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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

Lack of association between endothelial nitric oxide synthase (eNOS) *G894T* gene polymorphism and the risk of bladder cancer
A meta-analysis

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2022, 39(5):647–653

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OBJECTIVE **To assess the relationship of bladder cancer risk with eNOS *G894T* gene polymorphism using meta-analysis.** METHOD **The relevant articles were retrieved as of 30 August 2020 from PubMed and ScienceDirect. The associations were estimated using the calculation of pooled odds ratio (OR) and 95% confidence interval (CIs).** RESULTS **Four studies, covering a total of 834 cases and 16,80 control subjects, regarding the eNOS *G894T* gene variant and the risk of bladder cancer were identified and used in our analysis. Our pooled calculation revealed no overall association between the risk of bladder cancer and eNOS *G894T* gene polymorphism in all genetic modes, including G allele (OR: 0.81 [95% CI: 0.63–1.05], p=0.11), T allele (OR: 1.23 [95% CI: 0.95–1.58], p=0.11), GG genotype (OR: 0.52 [95% CI: 0.25–1.06], p=0.07), GT genotype (OR: 1.59 [95% CI: 0.85–2.98], p=0.143), and TT genotype (OR: 1.15 [95% CI: 0.76–1.73], p=0,51).** CONCLUSIONS **Our meta-analysis revealed that the gene polymorphism of eNOS *G894T* does not affect the risk of bladder cancer.**

Απουσία συσχέτισης μεταξύ πολυμορφισμού του γονιδίου *G894T* της συνθετάσης του ενδοθηλιακού οξειδίου του αζώτου (eNOS) και του κινδύνου καρκίνου της κύστης: Μια μετα-ανάλυση

Περίληψη στο τέλος του άρθρου

Key words

Bladder cancer
eNOS *G894T*
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Meta-analysis

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Bladder cancer continues to be a global problem, and is reported as one of the most common forms of cancer worldwide.[1] Several factors may contribute to the development of bladder cancer, of which tobacco exposure is recognized as the leading factor.[2] Other risk factors may also contribute to the development of bladder cancer, including exposure to carcinogenic agents in particular industries, schistosomiasis, and also genetic factors.[3,4]

In the context of genetics, certain molecules that may affect the physiological process, exerting cytotoxic effects are proposed as affecting the risk of bladder cancer. One of these is nitric oxide (NO).[5,6] NO synthase (NOS) has three isoforms, derived from different genes.[7] The NOS isoforms that produce NO in the vascular endothelium are defined as endothelial NOS (eNOS).[7,8] eNOS plays a role as a catalyst in NO formation, and is encoded by a gene located on chromosome 7q35–36.[8] eNOS can produce NO by the substitution of L-arginine by L-citrulline, and regulates the expression of the proinflammatory molecules.[9]

An eNOS gene polymorphism that is capable of modifying the transcription and producing NO has been identified: a guanine to thymine polymorphism at position 894 or G894T variation (rs1799983) in exon 7.[10] The *G894T* gene affects the susceptibility to develop various cancers, including prostate and laryngeal cancer.[11,12] Because of lack of evidence regarding eNOS *G894T* and bladder cancer, the understanding regarding eNOS *G894T* in the development of bladder cancer has remained unclear. Therefore, in this

meta-analysis, we aimed to investigate the relationship between the risk of bladder cancer and the gene variant of eNOS *G894T*.

MATERIAL AND METHOD

Study designs

This meta-analysis study was conducted in August 2020 to gain evidence about eNOS gene polymorphisms (eNOS *G894T*) as a factor possibly associated with bladder cancer. We followed the checklist from Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines to guide the protocols of our study.[13] The association was estimated using the calculation of pooled odds ratio (OR) and 95% confidence interval (CI).

Search strategy and data extraction

The literature search was conducted in PubMed and ScienceDirect as of 30 August 2020, using the combination of keywords: "Endothelial nitric oxide synthase or eNOS" and "gene polymorphism or *G894T*" and "bladder cancer or bladder neoplasm". The key words were adapted from medical subject heading (MeSH). We restricted the publication language to English.

Three authors (KR, AP, BS) selected eligible studies independently. Data extraction comprised the first author's name, publication year, ethnicity, country, the frequency of genotype and allele in both cases and control subjects, and Hardy-Weinberg equilibrium (HWE) calculation. Any discrepancy was resolved through discussion among authors, especially with the senior researcher (JKF).

Eligibility criteria

The inclusion criteria were: cross-sectional studies, randomized-controlled trials (RCTs), cohort studies and retrospective studies, evaluating the relation between the risk of bladder cancers and eNOS *G894T* polymorphism. Studies were excluded if they met the exclusion criteria: irrelevant title or abstract, commentary, review, family-based study, incomplete data, duplication of study data, and indication of deviation from HWE.[14]

Assessment of the methodological quality

All the studies included were evaluated by three authors (KR, AP, BS), using the 9-point Newcastle-Ottawa Scale (NOS) as standard quality control. The structure of this assessment consisted of the selection (3 checklist items), the comparability (1 checklist item), and the exposure (3 checklist items). The NOS score ranged between zero and nine: zero to three indicated low quality, four to six indicated moderate quality, and seven to nine suggested high quality.[15]

Statistical analysis

Three authors (KR, AP, BS) performed analysis independently using the comprehensive meta-analysis software (CMA version 3.3.070, New Jersey, USA) and review manager (Revman Cochrane version 5.3, London, UK). The association and OR and 95% CI between the risk of badder cancer and eNOS *G894T* polymorphism were calculated by a Z test, and a Q test was used to determine the random or fixed effect model in heterogeneity assessment. The fixed effect model was selected if no heterogeneity among the studies was found ($p>0.10$), while the random effect model was selected if heterogeneity was observed among studies ($p<0.10$). Additionally, the Egger test was conducted to determine publication bias ($p<0.05$). The genetic models of eNOS *G894T* gene polymorphism included T versus G; G versus T; TT versus GG+GT; GT versus GG+TT; and GG versus GT+TT.

RESULTS

Eligible studies

Four eligible studies were included in the meta-analysis. As shown in figure 1, 124 potential studies were identified from PubMed and ScienceDirect, of which we excluded 116 studies because of irrelevant titles and abstracts. Finally, only four studies were included because the remaining studies did not meet the eligibility criteria. The quality assessment of all the selected studies showed moderate to high quality (NOS=5–8). Table 1 presents the baseline characteristics of the four studies included in our analysis.

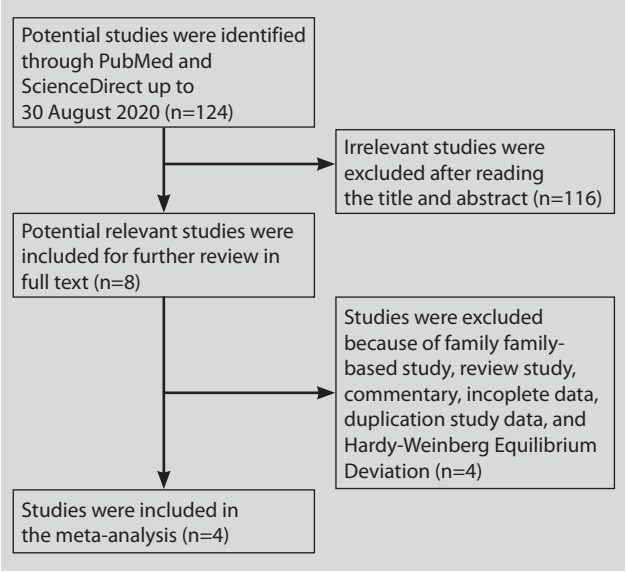

Figure 1. Bladder cancer and eNOS *G894T* polymorphism: A flow diagram of the literature search strategies for the meta-analysis.

Table 1. Bladder cancer and eNOS *G894T* polymorphism: Characteristics of the four relevant studies included in the meta-analysis.

Author and year	Case				Control				Ethnicity	Genotyping	X^2 HWE	NOS [S/C/E]	Conflict
	GG	GT	TT	N	GG	GT	TT	N					
Polat et al,[17] 2015	7	59	9	75	48	75	20	143	Caucasian	PCR	1.178	8 [4/1/3]	Significant association
Tsay et al,[19] 2019	343	80	8	431	676	176	10	862	Asian	PCR	0.148	5 [3/1/1]	No significant association
Ryk et al,[16] 2011	128	106	28	262	75	62	13	150	Caucasian	PCR	0.001	6 [3/1/2]	No significant association
Verim et al,[18] 2013	7	49	10	66	31	44	13	88	Caucasian	PCR	0.167	6 [3/2/1]	Significant association

NOS: Newcastle-Ottawa Scale, S: Selection, C: Comparability, E: Exposure, HWE: Hardy-Weinberg equilibrium, PCR: Polymerase chain reaction

Data synthesis

Four selected studies of eNOS *G894T*, covering a total of 834 cases and 1,680 control subjects, were registered in our analysis. Table 2 summarizes the main findings. No significant association was found between bladder cancer and eNOS *G894T* gene polymorphism in G allele (OR: 0.81 [95% CI: 0.63–1.05], p=0.11), T allele (OR: 1.23 [95% CI: 0.95–1.58], p=0.11), GG genotype (OR: 0.52 [95% CI: 0.25–1.06], p=0.07), GT genotype (OR: 1.59 [95% CI: 0.85–2.98], p=0.143), and TT genotype (OR: 1.15 [95% CI: 0.76–1.73], p=0,51).

Heterogeneity among studies

Heterogeneity was observed among the studies in all genotypes and alleles (T versus G; G versus T; TT versus GG+GT; GT versus GG+TT; and GG versus GT+TT) (figures 2, 3). Table 2 presents the evidence of heterogeneity in our study. The model of random effect was selected to assess the relationships in all genetic models.

Potential publication bias

We assessed the publication bias within all of the included studies using Egger's test and Funnel plot. Publication bias was detected in the TT genotype (p<0.001) (fig. 4), but, otherwise, no publication bias was detected in the other genetic models.

DISCUSSION

We identified four studies assessing the association between the risk of bladder cancer and eNOS *G894T* polymorphism. Two studies reported a significant association between the *G894T* gene and bladder cancer, while two other studies failed to detect an association.[16–19] Our meta-analysis indicated that, overall, no association was observed between the gene polymorphism of *G894T* and the risk of bladder cancer. The role of eNOS *G894T* gene polymorphism in the development of bladder cancer remains debatable. Theoretically, eNOS is proposed to affect the pathological process in various cancers through angiogenesis and metastasis.[9] One study also reported that the eNOS *G894T* gene was found in the individuals of both Caucasian and Asian origin susceptible to cancer,[20] but the involvement of eNOS *G894T* in the development of cancer was reported for different types of cancer, specifically breast and prostate cancer.[21–23]

Table 2. The relationship of bladder cancer with eNOS *G894T* polymorphism in summary.

Allele and genotype	NS	Model	Value		OR	95% CI	pH	pE	p
			Case (%)	Control (%)					
Overall analysis									
G versus T	4	Random	75.78	81.13	0.82	0.63–1.05	0.091	0.187	0.11
T versus G	4	Random	24.22	18.87	1.23	0.95–1.58	0.091	0.187	0.11
GG versus GT+TT	4	Random	58.15	66.77	0.52	0.25–1.06	<0.000	0.651	0.07
GT versus GG+TT	4	Random	35.25	28.72	1.59	0.85–2.98	<0.000	0.580	0.143
TT versus GG+GT	4	Random	6.59	4.51	1.15	0.76–1.73	0.761	<0.000	0.509

pE: p egger, pH: p heterogeneity, CI: Confidence interval, OR: Odds ratio, NS: Number of studies

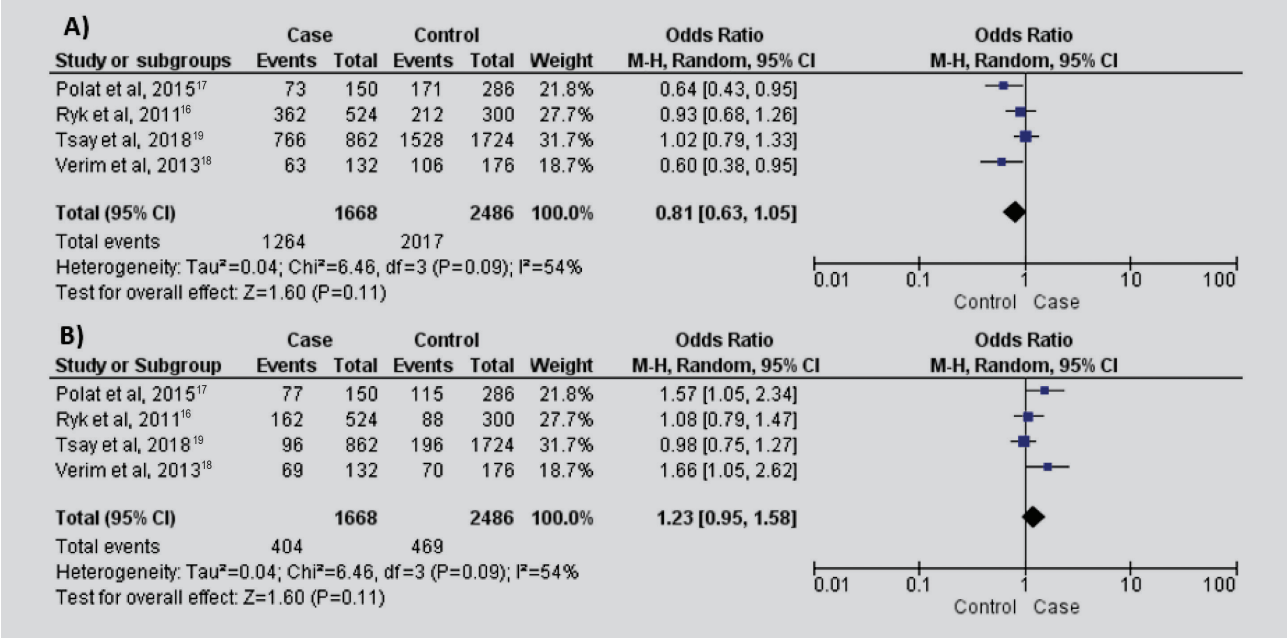

Figure 2. The relationship of bladder cancer with eNOS *G894T* polymorphism in forest plot: 2A (G versus T), and 2B (T versus G). Overall, no relationship.

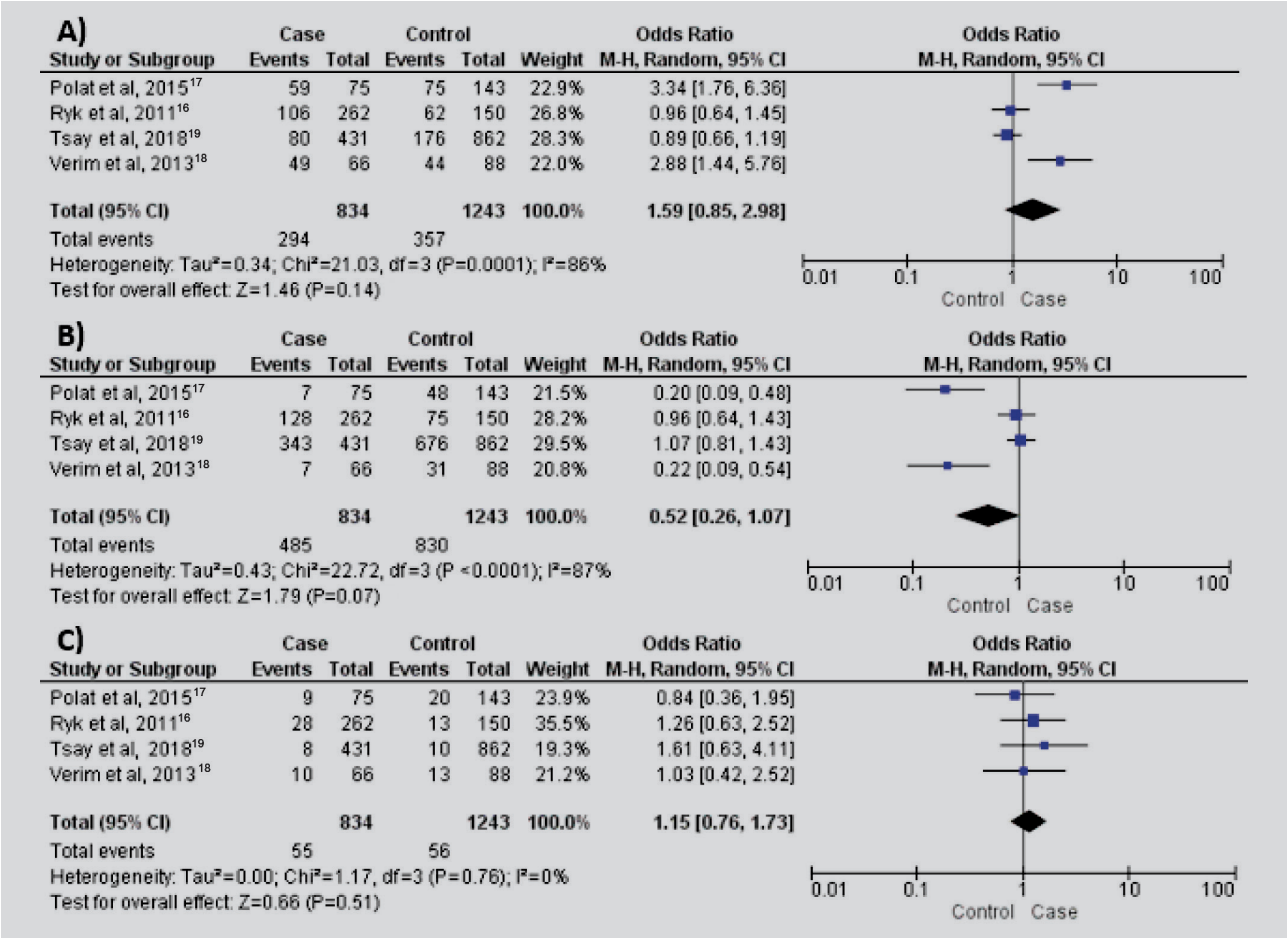

Figure 3. The relationship of bladder cancer with eNOS *G894T* polymorphism in forest plot: 3A (GG versus GT+TT), 3B (GT versus GG+TT), and 3C (TT versus GG+GT): Overall, no relationship.

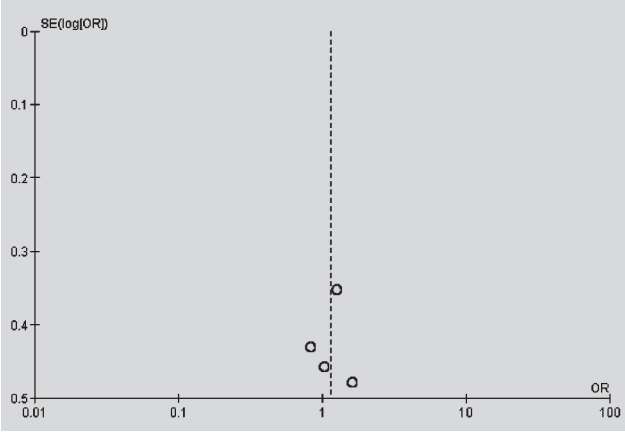

Figure 4. The association of eNOS *G894T* and risk of bladder cancer in Funnel plot.

This study might not confirm the relationship between eNOS *G894T* gene polymorphism and bladder cancer, clearly due to limitation of data. In addition, single-gene polymorphism, alone, might not be adequate to describe the potential relationship in the context of bladder cancer without considering the holistic picture.[20] Several other factors that might govern the gene variant in the case of bladder cancer should be considered. One study revealed that occupational exposures contributed to the risk of bladder cancer disease in certain populations, such as laboratory technicians and workers with industrial machinery.[24] Older age may escalate the risk, and the incidence becomes higher at the peak age of 85 years.[25] Hormones were also a contributing factor among women with menopause at an earlier age.[26,27] The risk of bladder cancer can be minimized by a higher intake of total whole grain and dietary fiber, and by smoking cessation in smokers.[28,29] The findings of our meta-analysis need to be clarified by further studies.

Our meta-analysis assessed the potential role of eNOS *G894T* in the development of bladder cancer, based on the findings of four studies. This analysis failed to confirm the role of eNOS *G894T* in the case of bladder cancer, as the evidence was insufficient. These findings should not be regarded as final. Describing the association between gene polymorphism and specific disease is complex, and may involve a wide variety of factors. Further studies, including only RCTs, or with larger sample size and involving gene-gene interaction or gene-environment interaction are warranted to reveal the real association between the gene variant of eNOS *G894T* and the risk of bladder cancer.

Potential confounding biases in this study that might affect the results were the non-randomized design and the small sample sizes. Two of these studies reported a significant association between the *G894T* gene with bladder cancer while the two other studies found no association, suggesting that the population for our meta-analysis was insufficient to reach definite conclusions. Therefore, further study with better design and larger sample sizes are required.

In conclusion, our meta-analysis revealed that the gene polymorphism of eNOS *G894T* does not have an association with the risk of bladder cancer, but these findings should be re-clarified in the near future by studies with larger sample sizes.

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ΠΕΡΙΛΗΨΗ

Απουσία συσχέτισης μεταξύ πολυμορφισμού του γονιδίου *G894T* της συνθετάσης του ενδοθηλιακού οξειδίου του αζώτου (eNOS) και του κινδύνου καρκίνου της κύστης: Μια μετα-ανάλυση

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Αρχεία Ελληνικής Ιατρικής 2022, 39(5):647–653

ΣΚΟΠΟΣ Μετα-ανάλυση για την εκτίμηση της σχέσης μεταξύ του κινδύνου καρκίνου της ουροδόχου κύστης και του πολυμορφισμού του γονιδίου *G894T* της eNOS. **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Τα σχετικά άρθρα ανακτήθηκαν στις 30 Αυγούστου 2020 από το PubMed και το ScienceDirect. Η συσχέτιση εκτιμήθηκε, εφαρμόζοντας τον υπολογισμό του συντελεστή απόδοσης (OR) και του 95% διαστήματος εμπιστοσύνης (CI). **ΑΠΟΤΕΛΕΣΜΑΤΑ** Συμπεριλήφθηκαν 4 μελέτες

(834 περιπτώσεις και 1.680 μάρτυρες) σχετικά με την παραλλαγή γονιδίου *G894T* της eNOS και τον κίνδυνο καρκίνου της ουροδόχου κύστης. Δεν βρέθηκε συσχέτιση μεταξύ του κινδύνου καρκίνου της ουροδόχου κύστης και του πολυμορφισμού του γονιδίου *G894T* της eNOS σε όλους τους γενετικούς τρόπους, περιλαμβανομένου του αλληλίου G (OR: 0,81 [95% CI: 0,63–1,05], p=0,11), του αλληλίου T (OR: 1,23 [95% CI: 0,95–1,58], p=0,11), του γονοτύπου GG (OR: 0,52 [95% CI: 0,25–1,06], p=0,07), του γονοτύπου GT (OR: 1,59 [95% CI: 0,85–2,98], p=0,143), και του γονοτύπου TT (OR: 1,15 [95% CI: 0,76–1,73], p=0,51). **ΣΥΜΠΕΡΑΣΜΑΤΑ** Η μελέτη αποκάλυψε ότι ο γονιδιακός πολυμορφισμός του *G894T* της eNOS δεν επηρεάζει τον κίνδυνο εμφάνισης καρκίνου της ουροδόχου κύστης.

Λέξεις ευρετηρίου: Γονιδιακός πολυμορφισμός, eNOS, *G894T*, Καρκίνος ουροδόχου κύστης

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