RESEARCH ARTICLE

Association of GSTO1 A140D and GSTO2 N142D Gene Variations with Breast Cancer Risk

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Abstract

Background: Polymorphisms in glutathione S-transferase (GST) genes may contribute to breast cancer risk. The aim of this study was to investigate any association of two common GSTO1 A140D and GSTO2 N142D gene polymorphisms with breast cancer risk in an Iranian population followed by a protein structure analysis. **Materials and Methods:** In the case-control study, 303 subjects comprising 153 women with breast cancer and 150 healthy controls were included. Genotypes of GSTO1 A140D and GSTO2 N142D polymorphisms were assessed by PCR-RFLP. Bioinformatics tools were employed to evaluate the damaging effects of A140D and N142D on the structures of GSTO1 and GSTO2 proteins. **Results:** Our genetic association study revealed that the GSTO1 A140D polymorphism was associated with breast cancer in a dominant model (OR= 1.75, 95%CI= 1.07-2.86, p= 0.026). Also, the A allele was significantly associated with breast cancer risk (OR= 1.69, 95%CI= 1.09-2.60, p= 0.018). With regard to the N142D polymorphism, there were significant associations between the GG genotype (OR= 2.20, 95%CI= 1.14-4.27, p= 0.019) and the G allele (OR= 1.47, 95%CI= 1.06-2.05, p= 0.021) and risk of breast cancer. Structural analysis revealed that A140D and N142D polymorphisms cause changes in both primary and secondary structures of GSTO1 and GSTO2, respectively. **Conclusion:** Based on our results, GSTO1 A140D and GSTO2 N142D polymorphisms could be genetic risk factors for breast cancer, but further studies with larger sample sizes focusing on different ethnicities are needed to obtain more comprehensive results.

Keywords: Breast cancer- Glutathione S-transferase- genetic polymorphism- bioinformatics

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Introduction

Breast cancer (OMIM: 114480) is one of the most common cancers in women worldwide (Soleimani et al., 2017), and its incidence is growing in developed and developing countries (Bandera et al., 2015). The causes of breast cancer is still not understood well, however numerous risk factors are well-known, containing life-style, high estrogen exposure, and genetic factors (Dumitrescu and Cotarla, 2005; Sharif et al., 2016; Khamechian et al., 2013). Since human breast cancer results from genetic-environmental interactions, genetic factors need to be identified for a more accurate evaluation of overall breast cancer risk. Since breast cancer arises from interactions between environmental and genetic factors, identification of genetic factors could be valuable for more precise assessment of the overall risk of breast cancer. Polymorphisms in genes involving in susceptibility to breast cancer play more important role in tumorigenesis when joint with environmental factors (Rothman et al., 2001).

Glutathione S-transferases (GSTs) are suitable candidate to study of a possible association with risk of breast cancer. GSTs, a group of phase II detoxifying enzymes, are involved in the conjugation of glutathione to a wide range of electrophilic agents (Tew, 1994). Therefore, GSTs protect the cells against environmental carcinogens (Balendiran et al., 2004). According to amino acid sequence, mammalian cytosolic GSTs are classified to seven groups which known as GSTA, GSTM, GSTP, GSTS, GSTT, GSTO, and GSTZ. All seven human GSTs display some common genetic variations and since these functional polymorphisms are dubious to change the risk of cancers including breast cancer, GSTs genetic polymorphisms have been exposed on the genetic association studies with breast cancer risk (Andonova et al., 2010). Meanwhile, the main part of studies have been focused on the association of GSTM1, GSTT1, and GSTP1 common polymorphisms with breast cancer risk (Egan et al., 2004). However, there are a few studies investigating the association of other classes of GSTs with breast cancer risk. For example there are

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five studies investigating the association of GSTO gene polymorphisms with breast cancer risk that the results of these studies are controversial (Xu et al., 2014). The aim of this study was to investigate the association of GSTO1 A140D (rs4925; p.Ala140Asp; c.335C>A) and GSTO2 N142D (rs156697; p.Asn142Asp; c.424A>G) gene single nucleotide polymorphisms (SNPs) with breast cancer risk in an Iranian population.

Materials and Methods

Subjects

The present study was performed in case and control groups. The case group was comprised of 153 women with breast cancer (mean age 56.37±9.81 years) who had referred to Shahid Beheshti Hospital (Kashan, Iran) during 2015-2016. Breast cancer was approved by histological tests for all cases. It should be mentioned that just case subjects with newly incident breast cancer were selected. Controls including 150 age-matched women (mean age 58.45±11.59 years) were selected from peoples who contributed in a native screening plan, and they did not present any positive signs and familial history of any malignancy such as breast cancer. Informed consent was obtained from all participants of this project. Approval of the Local Ethics Committee was obtained from Kashan University of Medical Sciences. Finally, about 2ml blood was obtained from all subjects into sterile tubes containing EDTA.

SNPs genotyping

Total genomic DNA was isolated from blood samples using DNGplus buffer (CinnaGen Co., Tehran, Iran) according to the protocol of manufacturer. The GSTO1 A140D and GSTO2 N142D SNPs genotyping was performed by PCR-RFLP method according to previous report (Marahatta et al., 2006). To confirm primers sequences, we deduced the genomic sequences of GSTO1 and GSTO2 from NCBI databank. The primers sequences that presented in Table 1 were checked around the polymorphic positions by Oligo6 software (Molecular Biology Insights, Inc., Cascade, CO, USA) and then were ordered from CinnaGen Company (CinnaGen, Tehran, Iran). PCR was carried out in 25µl total volume containing 2.5µl 10X PCR buffer, 0.75µl MgCl2 (stock: 50 mM), 0.3 µl dNTPs mix (stock: 10 mM), 0.5 µl each of forward and reverse primer (stock: 100pM), 5 µl Betaine (stock: 5M), 0.3 µl SmarTaq polymerase (stock: 5 u/µl), and 50 ng of genome as template (All PCR reagents were purchased from CinnaGen). PCR performed in Eppendorf thermal cycler set (Eppendorf AG, Hamburg, Germany) with the conditions introduced in Table 1. To ensure the accuracy of amplification procedure, PCR products were detected by electrophoresis in 1% agarose gel stained with ethidium bromide. The length of PCR products for each specific primers are presented in Table 1. The Cac8I and MboI restriction enzymes (Fermentas Co., Leon-Rot, Germany) were employed for GSTO1 A140D and GSTO2 N142D SNPs, respectively. About 0.1µg of PCR products were treated by 5 units of aforementioned restriction enzymes. After digestion process, the genotypes of samples were detected on 2% agarose gel. The digestion conditions and pattern of each genotypes in electrophoresis were detailed in Table 2. The accuracy of PCR-RFLP method was checked by repeating the test for 2% of samples.

Statistical analysis

The numerical parameters were analyzed by independent t-test. The Hardy-Weinberg equilibrium was analyzed by Chi-square test, also the same test was used to compare the difference of allele and genotype frequencies between case and control groups. Also, odd ratios (ORs) 95% confidence interval (CI) were estimated to measure the strength of association between GSTO1 A140D and GSTO2 N142D SNPs and breast cancer risk. A p-value less than 0.05 was considered statistically significant. All of statistical analyses were done by SPSS ver.16 software package (SSPS Inc., IBM Corp Armonk, NY, USA).

Structural analysis

Some bioinformatics tools were employed to analyze the effects of A140D and N142D SNPs on the structures and functions of GSTO1 and GSTO2, respectively. For this purpose, the coding sequence of these to proteins was obtained from NCBI databases and translated to amino acid sequence. The physicochemical properties of GSTO1 and GSTO2 were assessed by ProtParam web server (http://web.expasy.org/protparam/) after A140D and N142D substitutions, respectively. The changes in secondary structure of the GSTO1 and GSTO2 were assessed by Garnier-Osguthorpe-Robson (GOR), Choue-Fasman (CF), and Neural Network (NN) methods (http://cib.cf.ocha.ac.jp/bitool/MIX/). The 3D structures of GSTO1 and GSTO2 proteins were obtained from RCSB Protein Data Bank (www.rcsb.org). The Accelrys DS Visualiser 1.7 software was used to analyze the location of mentioned SNPs on 3D structure of proteins.

Results

Genetic association analysis

Our data revealed that the distribution of genotype frequencies for both A140D ($\chi 2 = 0.22$, p= 0.64) and N142D ($\chi 2 = 0.36$, p= 0.55) SNPs was consistent to

Table 1. The Primer Sequences and PCR Conditions*

Gene	SNP (rs No.)	Primer name	5' to 3' oligonucleotide	PCR conditions	PCR prod.
GSTO1	A140D (rs4925)	F1	5'- GAACTTGATGCACCCTTGGT	94°C 5′, 94°C 45″	254 bp
		R1	5'- TGATAGCTAGGAGAAATAATTAC	60°C 45″, 72°C 45″	
GSTO2	N142D (rs156697	F2	5'- AGGCAGAACAGGAACTGGAA	35 cycles, 72°C 5′	185 bp
		R2	5'- GAGGGACCCCTTTTTGTACC		

*Primers reported by Marahatta et al., 2006.

Table 2. Digestion Conditions and Pattern of Each Genotypes

Gne	SNP (rs No.)	SNP	Restriction enzyme	Digestion products (bp)		
				Wild genotype	Mutant genotype	Heterozygote genotype
GSTO1	A140D (rs4925)	A/C	Cac8I	186, 68	254	254, 186, 68
GSTO2	N142D (rs156697	G/A	MboI	185	121, 64	185, 121, 64

Figure 1. Secondary Structure of GSTO1 and GSTO2. The secondary structure of GSTO1 for 140A (up) and 140D (down) phenotypes (A). The secondary structure of GSTO2 for 142N (up) and 142D (down) phenotypes (A).

Hardy-Weinberg criteria in control group. The distribution of alleles and genotypes frequencies of A140D and N142D are detailed in Tables 3 and 4, respectively. With regard to GSTO1 A140D polymorphism, the frequencies of CC, CA, and AA genotypes in control group were 74.67%, 24.00%, and 1.33%, respectively. While these

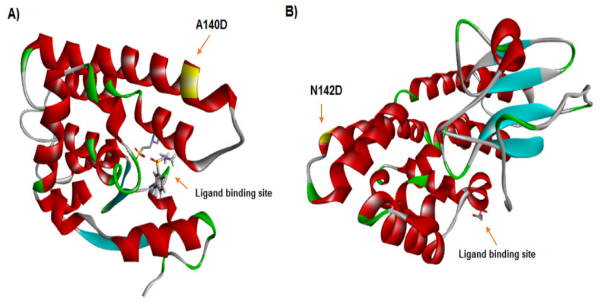

Figure 2. The Three Dimensional Structure of GSTO1 and GSTO2. The A140D and N142D substitutions are located far from GSTO1 (A) and GSTO2 (B), respectively

ratios in case group were 62.75%, 33.33%, and 3.92%, respectively. Therefore, we observed a significant association between A140D and breast cancer risk in a dominant genetic model (CA+AA vs. CC; OR= 1.75, 95%CI= 1.07-2.86, p= 0.026). Also, a difference of CA genotype was observed between case and control groups, but it was not statistically significant (OR= 1.65, 95%CI= 1.00-2.74, p= 0.052). Analysis of allele frequencies in case and control groups revealed that there is a significant association between A allele and breast cancer susceptibility (OR= 1.69, 95%CI= 1.09-2.60, p= 0.018). With regard to N142D polymorphism, the frequencies of AA, AG, and GG genotypes were 44.67%, 42.67%, and 12.66%, respectively. But, these ratios were 36.60%, 40.52%, and 22.88% for case group, respectively. So, we

Table 3. Genotype and Allele Frequencies of GSTO1 A140D in Both Cases and Controls

Genotype/ Allele	No. and Percentage		OR (95% CI)	p value
	Control (n=150)	Case (n=153)		
CC	112 (74.67%)	96 (62.75%)	-	-
CA	36 (24.00%)	51 (33.33%)	1.65 (1.00-2.74)	0.052
AA	2 (1.33%)	6 (3.92%)	3.50 (0.69-17.75)	0.13
CA+AA	38 (25.33%)	57 (37.25%)	1.75 (1.07-2.86)	**0.026**
C	260 (86.67%)	243 (79.41%)	-	-
A	40 (13.33%)	63 (20.59%)	1.69 (1.09-2.60)	**0.018**

OR, odds ratio; CI, confidence interval; Significant differences between the case and control groups are bolded.

Table 4. Genotype and Allele Frequencies Of GSTO2 N142D in Both Cases and Controls

Genotype/ Allele	No. and Percentage		OR (95% CI)	p value
	Control (n=150)	Case (n=153)		
AA	67 (44.67%)	56 (36.60%)	-	-
AG	64 (42.67%)	62 (40.52%)	1.16 (0.70-1.91)	0.561
GG	19 (12.66%)	35 (22.88%)	2.20 (1.14-4.27)	**0.019**
AG+GG	83 (55.33%)	97 (63.40%)	1.40 (0.88-2.22)	0.153
A	198 (66.00%)	174 (56.86%)	-	-
G	102 (34.00%)	132 (43.14%)	1.47 (1.06-2.05)	**0.021**

OR, odds ratio; CI, confidence interval; Significant differences between the case and control groups are bolded.

Table 5. Physicochemical Properties for Wild types and Mutant Types of GSTO1 and GSTO2 Proteins

Protein phenotype	Molecular weight (Da)	Theoretical pI	Estimated half-life	Instability index	Aliphatic index
GSTO1					
Normal	27,565.86	6.24	30 hours	45.75	80.54
Mutant	27,609.87	5.89	30 hours	45.75	80.12
GSTO2					
Normal	28,253.81	7.51	30 hours	44.02	87.08
Mutant	28,254.79	6.97	30 hours	44.63	87.08

found a significant association between GG genotype and breast cancer risk (OR= 2.20, 95% CI= 1.14-4.27, p= 0.019). Moreover, there was a significant association between G allele and risk of breast cancer (OR= 1.47, 95%CI= 1.06-2.05, p= 0.021).

Bioinformatics analysis

The results of ProtParam web server are detailed in Table 5. The data revealed that molecular weights of normal GSTO1 and GSTO2 are 27565.86 and 28253.81 Da, respectively. These scores for mutant types are 27609.87 and 28254.79 Da. Theoretical pI for normal phenotypes of GSTO1 and GSTO2 were predicted 6.24 and 7.51 whereas these ratios for mutant phenotypes reduced to 5.89 and 6.97. Half-life for all phenotypes were estimated 30 hours in mammalian cells. Also, instability index for GSTO1 doesn't change after A140D substitution. But, this parameter increases in GSTO2 after N142D mutation. Aliphatic index of normal GSTO1 was predicted 80.54 and 80.12 for 140A and 140D phenotypes, respectively while this parameter doesn't alter after N142D substitution. The data from secondary structure analysis revealed that this structure of GSTO1 and GSTO2 change around the A140D and N142D positions, respectively (Figure 1). But, analysis of three dimensional structures of GSTO1 and GSTO2 showed that both A140D and N142D SNPs are far from ligand binding sites (Figure 2).

Discussion

In this study we investigated the association of two common GSTO1 A140D and GSTO2 N142D gene polymorphisms with breast cancer susceptibility in an Iranian population. Our data revealed that there is a significant association between GSTO1 A140D and breast cancer risk in a dominant genetic model. Also, A allele showed a significant association with breast cancer. Moreover, GG genotype and G allele of GSTO2 N142D polymorphism were significantly associated with breast cancer risk in our study population. There are some similar studies investigating GSTO1 A140D and GSTO2 N142D gene polymorphisms with breast cancer in different ethnicities. For example, Chariyalertsak et al., (2009) reported that there were no significant associations between GSTO1 A140D and GSTO2 N142D variations and breast cancer risk in Thai population. But, they reported that GSTO1-A140/A140 genotype was significantly associated with advanced-stage of breast cancer. Andonova et al., (2010) reported that there is no significant association between mentioned SNPs and breast cancer risk in Germany population. In addition, Masoudi et al., (2010) reported that there is no significant association between GSTO2 N142D polymorphism and risk of breast cancer in an Iranian population. But, Sohail et al., (2013) reported significant associations between GSTO2 N142D polymorphism and breast cancer risk in Pakistani population (Sohail et al., 2013). The inconsistent results from different studies may be due to differences in ethnicities and environmental factors. Another cause of this inconsistency may arise from the small sample sizes, particularly in the case group, unsuitable for studies of genetic association (Karimian and Hosseinzadeh Colagar, 2016; Mazaheri et al., 2017; Rafatmanesh et al., 2017). But, results of pooled data from a meta-analysis revealed that there no significant associations between GSTO1 A140D and breast cancer risk. While the meta-analysis showed a significant association between GSTO2 N142D and breast cancer risk in a homozygote co-dominant model (Xu et al., 2014).

Dissimilar other GST molecules, there is a cysteine active site in GSTO structure that it can produce a disulfide bond with glutathione and shows some activities such as thiol transferase and GSH-dependent dehydroascorbate reductase (Girardini et al., 2002). The GSTO is expressed in many healthy tissues such as heart, liver, pancreas, colon, ovary, prostate, spleen, and breast. This wide range distribution of GSTO expression could explain the main biological roles of this enzyme (Whitbread et al., 2003). GSTO has a crucial role in cell signaling and overexpression of this gene may stimulate cancer development via involvement in apoptosis process (Wang et al., 2005). Moreover, GSTO induces the activation of interleukin-1β as a pro-inflammatory cytokine (Laliberte et al., 2003).

Non-synonymous SNPs (nsSNPs) are causes of amino acid replacements by nucleotide alterations in coding sequence of genes, and they could affect protein function and structure (Nikzad et al., 2015; Karimian and Colagar, 2016). Experimental assessment of nsSNPs effects on protein function and structure could be a difficult procedure. But, in silico tools are a useful way to evaluate the structural effects of these genetic variations (Raygan et al., 2016; Karimian et al., 2015). In addition, these bioinformatics tools could be useful in evaluation of SNPs existing on promoter regions and/or non-coding sequences. These variations can influence the gene expression, RNA splicing, and mRNA structure (Jamali et al., 2016; Karimian et al., 2017). We showed that GSTO1 A140D and GSTO2 N142D nsSNPs can

influence primary and secondary structure of proteins. Therefore, pathogenic effects of aforementioned nsSNPs may arise from these changes in primary and secondary structure of protein. But, further structural analysis on mRNA structure and RNA splicing will be useful to obtain more accurate results.

In conclusion, our study revealed that GSTO1 A140D and GSTO2 N142D polymorphisms can increase the risk of breast cancer and they could be considered as genetic risk factors. But, further studies in different populations with larger sample sizes are needed to obtain more accurate results. There are some limitations in our study which should be mentioned. For example, we did not considered gene-gene and gene-environmental interactions which may modulate any genetic association results. Also, our study had been focused on a limited Iranian population. Therefore, further studies in different Iranian ethnicities with considered to gene-gene and gene-environmental are needed to achieve more accurate results.

Declaration

The authors report no financial or commercial conflicts of interest.

References

Andonova IE, Justenhoven C, Winter S, et al (2010). No evidence for glutathione S-transferases GSTA2, GSTM2, GSTO1, GSTO2, and GSTZ1 in breast cancer risk. *Breast Cancer Res Treat*, **12**1, 497-502.

Balendiran GK, Dabur R, Fraser D (2004). The role of glutathione in cancer. *Cell Biochem Funct*, **22**, 343-52.

Bandera EV, Maskarinec G, Romieu I, John EM (2015). Racial and ethnic disparities in the impact of obesity on breast cancer risk and survival: a global perspective. *Adv Nutr*, **6**, 803-19.

Dumitrescu RG, Cotarla I (2005). Understanding breast cancer risk- where do we stand in 2005?. *J Cell Mol Med*, **9**, 208-21.

Egan KM, Cai Q, Shu XO, et al (2004). Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai breast cancer study and meta-analysis. *Cancer Epidemiol Biomarkers Prev*, **13**, 197-204.

Girardini J, Amirante A, Zemzoumi K, Serra E (2002). Characterization of an omega-class glutathione S-transferase from Schistosoma mansoni with glutaredoxin-like dehydroascorbate reductase and thiol transferase activities. *Eur J Biochem*, **269**, 5512-21.

Jamali S, Karimian M, Nikzad H, Aftabi Y (2016). The c.-190 C> A transversion in promoter region of protamine1 gene as a genetic risk factor for idiopathic oligozoospermia. *Mol Biol Rep*, **43**, 795-802.

Karimian M, Colagar AH (2016). Association of C677T transition of the human methylenetetrahydrofolate reductase (MTHFR) gene with male infertility. *Reprod Fertil Dev*, **28**, 785-94.

Karimian M, Hosseinzadeh Colagar A (2016). Methionine synthase A2756G transition might be a risk factor for male infertility: Evidences from seven case-control studies. *Mol Cell Endocrinol*, **425**, 1-10.

Karimian M, Hosseinzadeh Colagar A (2017). Human MTHFR-G1793A transition may be a protective mutation against male infertility: a genetic association study and in silico analysis. *Hum Fertil*, **8**, 1-9.

Karimian M, Nikzad H, Azami-Tameh A, et al (2015). SPO11-C631T gene polymorphism: association with male infertility and an in silico-analysis. *J Family Reprod Health*, **9**, 155-63.

Khamechian T, Alizargar J, Mazoochi T (2013). Prevalence of depression in patients with Cancer. *Middle East J Sci Res*, **15**, 1311-5.

Laliberte RE, Perregaux DG, Hoth LR, et al (2003). Glutathione s-transferase omega 1-1 is a target of cytokine release inhibitory drugs and may be responsible for their effect on interleukin-1beta posttranslational processing. *J Biol Chem*, **278**, 16567-78.

Marahatta SB, Punyarit P, Bhudisawasdi V, et al (2006). Polymorphism of glutathione Stransferase omega gene, risk of cancer. *Cancer Lett*, **236**, 276-81.

Mazaheri M, Karimian M, Behjati M, Raygan F, Colagar AH (2017). Association analysis of rs1049255 and rs4673 transitions in p22phox gene with coronary artery disease: A case-control study and a computational analysis. *Ir J Med Sci*, **14**, 1-8.

Nikzad H, Karimian M, Sareban K, Khoshsokhan M, Hosseinzadeh Colagar A (2015). MTHFR-Ala222Val and male infertility: a study in Iranian men, an updated meta-analysis and an in silico-analysis. *Reprod Biomed Online*, **31**, 668-80.

Olsen A, Autrup H, Sørensen M, Overvad K, Tjønneland A (2008). Polymorphisms of glutathione S-transferase A1 and O1 and breast cancer among postmenopausal Danish women. *Eur J Cancer Prev*, **17**, 225-9.

Rafatmanesh A, Nikzad H, Ebrahimi A, Karimian M, Zamani T (2017). Association of the c.-9C> T and c. 368A> G transitions in H2BFWT gene with male infertility in an Iranian population. *Andrologia*, Doi: 10.1111/and.12805.

Raygan F, Karimian M, Rezaeian A, Bahmani B, Behjati M (2016). Angiotensinogen-M235T as a risk factor for myocardial infarction in Asian populations: a genetic association study and a bioinformatics approach. *Croat Med J*, **57**, 351-62.

Rothman N, Wacholder S, Caporaso NE, et al (2001). The use of common genetic polymorphisms to enhance the epidemiologic study of environmental carcinogens. *Biochim Biophys Acta*, **1471**, 1-0.

Sharif A, Kheirkhah D, Sharif MR, Karimian M, Karimian Z (2016). ABCB1-C3435T polymorphism and breast cancer risk: a case-control study and a meta-analysis. *J BUON*, **21**, 1433-41.

Soleimani Z, Kheirkhah D, Sharif MR, et al (2017). Association of CCND1 Gene c. 870G> A Polymorphism with Breast Cancer Risk: A Case-ControlStudy and a Meta-Analysis. *Pathol Oncol Res*, **23**, 621-31.

Tew KD (1994). Glutathione-associated enzymes in anticancer drug resistance. *Cancer Res*, **54**, 4313-20.

Wang L, Xu J, Ji C, et al (2005). Cloning, expression and characterization of human glutathione S-transferase Omega 2. *Int J Mol Med*, **16**, 19-27.

Whitbread AK, Tetlow N, Eyre HJ, Sutherland GR, Board PG (2003). Characterization of the human Omega class glutathione transferase genes and associated polymorphisms. *Pharmacogenetics*, **13**, 131-44.

Xu YT, Wang J, Yin R (2014). Genetic polymorphisms in glutathione S-transferase omega (GSTO) and cancer risk: a meta-analysis of 20 studies. *Sci Rep*, **4**, 6578.