RESEARCH ARTICLE

Nitric Oxide Synthase 3 Gene Variants and Colorectal Cancer: a Meta-Analysis

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Abstract

Background: Colorectal cancer (CRC) is the worldwide disease which causes enormous losses every year. Recent studies suggested that environmental and gene factors might be the etiologies in increasing the risk of morbidity. Nitric oxide synthase 3 (NOS3) gene polymorphisms are said to be associated with CRC risk but the conclusion is still controversial. Materials and Methods: Pubmed and HuGENet databases up to December 2013 were used in this meta-analysis. Three different certain genotypic models were applied, namely dominant (AA+AC versus CC), recessive (AA versus AC+CC), per-allele analysis (A *vs* C). In addition, information on tumor sites and pathologic stages was collected. The strength of associations was assessed through combining odds ratio (OR) and 95% confidence interval (CI). Results: Finally, five and three studies about the rs1799983 and rs2070744 were covered in the analysis with 2,745 cases and 2,478 controls. Three models were applied, but no significant association was found for NOS3 G894T/rs1799983 (dominant: OR=0.999, 95%CI=0.797-1.253, I^2=63.8%; recessive: OR=0.924, 95%CI=0.589-1.450, I^2=59.3%; allele analysis: OR=0.979, 95%CI=0.788-1.216, I^2=74.9%) and T-786C/rs2070744 (dominant: OR=1.138, 95%CI=0.846-1.530, I^2=67.9%; recessive: OR=0.956, 95%CI=0.708-1.291, I^2=0.0%; allele analysis: OR=1.110, 95%CI=0.865-1.425, I^2=69.4%). The same results were also obtained for tumor sites and pathologic stage subgroups. After further analyzing the NOS3 gene, rs1799983 as the tag- and functional SNP was presented. Conclusions: On the basis of this meta-analysis and the characteristics of the NOS3 gene, we suggested rs1799983 might be a key locus associated with CRC risk. Further prospective studies were needed to make more comprehensive explanation of the associations.

Keywords: Nitric oxide synthase 3 - polymorphism - colorectal cancer

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Introduction

Colorectal cancer (CRC) is the worldwide disease affecting a large number of populations. As a leading malignancy and major cause of mortality, the morbidity increases year by year. In 2009, CRC was the fourth most common cancer in men and the third most common cancer in women worldwide (Center et al., 2009). After one year, Cunningham D et al announce the incidences pulled them up one place among each sex, which indicated more than 1 million individuals would develop colorectal cancer (Cunningham et al., 2010). Otherwise, the prevalence of CRC varied greatly from country to country. Among Jordanian, it was the first most common form of cancer affecting male population and it accounted for 12.7% and 10.5% of all newly diagnosed male and female cancers respectively in 2009 (Tarawneh et al., 2009). At the same time, in United States, about 150,000 new cases of colorectal cancer and 51,000 deaths were expected (Jemal et al., 2010). In addition, 6.5% and 4.6% of the total cancer in urban and rural areas had been revealed in China (Zhao et al., 2010).

Lifestyles and several genetic factors were said to be associated predominantly with CRC risk (Ames, 1983; Pfeifer et al., 2002). As related genes, nitric oxide synthase 3 (NOS3) located on 7q36 in humane was identified to play a key role in angiogenesis and many diseases (Dan et al., 2013; Verim et al., 2013; Brankovic et al., 2013) with endothelial NOS (eNOS) encoded. In living organisms, nitric oxide (NO) taking part in many physiological and pathophysiological processes including cell growth, apoptosis, neurotransmission, and immunological regulation was produced with eNOS catalyzed (Kelly et al., 1996; Razavi et al., 2005). In 2012, Aguilar-Melero P et al (Aguilar-Melero et al., 2012) indicated the significant association of NOS3 and cell survival in a hepatoma cell line. While Oh et al. (2013) observed the expression of endothelial nitric oxide synthase was significantly higher in the uterus with leiomyoma or adenomyosis. When investigating the functions of the NOS3 gene in cancers, Terrazzino et al. (2012) suggested NOS3 might be the marker for predicting acute skin toxicity in breast cancer patients receiving radiotherapy. In addition, in 1996, Moochhala et al (1996) concluded NOS was present and

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active within the epithelium of the normal colon, while less activity in colonic neoplasms. However, Yagihashi et al. (2000) discovered in human colorectal cancers the enhanced expression of iNOS and eNOS were presented.

Recently, there were increasing studies focused on polymorphisms of NOS3 gene and CRC risk attempting to discover the genetic factors. Among the various SNPs, three putatively functional, common polymorphisms (G894T, 4a4b, T-786C) associated with cardiovascular diseases were identified (Fatini et al., 2004). Although, there were many studies suggested the correlation in the NOS3 polymorphisms and CRC risk, the conclusions were controversial. In order to study the real function of the NOS3 polymorphisms in CRC risk, this study was conducted with two SNP (G894T/rs1799983, T-786C/rs2070744) involved.

Materials and Methods

Search strategy and data extraction

PubMed and HuGENet were combined in this study with the keywords: 'Endothelial NOS', 'Endothelial nitric oxide synthase', 'eNOS, 'nitric oxide synthase 3', 'NOS3', 'constitutive NOS', 'cNOS', 'colorectal cancer', 'colorectal carcinoma', 'colon cancer', 'colon carcinoma', 'rectal carcinoma', 'adenocarcinoma', 'CRC', 'polymorphism'. The latest search was performed in December 2013. The languages of articles were restricted to English. All articles in the meta-analysis were published in the primary literature and not replicated with other studies. The other papers were discovered according to the references in the included studies. The inclusion criteria were as follows: (1) the studies were performed as case-control, cohort or case-only studies of the association between the NOS3 polymorphism and risk of colorectal cancer. (2) studies must provide the total number of samples, and also the number of cases and controls for separate genotypes. (3) the cases were the CRC patients regardless of tumor stage or histological, and healthy patients or those without colorectal cancer were defined as controls.

In addition, studies were restricted as follows for exclusion criteria: (1) the classifications in studies made it hard to screen cases and controls. (2) studies didn't gotten genotype data which can be used in the study. (3) studies didn't focus on the CRC risk and gene polymorphism but the chemotherapy, the survival rate or other factors. The flow diagram of the retrieval was showed in Figure 1.

The following data was recorded for each study: author, year of publication, country of origin, the race of the samples (categorized as Caucasian and Asian), number of cases and controls for each studies, the methods of the detection, kinds of cases and controls (categorized as CRC, health, other disease and noncancerous). (Table 1)

Statistical analysis

According to the studies included in our meta-analysis, the genotype data were used to test Hardy-Weinberg equilibrium (HWE; $p>0.05$) in control group (Egger et al., 1997). In order to present the comprehensive results of associations, three different certain genotypic models were applied, namely dominant (AA+AC versus CC), recessive (AA versus AC+CC), per-allele analysis (A vs C). In addition, information of tumor sites and pathologic stages were collected. The sites were defined as colon and rectum. As for pathologic stages, two groups (T≤2 and T>2) were involved with the dominant genotypic model (AA+AC versus CC) in the following calculation. The effect of the association was revealed by the odds ratio (OR) with a corresponding 95% confidence interval (CI). The pooled OR was estimated according to the individual ORs. I^2 might be a standard to distinguish the fixed effect from random effects (I^2 <50% referred to the fixed effect, otherwise the random effect was used). Meanwhile, the effects incorporated an estimate of the inter-study variance and provided wider 95% confidence intervals (95%CI), if the results of the constituent studies differed among themselves. Then to estimated heterogeneity the chisquare based Q statistic ($p<0.10$ as the standard) was used (Lau et al., 1997), that represented the weighted sum of the squared difference between the overall effect sizes from each study. As for the resources of heterogeneity, we categorized subgroups which were collected together with similar characteristics (namely country, continent and ethnicity and so on). The country could be classified into 5 in total: Spain, China, South Korea, Germany, and Turkish, which were categorized into three continents: Europe, Asia. As for the ethnic subgroups, there were two groups: Caucasian and Asian.

Publication bias was diagnosed both visually by using a funnel plot and Begg's unweighted regression test statistically. All of the allele frequencies were calculated for studies reporting only genotype data. Stata 9.0 (Stata Corporation, USA) was used to analyze, and all P values were two-tailed.

After the traditional meta-analysis, we tried to describe the loci we concerned. According to SNPinfo (http://manticore.niehs.nih.gov/snpinfo/snptag.htm), tagSNPs were identified, with common SNPs were involved (minor allele frequency, MAF≥0.05; r2≥ 0.8) among

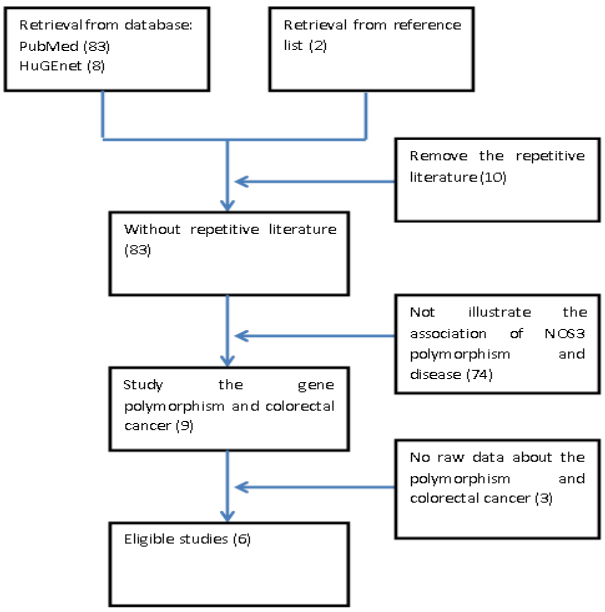

Figure 1. The Flow Diagram for the Literature Screening of Our Meta-analysis

the CHB+CHD+CEU+JPT population. Meanwhile, the functional SNPs were also predicted (http://manticore.niehs.nih.gov/snpinfo/snpfunc.htm). Combine with the results of our study and the characteristics of these loci we included, our study could illustrate the real association of the NOS3 gene polymorphisms and CRC risk comprehensively which might pave way for the targeted genetic therapy of the CRC.

Results

Study characteristics

According to the PubMed and HuGENet database with associated key words, 93 articles were retrieved. When reading all the abstracts, we left over 9 papers with association of CRC risk and NOS3 gene involved (Conde et al., 2006; Ikeda et al., 2008; Funke et al., 2009a; Funke et al., 2009b; Yeh et al., 2009; Ho-Pun-Cheung et al., 2011; Kim et al., 2011; Arıkan et al., 2012; Jang et al., 2013), after excluding the articles that didn't satisfy our criteria. While reviewing all the papers, we removed 3 articles as follows: (1) two studies mainly described the radiotherapy and chemotherapy without raw data of genotypes in the CRC and control groups (Funke et al., 2009b; Ho-Pun-Cheung et al., 2011); (2) although one study investigated the candidate genes polymorphism and CRC risk, there was no data about the each allele (Ikeda et al., 2008). Then, 6 articles with the eligible data met the inclusion criteria were included (three had the data of rs2070744 and five about the rs1799983) (Conde et al., 2006; Yeh et al., 2009; Funke et al., 2009a; Kim et al., 2011; Arıkan et al., 2012; Jang et al., 2013), in which one study only collected the CRC samples (Kim et al., 2011). The characteristics and H-W for the studies were showed in the Table 1. As for two subgroups about tumor sites and pathologic stages, five sets of data could be extracted for rs2070744 and six for the rs1799983 (Yeh et al., 2009; Kim et al., 2011; Arıkan et al., 2012; Jang et al., 2013). Within the eligible studies, 3 was involved with Caucasian populations (Conde et al., 2006; Funke et al., 2009a; Arıkan et al., 2012), the others investigated Asian patients (Yeh et al., 2009; Kim et al., 2011; Jang et al., 2013). Their countries are the as follows: Spain, China, South Korea, Germany, and Turkish. Almost all the cases came from the hospital and were diagnosed with the biopsy. Nevertheless, we indicated the controls as the ones who had other diseases, a history of other cancers, or healthy volunteers.

Subgroup analyses

In order to discover the real association in the CRC risk and NOS3 polymorphisms (rs2070744 and rs1799983), two common models (dominant and recessive model) were applied in the analysis with the different effects (I^2 <50%: the fixed effects; I^2 >50%: the random effects). In addition, the per-allele analysis was showed in our study. In every statistical analysis, two different subgroups were divided by the races (Caucasian and Asian). All results were showed in the Table 2. The whole pooled results showed that there seemed no significant relationship in the NOS3 G894T/rs1799983 (dominant: OR=0.999, 95% CI=0.797-1.253, I^2=63.8%; recessive: OR=0.924, 95% CI=0.589-1.450, I^2=59.3%; allele analysis: OR=0.979, 95% CI=0.788-1.216, I^2=74.9%), T-786C/rs2070744 (dominant: OR=1.138, 95% CI=0.846-1.530, I^2=67.9%; recessive: OR=0.956, 95% CI=0.708-1.291, I^2=0.0%; allele analysis: OR=1.110, 95% CI=0.865-1.425, I^2=69.4%) polymorphism and CRC risk, which lead us to do the subgroup analysis. Even so, the positive results didn't emerge. The results suggested there was no association neither among the Caucasian nor Asian.

Table 1. Characteristic of the Eligible Studies

Author	years	country	race	sample	method	loci	case	control	case	control	H-W
Conde MC et al	2006	Spain	Caucasian	blood	PCR	rs2070744	CRC	controls	368	547	0.9779
Yeh CC et al	2009	China	Asian	blood	PCR	rs2070744	CRC	without cancer	683	726	0.8687
Jang MJ et al	2013	South Korea	Asian	leukocytes	PCR	rs2070744	CRC	without thrombotic diseases or cancer	528	509	0.8207
Kim YJ et al	2011	Korea	Asian	fresh colorectal tissue	PCR	rs2070744	CRC	NA	444	NA	NA
Conde MC et al	2006	Spain	Caucasian	blood	PCR	rs1799983	CRC	controls	355	538	0.09
Yeh CC et al	2009	China	Asian	blood	PCR	rs1799983	CRC	without cancer	702	728	0.744
Funke S et al	2009	Germany	Caucasian	whole-blood	PCR	rs1799983	CRC	without cancer	632	604	0.547
Arıkan S et al	2012	Turkish	Caucasian	blood	PCR	rs1799983	CRC	health	84	99	0.8996
Jang MJ et al	2013	South Korea	Asian	leukocytes	PCR	rs1799983	CRC	without thrombotic diseases or cancer	528	509	0.484
Kim YJ et al	2011	Korea	Asian	fresh colorectal tissue	PCR	rs1799983	CRC	NA	444	NA	NA

*PCR: Polymerase Chain Reaction; CRC: colorectal cancer

Table 2. Dominant Model (AA+AC *vs* CC), Recessive Model (AA *vs* AC+CC) and Allele Analysis (A *vs* C) in the two Groups (Asian and Caucasian) were Applied in this Meta-analysis

Loci		dominant (AA+AC *vs* CC)				recessive (AA *vs* AC+CC)				allele analysis (A *vs* C)			
		OR	95%CI	I^2	*p*	OR	95%CI	I^2	*p*	OR	95%CI	I^2	*p*
rs2070744	Asian	1.253	0.836-1.878	74.40%	0.0048	1.228	0.528-2.853	0.00%	0.973	1.224	0.871-1.719	68.90%	0.073
	Caucasian	0.939	0.701-1.258	NA	NA	0.922	0.668-1.272	NA	NA	0.948	0.786-1.143	NA	NA
	All	1.138	0.846-1.530	67.90%	0.044	0.956	0.708-1.291	0.00%	0.823	1.11	0.865-1.425	69.40%	0.038
rs1799983	Asian	1.132	0.689-1.858	82.80%	0.016	1.852	0.463-7.411	60.80%	0.11	1.158	0.706-1.900	85.30%	0.009
	Caucasian	0.922	0.695-1.223	55.50%	0.105	0.794	0.493-1.279	65.40%	0.055	0.886	0.680-1.155	73.30%	0.024
	All	0.999	0.797-1.253	63.80%	0.026	0.924	0.589-1.450	59.30%	0.043	0.979	0.788-1.216	74.90%	0.003

*A stands for the mutant type of the allele and C is the wild allele; *rs1799983 was the TagSNP and functional loci

Table 3. Two Groups (Tumor site; Pathologic stage) were Collected in the Meta-Analysis

		OR	95%CI	I^2	*p*
rs2070744					
Tumor site	Asian	1.024	0.802-1.308	0.00%	0.914
	Caucasian	NA	NA	NA	NA
	All	1.024	0.802-1.308	0.00%	0.914
Pathologic stage	Asian	1.027	0.756-1.395	0.00%	0.327
	Caucasian	NA	NA	NA	NA
	All	1.027	0.756-1.395	0.00%	0.327
rs1799983					
Tumor site	Asian	0.927	0.726-1.184	42.60%	0.175
	Caucasian	NA	NA	NA	NA
	All	0.927	0.726-1.184	42.60%	0.175
Pathologic stage	Asian	0.856	0.623-1.174	0.00%	0.395
	Caucasian	2.5	0.627-9.970	NA	NA
	All	0.91	0.670-1.237	31.50%	0.232

Tumor site: the sites were defined as colon and rectum; Pathologic stage: T≤2 and T>2; The genotype was presented in (AA+ AC *vs* CC); A stands for the mutant type of the allele and C is the wild allele

In order to understand the NOS3 gene more comprehensively, tagSNPs and functional loci in this gene were collected. The results suggested that rs1799983 was one of the tagSNPs which was said to be a representative single nucleotide polymorphism (SNP) in a region of the genome with high linkage disequilibrium. In addition, after predicting the functions of the SNPs in NOS3 gene, rs1799983 was also identified as non-synonymous mutation with splicing (ESE or ESS) function. However, the same features could not found for rs2070744.

Among the eligible studies we included, one collected only the CRC samples with the information of tumor sites and pathologic stage involved. After integrating all the eligible studies, tumor sites were identified as colon and rectum. The pathologic stage was also divided into two groups: T≤2 and T>2. Dominant genotypic model (AA +AC versus CC) was applied in the following statistical analysis. All the results were presented in Table 3, which did not supply the prominent results in any subgroups and pooled results.

Discussion

CRC was one of the terrible diseases with a large number of populations involved. In order to discover the pathogenesis, many studies had conducted on various aspects such as genetic, life styles and environment. Recently, some studies indicated the significant association in the genes polymorphisms and CRC risk (Chaleshi et al., 2013; Kassab et al., 2014). NOS3, as one of the key gene, was said to play a key role in cancers and angiogenesis. In order to discover the real correlation in NOS3 gene polymorphisms and CRC risk, this study was conducted with available studies on the basis of controversial conclusions. Although combining with powerful analysis, we could not find any association in the NOS3 polymorphisms (G894T/rs1799983, T-786C/rs2070744) and CRC risk, we suggested rs1799983 as the tag- and functional SNP could be more vital than rs2070744 for the development and progression of CRC, which could provide a promising genetic target for further researches about the NOS3 polymorphism and CRC risk.

In order to study the real association in NOS3 gene polymorphisms and CRC risk, the dominant, recessive and pre-allele analysis were conducted. In addition, the tumor sites and pathologic stages were also contained in our analysis. However, although with the powerful statistical analysis, we could not discover any associations in the two loci (G894T/rs1799983, T-786C/rs2070744) and CRC risk or the tumor sites and pathologic stages. According to the recent studies, the significant corrections in these two NOS3 polymorphisms were confirmed even though the reverse opinions presented, (Yeh et al., 2009; Jang et al., 2013) which promoted us to study these concerned polymorphisms further. After seeking for the essential characteristics of NOS3 gene, we found that rs1799983 was the non-synonymous mutation with splicing (ESE or ESS) function. On the basis of the function and key position, we supposed that the rs1799983 might play a key role in the development and progression of CRC. In 2013, the conclusion was identified by Jang MJ et al in Korean population (Jang et al., 2012). Rs1799983 was a G/T variation on human chromosome 7. In 2011, Ryk et al said it might influence bladder cancer risk (Ryk et al., 2011). Hildebrandt MA et al designs to study genetic variants and non-small cell lung cancer, which displayed a protective effect in pneumonitis risk (Hildebrandt et al., 2010). In addition, a meta-analysis was conducted by Yao et al in 2010, declaring that rs1799983 was associated with reduced breast cancer risk (Yao et al., 2010). Furthermore, rs1799983 was also said to be related to many other diseases such as recurrent miscarriage (Luo et al., 2013) and cardiovascular diseases (Fraga et al., 2013). Considering the important role of NOS3 gene in the cancers and vasculopathy, we suggested that as one of the promising SNP rs1799983 might be the key polymorphism of NOS3 gene influencing CRC risk which might be useful in the genetic target therapy. The further and deeper researches were needed in urgent.

With effective studies and statistical analysis, our study reveal there is no significant association in the CRC risk and NOS3 gene polymorphism. Nevertheless there are still some limitations of the eligible studies. (1) Regardless of the rigorous and extensive retrieval we had conducted, there might be some studies left out because of the shortness in every database, the restrictive language, or misunderstand the abstracts and so on, which would influence the results and lead to the bias. (2) Among the eligible studies, they included the samples came from various area with different inclusion criterions and definition of the samples. (3) In addition, only focusing on the relationship between the genetic polymorphism and the diseases, but ignoring the environment function was also a source of limitation.

In conclusions, CRC was a disease influencing a wide range of populations around the word. The pathogenesis had not been clear with many aspects involved. As one of the factors, genetic polymorphisms were said to play a key role in the CRC risk. NOS3 gene was identified to be associated with the CRC. After analyzing the available studies statistically, we proposed that rs1799983 might be the promising locus which could affect the development and progression of CRC. But the further studies were needed to confirm this.

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