherapy toxicities as controls; *(iii)* include full-text manuscrips only; *(iv)* describe useful allele and genotype frequencies for estimating an odds ratios and 95% confidence intervals (95%CIs). The major exclusion criteria were: *(i)* duplication of the previous publications; *(ii)* abstract, comment, and review; *(iii)* no sufficient data were reported.

Data extraction

Three investigators extracted all data independently, complied with the inclusion criteria listed above. Any disagreement was resolved by discussion until a consensus was reached between the investigators. The following information was collected from each publication: the first author's name, year of publication, country of study, primary tumor site, genotype frequency in cases (with high radiation-induced toxicity) and controls (with low radiation-induced toxicity), side effects analyzed in study and definition of radiation-induced side effects.

Statistical methods

Odds ratios and 95% confidence intervals (CIs) were pooled to evaluate the association between ATM Asp1853Asn or TP53 Arg72Pro polymorphisms and risk of radiotherapy adverse effects. The statistical significance of the ORs was determined using the Z test. For ATM Asp1853Asn, we estimated whether radiotherapy adverse effects risk was associated with the GA and AA genotypes, compared with the wild-type GG homozygote, respectively. For TP53 Arg72Pro, we evaluated whether radiotherapy adverse effects risk was associated with the GC and CC genotypes, compared with the wild-type GG homozygote, respectively. Subgroup analysis were performed by regarding type of cancer, region and time of appearance of side effects as group factors.

Heterogeneity across individual studies was investigated by the Cochran's chi-square Q test (a significance level of $p<0.05$). The fixed effect model and the random effect model based on the Mantel-Haenszel method and the Dersimonian-Laird method, respectively, were used to pool data from different studies. If the heterogeneity between studies was absent, these two models provided similar results; otherwise, it was more appropriate to adopt the random effect model.

The effect of publication bias was examined by Begg's funnel plots, we also adopted the liner regression approach proposed by Egger et al. All of P values were two-sided and all analyses were performed using the Stata software (version 11.0).

Results

Characteristics of studies

Figure 1 shows the study selection process. A total of 861 articles were retrieved to identify eligibility for our meta-analysis. According to the inclusion and exclusion criteria, 836 studies were excluded after screening the

titles and abstracts, 2 studies were excluded for irrelevant research content, 3 studies were excluded because we were unable to extract the data. Thus 20 full text articles with 1214 severe toxicities cases and 3654 lower toxicities controls were reviewed to evaluate the association of ATM Asp1853Asn or TP53 Arg72Pro polymorphisms with risk of radiotherapy adverse effects. Among the 20 eligible studies, 12 studies assessed the association between ATM Asp1853Asn and radiotherapy adverse effects and 9 articles studied the relationship between TP53 Arg72Pro and radiotherapy adverse effects. There were 5 studies of North Americans populations, 4 studies of Asian populations and 11 studies of Europeans populations. Of all the 20 studies, there were 19 English language articles and 1 Chinese language articles. Main characteristics of the 20 included publications were summarized in Table 1.

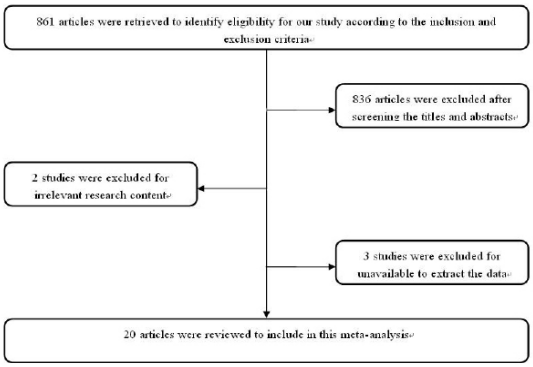

Figure 1. Flow Chart of the Eligible Study Selection Process

Meta-analysis results

As shown in Table 2, in the overall analysis, no significant associations between ATM Asp1853Asn and TP53 Arg72Pro polymorphisms and the risk of radiotherapy adverse effects were found. For ATM Asp1853Asn, the overall ORs of the variant GA heterozygous and AA homozygous for radiotherapy adverse effects was 0.95 (95%CI: 0.73-1.25, p=0.729 Figure 2) and 1.37 (95%CI: 0.31-6.05, p=0.471), respectively. The per-allele overall OR of the A variant for the risk of radiotherapy adverse effects was 1.11(95%CI: 0.77-1.59, p=0.592). When all of the eligible studies were pooled, there was no significant correlation in the dominant model(GA+AA *vs* GG: OR=1.09, 95%CI: 0.86-1.39, p=0.471). In the subgroup analysis of region, type of tumor and time of appearance of side effects, there was not any significant association between ATM Asp1853Asn and risk of radiotherapy adverse effects (data not shown). For TP53 Arg72Pro, the overall ORs of the variant GC heterozygous and CC homozygous for radiotherapy adverse reactions was 0.91 (95%CI: 0.71-1.15, p=0.426 Figure 3) and 0.75 (95%CI: 0.49-1.15, p=0.189), respectively. There was no evidence for the association between GC or CC genotype and the risk of radiotherapy adverse effects (GC+GG *vs* GG: OR=0.86, 95%CI: 0.70-1.05, p=0.136). The per-allele overall OR of the C variant for the risk of radiotherapy adverse effects was 0.89 (95%CI: 0.75-1.05, p=0.167). In the stratified analysis by region, we observed that variant C allele was associated with decreased radiotherapy adverse effects risk among Asian cancer patients (OR=0.71, 95%CI: 0.54-0.93, p=0.012 Figure 4). Similar result was not found in European population. We also performed subgroup analysis stratified by cancer type and time of appearance of side effects. But neither of these two stratified analysis was statistically significant (data not shown).

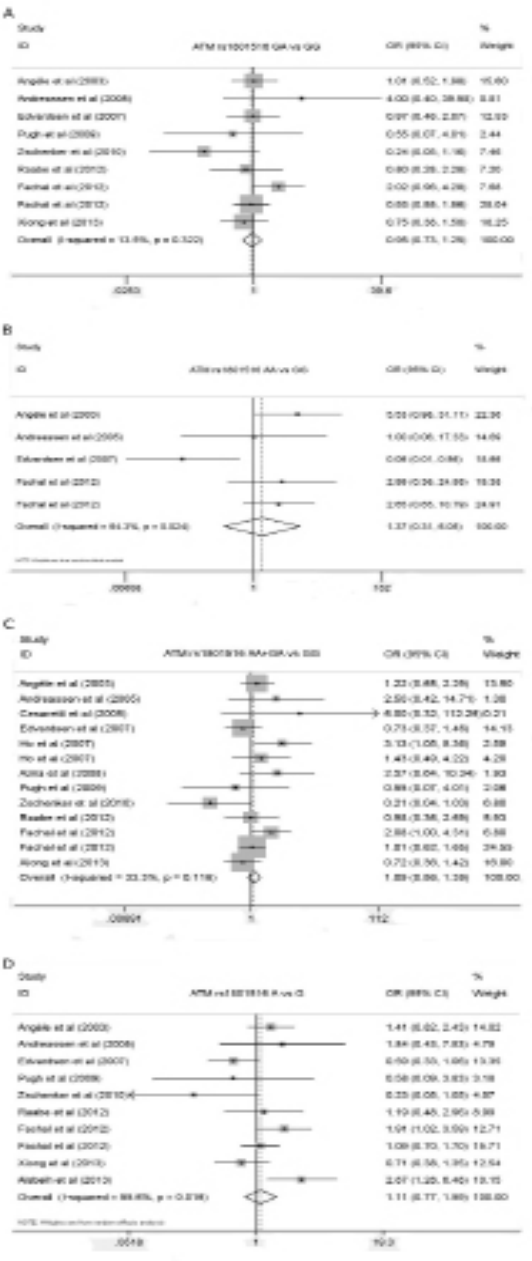

Figure 2. Forest Plots of the Association between Radiotherapy Adverse Toxicity Risk and ATM rs1801516 Polymorphism under 4 Different Comparison (A: GA *vs* GG; B: AA *vs* GG; C: GA+AA *vs* GG; D: A *vs* G)

Publication bias

Begg's funnel plot and Egger's test were performed to evaluate the publication bias of included literatures. As shown in Figure 5, the shapes of the funnel plots appeared symmetrical in all comparisons, indicating the absence of publication bias. We also used Egger's test to provide statistical evidence for the funnel plot

Table 1. Characteristics of Twenty Eligible Studies Included in this Meta-analysis of Associations between ATM Asp1853Asn and TP53 Arg72Pro Polymorphisms and Risk of Cancer Radiotherapy Adverse Effects

Study	Country	Primary tumor site	No. of patients		Length of follow-up	Side effects analyzed	Definition of RT-induced side effects
			Severe toxicity	Lower toxicity			
ATM Asp1853Asn (rs1801516)							
Angèle et al (2003)	France	breast	70	184	2 years	Early, early and late, or late normal tissue reactions	EORTC grade≥1
Andreassen et al (2006)	Denmark	breast	22	19	2.2-5.4 years	Late adverse effects including subcutaneous fibrosis	LENT/SOMA grade≥1
Cesaretti et al (2005)	USA	prostate	6	31	12-71 months	Acute and late rectal toxicities	RTOG grade≥1
Edvardsen et al (2007)	Norway	breast	189	50	7 years	Late adverse effects including telangiectasias	CTC and LENT/SOMA grade≥1
Ho et al (2007)	USA	breast	39	92	2-16 years	Late skin and subcutaneous tissue toxicities	RTOG grade 2-4
			51	80	3 months	Acute skin and subcutaneoustissue toxicities	
Azria et al (2008)	USA head and neck meningo	breast	16	18	2 years	Late toxicities including subcutaneous fibrosis and intracerebral edema	RTOG/EORTC grade≥3
Pugh et al (2009)	Canada	prostate	13	12	3 years	Late side effects	RTOG grade≥2
Zschenker et al (2010)	Germany	breast	17	52	9-20 years fibrosis	Late tissue effects including grade≥2	LENT/SOMA
Raabe et al (2012)	Germany	breast	46	37	42 days	Acute side effects including erythema	RTOG grade≥2
Fachal et al (2012)	Spain	prostate	34	664	3 months	Acute gastroinstestinal toxicity	CTCAE grade≥2
			111	587	3 months	Acute genitourinary toxicity	
Xiong et al (2013)	USA	lung	56	305	1.0-157.6 months	Radiation-induced pneumonitis	CTCAE grade≥3
Alsbeih et al (2013)	Saudi Arabia	head& neck	48	107	24-180 months	Late tissue effects including fibrosis	RTOG/EORTC grade≥3
TP53 Arg72Pro (rs1042522)							
Tan et al (2006)	Germany	breast	77	368	--	Acute side effects	CTC grade≥2c
Wang et al (2007)	China	nasopharynx	46	10	--	Acute injury of mucosa	RTOG grade≥2
Badie et al (2008)	UK	breast	14	11	--	Acute skin toxicities had moist desquamation	Most of the breast
Yang et al (2011)	China	lung	44	209	22 months	Radiation-induced pneumonitis	CTCAE grade≥2
Chang-Claude et al (2009)	Germany	breast	127	276	51 months	Late adverse effects including telangiectasia and fibrosis	RTOG/EORTC LENT/SOMA grade≥2
Ishikawa et al (2011)	Japan	cervix	58	150	3 months	Acute adverse reaction including diarrhea	CTC grade≥2
Terrazzino et al (2012b)	Italy	breast	89	196	-	Acute skin toxicities	RTOG grade≥2
Terrazzino et al (2012a)	Italy	breast	41	196	1 year	Late tissue toxicities including telangiectasia and subcutaneous fibrosis	LENT/SOMA grade≥2
Alsbeih et al (2013)	Saudi Arabia	head & neck	48	107	24-180 months	Late tissue effects including fibrosis	RTOG/EORTC grade≥3

*Abbreviations: EORTC, European Organization for Research and Treatment of Cancer; CTC, Common Toxicity Criteria; LENT/SOMA, Late Effects on Normal Tissue/ Subjective Objective Management Analytic system; RTOG, Radiation Therapy Oncology Group; CTCAE, Common Terminology Criteria for Adverse Effects; not given

symmetry. The greater the intercept deviation from zero in linear regression analysis, the greater the possibility for asymmetry. The results from this study showed that no evidence of publication bias was observed in any comparison model (all $p>0.05$).

Discussion

The acute or late side reactions in radiotherapy depend on several factors. The total dose, chemotherapy, surgery, hormone therapy are the treatment-related

Table 2. Main Results of Pooled ORs and 95%CIs for ATM Asp1853Asn and TP53 Arg72Pro Polymorphisms

Comparisons	Region	Test of association				Test of Heterogeneity			
		N	OR(95%CI)	Z	*p* value	Model	χ^2	*p* value	I^2(%)
ATM Asp1853Asn (rs1801516)									
GA *vs* GG	Overall	8	0.95(0.73-1.25)	0.35	0.729	Fixed	9.25	0.322	13.5
AA *vs* GG	Overall	4	1.37(0.31-6.05)	0.42	0.678	Random	11.21	0.024	64.3
AA *vs* GG+GA	Overall	4	1.30(0.30-5.70)	0.35	0.729	Random	11.21	0.024	64.3
GA+AA *vs* GG	Overall	11	1.09(0.86-1.39)	0.72	0.471	Fixed	18	0.116	33.3
A *vs* G	Overall	9	1.11(0.77-1.59)	0.54	0.592	Random	20.26	0.016	55.6
TP53 Arg72Pro (rs1042522)									
GC *vs* GG	Overall	7	0.91(0.71-1.15)	0.8	0.426	Fixed	4.13	0.659	0
CC *vs* GG	Overall	7	0.75(0.49-1.15)	1.31	0.189	Fixed	10.81	0.094	44.5
CC *vs* GG+GC	Overall	7	0.77(0.51-1.15)	1.27	0.203	Fixed	8.27	0.219	27.5
GC+CC *vs* GG	Overall	9	0.86(0.70-1.05)	1.49	0.136	Fixed	9.47	0.304	15.6
C *vs* G	Overall	7	0.89(0.75-1.05)	1.38	0.167	Fixed	10.96	0.09	45.2
	Europe	4	1.03(0.83-1.28)	0.27	0.789	Fixed	4.56	0.207	34.2
	Asia	3	0.71(0.54-0.93)	2.51	0.012	Fixed	1.83	0.4	0

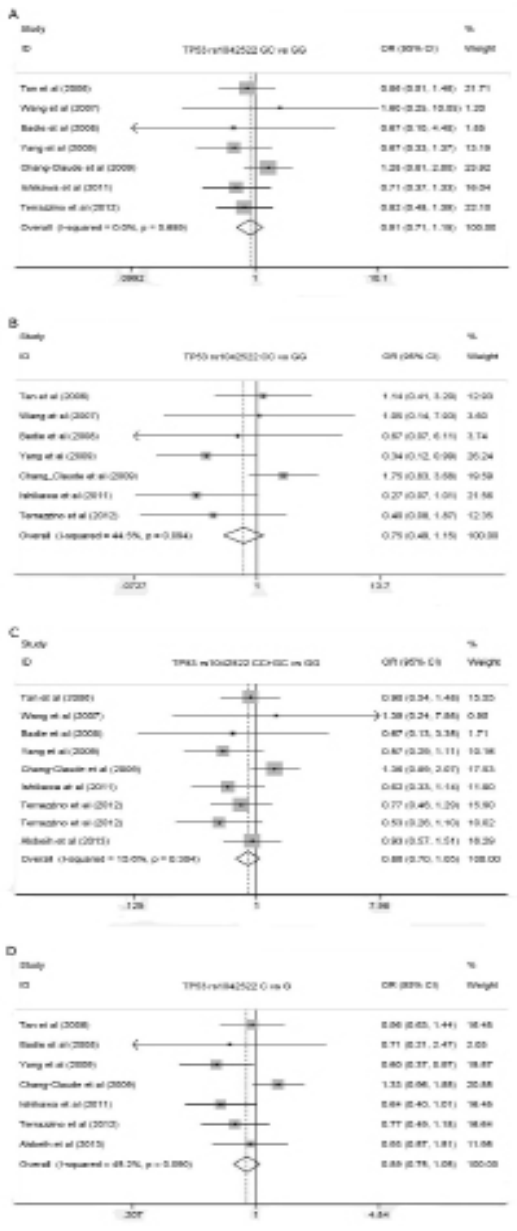

Figure 3. Forest plots of the Association between Radiotherapy Adverse Toxicity Risk and TP53 rs1042522 Polymorphism under 4 Different Comparison (A: GC *vs* GG; B: CC *vs* GG; C: GC+CC *vs* GG; D: C *vs* G)

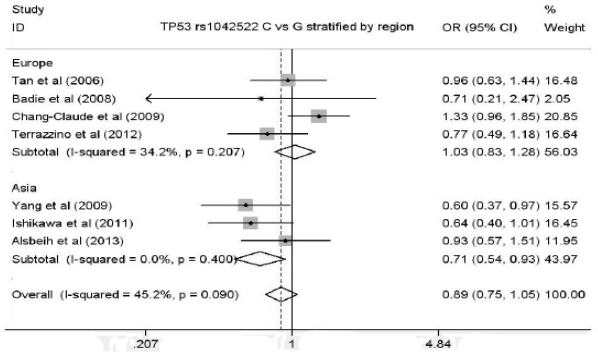

Figure 4. Forest Plot of Subgroup Analysis of TP53 rs1042522 by Geographical Region

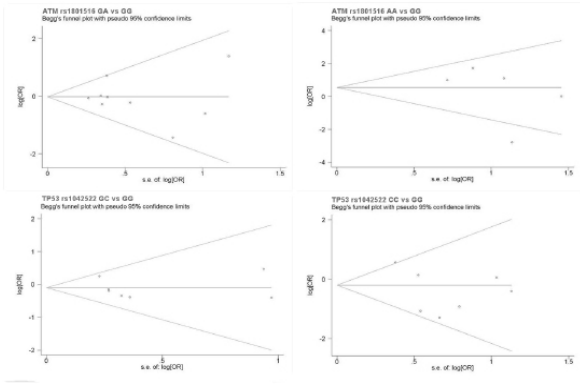

Figure 5. Funnel Plot of Associations between rs1801516 and rs1042522 and Risk of Radiotherapy Adverse Effects

factors. Patients' age, menopausal status, blood pressure, smoking habits, diseases are the patient-related factors. Besides all these extrinsic factors, the variation of radiotherapy side reactions among individual patients could be partially due to intrinsic differences in cellular radiosensitivity on a genetic basis (Turesson et al., 1996). According to previous clinical cases, A-T patients have been reported to show enhanced sensitivity to ionizing radiotherapy (Gotoff et al., 1967; Morgan et al., 1968). Also experimental study manifests that A-T cells are more sensitive to the chromosome breaking effects of ionizing radiation. It has been shown that cells derived

from heterozygous individuals exhibit an intermediate degree of radiosensitivity between those of wild-type and homozygously mutated cells derived from people with A-T (Taylor et al., 1975). Several studies have screened the ATM gene in patients who displayed clinically abnormal radiosensitivity. The results of these studies suggest variants in the ATM gene could affect the function of encoded protein and in turn influence the development of radiotherapy adverse effects. Consequently, to well understand the associations between crucial genetic variants and the risk of radiotherapy toxicities not only play an important role in selecting individualized optimal treatment for cancer patients, but also can prevent possible side reactions and improve the prognosis of cancer patients.

After analyzing the risk of toxicities of 20 selected articles on cancer patients treated with radiation therapy, it was concluded that ATM Asp1853Asn and TP53 Arg72Pro polymorphisms did not contribute to the development of radiation-induced side effects in the overall analysis. Data from the literatures we included in this meta-analysis did not exhibit statistically significant heterogeneity in the majority of the comparison models. We summarized all the possible factors that might affect the heterogeneity between studies: type of tumor, type of adverse reactions, time of appearance of the side reactions, region, sample size, sex, treatment parameters such as total dose, the dose per fraction, schedule of treatment, and treatment volume. Considering the factors available for us to analyze the cause of heterogeneity, we conducted subgroup analysis of region, cancer type and time of appearance of side effects. For ATM Asp1853Asn, the subgroup analysis of region, tumor type and time of appearance of side effects showed no significant association with risk of severe radiotherapy toxicity. As for TP53 Arg72Pro, the subgroup analysis by region indicated variant C allele was associated with decreased radiotherapy adverse effects risk among Asian cancer patients. As far as we concerned, ethnic differences may have an impact on these associations, since the distributions of the ATM Asp1853Asn and TP53 Arg72Pro allele frequencies were different between various ethnic populations. For ATM Asp1853Asn, the variant A allele frequency was extensively higher among Caucasians (18.8%) than Asians (1.2%). As for TP53 Arg72Pro, the variant C allele frequency was higher among Asians (48.9%) than Caucasians (23.3%). Furthermore, patients from the same area shared a lot common environmental exposures such as diet, lifestyle. This might suggest a potential role for ethnic differences in genetic backgrounds as well as environmental exposures. The large differences in the variant allele frequencies between Caucasians and Asians may be an important cause of different risk of radiotherapy-induced side effects between different population groups.

In some tissues, quite a lot of damage after irradiation may be tolerable, especially if the tumor is under reasonable control. For example, in the lung, a small amount of fibrosis is well endured and is commonly present after radiotherapy. However, in the central nervous system, the consequences can be severe(Stone et al., 2003). As the type of tissues exposed to radiation could affect the severity of radiotherapy side effects, we also conducted subgroup analysis of tumor type. The twenty studies included in this meta-analysis contained 7 tumor types: breast, prostate, head and neck, meningo, lung, nasopharynx, cervix. Only two studies investigated the radiotherapy-induced adverse reactions of head and neck tumor, mainly focused on the late toxicities including subcutaneous fibrosis and intracerebral edema. Compared with other tumor type such as breast, lung and prostate, head and neck cancer patients under the same treatment dose experienced much more dangerous concequences. Patients maintained grade 3 late toxicities were refered to severe toxicities using RTOG/EORTC grading system. But the result of subgroup analysis of tumor type suggested no association between these two SNPs and the risk of radiotherapy-induced toxicities. It was possibly due to the small smple size. The number of cancer patients from this two studies was 189 only accounting for 4 percent of the total number of patients. Thus, it might lower the statistical power to have a significant correlation.

Higher doses can cause varing side effects during treatment or in the months or years following treatment. Acute effects are those that observed during the course of treatment and late effects occur months to years after radiation exposure. Regarding the time of appearance of the radiotherapy toxicities as a potential confounding factor, we stritified the studies into two layers, acute side effects and late side effects, to explore the association between ATM Asp7253Asn and TP53 Arg72Pro polymorphisms and the risk of radiotherapy-induced toxicities. Unfortunately, we did not find any statistically significant relation. According to previous studies, the nature of acute or late reactions in a patient seems to be influenced by radiation fractions. Late effects are generally more sensitive to changes in fraction dose, and less sensitive to changes in overall treatment time than early responses(Thames et al., 1982). As our data presented here were based upon unadjusted estimates, we were unable to get the original individual patients' radiation therapy treatment schedule for further research.

Another factor may affect the results of our meta-analysis was the grading system used in these studies. Patients' side reactions were evaluated according to different grading system. Articles we included in this study adopted 5 grading system: EORTC, CTC, LENT/SOMA, RTOG and CTCAE. The definitions of radiotherapy-induced side effects grade were not same among each study. Because we were unable to check the original individual patients' radiation therapy records, we could not achieve to reassess the patients' radiotherapy toxicities using the same grading scale.

All in all, it is noteworthy that this is the first quantitatively systematical evaluation of the effect of ATM Asp1853Asn and TP53 Arg72Pro polymorphisms on the risk of radiotherapy side effects. Our study indicates that TP53 Arg72Pro C allele might be a protective factor of radiotherapy-induced adverse effects among cancer patients from Asia. Lack of the original individual patients' treatment records we are unable to conduct a more precise analysis with estimates adjusted according to covariates such as total dose, the dose per fraction, schedule of

treatment, and treatment volume. This is a limitation of our study. Moreover, additional large prospective studies considering treatment-related factors and patients' lifestyle environmental exposures are essential to confirm our findings.

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