RESEARCH ARTICLE

The Interleukin-18 Promoter -607C>A Polymorphism Contributes to Nasopharyngeal Carcinoma Risk: Evidence from a Meta-analysis Including 1,886 Subjects

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Abstract

The interleukin-18 promoter -607C>A gene polymorphism may be related to nasopharyngeal carcinoma (NPC) risk but the results of individual studies remain conflicting. A meta-analysis including 1,886 subjects from five individual studies was therefore performed to provide a more accurate estimation. Pooled odds ratios (ORs) and their corresponding 95% confidence intervals (95% CIs) were evaluated by fixed- or random-effects models. A significant relationship between interleukin-18 promoter -607C>A gene polymorphism and NPC was found in a dominant genetic model (OR: 1.351, 95% CI: 1.089-1.676, P=0.006, $P_{heterogeneity}$=0.904), a homozygote model (OR: 1.338, 95% CI: 1.023-1.751, P=0.034, $P_{heterogeneity}$=0.863), and a heterozygote model (OR: 1.357, 95% CI: 1.080-1.704, P=0.009, $P_{heterogeneity}$=0.824). No significant association was detected in either an allelic genetic model (OR: 1.077, 95% CI: 0.960-1.207, 0.207, $P_{heterogeneity}$=0.844) or a recessive genetic model (OR: 1.093, 95% CI: 0.878-1.361, P=0.425, $P_{heterogeneity}$=0.707). In conclusion, a significant association was found between interleukin-18 promoter -607C>A gene polymorphism and NPC risk. Individuals with the C allele of interleukin-18 promoter -607C>A gene polymorphism have a higher risk of NPC development.

Keywords: Nasopharyngeal carcinoma - interleukin-18 - polymorphism - meta-analysis - risk

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Introduction

Nasopharyngeal carcinoma (NPC) is endemic in southern China where genetic abnormalities and Epstein–Barr virus (EBV) infection are critical in the pathogenesis of the disease (Chan et al., 2002). There were an estimated 84,400 incident cases of NPC and 51,600 deaths in 2008, representing about 0.7% of the global cancer burden, and the disease may be considered one of the rarer cancer forms globally, ranking as the 24 th most frequently diagnosed cancer form worldwide and 22 nd within the developing world. The global statistics by world region reveal the distinct features of its descriptive epidemiology, however, and the contrasting geographical and ethnic variations in the distribution of incidence worldwide (DeSantis et al., 2013).

NPC is more frequent in males than females in both the developing and developed world, with incidence rates commonly 2 to 3 times higher in males in higher resource countries, with male-to-female rate ratios often considerably higher in developing regions. The geographical disparities in the burden of NPC in relation to resource are noteworthy, with an estimated 92% of new cases occurring within economically developing countries (Jemal et al., 2011). According to world area, incidence rates are highest in South-Eastern Asia, in both sexes, with the disease being the sixth most common among males in the region. Indeed in global terms, the 3 highest national incidence rates are estimated in Malaysia, Indonesia, and Singapore, where rates are high among the Chinese and Malay populations (Chan et al., 2002). Elsewhere in Asia, high incidence rates are observed in a number of provinces in South-Eastern China, including Guangdong and Hong Kong, and in other parts of Southern Asia (the Philippines and Thailand).

NPC has viral, environmental, and genetic components to its etiology. Migrants from high- to low-risk countries retain incidence rates intermediate to natives of their host country and their country of origin, implicating a role for environmental and/or genetic factors. Many studies have reported increased risks associated with single nucleotide polymorphisms (SNPs) of interleukin-18 (IL-18) gene (Dinarello et al., 2013). Interleukin-18 (IL-18), a recently described member of the IL-1 cytokine superfamily, is now recognized as an important regulator of innate and acquired immune responses. IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases (Ji et al., 2013; Song et al., 2013), in a variety of cancers (Srivastava et al., 2010), and in the context of numerous infectious diseases. There are three SNPs in the promoter region of

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IL-18: -137, -607, and –656, relative to the transcriptional start site. The G>C substitution at position –137 abolishes a histone 4 transcription factor-1 nuclear factor binding site, and the C to A substitution at position –607 disturbs a cyclic adenosine monophosphate responsive element protein-binding site. Cloning and transcriptional analysis showed that these two SNPs altered the IL-18 expression level. Additionally, polymorphisms of IL-18 have been demonstrated with risk of various inflammation associated diseases (Bombardieri et al., 2007), for example, rheumatoid arthritis (Fariasa et al., 2013; Song et al., 2013) and atopic asthma (Ma et al., 2012). The association of -607C>A polymorphism with cancer risk has been investigated in several studies, while the conclusion is still inconclusive. For example, Pratesi et al. found that carriers of the -607C variant allele were associated with a significantly increased risk of nasopharyngeal cancer (Pratesi et al., 2006), but Pan et al. found no association between -607C>A polymorphism and nasopharyngeal cancer risk (Pan et al., 2013).

To ascertain the relationship between IL-18 -607C>A polymorphism and cancer risk, we performed this meta-analysis by pooling all eligible studies.

Materials and Methods

Publication Search and Inclusion Criteria

The following keywords were searched in electronic databases such as Embase, PubMed, Web of Science, China Biological Medicine Database, Wanfang Database and China National Knowledge Infrastructure: "cancer of nasopharynx", "nasopharyngeal cancer", "nasopharyngeal carcinoma", "carcinoma of nasopharynx", "pharyngeal neoplasms", "nasopharyngeal neoplasms", "IL-8", "interleukin-8", "genetic variation", and "polymorphism." Other relevant studies were also found in the indexed references of the retrieved literatures. The last research was updated on October 20, 2013, with publication years ranging from 2006 to 2013.

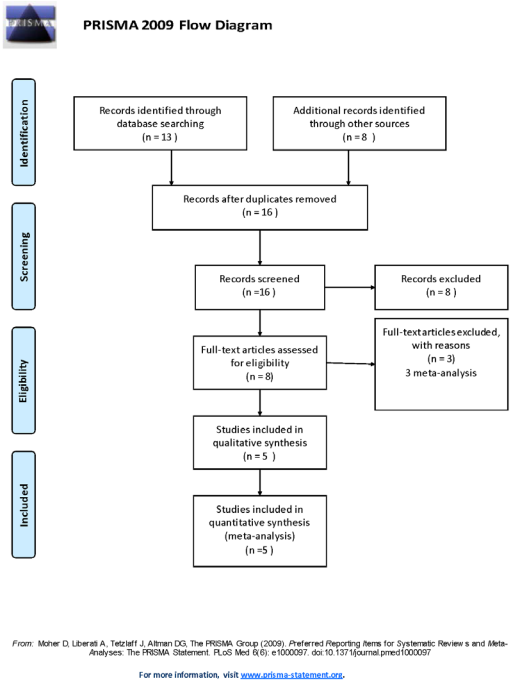

Figure 1. Flow Chart of the Study Selection Process

The studies were selected based on the following inclusion criteria: studies that evaluate interleukin-18 promoter -607C>A gene polymorphism and NPC, studies that diagnosis of cancer was confirmed by a histopathological analysis, case-control or cohort studies published in official journals, and studies that conform to the Hardy-Weinberg equilibrium (HWE). All records were selected by two authors independently according to the inclusion criteria and reached consensus on each record.

Data extraction

The data were abstracted according to a standard protocol. Studies that did not follow the inclusion criteria, those considered double publications, or those that provided inadequate data were excluded. If the same data appeared in different studies, the data were used only once. The abstracted data comprised the following items: the first author's name, publication year, region, number of genotypes, genotyping, study design, matching criteria, total number of cases and controls and HWE.

Statistical analyses

Five genetic models were used, including allelic (distribution of A allelic frequency of interleukin-18 promoter -607C>A gene polymorphism, allelic model: A allele vs. C allele), recessive (AA vs. CC+CA), dominant (CA+AA vs. CC), homozygous (AA vs. CC), and heterozygous (CA vs. CC) (Thakkinstian et al., 2005) models. The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to compare the association between interleukin-18 promoter -607C>A gene polymorphism and NPC. Chi-square-based Q-tests were used to calculate the heterogeneity between the individual studies with significance set at the $P<0.05$ level (Cochran 1968). The random-effect model was used to assess the pooled OR (DerSimonian and Laird method) if there was heterogeneity among the individual studies (Mantel et al., 1959). Otherwise, the fixed-effect model was used (the Mantel-Haenszel method). The pooled OR was determined through Z test with significance set at the $P<0.05$ level.

Fisher's exact test was used to evaluate the HWE, and significance was set at the $P<0.05$ level. The funnel plot was used to estimate the potential publication bias (Stuck et al., 1998). Egger's linear regression test on the natural logarithm scale of the OR was used to assess the funnel plot asymmetry with significance set at the $P<0.05$ level (Egger et al., 1997). STATA 12.0 software was used to perform the statistical analyses (Stata Corp, College Station, TX, USA).

Results

Characteristics of eligible studies

Of the 21 articles that were initially identified in the search strategy, 16 studies were removed, including 5 duplicates, 8 studies during the title/abstract review, and 3 studies (Mi et al., 2011; Wang et al., 2013; Yang et al., 2013) during the full-text review (Figure 1). Five studies satisfied all of the criteria and were included in this report

Table 1. Characteristics of Studies of Il-18 -607 (C/A) Polymorphism Included in This Pooled Analysis

Author	Year	Region	Ethnicity	NPC			Control			Genotyping	Study design	Matching criteria	Sample size	HWE
				AA	CA	CC	AA	CA	CC				(NPC/Control)	
Pratesi	2006	Italy	European	21	42	26	23	64	43	PCR-RFLP	Case-control	Age, sex, ethnicity	89/130	Yes
Farhat	2008	Tunisia	African	28	94	41	34	77	53	PCR-RFLP	Case-control	Age, sex, ethnicity	163/164	Yes
Nong	2009	China	Asian	71	132	47	68	133	69	PCR-RFLP	Case-control	Age, sex, ethnicity	250/270	Yes
Du	2012	China	Asian	34	80	36	40	93	47	PCR-RFLP	Case-control	Age, sex, ethnicity	150/180	Yes
Pan	2013	China	Asian	53	97	40	51	93	56	PCR-RFLP	Case-control	Age, ethnicity	190/200	Yes

NPC, nasopharyngeal carcinoma; IL-18, interleukin-18; HWE, Hardy-Weinberg equilibrium

Table 2. Meta-analysis of the Association Between the Il-18 Promoter -607 C/A Polymorphism and Npc

Polymorphism	Population	Number of studies	Test of association			Test of heterogeneity		
			OR	95%CI	*P* value	Model	*P* value	I^2
A allele	Overall	5	1.077	0.960-1.207	0.207	FEM	0.991	0.00%
	Asian	3	1.079	0.942-1.236	0.27	FEM	0.913	0.00%
AA versus CC	Overall	5	1.338	1.023-1.751	0.034	FEM	0.863	0.00%
	Asian	3	1.386	1.006-1.910	0.046	FEM	0.292	0.00%
CA versus CC	Overall	5	1.357	1.080-1.704	0.009	FEM	0.824	0.00%
	Asian	3	1.355	1.025-1.792	0.033	FEM	0.712	0.00%
AA versus CA+CC	Overall	5	1.093	0.878-1.361	0.425	FEM	0.707	0.00%
	Asian	3	1.124	0.870-1.452	0.37	FEM	0.916	0.00%
CA+AA versus CC	Overall	5	1.351	1.089-1.676	0.006	FEM	0.904	0.00%
	Asian	3	1.364	1.047-1.776	0.022	FEM	0.66	0.00%

IL-18, interleukin-18; OR, odds ratio; CI, confidence interval; FEM, fixed effects model

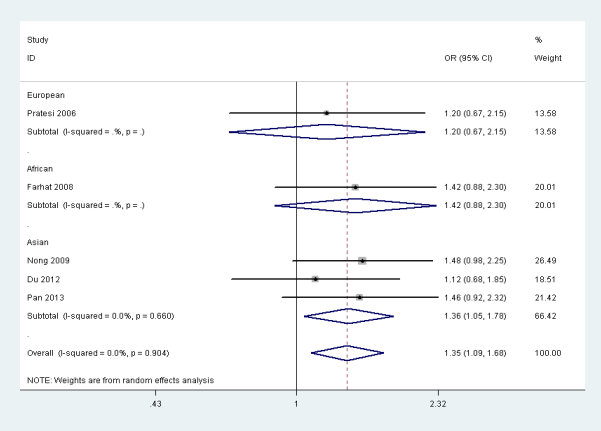

Figure 2. Forest Plot of NPC Associated with Il-18 Promoter -607 C/A Gene Polymorphism under an Dominate Genetic Model

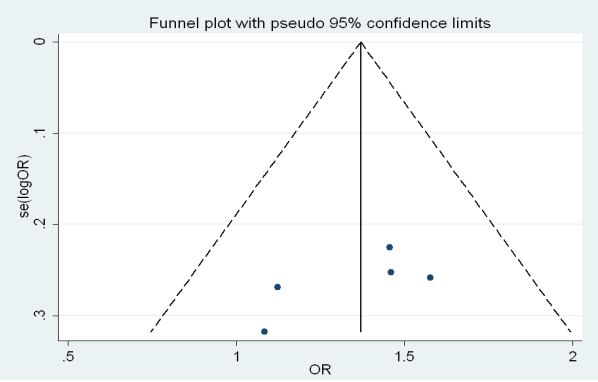

Figure 3. Funnel Plot for Studies of the Association of Npc and Il-18 Promoter -607 C/A Gene Polymorphism

(Pratesi et al., 2006; Farhat et al., 2008; Nong et al., 2009; Du, 2012; Pan, 2013). Three studies were conducted in China, one conducted in Italy and one performed in Tunisia. No study was discarded for deviating from the

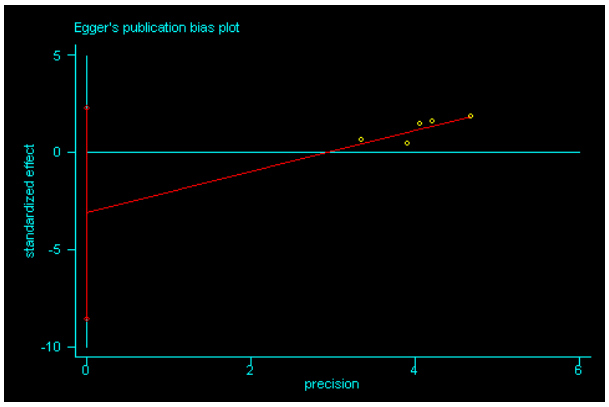

Figure 4. Egger Plot for Studies of The Association of NPC and Il-18 Promoter -607 C/A Gene Polymorphism

HWE. The data were extracted from 942 NPC cases and 944 controls (Table 1, Figure 1).

Pooled analysis

A significant association between the interleukin-18 promoter -607C>A gene polymorphism and NPC was found in a dominant genetic model (OR: 1.351, 95% CI: 1.089-1.676, P=0.006, $P_{heterogeneity}$=0.904), a homozygote model (OR: 1.338, 95% CI: 1.023-1.751, P=0.034, $P_{heterogeneity}$=0.863), a heterozygote model (OR: 1.357, 95% CI: 1.080-1.704, P=0.009, $P_{heterogeneity}$=0.824) as shown in Table 2 and Figure 2 .

The results from the three genetic models (a dominant genetic model, a homozygote model and heterozygote model) were positive and the heterogeneity between the individual studies did not exist which indicated that there was an intensively positive association between interleukin-18 promoter -607C>A allele and NPC risk.

No significant association between them was detected both in an allelic genetic model (OR: 1.077, 95% CI: 0.960-1.207, 0.207, $P_{heterogeneity}$=0.844) and a recessive

genetic model (OR: 1.093, 95% CI: 0.878-1.361, P=0.425, $P_{heterogeneity}$=0.707).

Bias diagnosis

The publication bias of the studies was assessed using the funnel plot and Egger's test. Publication bias was not seen in the funnel plot (Figure 3). No statistically significant difference was found in the Egger's test (P=0.164), indicating low publication bias in the current meta-analysis (Figure 4).

Discussion

The present study suggested a significant association between the interleukin-18 promoter -607C>A gene polymorphism and NPC in a dominant genetic model (OR: 1.351), a homozygote model (OR: 1.338) and a heterozygote model (OR: 1.357). The C allele of the interleukin-18 promoter -607C>A gene may be the susceptibility gene for NPC. This result was the strength of this meta-analysis.

Nasopharyngeal carcinomas are very different from other head and neck cancers because of their specific multifactorial etiology (Chan et al., 2002), their geographical distribution and their high sensitivity to radiotherapy and chemotherapy induction. NPC has a complex etiology involving a consistent association with the Epstein–Barr virus (EBV) regardless of the patient's geographical origin as well as environmental and hereditary factors. Other features of NPCs are their high level of leukocyte infiltration among tumor cells. These infiltrating leukocytes are principally composed of T lymphocytes and macrophages. Many studies suggest that this leukocyte infiltration process may be promoted by the constitutive secretion by nasopharyngeal carcinoma tumor cells of a pro-inflammatory cytokine, the IL-18. There is growing evidence suggesting that IL-18 levels may affect individual to virus-associated neoplastic and that single nucleotide polymorphisms (SNPs) within the gene may influence its production.

It has been proposed that inflammation is a risk factor of tumorigenesis. Preclinical studies have shown that IL-18, a key cytokine in immune response, has a controversial role in cancer development (Srivastava et al., 2010). Evidence suggests that IL-18 can promote cell death and tumor progression by activation of immune response and natural killers and could be used in anti-cancer gene therapy. However, under some experimental conditions, tumor cells may also escape immune recognition, increasing adherence to the microvascular wall through IL-18-dependent mechanism. Experimental evidence has also showed that IL-18 could promote metastasis by inducing cell adhesion molecules and matrix metallic proteinases. Thus, the correlation between IL-18 and cancer seems complex and may be in a tissue-specific manner.

Overall, a significant association exists between -607C/A polymorphisms in IL-18 gene promoter and cancer risk (Mi et al., 2011). This finding indicates that the genetic variant in IL-18 gene promoter region may crucially modify the susceptibility of cancers (Yang et al., 2013). The C to A substitution at position –607 disrupts a consensus cAMP-responsive element protein-binding site, causing altered transcription factor binding and gene expression. IL-18 serum levels have been reported to be elevated in a variety of cancers compared with control group. Hence, the -607C/A polymorphisms in IL-18 gene promoter may modify the susceptibility of cancers though changing the expression of IL-18 gene. The mechanism needs further investigation.

Meta-analysis is a retrospective research that is subject to the methodological deficiencies of the included studies and several specific details merit consideration in the current meta-analysis. A first consideration is that our results are based on unadjusted estimates and a more precise analysis stratified by different lifestyle related habits and different grades of NPC could be performed if individual data were available. A second consideration is that large-scale studies on the relationship between interleukin-18 promoter -607C>A gene polymorphism and NPC are still inadequate. IL-18 is influenced not only by interleukin-18 promoter -607C>A gene polymorphism, but also by environmental factors, such as the concentration of blood sugar, insulin, triglycerides, and so on. Nevertheless, the total number of subjects included in this part of the analysis comprises the largest sample size so far. Finally, as with any meta-analysis of published results, the quality of our meta-analysis depends on that of individual studies. Ideally we would like to pool individual level data. However this is not possible for the present study. These considerations may distort our results.

To conclude, our meta-analysis demonstrated an association between interleukin-18 promoter -607C>A gene genotype and NPC risk. Nevertheless, large-scale and well-designed studies are needed to investigate gene–gene and gene–environment interactions on interleukin-18 promoter -607C>A gene polymorphisms and NPC risk, which may eventually lead to better comprehensive understanding of the possible roles in tumorigenesis.

To the best of our knowledge, this is the first meta-analysis to evaluate the relationship between the IL-18 gene–670A>C polymorphism and NPC risk.

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