RESEARCH COMMUNICATION

Association Between the Ku70 -1310C/G Promoter Polymorphism and Cancer Risk: a Meta-analysis

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Abstract

Ku70 plays an important role in DNA double-strand break repair. Studies revealing conflicting results on the role of the Ku70-1310C/G promoter polymorphism on cancer risk led us to perform a meta-analysis to investigate this relationship. Ten case-control studies with 2566 cases and 3058 controls were identified. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of associations. The overall results suggested no association between the Ku70-1310C/G promoter polymorphism and total cancer risk. However, on stratified analysis, significantly increased risks were observed among the Asian population (GG vs. CC: OR= 1.50, 95%CI= 1.10-2.06; GG vs. CC/CG: OR=1.47, 95%CI= 1.07-2.01) and population-based case-control studies (GG vs. CC: OR= 1.57, 95%CI= 1.12-2.22; CG vs. CC: OR=1.35, 95%CI= 1.11-1.64; CG/GG vs. CC: OR= 1.37, 95%CI= 1.14-1.65). Additionally, variant genotypes were associated with a significantly increased breast cancer risk (GG vs. CC: OR= 1.80, 95%CI= 1.26-2.56; GG vs. CC/CG: OR=1.40, 95%CI= 1.01-1.95).

Keywords: Ku70 - polymorphism - cancer - meta-analysis - carcinogenesis

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Introduction

By affecting genomic stability, DNA damage may induce abnormal cell proliferation, differentiation and apoptosis, which finally leads to carcinogenesis. As the major opponent of genetic injury, DNA repair mechanisms are essential in preventing tumor initiation and progress (Shiraishi et al., 2010).

DNA double-strand breaks (DSB) are the most serious type of DNA damage (Wood et al., 2001) that can be repaired by DNA DSB repair system. DNA DSB repair system consists of two sub-pathways, among which nonhomologous end-joining (NHEJ) is predominant in humans (Khanna and Jackson, 2001). The central factor of NHEJ is DNA-dependent protein kinase (DNA-PK), composed of DNA-PK catalytic subunit (DNA-PKcs) and Ku70/Ku80 heterodimer (Pfeiffer et al., 2000). Ku70, the product of the Ku70 gene (also named XRCC6 gene), is proposed to be a caretaker protein, which suppresses chromosomal rearrangements and maintains genome integrity (Tseng et al., 2009). A potentially functional polymorphism in the promoter region of Ku70 is described as -1310C/G (rs2267437) (Sobczuk et al., 2010).

Various case-control studies have investigated the association between the risk of human cancer and Ku70 -1310C/G promoter polymorphism; however, the findings have been conflicting. Furthermore, due to limitations in sample size, it may be insufficient to determine such an association from a single study. To our knowledge, there is currently no meta-analysis for the relationship between Ku70 -1310C/G promoter polymorphism and cancer risk. Therefore, a meta-analysis of all related case-control studies was implemented to determine quantitatively the potential heterogeneity and study bias in the current literature, and develop a more accurate representation of the association between Ku70 -1310C/G promoter polymorphism and human cancer risk.

Materials and Methods

Literature search strategy

Two electronic databases (Embase http://www.embase.com/ and Medline http://www.nlm.nih.gov/bsd/pmresources.html) were searched for all relevant reports (last search was updated on October 31th, 2011) using the following key words: "Ku70" or "XRCC6", "polymorphism" or "haplotype", "carcinoma" or "cancer" or "carcinogenesis" or "tumor". The searching was limited to English language papers and studies conducted on human subjects. Additional studies were identified by a manual search of the references of original studies. The ''Related Articles'' option in NCBI's PubMed source (www.ncbi.nlm.nih.gov/pubmed/) was also used to search for potentially relevant articles.

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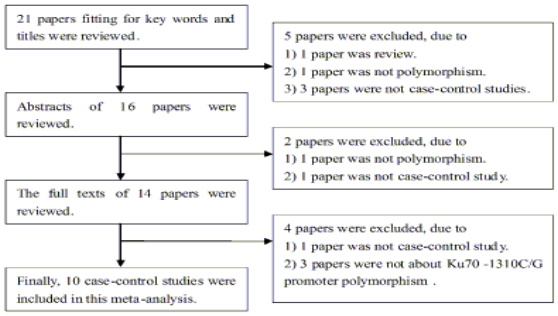

Figure 1. Flowchart for the Primary Studies Selection in this Meta-analysis

Inclusion and exclusion criteria

The details of inclusion criteria were studies that: (a) used a case-control design; (b) illustrated the relationship between Ku70 -1310C/G promoter polymorphism and risk of cancer; (c) provided the total number of cases and controls; (d) provided available genotype frequency in case and control group, respectively. The major exclusion criteria were as follows: (a) duplicate data; (b) abstract, comment, review or editorial; (c) insufficient data. The process of paper selection is shown in the flowchart (Figure 1). Eventually, 10 case-control studies with 2,566 cases and 3,058 controls were included in this meta-analysis.

Data extraction

All data were extracted independently according to the pre-specified selection criteria. Disagreements were resolved by discussion with coauthors. The following data were extracted from each study: name of first author, year of publication, source of control group, number of cases and controls, study results, ethnicity, countries and type of cancers. Ethnicity was categorized as Caucasian or Asian. For studies including subjects of different ethnic groups, data were extracted separately for each ethnic group, where possible. According to source of control groups, the studies were sorted as hospital-based control (HBC) (controls from hospitalized patients) or population-based control (PBC) (controls from healthy population).

Statistical analysis

The strength of the association between Ku70 -1310C/G promoter polymorphism and the risk of cancer was measured by odds ratios (ORs) and 95% confidence intervals (CIs). In this meta-analysis, GG or CG was first compared with CC. Then, the risks of GG vs. C carriers (CC/CG), and G carriers (CG/GG) vs. CC for cancers were evaluated in dominant (GG vs. CC/CG) and recessive (CG/GG vs. CC) models, respectively. In consideration of the possibility of statistical heterogeneity across the studies, a statistical test for heterogeneity was performed based on the Q-test. The summary OR estimate of each study was calculated by the fixed effects model (the Mantel-Haenszel method) if the P value of the Q-test was greater than 0.10, which indicated no significant heterogeneity among the studies (Mantel and Haenszel, 1959). Otherwise, the random effects model (the DerSimonian and Laird method) was used (DerSimonian and Laird, 1986). Subgroup analyses were also performed according to ethnicity, type of cancer, and source of control. In addition, Funnel plots and Egger's linear regression were used to diagnose a potential publication bias (Egger et al., 1997). For the control group in each study, the allelic frequency was calculated and the observed genotype frequencies of the Ku70 -1310C/G promoter polymorphism were assessed for Hardy-Weinberg equilibrium using the χ^2 test; $P<0.05$ was considered to be statistically significant (Grover et al., 2010). Sensitivity analyses were performed to assess the stability of the results. All statistical analyses were conducted using STATA 11.0 (StataCorp, College Station, Tex). All statistical tests were two-sided.

Results

Characteristics of studies

A total of 10 eligible studies, involving 2566 cases and 3058 controls, were included in our meta-analysis. Of these, 7 studies focused on an Asian population, with the remaining 3 studies focused on a Caucasian population. Furthermore, 7 studies were classified as HBC studies, while the 3 were PBC studies. Four studies examined breast cancer, while the other 6 studies investigated gastric, hepatocellular, lung, head and neck, oral and bladder cancers. With the exception of one study, the distribution of genotypes in the controls was consistent with Hardy-Weinberg equilibrium in all studies. The details are listed in Table 1.

Quantitative synthesis

The Q-test of heterogeneity for all populations was always significant, so we conducted analyses using the random effects model. As shown in Table 2, there

Table 1. Characteristics of Primary Studies in the Meta-analysis

First author	Year	Cancer	Country	Ethnicity	SOC[a]	Case	Con	CC Case/Con	CG Case/Con	GG Case/Con	HWE
Yang	2011	Gastric cancer	China	Asian	HCC[b]	136	560	95/383	37/167	4/10	0.088
He	2011	Breast cancer	China	Asian	HCC	293	301	141/179	127/113	25/9	0.075
Li	2011	Hepatocellular cancer	China	Asian	PCC[c]	675	667	433/457	207/184	35/26	0.173
Tseng	2009	Lung Cancer	China	Asian	HCC	150	151	140/138	9/11	1/2	0.005
Willems	2009	Breast cancer	Belgian	Caucasian	PCC	206	171	59/71	107/73	40/27	0.263
Werbrouck	2008	Head and neck cancer	Belgian	Caucasian	HCC	152	157	67/59	71/74	13/24	0.920
Bau	2008	Oral cancer	China	Asian	HCC	318	318	227/214	83/98	8/6	0.168
Wang	2008	Bladder cancer	China	Asian	HCC	213	235	129/149	71/74	13/12	0.481
Willems	2008	Breast cancer	Belgian	Caucasian	PCC	169	119	44/45	94/54	31/20	0.581
Fu	2003	Breast cancer	China	Asian	HCC	254	379	192/261	55/106	7/12	0.758

[a]Source of case–control study; [b]Hospital-based case–control study; [c]Population-based case–controls study

Table 2. Meta-analysis of the Ku70 -1310C/G Promoter Polymorphism and Cancer Risk Association

Polymorphism	Study	Sample Size Case	Control	N[a]	Test of Association OR (95% CI)	Z	P value	Model[b]	Test of Heterogeneity χ^2	P value	I^2 (%)
GG versus CC	Overall	1704	2104	10	1.33(0.94-1.89)	1.60	0.109	R	15.89	0.069	43.4%
Ethnicity	Asian	1450	1858	7	1.50(1.10-2.06)	2.53	0.012	F	7.36	0.289	18.4%
	Caucasian	254	246	3	1.13(0.52-2.49)	0.31	0.757	R	7.95	0.019	74.8%
Cancer type	Breast cancer	539	624	4	1.80(1.26-2.56)	3.21	0.001	F	5.74	0.125	47.7%
	Other cancers	1165	1480	6	1.08(0.77-1.51)	0.44	0.661	F	6.54	0.257	23.6%
Souce of control	HCC[c]	1062	1458	7	1.16(0.65-2.08)	0.50	0.616	R	14.25	0.027	57.9%
	PCC[d]	642	646	3	1.57(1.12-2.22)	2.58	0.010	F	0.31	0.855	0.0%
CG versus CC	Overall	2388	2910	10	1.08(0.89-1.32)	0.77	0.441	R	21.13	0.012	57.4%
Ethnicity	Asian	1946	2534	7	1.00(0.82-1.22)	0.02	0.985	R	11.81	0.066	49.2%
	Caucasian	442	376	3	1.38(0.85-2.25)	1.29	0.198	R	6.04	0.049	66.9%
Cancer type	Breast cancer	819	902	4	1.31(0.84-2.03)	1.20	0.230	R	13.41	0.004	77.6%
	Other cancers	1569	2008	6	1.00(0.86-1.17)	0.04	0.965	F	4.83	0.437	0.0%
Souce of control	HCC[c]	1444	2026	7	0.95(0.81-1.11)	0.68	0.494	F	10.01	0.124	40.1%
	PCC[d]	944	884	3	1.35(1.11-1.64)	3.00	0.003	F	3.47	0.176	42.4%
GG versus CCCG	Overall	2565	3070	10	1.22(0.97-1.55)	1.71	0.088	F	12.22	0.201	26.4%
Ethnicity	Asian	2039	2623	7	1.47(1.07-2.01)	2.40	0.016	F	5.56	0.474	0.0%
	Caucasian	526	447	3	0.98(0.70-1.39)	0.12	0.905	F	4.11	0.128	51.4%
Cancer type	Breast cancer	922	970	4	1.40(1.01-1.95)	2.00	0.046	F	5.37	0.146	44.2%
	Other cancers	1643	2100	6	1.07(0.77-1.49)	0.40	0.690	F	5.90	0.316	15.3%
Souce of control	HCC[c]	1515	2113	7	1.18(0.70-2.00)	0.63	0.528	R	11.91	0.064	49.6%
	PCC[d]	1050	957	3	1.26(0.92-1.73)	1.41	0.157	F	0.23	0.893	0.0%
CGGG versus CC	Overall	2565	3058	10	1.09(0.89-1.35)	0.85	0.394	R	25.64	0.002	64.9%
Ethnicity	Asian	2039	2611	7	1.03(0.83-1.28)	0.24	0.814	R	15.05	0.020	60.1%
	Caucasian	526	447	3	1.32(0.76-2.30)	0.98	0.329	R	8.67	0.013	76.9%
Cancer type	Breast cancer	922	970	4	1.35(0.86-2.11)	1.30	0.192	R	15.27	0.002	80.3%
	Other cancers	1643	2088	6	1.02(0.88-1.18)	0.22	0.822	F	6.42	0.268	22.1%
Souce of control	HCC c	1515	2101	7	0.95(0.75-1.21)	0.43	0.666	R	14.49	0.025	58.6%
	PCC d	1050	957	3	1.37(1.14-1.65)	3.30	0.001	F	3.23	0.199	38.1%

[a]Number of comparisons; [b]Random-effects model was used when P value for heterogeneity test < 0.10; otherwise, fix-effects model was used; [c]HCC hospital-based case–control study; [d]PCC population-based case–controls study

was no association between Ku70 -1310C/G promoter polymorphism and risk of cancer; the ORs (95% CIs) were 1.33 (0.94-1.89) for GG vs. CC, 1.08 (0.89-1.32) for CG vs. CC, 1.22 (0.97-1.55) for GG vs. CC/CG, and 1.09 (0.89-1.35) for CG/GG vs. CC.

Subgroup analyses

Subgroup analyses were conducted according to ethnicity, type of cancer, and source of control. In the stratification analyses for ethnicity, a significantly increased risk was associated with the variant genotype GG in both homozygote and dominant models among Asians (GG vs. CC: OR= 1.50, 95%CI= 1.10-2.06 and GG vs. CC/CG: OR= 1.47, 95%CI= 1.07-2.01). However, there was no significantly elevated risk for Caucasians with this polymorphism. According to the type of cancer, in both homozygote and dominant models, we found that Ku70 -1310C/G promoter polymorphism was associated with an increased risk of breast cancer (GG vs. CC: OR= 1.80, 95%CI= 1.26-2.56 and GG vs. CC/CG: OR= 1.40, 95%CI= 1.01-1.95) (Figure 2). However, no significantly elevated risk of other cancers associated with this polymorphism was shown in overall comparisons. When analyzing for source of control, an association was observed among the PBC studies (GG vs. CC: OR= 1.57, 95%CI= 1.12-2.22; CG vs. CC: OR= 1.35, 95%CI= 1.11-1.64 and CG/GG vs. CC: OR= 1.37, 95%CI= 1.14-1.65). However, no significantly increased risk of this polymorphism was found among HBC studies. The details

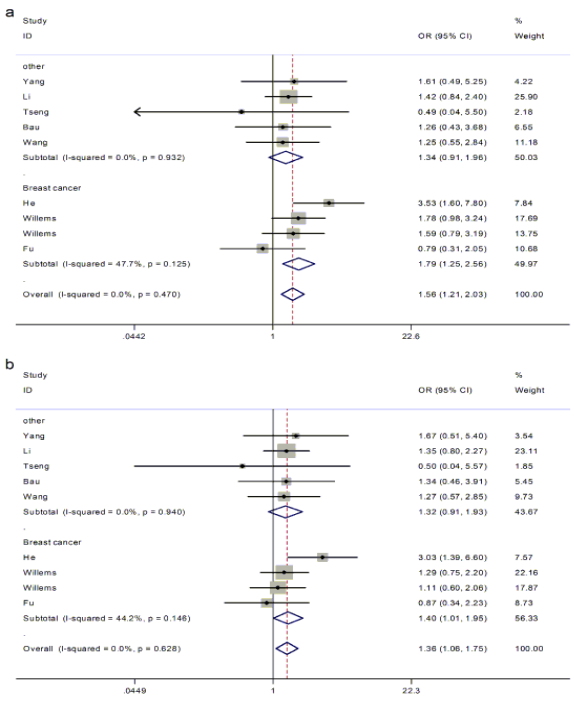

Figure 2. Forest plot Showing the Association Between the Ku70 -1310C/G Promoter Polymorphism and Risk of Breast Cancer. (a) Ku70 -1310C/G promoter polymorphism was associated with the risk of breast cancer in homozygote. (b) Ku70 -1310C/G promoter polymorphism was associated with the risk of breast cancer in dominant model. The fixed-effects model was used

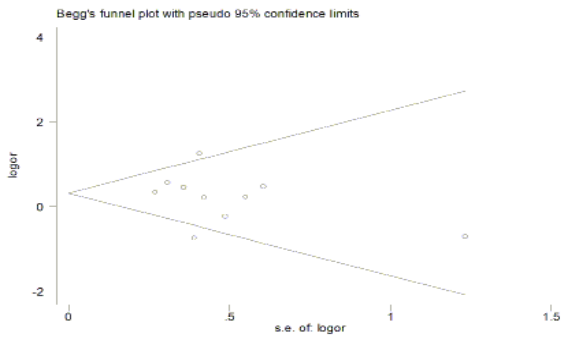

Figure 3. Begger's Funnel Plot for Publication Bias Test, GG vs. CC; each Point Represents a Separate Study for the Indicated Association. Log [OR]: natural logarithm of OR. Horizontal line represents size of effect

are listed in Table 2.

Test of heterogeneity and sensitivity analyses

Test of heterogeneity and sensitivity analyses were performed to assess the stability of the results. As shown in Table 2, no significant heterogeneity between the studies was observed in all comparisons. To reflect the influence of the individual dataset to the pooled ORs, a single study involved in this meta-analysis was deleted each time, and the corresponding pooled ORs were not considerably altered.

Publication bias

To assess the publication bias of the literature, Begger's funnel plot and Egger's test were performed. As shown in Figure 3, the shapes of the Begger's funnel plots did not indicate any evidence of obvious asymmetry in homozygote model. Thus, Egger's test was used to provide statistical evidence of funnel plot symmetry for each model. These tests also did not show any evidence of a publication bias (GG vs. CC: t = -0.73, P = 0.486; CG vs. CC: t = -0.16, P = 0.874; G carrier vs. CC: t = -0.42, P = 0.687; C carrier vs. GG: t = 0.29, P = 0.776).

Discussion

Meta-analyses based on gene polymorphisms have been widely performed in the past few decades to assess the association between a particular gene and cancer risk. A meta-analysis grouping various cancer types can accurately determine the relationship between a particular gene and cancer risk, with the help of subgroup analyses to solidify these associations. In the present study, we performed a meta-analysis based on 10 case-control studies involving 2566 cases and 3058 controls. We found that in the overall studies, there was no association between Ku70 -1310C/G promoter polymorphism and total cancer risk. However, in the stratification analyses for ethnicity, a significantly increased total cancer risk was associated with the variant genotype in both homozygote model (GG vs. CC) and dominant model (GG vs. CC/CG) among Asian subjects. In addition, according to the types of cancer, we also found a significantly increased risk of breast cancer for both homozygote and dominant models. Additionally, when analyzing for source of control, an association was observed among PBC studies for GG versus CC, CG versus CC and G carrier versus CC (Table 2).

Ku70 is important during the process of DNA double-strand break repair (DSBR) and maintains genomic integrity. Furthermore, as a heterodimer, its cell surface expression regulates cell adhesion and invasion (Muller et al., 2005). An increased risk of several types of cancers is associated with genetic variations within human Ku70 (Pucci et al., 2001). In breast cancer, several tumor suppressors, such as Kruppel-like transcription factors, are known to bind to the first putative CACCC box of the Ku70 promoter, which lies adjacent to the Ku70 -1310C/G (Hosoi et al., 2004). Within the Kruppel-like binding site and its adjacent sequences, a single nucleotide substitution can alter transcription factor binding activity. Alternatively, the functional role of the SNP may be due to its association with Ku70 transcriptional expression and the ensuing DNA repair (Willems et al., 2008).

Our results showed that the G allele was a risk allele for susceptibility to total cancer among Asians, but not among Caucasians. Although these results are preliminary, this risk may be attributed to the differences in the genetic background and/or the environmental life-style between these two populations (Hirschhorn et al., 2002). Furthermore, disease susceptibility may also vary with the different genetic background (Parkin et al., 1993).

A significant increase in the association between Ku70 -1310C/G promoter polymorphism and total risk of cancer was observed among PBC studies (GG vs. CC, GC vs. CC and G carrier vs. CC), but not HBC studies. The reason for this disparity may be explained by the presence of certain diseases in the control group patients of the HBC studies; these patients may show a different genetic susceptibility from the general population (Kopec and Esdaile, 1990), particularly when the genotypes under investigation are associated with the disease-related conditions. Therefore, HBC studies have inherent defects in selection bias.

In this meta-analysis, we observed an association between Ku70 -1310C/G promoter polymorphism and the risk of breast cancer, but this did not affect the lack of association between Ku70 -1310C/G promoter polymorphism and risk of total cancer. This may be due to the limited sample size of breast cancer research in the cohort, which may not bias the total relationship between total cancer risk and Ku70 -1310C/G promoter polymorphism.

Several limitations of our meta-analysis should be addressed. First, only papers written in English were included. Studies published in other languages were not included, which may bias the results. Second, further evaluation was limited due to the lack of original data, because the interactions between gene-gene, gene-environment, and even different polymorphic loci of the same gene may modulate cancer risk (Gu et al., 2009). Third, the numbers of published studies were not sufficiently large for a comprehensive analysis; consequently, this study may not have adequate power to detect the possible risk for Ku70 -1310C/G promoter polymorphism. Fourth, one study involved in our analysis was not consistent with Hardy-Weinberg equilibrium; it

was not excluded so as to maintain the integrality of the research about Ku70 -1310C/G promoter polymorphism. However, the data extracted from it may bias our final results. Finally, the observed significant ORs in some studies with a small sample size may provide a false association. Therefore, large and well-designed case-control studies are needed.

In conclusion, this meta-analysis showed some evidence of the Ku70 -1310C/G promoter polymorphism and cancer risk, supporting the hypothesis that the Ku70 -1310C/G promoter polymorphism may be a low-penetrance susceptibility marker of cancer. Due to the relatively small sample size, this result should be regarded as preliminary. Additional studies are needed to validate the possible ethnic differences that lead to an increased risk of cancer, and to understand the association between the Ku70 -1310C/G promoter polymorphism and cancer risk.

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