REVIEW

Glutathione S–Transferase Polymorphisms (GSTM1, GSTT1, GSTP1) and Male Factor Infertility Risk

A Pooled Analysis of Studies

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Purpose: To determine the role of glutathione S-transferases (GSTs; GSTM1, GSTT1, and GSTP1) gene polymorphisms in susceptibility to male factor infertility.

Materials and Methods: We report a pooled analysis of 11 studies on the association of GSTM1, GSTT1, and GSTP1 polymorphisms and male factor infertility, including 1323 cases and 1054 controls.

Results: An overall significant association was determined between the GSTM1 null genotype [odds ratio (OR), 2.74; 95% confidence interval (CI), 1.72 to 3.84; $P = .003$], GSTT1 null genotype (OR, 1.54; 95% CI, 1.43 to 3.47; $P = .02$), and male factor infertility. The GSTP1 Ile/Val genotype had overall protective effect against development of infertility (OR, 0.48; 95% CI, 0.27 to 0.77), while there was significant heterogeneity between studies. In sensitivity analysis, two studies were excluded; the association and direction between GSTM1 and GSTT1 null genotypes and GSTP1 Ile/Val genotype and male infertility remained unchanged. There was no significant interaction between smoking status and studied genotypes on male infertility risk ($P = .26$).

Conclusion: These results demonstrated that amongst populations studied to date, GSTM1 and GSTT1 null genotypes are associated with strong and modest increase in the risk of male infertility, respectively. On the contrary, GSTP1 Ile/Val genotype has protective effect.

Keywords: glutathione S-transferase, single-nucleotide polymorphisms, infertility, male, genetic, risk assessment

INTRODUCTION

Worldwide, roughly 1 of 10 couples suffers from primary or secondary infertility.[1] The cause of infertility is still not determined in at least 30% of cases, which are considered as idiopathic infertility.[2] The overall prevalence of infertility in Iran is 8%.[3] In half of infertile couples, a male factor is implicated.[4] There is growing evidence that a significant number of male infertility is due to gene mutations and single-nucleotide polymorphisms (SNP).[5-8]

An increasing number of studies are determining the associations between candidate genes polymorphisms and male factor infertility risk,[9-12] which has also a practical implication. By determining the underlying genetic basis of infertility, it would be possible to diagnose the causes of infertility and provide effective treatment modalities. In this review, we address and discuss the evidence reported up to date on the association between male factor infertility and genetic polymorphisms of GST genes.

Gene and Gene Function

Glutathione S-transferases (GST, EC 2.5.1.18) constitute a superfamily of multifunctional enzymes that detoxify products of oxidative stress, environmental substances, and reactive electrophiles.[13,14] Glutathione S-transferases also inactivate carcinogens by catalyzing the conjugation of electrophiles to glutathione.[15] DNA damage due to endogenously formed lipid peroxidase can be prevented by GSTs.[16]

GST isoenzymes have been assigned to eight separate classes, including α (Alpha), μ (Mu), κ (kappa), ω (Omega), π (Pi), σ (Sigma), θ (Theta), and ζ (Zeta), which are encoded by the GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT, and GSTZ genes, respectively.[17,18] In addition, each class includes several genes and isoenzymes.[19] Polymorphisms have been reported in the GSTM1, GSTT1, and GSTP1 genes coding for GSTs enzymes in the Mu, Theta, and Pi classes, respectively.

In humans, the GSTM1 gene is polymorphic and mapped on chromosome 1p13.3.[20] GSTM1 has a common functional variant (null versus present). The frequency of this null varies between 23% and 63%, depending on the population studied.[21] Individuals with homozygous deletion of the GSTM1 locus have no enzymatic functional activity of the cytosolic enzyme GST-μ.[22] The homozygous deletion of GSTM1 has been shown to be associated with an increased risk of male infertility.[23-25]

The human Theta class of GSTs (GSTT) is comprised of two subunits, GSTT1 and GSTT2, both of which are located on chromosome 22q11.[26] The polymorphism in the GSTT1 gene loci is also caused by a gene deletion and brings about in virtual absence of enzyme activity in persons with the null genotype.[27] The association between both GSTT1 null genotype[23,24] and nondeletion genotype of the GSTT1 gene[28] has also been reported.

GSTP1 is a 2.8-kb gene located on chromosome 11q13.[29] Two genetic variants have been reported in GSTP1 gene. A single-nucleotide polymorphism at position 313 in GSTP1 converts an adenine to a guanine (A→G).[30] The resulting isoleucine to valine substitution in codon 105 of exon 5 ($Ile^{105} \rightarrow Val^{105}$) significantly lowers GST enzyme activity.[31] Another SNP at codon 114 leads to alanine (Ala) to Val transition, which results in a significant difference in catalytic activity.[32] In a study by Safarinejad and colleagues, GSTP1 Ile/Val genotype had significant negative association with male infertility.[23] But in a study by Tang and associates, GSTP1 allelic variation was not significantly different between the cases and controls.[33]

GST and Male Infertility Risk

Excessive reactive oxygen species (ROS) have been suggested to be one of the major contributory factors resulting in male infertility via oxidative DNA damages.[34] Glutathione S-transferase is one of the human defense mechanisms opposing the deleterious effects of oxidative stress.[35] Glutathione S-transferases gene polymorphisms could impair the capability of defense against oxidative stress and result in the development of some cancers.[36] One of the determinant factors of susceptibility of spermatozoa to oxidative damage is GSTM1 polymorphism.[25] DNA fragmentation in human sperm can be modulated by GSTM1 gene polymorphism.[37]

Objective

There have been various studies in the literature regarding the association of GST (M1, T1, and P1) polymorphisms with male infertility[23-25,27,33,38-43] (Table 1). There is sig-

Table 1. Summary of published studies on the association between GSTM1, GSTT1, and GSTP1 polymorphisms and male infertility.

First author (Ref no.)	Publication year	Cases	Controls	Country	Ethnicity	Studied gene(s)
Tang [33]	2012	65	30	China	Asian	GSTM1, GSTT1, GSTP1
Safarinejad[23]	2010	166	166	Iran	Asian	GSTM1, GSTT1, GSTP1
Tirumala[40]	2010	42	43	India	Asian	GSTM1
Polonikov[28]	2010	203	227	Russia	Caucasian	GSTM1, GSTT1
Ichioka[41]	2009	274	101	Japan	Asian	GSTM1, GSTT1
Wu[42]	2009	63	54	China	Asian	GSTT1
Finotti[24]	2009	128	105	Brazil	Caucasian	GSTM1, GSTT1
Aydos[38]	2009	110	105	Turkey	Caucasian	GSTM1
Wu[43]	2008	78	103	China	Asian	GSTT1
Aydemir[25]	2007	52	60	Turkey	Caucasian	GSTM1
Chen[39]	2002	142	60	China	Asian	GSTM1

nificant heterogeneity among the studies, and no pooled analysis was performed. We carried out a pooled analysis to determine the overall effect of GST (M1, T1, and P1) polymorphisms on male infertility risk.

MATERIALS AND METHODS

Data Collection

A MEDLINE literature search for case–control studies published between 1972 and May 1, 2012 on the association of GST polymorphisms (GSTM1, GSTT1, and GSTP1) and male factor infertility was conducted using the search terms of "GST OR Glutathione S-transferase AND polymorphism OR polymorphisms AND variant OR variants AND infertility OR infertile AND male", yielding 11 results.

Statistical Analysis

Adjusted odds ratios (OR) and 95% confidence intervals (CI) for GSTM1, GSTT1, and GSTP1 were calculated using unconditional logistic regression model, including study, age, and smoking status as covariates. Interactions were examined between GSTM1, GSTT1, and GSTP1 genotypes and smoking status. Heterogeneity was determined with a Q-statistic and P values < .05 were calculated using ORs from the individual included studies. To assess publication bias between the pooled studies, Egger's and Begg's tests were carried out. When there is heterogeneity between studies, the pooled analysis was restricted to those studies without significant heterogeneity and then sensitivity analysis was done.

All statistical analyses were performed using STATA statistical software, version 8.0, random effects model.

RESULTS

The results of the pooled analysis are summarized in Tables 2 to 4. There was a significant positive overall association between GSTM1 null genotype (OR, 2.47; 95% CI, 1.72 to 3.84; Trend P = .003), GSTT1 null genotype (OR, 1.54; 95% CI, 1.43 to 3.47; Trend P = .02), and male infertility. The association between GSTM1 null genotype and male infertility was stronger. However, significant hetero-

Table 2. Association between GSTM1 and male infertility in the pooled analysis.

Genotype	Adjusted Odds Ratio*	Trend *P*
Overall		.003
Present	1.00 (Ref)	
Null	2.47 (1.72 to 3.84)	
Never smoker		.004
Present	1.00 (Ref)	
Null	2.21 (1.52 to 3.57)	
Ever smoker		.002
Present	1.00 (Ref)	
Null	2.88 (1.91 to 4.16)	
Infertility		.002
Present	1.00 (Ref)	
Null	2.71 (1.84 to 3.92)	

*Odds Ratios are adjusted for study, age, race, and smoking history.

Table 3. Association between GSTT1 and male infertility in the pooled analysis.

Genotype	Adjusted Odds Ratio*	Trend *P*
Overall		.02
Present	1.00 (Ref)	
Null	1.54 (1.43 to 3.47)	
Never smoker		.03
Present	1.00 (Ref)	
Null	1.36 (1.57 to 3.12)	
Ever smoker		.01
Present	1.00 (Ref)	
Null	1.72 (1.63 to 3.74)	
Infertility		.01
Present	1.00 (Ref)	
Null	1.68 (1.57 to 3.69)	

*Odds Ratios are adjusted for study, age, race, and smoking history.

geneity was observed for both GSTM1 null genotype (P = .007) and GSTT1 null genotype (P = .02). No evidence of publication bias was detected for the GSTM1 genotype (P = .62) nor for the GSTM1 genotype (P = .24).

On the other hand, GSTP1 polymorphism had overall protective effect against development of male infertility, with an overall trend P value of .002. Men with Ile/Val genotype had overall 52% decreased risk for development of infertility (OR, 0.48; 95% CI, 0.27 to 0.77; Trend P = .002). Significant heterogeneity was also observed for GSTP1 (P = .01), with no evidence of publication bias (P = .12).

The sensitivity analysis demonstrated that exclusion of two studies (Tang and colleagues[33] and Ichioka and associates[41] from the analysis decreased the evidence of heterogeneity (GSTM1: P = .02; GSTT1: P = .04; and GSTP1: P = 1.0). After performing sensitivity analysis, there was still no evidence of publication bias for GSTM1 null genotype (P = .81) and GSTP1 Ile/Val genotype (P = 1.0), but there was evidence of publication bias for GSTT1 null genotype (P = .04).

The analysis was then restricted to infertility; similar results were obtained for the GSTM1 null genotype (OR, 2.71; 95% CI, 1.84 to 3.92), GSTT1 null genotype (OR, 1.68; 95% CI, 1.57 to 3.69), and the GSTP1 Ile/Val genotype (OR, 0.54; 95% CI, 0.37 to 0.81).

A significant positive association between the GSTM1 null genotype and infertility was observed in both never-smokers (OR, 2.21; 95% CI, 1.52 to 3.57; Trend P = .004) and ever smokers (OR, 2.88; 95% CI, 1.91 to 4.19; Trend P = .002). Among never and ever-smokers, there was also significant association between the GSTT1 null

Table 4. Association between GSTP1 and male infertility in the pooled analysis.

Genotype	Adjusted Odds Ratio*	Trend *P*
Overall		.002
Ile/Ile	1.00 (Ref)	
Ile/Val	0.48 (0.27 to 0.77)	
Val/Val	1.81 (0.34 to 7.85)	
Ile/Val or Val/Val	0.52 (0.31 to 0.81)	
Never smoker		.03
Ile/Ile	1.00 (Ref)	
Ile/Val	0.52 (0.33 to 0.80)	
Val/Val	1.84 (0.37 to 7.42)	
Ile/Val or Val/Val	0.58 (0.38 to 0.87)	
Ever smoker		.01
Ile/Ile	1.00 (Ref)	
Ile/Val	0.50 (0.31 to 0.79)	
Val/Val	1.82 (0.35 to 37.66)	
Ile/Val or Val/Val	0.54 (0.34 to 0.84)	
Infertility		.01
Ile/Ile	1.00 (Ref)	
Ile/Val	0.54 (0.37 to 0.81)	
Val/Val	1.80 (0.41 to 7.12)	
Ile/Val or Val/Val	0.57 (0.36 to 0.86)	

* Odds Ratios are adjusted for study, age, race, and smoking history.

genotype and infertility (Never: OR, 1.36; 95% CI, 1.57 to 3.12; Trend P = .03, Ever: OR, 1.72; 95% CI, 1.63 to 3.74; Trend P = .01). The direction and significance remained unchanged for GSTP1 genotypes when examining the interactions between different genotypes and smoking status (Table 4). No significant interaction was observed between smoking and the GSTM1 and GSTT1 null genotypes in both the analysis restricted to the homogeneous studies and in the full-pooled analysis.

DISCUSSION

This pooled analysis, containing 1054 controls and 1323 cases from 11 studies, demonstrates an overall association between GSTM1 and GSTT1 null genotypes and GSTP1 Ile/Val genotype and male factor infertility. We did not find publication bias; however, there was some heterogeneity between included studies.

This pooled analysis demonstrated significant positive associations between GSTM1 and GSTT1 null genotypes and male infertility; while significant inverse association was observed for GSTP1 Ile/Val genotype. Furthermore, we did not find a significant interaction between smoking and GSTM1 and GSTT1 null genotypes. The direction of the interaction is the same to the expected effect.

Strength of our pooled analysis is that the collection of data from various studies may well provide better statistical power necessary to detect any presence of association. Blettner and coworkers believe that the pooled analysis has advantages over meta-analysis.[44] Meta-analysis allows adjustment for potential confounding factors, evaluation of interactions, and testing for stratification. Using meta-analysis, we can adjust for potential confounding factors, assess the interactions, and examine for stratification. Pooled analysis has also its own advantages, including higher study power, which allows a full evaluation of effect modification within the specific data set,[45] adequate numbers of patients with sufficient follow-up, enough power essential to draw conclusions,[46] and being a powerful tool for human population studies.[47]

In the present study, we were able to adjust for age, ethnicity, and smoking status. Furthermore, we tested for interactions between smoking status and GSTM1, GSTT1, and GATP1 genotypes; and stratified our results by smoking history, which might not be possible with meta-analysis.

However, this pooled review is not without limitations. First, this study contains 11 studies; 7 of which report on Asian populations[23,33,39-43] and 4 on Caucasian subjects.

[24,25,28,38] Currently, there is no published literature investigating the association between the GSTM1, GSTT1, and GSTP1 polymorphisms and male infertility in ethnicities other than Asian and Caucasian. Second, there were only two studies addressing the association between GSTP1 genotypes and male infertility.

CONCLUSION

We demonstrated an association between GSTM1, GSTT1, and GSTP1 polymorphisms and male factor infertility, and no interaction between smoking history and studied genotypes. Further population-based studies are warranted to confirm the conclusion on the role of GSTM1, GSTT1, and GSTP1 in male infertility risk.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. World Health Organization. In: Vayena E, Rowe PJ, Griffin PD, eds. *Medical, Ethical and Social Aspects of Assisted Reproduction (2001, Geneva), Current Practices and Controversies in Assisted Reproduction: Report of a WHO Meeting*. Geneva, Switzerland: WHO Publications; 2002.
2. Nieschlag E. Scope and goals of andrology. In: Nieschlag E, Behre HM, eds. *Andrology: Male Reproductive Health and Dysfunction*. 2 ed. Heidelberg: Springer-Verlag; 2000:1-8.
3. Safarinejad MR. Infertility among couples in a population-based study in Iran: prevalence and associated risk factors. Int J Androl. 2008;31:303-14.
4. Brugh VM, 3rd, Lipshultz LI. Male factor infertility: evaluation and management. Med Clin North Am. 2004;88:367-85.
5. Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. Nat Med. 2008;14:1197-213.
6. Safarinejad MR, Shafiei N, Safarinejad S. The role of endothelial nitric oxide synthase (eNOS) T-786C, G894T, and 4a/b gene polymorphisms in the risk of idiopathic male infertility. Mol Reprod Dev. 2010;77:720-7.
7. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril. 2010;93:1-12.
8. Safarinejad MR, Shafiei N, Safarinejad S. Association of polymorphisms in the estrogen receptors alpha, and beta (ESR1, ESR2) with the occurrence of male infertility and semen parameters. J Steroid Biochem Mol Biol. 2010;122:193-203.
9. Vicdan A, Vicdan K, Gunalp S, et al. Genetic aspects of human male infertility: the frequency of chromosomal abnormalities and Y chromosome microdeletions in severe male factor infertility. Eur J Obstet Gynecol Reprod Biol. 2004;117:49-54.
10. Safarinejad MR, Shafiei N, Safarinejad S. Association of the (TAAAA)n repeat and Asp327Asn polymorphisms in the sex hormone-binding globulin (SHBG) gene with idiopathic male infertility and relation to serum SHBG concentrations. J Steroid Biochem Mol Biol. 2011;123:37-45.
11. Maduro MR, Lamb DJ. Understanding new genetics of male infertility. J Urol. 2002;168:2197-205.
12. Safarinejad MR, Shafiei N, Safarinejad S. Relationship between genetic polymorphisms of methylenetetrahydrofolate reductase (C677T, A1298C, and G1793A) as risk factors for idiopathic male infertility. Reprod Sci. 2011;18:304-15.
13. Sanyal S, Festa F, Sakano S, et al. Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis. 2004;25:729-34.
14. Schnakenberg E, Breuer R, Werdin R, Dreikorn K, Schloot W. Susceptibility genes: GSTM1 and GSTM3 as genetic risk factors in bladder cancer. Cytogenet Cell Genet. 2000;91:234-8.
15. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol. 1995;30:445-600.
16. Hurst R, Bao Y, Jemth P, Mannervik B, Williamson G. Phospholipid hydroperoxide glutathione peroxidase activity of human glutathione transferases. Biochem J. 1998;332 (Pt 1):97-100.
17. Lo HW, Ali-Osman F. Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance. Curr Opin Pharmacol. 2007;7:367-74.
18. Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. Mutat Res. 2001;482:21-6.
19. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology. 2000;61:154-66.

20. Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev. 1997;6:733-43.
21. Garte S, Gaspari L, Alexandrie AK, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev. 2001;10:1239-48.
22. Zhong S, Howie AF, Ketterer B, et al. Glutathione S-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. Carcinogenesis. 1991;12:1533-7.
23. Safarinejad MR, Shafiei N, Safarinejad S. The association of glutathione-S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) with idiopathic male infertility. J Hum Genet. 2010;55:565-70.
24. Finotti AC, Costa ESRC, Bordin BM, Silva CT, Moura KK. Glutathione S-transferase M1 and T1 polymorphism in men with idiopathic infertility. Genet Mol Res. 2009;8:1093-8.
25. Aydemir B, Onaran I, Kiziler AR, Alici B, Akyolcu MC. Increased oxidative damage of sperm and seminal plasma in men with idiopathic infertility is higher in patients with glutathione S-transferase Mu-1 null genotype. Asian J Androl. 2007;9:108-15.
26. Tan KL, Webb GC, Baker RT, Board PG. Molecular cloning of a cDNA and chromosomal localization of a human theta-class glutathione S-transferase gene (GSTT2) to chromosome 22. Genomics. 1995;25:381-7.
27. Pemble S, Schroeder KR, Spencer SR, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J. 1994;300 (Pt 1):271-6.
28. Polonikov AV, Yarosh SL, Kokhtenko EV, Starodubova NI, Pakhomov SP, Orlova VS. The functional genotype of glutathione S-transferase T1 gene is strongly associated with increased risk of idiopathic infertility in Russian men. Fertil Steril. 2010;94:1144-7.
29. Kano T, Sakai M, Muramatsu M. Structure and expression of a human class pi glutathione S-transferase messenger RNA. Cancer Res. 1987;47:5626-30.
30. Ahmad H, Wilson DE, Fritz RR, et al. Primary and secondary structural analyses of glutathione S-transferase pi from human placenta. Arch Biochem Biophys. 1990;278:398-408.
31. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis. 1998;19:275-80.
32. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem. 1997;272:10004-12.
33. Tang K, Xue W, Xing Y, et al. Genetic polymorphisms of glutathione S-transferase M1, T1, and P1, and the assessment of oxidative damage in infertile men with varicoceles from northwestern China. J Androl. 2012;33:257-63.
34. Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. J Androl. 2002;23:737-52.
35. Awasthi YC, Sharma R, Singhal SS. Human glutathione S-transferases. Int J Biochem. 1994;26:295-308.
36. Ryberg D, Skaug V, Hewer A, et al. Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. Carcinogenesis. 1997;18:1285-9.
37. Rubes J, Selevan SG, Sram RJ, Evenson DP, Perreault SD. GSTM1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res. 2007;625:20-8.
38. Aydos SE, Taspinar M, Sunguroglu A, Aydos K. Association of CYP1A1 and glutathione S-transferase polymorphisms with male factor infertility. Fertil Steril. 2009;92:541-7.
39. Chen SS, Chang LS, Chen HW, Wei YH. Polymorphisms of glutathione S-transferase M1 and male infertility in Taiwanese patients with varicocele. Hum Reprod. 2002;17:718-25.
40. Tirumala Vani G, Mukesh N, Siva Prasad B, et al. Role of glutathione S-transferase Mu-1 (GSTM1) polymorphism in oligospermic infertile males. Andrologia. 2010;42:213-7.
41. Ichioka K, Nagahama K, Okubo K, Soda T, Ogawa O, Nishiyama H. Genetic polymorphisms in glutathione S-transferase T1 affect the surgical outcome of varicocelectomies in infertile patients. Asian J Androl. 2009;11:333-41.
42. Wu Q, Xing J, Xue W, Sun J, Wang X, Jin X. Influence of polymorphism of glutathione S-transferase T1 on Chinese infertile patients with varicocele. Fertil Steril. 2009;91:960-2.
43. Wu QF, Xing JP, Tang KF, et al. Genetic polymorphism of glutathione S-transferase T1 gene and susceptibility to idiopathic azoospermia or oligospermia in northwestern China. Asian J Androl. 2008;10:266-70.
44. Blettner M, Sauerbrei W, Schlehofer B, Scheuchenpflug T, Friedenreich C. Traditional reviews, meta-analyses and pooled analyses in epidemiology. Int J Epidemiol. 1999;28:1-9.

45. Friedenreich CM. Commentary: improving pooled analyses in epidemiology. Int J Epidemiol. 2002;31:86-7.

46. Kusters M, Valentini V, Calvo FA, et al. Results of European pooled analysis of IORT-containing multimodality treatment for locally advanced rectal cancer: adjuvant chemotherapy prevents local recurrence rather than distant metastases. Ann Oncol. 2010;21:1279-84.

47. Chen J, Germer S, Higuchi R, Berkowitz G, Godbold J, Wetmur JG. Kinetic polymerase chain reaction on pooled DNA: a high-throughput, high-efficiency alternative in genetic epidemiological studies. Cancer Epidemiol Biomarkers Prev. 2002;11:131-6.