Association between FEN1 Polymorphisms -69G>A and 4150G>T with Susceptibility in Human Disease: A Meta-Analysis

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Abstract

Background: As a DNA repair protein, flap endonuclease 1 is a key enzyme in maintaining genomic instability and preventing carcinogenesis. Two single nucleotide polymorphisms (SNPs), -69G>A and 4150G>T are associated with DNA damage. This meta-analysis is to evaluate the genetic effects of FEN1 gene SNPs (-69G/A and 4150G/T) and the susceptibility to diseases, including glioma risk, breast cancer, lung cancer, keratoconus (KC) and fuchs' endothelial corneal dystrophy (FECD).

Methods: A literature search of PubMed and Embase was conducted to identify all eligible published studies. Five case-control studies were included with a total of 5612 cases and 6703 controls in this meta-analysis. Crude odds ratios (ORs) with their corresponding confidence intervals (95%CI) were used to assess the strength of the association.

Results: The FEN1 -69G/A and 4150G/T polymorphisms were significantly associated with the disease risk. Our meta-analysis showed the FEN1 -69GG genotype was correlated to increase risk for the contained diseases compared with the -69AG genotype (OR=0.77, 95%CI=0.71~0.83). Moreover, the FEN1 4150GG genotype could increase diseases risk compared with the 4150TG genotype (OR=0.81, 95%CI=0.75~0.87).

Conclusion: The variant genotypes of the FEN1 -69G/A and FEN1 4150G/T polymorphisms may be associated with diseases susceptibility. However, more studies are needed to detect the disease risk in different ethnic populations.

Keywords: Flap endonuclease 1, Genetic variants, SNPs, Susceptibility, Disease

Introduction

Genome stability is critical to cell survival and normal cell growth such as genome instability caused numerous human cancers (1). It ensures that replication, repair and recombination occur with high fidelity (2). There are still many unknown details of DNA metabolic, because of the multiple functions of proteins that cause diseases. Flap endonuclease1 (FEN1) is one of the DNA metabolism enzyme that possesses multiple functions. FEN1 is a member of the RAD2 structure-specific nuclease family (3). It is best known for its essential roles in the penultimate steps of Okazaki

fragment maturation and 5´-flap removal during long-patch base excision repair (BER) (4, 5). Besides, FEN1 has also been implicated in other major DNA metabolic pathways, including rescue of stalled DNA replication forks, maintenance of telomere stability and apoptotic fragmentation of DNA (6, 7). In addition, FEN1 is also a 5´ exonuclease (EXO) in eliminating heterologous sequences at DNA damage site and facilitating DNA repair by homologous recombination (8). The third activity of FEN1 has been identified is gap-dependent endonuclease (GEN) activity,

which is critical in resolving stalled replication fork (6, 7). FEN1 haploinsufficiency appears to lead to genome instability and carcinogenesis in mice, indicating that the FEN1 gene product is important for development and its expression might be necessary for cell survival or proliferation (9). The expression of its mRNA is up regulated in small cell lung cancer and the protein is high expressed in sorts of lung cells comparing with normal lung cells (10).FEN1 is highly expressed in gastric tumor cell lines compared with normal cell lines (11).

A group of mutations in human FEN1 is associated with several cancers and cause nuclease activity deficiency (12). The disease susceptibility may be increased due to the aberrant expression of FEN1. Therefore, we thought the gene polymorphisms also could affect the risk of diseases. Both -69G/A (rs174538, in the gene promoter region) and 4150G/T(rs4246215, in gene 3′-UTR) SNPs could influence gene expression in vivo (13, 14). Then we identified two SNPs, -69G/A and 4150G/T to analyze the association between these variant and disease risk. Our study included nine diseases in Asians and Caucasians: glioma risk, breast cancer, lung cancer, hepatocellular carcinoma (HCC), esophageal cancer (EC), gastric cancer (GC), colorectal cancer (CRC), keratoconus (KC) and fuchs' endothelial corneal dystrophy (FECD). Cancer is one of serious disease that threatening human health.

Hence, we performed this meta-analysis from these studies and hope it might be potentially important for early identification of individuals with diseases.

Materials and Methods

Search strategy
An extensive literature search for relevant studies was conducted on PubMed and Embase by using the following terms: {"FEN1" or "flap endonuclease 1"} and "disease risk" and {"SNPs" or "polymorphisms" or "variation"}.

Selection criteria
1)   The case-control studies focus on the rela-

tionship between FEN1 gene polymorphisms (including -69G/A and 4150G/T) and disease; 2) studies published from 2009 to 2014; (3) the method of genotype detection was PCR-RFLP; 4) the methods of statistics were appropriate and the data were reliable.

Exclusion criteria
1) Articles excluded based on screening of titles and abstracts, 2) The data in these studies were not exact; 3) studies that had no control group.

Statistical analysis
The ORs with their corresponding 95%CIs were calculated under five genetic models: the allele model, the homozygous model, the heterozygous model, the dominant model, and the recessive model were used to estimating the associations between FEN1 genotypes and risk of the included diseases. Genotype distributions of FEN1 polymorphisms between patients and controls were examined by chi-square test. The significance of the combined OR was determined by Z test and $P<0.05$ was considered as significant. We estimated the degree of heterogeneity among studies using the Q-statistic of Review-Manager software, which is considered significant at $P_H<0.05$. In addition, the I^2 test was used to quantify the heterogeneity (15, 16). When $P_H<0.05$, we used random effects model, else we used fixed-effects model. After that, we constructed the funnel plot to investigate whether publication bias might affect the validity of the estimates. The symmetry of the funnel plot was further evaluated by Egger's linear regression test. All the analyses were calculated using the Review-Manager 5.2 (Cochrane Library Software, Oxford, UK) and Stata 12.0 (Stata Corp, TX, USA) software.

Results

Included studies
Fifteen case-control studies were searched. Five studies in Asians and Caucasians with 5612 cases and 6703 controls were involved (13, 14, 17-19). Publication year ranged from 2009 to 2014. One

study had four different types of diseases: HCC, EC, GC, and CRC. Another study with Caucasian population had two types of disease: KC and FECD, but it only had one control group, so we combined these two diseases for analyzing (Table 1).

Table 1: Characteristics and methodological quality of studies included in meta-analysis

Reference Number	Country/Ethnicity	Disease Type	Genotyping	-69G>A(rs174538)		4150G>T(rs4246215)	
				case AA/GA/GG	control AA/GA/GG	case TT/GT/GG	control TT/GT/GG
13	China/ Asian	glioma risk	PCR-RFLP	35/122/160	130/356/316	34/120/160	130/363/309
14	China/ Asian	HCC	PCR-RFLP	68/290/290	134/334/270	73/295/280	129/335/274
17	China/ Asian	EC	PCR-RFLP	62/249/244	128/331/263	58/255/225	125/336/262
		GC		41/182/189	76/209/169	42/177/183	76/212/166
		CRC		28/104/104	54/144/109	27/102/100	52/145/109
		Breast cancer		116/437/547	246/639/515	134/449/517	246/651/503
18	China/ Asian	Lung cancer	PCR-RFLP TaqMan	253/796/791	354/880/724	271/815/754	358/883/717
19	Poland/ Caucasian	KC, FECD	PCR-RFLP	29/262/213	17/178/127	53/239/212	16/157/149

Association between FEN1 gene polymorphisms (-69G/A and 4150G/T) and susceptibility to disease

The studies on Table 2 were combined to show the result of FEN1 -69G/A and 4150G/T polymorphisms with disease risk, in which diseases were including breast cancer, KC, FECD, glioma risk, HCC, EC, GC, CRC and lung cancer. For the -69G/A polymorphisms, A vs. G: OR=0.74, 95%CI=0.70~0.78, P_H=0.05; AA vs. GG: OR=0.60, 95%CI=0.43~0.83, P_H<0.001; AG vs. GG: OR=0.77, 95%CI=0.71~0.83, P_H=0.41; (AA+AG) vs. GG: OR=0.71, 95%CI=0.66~0.76, P_H=0.17; AA vs. (AG+GG): OR=0.63, 95%CI=0.57~0.70, P_H=0.29. The result from the forest plot showed that FEN1 -69GG was significant associated with diseases (*P*<0.0001). (P_H: *P* value for the heterogeneity test, Fig. 1)
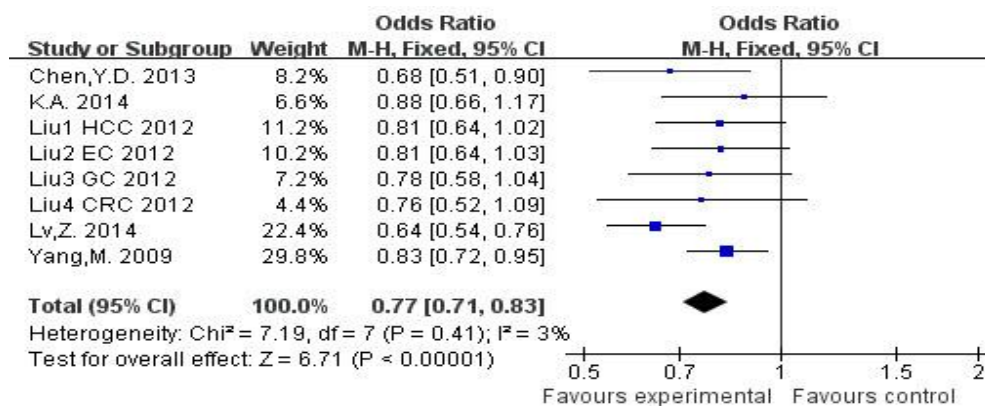

Fig. 1: Forest plot for FEN1 -69G/A polymorphisms and disease risk in the heterozygous model

For the association between the 4150G/T polymorphisms and disease risk, T vs. G: OR=0.79, 95%CI=0.71~0.89, P_H<0.001; TT vs. GG: OR=0.63, 95%CI=0.50~0.80, P_H<0.001; TG vs. GG: OR=0.81, 95%CI=0.75~0.87, P_H=0.07; (TT+TG) vs. GG: OR=0.76, 95%CI=0.67~0.87, P_H=0.005; TT vs. (TG+GG): OR=0.71, 95%CI=0.58~0.86, P_H=0.004. The results revealed that the GG of 4150G/T polymorphisms may be associated with an increasing risk of the

glioma risk, breast cancer, lung cancer, KC and FECD (*P*<0.00001, Fig. 2).

Evaluation of heterogeneity and publication bias

Funnel plot and Egger's test were performed to access the publication bias of literature. The results did not show any evidence of publication bias for FEN1 -69G/A (t=0.03, *P*=0.975; Fig. 3A) or FEN1 4150G/T (*t*=-0.08, *P*=0.937; Fig. 3B).
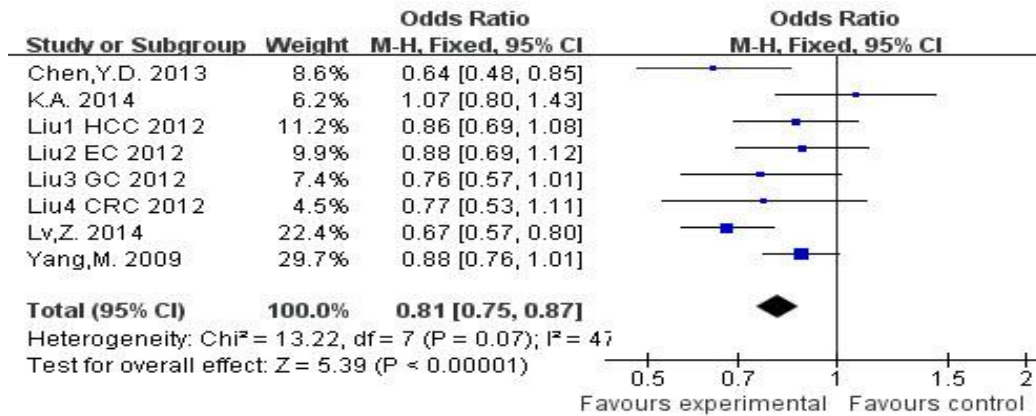

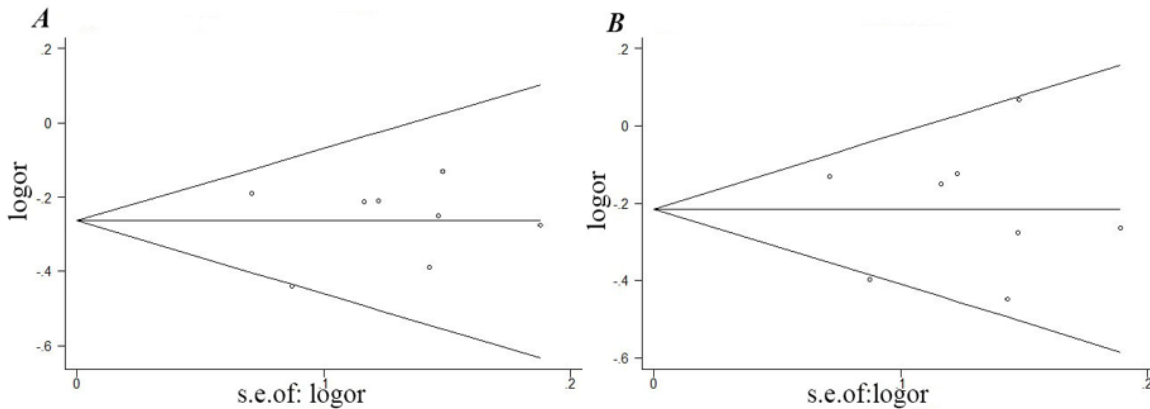**Fig. 2:** Forest plot for FEN1 4150G/T polymorphisms and disease risk in the heterozygous model

Fig. 3: Begg's funnel plots with pseudo 95% confidence limits of FEN1 -68G/A and 4150G/T polymorphisms in the heterozygous model. (A: -69G/A polymorphisms; B: 4150G/T polymorphisms)

Discussion

Our study is the first meta-analysis that reveals the effects of FEN1 -69G/A and 4150G/T polymorphisms to the diseases susceptibility which including the glioma risk, breast cancer, lung cancer, KC

and FECD. FEN1 could increase the susceptibility of cancers (20-23). By analyzing we got that for the -69G/A, the G allele could increase the risk compared with A allele in Asians and Caucasians, and for the 4150G/T, the G allele could increase the risk. About 90% of human DNA sequence

variants are single nucleotide polymorphisms (SNPs) (24). Testing the SNPs is helpful to know complex diseases such as we can get the relationship between the SNPs and relative diseases by using the case-control method (25). As a DNA structure-specific endonuclease, FEN1 plays pivotal roles in maturation of Okazaki fragments, long-patch base excision repair, restarting of stalled replication forks and telomere maintenance, and FEN1 is required for normal cell growth and proliferation (26, 27). Absence of FEN1 expression causes genome instability, for the homozygous, it causes embryonic lethality in mice, and for the heterozygous, it results in decreased survival rates (28). Involved studies confirmed that the -69G>A and 4150G>T have been associated with an increased frequency of serious diseases (13~, 14, 17~19). The -69G>A polymorphism happens in the promoter region, and the 4150G>T occurs in the 3' untranslated region. Both of these polymorphisms can decrease the mRNA expression of FEN1 (16). These SNPs may contribute to disease risk, and could be used as markers for detection of those involved diseases. A lack of FEN1 in haploin sufficient cells could cause the rapid tumor progression (9).

Some limitations of this meta-analysis should be acknowledged. First, the number of the involved studies and populations were limited, and because this is a meta-analysis, so we did not know actual information at the patient levels, it has limited our statistical power to the relationship between gene and environment when analyzing. Secondly, though there were nine different diseases were involved, it is also not enough. The language we selected only in English, so we lacked kinds of other studies.

Although some parts were limited, our results did not show any publication bias by testing using Begg's funnel plot and Egger's testing (19), so this meta-analysis still had some strength.

Conclusion

Our results do support the hypothesis that the SNPs in FEN1 -69G/A and 4150G/T may serve as biological markers of some diseases. Larger and more kinds of populations are needed to confirm our finding.

Ethical considerations

Acknowledgements

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