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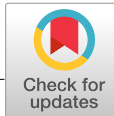

RESEARCH ARTICLE

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Arg399Gln substitution in XRCC1 as a prognostic and predictive biomarker for prostate cancer: Evidence from 8662 subjects and a structural analysis

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Abstract

Background The Arg399Gln polymorphism in the X-ray repair cross-complementing group 1 gene (*XRCC1*) may alter the risk of prostate cancer (PCa). The present study aimed to investigate the association of the *XRCC1*-Arg399Gln polymorphism with PCa risk in an Iranian population, as followed by a meta-analysis and an in silico analysis.

Methods In a case–control study, 360 subjects were included (180 men with PCa and 180 healthy controls). *XRCC1*-Arg399Gln genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism method. In the meta-analysis, 14 eligible studies were included to which our case–control data were added to estimate the pooled odds ratios. Some bioinformatics tools were employed to evaluate the effects of Arg399Gln substitution on molecular aspects of the XRCC1 protein.

Results Our case–control study revealed a significant association between the *XRCC1*-Arg399Gln polymorphism and PCa risk. The data from overall meta-analysis showed significant associations between the mentioned polymorphism and PCa risk in allelic and recessive genetic models. In addition, we observed statistically significant associations in stratified analyses by ethnicity, sample size and source of controls. Our in silico analysis showed that Arg399Gln substitution could be damaging with respect to the function and structure of the XRCC1 protein.

Conclusions Based on these results, the *XRCC1*-Arg399Gln polymorphism might be a risk factor for PCa and it could be considered as a prognostic and predictive biomarker for susceptible men.

KEYWORDS

in silico analysis, meta-analysis, prostate cancer, *XRCC1* gene

1 | INTRODUCTION

Prostate cancer (PCa) is amongst the most common cancers among males.[1] This cancer is the second cause of death of males and its occurrence increases with an increase in age.[2] Although previous studies have been shown that diet, cigarette smoking, race, infection and ultraviolet light could increase PCa, the exact cause of PCa remains unclear.[3,4] Despite possessing these risk factors, PCa might not occur, which could be a result of the presence of genetic factors. Thus genetic factors in combination with environmental players are

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involved in the development of PCa.[5,6] Malignant transformation of prostate cells is always associated with genetic alterations such as deletions, inversion and point mutations.[7] The association between genetic factors such as several single nucleotide polymorphisms (SNPs) with various cancers such as PCa, leukemia, colorectal cancer, breast cancer, and so on, has been reported.[8-11] Several lines of investigations have shown the relationship between SNPs of the genes involved in DNA repair and cancer susceptibility. Repair capacity of DNA is also involved in pathogenesis of PCa.[8,12,13]

The X-ray repair cross-complementing group 1 gene (*XRCC1*), located on chromosome 19 (19q13) with 17 exons, plays a fundamental role in DNA repair.[14] This gene encodes a protein with 633 amino acids that plays an essential role in repair of mutated DNA. Arg280His (ID: rs25489) and Arg399Gln (ID: rs25487) are two common variations of *XRCC1* gene. The functional impacts of these polymorphism have not been fully understood. Based on the SNP database of the NCBI, the Arg280His variation is a three-allelic (A/C/G) polymorphism.[13] Several reports have indicated that the Arg399Gln polymorphism is a risk factor for Pca, although some controversy remains.[15-19] Prostate malignancy is a disorder in elderly men and its occurrence is increasing in Iran.[20] To our knowlege, no studies have investigated the association of *XRCC1* gene polymorphisms with PCa risk in Iranian population. Thus, we aimed to evaluate the association between Arg399Gln polymorphism and PCa in a case–control study. Then, the genetic association of this polymorphism with PCa would be evaluated via a meta-analysis. Finally, the effects of this polymorphism on the structure and function of the XRCC1 protein would be assessed using bioinformatics tools.

2 | MATERIALS AND METHODS

2.1 | Case–control study

The present study comprised a case–control study. We recruited 180 PCa subjects and 180 controls from Kashan city (Isfahan province, Iran) between 2014 and 2016. The PCa participants were histopathologically confirmed as PCa at the Shahid Beheshti hospital. The healthy subjects were randomly selected from men who referred to the same hospital for health check-up. Control subjects with serum prostate-specific antigen (PSA) > 2.5 ng/ml were excluded from the study. Written informed consent was obtained from all subjects. Finally, 2 ml of blood was collected from all participants into complete blood count tubes containing the anticoagulant ethylenediaminetetracetic acid. The research protocols and experimental tests were approved by the Medical Ethic Committee of the Research Council of Kashan University of Medical Sciences in December 2016 (Ref no. 95103).

DNA extraction from blood samples was performed by DNGplus (CinnaGen, Tehran, Iran) in accordance with a standard protocol. *XRCC1*-Arg399Gln SNP genotyping was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Accordingly, the total *XRCC1* gene sequence was obtained from NCBI. The Arg399Gln polymorphic region was detected on the *XRCC1* gene and then specific primers were designed using Oligo7 software (https://www.oligo.net). The sequences of the forward and reverse primers were 5′-CCTCAGATCACACCTAACTGGC-3′ and 5′-CTAACACTTGTTCTCCCACCCC-3′, respectively. PCR was carried out in total volume of 25 μl containing 12.5 μl of PCR Master Mix (2×), each primer at a concentration of 0.5 μM and 50 ng of DNA template. PCR was performed in a peqSTAR thermal cycler (PeqLab, Erlangen, Germany) with the program: initial denaturation in 94°C for 5 min, with 35 repetitive cycles containing a denaturation step at 94°C for 45 s, an annealing step at 58°C for 45 s, and an extension step at 72°C for 45 s, which was followed by extension in 72°C for 7 min. All of the PCR reagents were obtained from Fermentas (Sankt Leon-Rot, Germany). Amplified fragments were treated by *Msp*I (Fermentas) restriction enzyme using a standard protocol. Finally, the digested mixtures were electrophoresed on 1% agarose gel for genotype detection. The samples with the AA genotype showed a 440-bp fragment on the agarose gel. Samples with the GG genotype show two 158- and 282-bp fragments, whereas the GA genotype revealed three 440-, 158- and 282-bp fragments on the agarose gel. To confirm the PCR-RFLP procedure, some samples with different genotypes were sequenced by CinnaGen.

2.2 | Meta-analysis

An electronic search was condicted utilizing the PubMed, EMBASE, Google Scholar and ScienceDirect databases (dated up to 20 October 2017) by two of the researchers independently. Keywords, including X-ray repair cross-complementing or XRCC1, and SNP or polymorphism or mutation or variants, and prostate cancer, were used to identify relevant articles. Also, the references of collected articles were revised to find possible eligible studies. Eligible studies had to meet the inclusion criteria: (i) case–control studies with the human origin designed to examine the association of *XRCC1*-Arg399Gln with PCa risk and (ii) existing data on the genotype frequencies in both cases and controls. Two two of the researchers independently screened and selected eligible studies with respect to the final meta-analysis. Disagreements were solved by consultation. For the next step, the relevant data were extracted from all of the included studies.

2.3 | In silico analysis

The effects of Arg399Gln substitution on the XRCC1 protein were evaluated using bioinformatics tools. First, the peptide sequence of XRCC1 was deduced from the ExPASy database (https://www.expasy.org) and the location of the Arg399Gln polymorphism was determined on the XRCC1 sequence. The impact of Arg399Gln on the physicochemical characteristics of the protein was evaluated using the ProtParam webserver (https://web.expasy.org/protparam). The effects of the substitution on the secondary structure of protein were assessed by the Chou–Fasman method. Then, the FASTA sequence of the mentioned peptide was converted to Protein Databank format for both wild and mutant protein using the PHYRE2 protein fold recognition server (www.sbg.bio.ic.ac.uk/phyre2).[21] The Ramachandran and hydrophobicity plots were obtained using Discovery Studio Visualization – Accelrys software (http://accelrys.com/products/collaborative-science/biovia-discovery-studio). In addition, the molecular effects of Arg399Gln substitution on protein function were evaluated using

the Polyphen2 webserver (genetics.bwh.harvard.edu/pph2) for both HumDiv and HumVar models.[22]

2.4 | Statistical analysis

In the case–control study, a chi-squared test was used to evaluate Hardy–Weinberg equilibrium (HWE), differences in allele and genotype frequencies, and other qualitative variables between case and control groups. Association of the *XRCC1*-Arg399Gln polymorphism with the risk of PCa was estimated by odds ratios (ORs) with 95% confidence intervals (95% CI). In addition, an independent *t*-test was used to compare numerical variables between case and control groups. $p < 0.05$ was considered statistically significant. These analyses were performed using SPSS, version 19 (IBM Corp., Armonk, NY, USA).

In the meta-analysis, the pooled data were employed to evaluate the strength of the association of *XRCC1*-Arg399Gln SNP with PCa risk by utilizing ORs with 95% CIs in five allelic (G versus A), homozygote co-dominant (GG versus AA), heterozygote co-dominant (AG versus AA), dominant (AG + GG versus AA) and recessive (GG versus AA +AG) genetic models. Moreover, a stratified meta-analysis was performed for ethnicity, sample size, source of control and HWE status. Heterogeneity among the included studies was evaluated by I^2 score and a chi-square-based *Q*-statistic test, with $p > 0.10$ being considered statistically significant. In these cases, the ORs were pooled in a random-effects model; otherwise, the fixed-effects model was employed.[23,24] Potential publication bias was evaluated by funnel plots and Egger's regression test. In addition, a sensitivity examination was performed to measure the stability of the association results. These analyses were performed using Open Meta Analyst (www.cebm.brown.edu/openmeta) and Comprehensive Meta-Analysis (https://www.meta-analysis.com) software.

3 | RESULTS

3.1 | Case–control analysis

Some demographic and clinical features of the study population are shown in Table 1. We found no statistically significant differences for body mass index, age and smoking status between the case and control groups. Furthermore, Gleason scores and PSA levels are shown in Table 1.

We calculated the power of our study with regard to the dominant model (AG + GG versus AA) and this was estimated to be more than 80%. The distribution of the genotype and allele frequencies of the case–control study is shown in Table 2. The genotype distribution revealed that HWE remains in both the case ($\chi^2 = 0.023$, $p = 0.879$) and control ($\chi^2 = 1.111$, $p = 0.292$) groups. The genotype analysis revealed that AG (OR = 1.82, 95% CI = 1.17–2.83, $p = 0.008$) and GG (OR = 2.12, 95% CI = 1.04–4.34, $p = 0.040$) genotypes are associated with PCa risk in our study population. Moreover, carriers of the G allele are at high risk for PCa (OR = 1.87, 95% CI = 1.23–2.85, $p = 0.003$). Allele analysis showed that there is a significant association between the G allele and PCa risk (OR = 1.60, 95% CI = 1.16–2.20, $p = 0.004$).

TABLE 1 Demographic and clinical features of study population

Variables	Case (*n* = 180)	Control (*n* = 180)	*p* value
Age (years)			
Range	41–86	51–88	
Mean ± SD	64.73 ± 12.28	66.67 ± 7.46	0.071
BMI (kg/m^2)			
Mean ± SD	23.08 ± 2.72	23.63 ± 2.55	0.051
Total PSA (ng/ml)			
Mean ± SD	110.51 ± 57.64	1.25 ± 0.75	<0.0001
Smoking			
Yes	112	123	0.223
No	68	57	
Gleason grade			
< 7	50	-	-
7	41	-	-
> 7	89	-	-

BMI, body mass index.

3.2 | Meta-analysis

Eighty-one relevant papers were identified by the charted search strategy. In accordance with the inclusion criteria, 16 sets of data from fourteen articles[1-5,15-19,25-28] were selected for meta-analysis. Then, our case–control data were added to the meta-analysis. A flow diagram of the study selection procedure and exact causes for exclusions is shown in Figure 1. In total, 8662 subjects including 4307 cases of PCa and 4355 healthy controls, were included in the meta-analysis. The year of publication ranged from 2002 to 2012. Details of eligible studies, including ethnicity, sample size, allele and genotype frequencies, p_{HWE} of control groups, source of controls, and genotyping method, are provided in Table 3. Among all of the studies, one study did not separate GG and AG genotypes.[2] In addition, two studies did not report the ethnicity of their population studies.[2,25] Furthermore, two studies investigated both Caucasian and African-American populations.[1,19]

An assessment of the association between the *XRCC1*-Arg399Gln polymorphism and PCa risk is provided in Table 4. Through the overall analyses, significant associations were observed between *XRCC1*-Arg399Gln and PCa susceptibility in allelic (OR = 1.19, 95% CI = 1.070–1.32, $p = 0.001$) and recessive (OR = 1.15, 95% CI = 1.04–1.28, $p = 0.007$) models (Figure 2). Stratified analysis by ethnicity indicated a significant increased risk for PCa in Caucasian population (G versus A: OR = 1.22, 95% CI = 1.01–1.48, $p = 0.039$). Also, stratified analysis by sample size revealed a significant association between Arg399Gln and PCa in studies with a sample size smaller (GG versus AA+AG: OR = 1.22, 95% CI = 1.04–1.45, $p = 0.017$) or greater (G versus A: OR = 1.32, 95% CI = 1.13–1.55, $p < 0.001$) than 400 subjects. Moreover, stratified analysis by source of controls showed a significant association between Arg399Gln and PCa in studies with population- (GG versus AA+AG: OR = 1.14, 95% CI = 1.01–1.29, $p = 0.028$) and hospital-based (G versus A: OR = 1.29, 95% CI = 1.13–1.48, $p < 0.001$) control subjects. When a study with a p_{HWE} of control group less than 0.05[18] was removed from the meta-analysis, we observed a significant association between the polymorphism and PCa in allelic (OR = 1.19,

TABLE 2 Genotype and allele frequencies of the *XRCC1*-Arg399Gln in case and control groups

Genotype/allele	*n* (%) Control (*n* = 180)	Case (*n* = 180)	OR (95% CI)	*p* value
AA	101 (56.11%)	73 (40.55%)	-	-
AG	64 (35.56%)	84 (46.67%)	1.82 (1.17–2.83)	**0.008**
GG	15 (8.33%)	23 (12.78%)	2.12 (1.04–4.34)	**0.040**
AG + GG	79 (43.00%)	107 (59.45%)	1.87 (1.23–2.85)	**0.003**
A	266 (73.89%)	230 (63.89%)	-	-
G	94 (26.11%)	130 (36.11%)	1.60 (1.16–2.20)	**0.004**

Significant differences between case and controls are shown in bold.

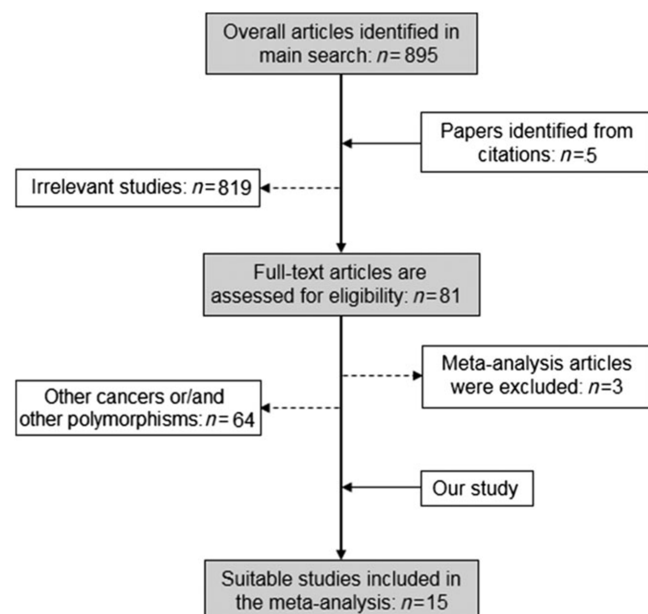

FIGURE 1 Flow chart of the study selection

95% CI = 1.06–1.32, p = 0.003) and recessive (OR = 1.16, 95% CI = 1.05–1.30, p = 0.006) models. Overall, there were true inter-study heterogeneities in allelic ($p_{heterogeneity}$ = 0.009, I^2 = 50%), homozygote co-dominant ($p_{heterogeneity}$ = 0.071, I^2 = 37%), homozygote co-dominant ($p_{heterogeneity}$ < 0.001, I^2 = 62%) and dominant ($p_{heterogeneity}$ > 0.001, I^2 = 88%) models. Similarly, heterogeneities remained in the stratified analyses (see Supporting information, Table S1).

For overall and stratified analyses, the funnel plots appeared to be symmetric in all genetic models (for allelic and recessive models, Figure 2). Also, we did not observe any publication bias using Egger's test (see Supporting information, Table S1). We evaluated the strength of the pooled ORs by removing a study at each analysis. No significant change of the pooled ORs was observed when each of the studies was omitted (data not shown). These results show that our meta-analysis was exactly robust.

3.3 | Structural analysis

Some bioinformatics tools were employed to evaluate the effects of Arg399Gln on molecular aspects of the XRCC1 protein. The data from ProtParam revealed that the molecular weights of wild and mutant types of XRCC1 were 69497.53 and 69525.59 Da, respectively. Theoretical pI was predicted to be 5.93 and 6.02 for wild and mutant proteins, respectively. The instability index was computed to be 63.35 for the 399Gln phenotype and 64.25 for 399Arg. Furthermore, the aliphatic index of wild and mutant proteins was predicted to be 62.89. This polymorphism could alter hydrophobicity and the Ramachandran plots of XRCC1 (see Supporting information, Figure S1). The location of the Arg399Gln polymorphism was predicted on a helix domain in the three-dimensional structure of XRCC1 (Figure 3). The effects of Arg399Gln on the secondary structure of XRCC1 were evaluated by the Chou–Fasman method. As shown in Figure 3, Arg399Gln substitution could change the secondary structure of XRCC1 around the mentioned polymorphism. The data from the Polyphen2 webserver showed that Arg399Gln substitution is probably damaging for both HumDiv (score = 0.979; sensitivity = 0.76; specificity = 0.96) and HumVar (score = 0.536; sensitivity = 0.82; specificity = 0.82) models (see Supporting information, Figure S2).

4 | DISCUSSION

In the present study, we first evaluated the association of *XRCC1*-Arg399Gln with PCa in an Iranian population. Our data revealed that there is a significant association between the mentioned SNP and PCa risk. However, there are some studies with inconsistent results in this regard. For example, Hamano *et al.*[16] reported that there is no significant association between *XRCC1*-Arg399Gln and PCa risk, whereas Berhane *et al.*[4] reported significant associations. Therefore, we performed a meta-analysis with the existing publications in this area to obtain a more accurate conclusion. The results of the meta-analysis demonstrated significant associations between Arg399Gln and PCa risk. Heterogeneity analysis revealed true heterogeneities among the studies included in the meta-analysis. Therefore, we employed a random-effects model with a wider confidence interval to calculate pooled ORs. Differences between the results of individual studies may arise from the sample size and the different ethnic origin of the people included in the cited articles, as well as geographical and environmental factors. A meta-analysis revealed there was no publication bias. Also, after the sensitivity test, no significant change of the pooled ORs was found. Such evidence indicates that the results of the meta-analysis are robust and reliable.

DNA repair mechanisms play a crucial role in the repair of multiple DNA damage triggered by exogenous or endogenous causes, such as single-strand breaks, oxidative DNA damage, non-bulky adducts or

TABLE 3 Characteristics of included studies in meta-analysis

Country (ethnicity)	Sample size (case/control)	Allele frequencies				Genotype frequencies						P HWE[a]	Source of control	Genotyping method	Study (year)
		Case		Control		Case			Control						
		G	A	G	A	GG	AG	AA	GG	AG	AA				
Italy (Caucasian)	76/182	48	104	141	232	9	30	37	27	78	77	0.325	PB	PCR-RFLP	Van Gils *et al.* (2002)
America (Caucasian)	637/480	418	548	320	640	72	274	291	56	208	216	0.583	HB	PCR-RFLP	Rybicki *et al.* (2004)
China (Asian)	155/243	223	87	363	123	85	53	17	132	99	12	0.226	PB	MALDI-TOF	Ritchey *et al.*5 (2005)
America (Caucasian)	228/217	162	294	129	305	29	104	95	21	87	109	0.552	HB	PCR-RFLP	Chen *et al.* (2006)
America (African-American)	123/115	36	210	34	196	3	30	90	3	28	84	0.718	HB	PCR-RFLP	Chen *et al.* (2006)
Japan (Asian)	165/165	93	237	89	241	15	63	87	10	69	86	0.428	HB	PCR-RFLP	Hirata *et al.* (2007)
China (Asian)	207/235	113	301	92	378	14	85	108	10	72	153	0.679	PB	PCR-RFLP	Xu *et al.* (2007)
Japan (Asian)	142/119	198	86	166	72	72	54	16	58	50	11	0.962	PB	PCR-RFLP	Hamano *et al.* (2008)
America (Caucasian)	1257/1240	1620	894	1552	928	522	576	159	481	590	169	0.575	PB	SNPlex™	Agalliu *et al.* (2010)
America (African-American)	144/82	243	45	133	31	103	37	4	53	27	2	0.503	PB	SNPlex™	Agalliu *et al.* (2010)
America (Unknown)	190/195	52	141	36	161	88		102	68		127	NS	PB	MALDI-TOF	Zhang *et al.* (2010)
India (Asian)	171/200	207	135	249	151	78	51	42	83	83	34	0.098	PB	PCR-RFLP	Mandal *et al.* (2010)
Brazil (Unknown)	172/172	198	146	167	141	73	52	47	65	73	34	0.107	HB	PCR-RFLP	Kuasne *et al.* (2011)
India (Asian)	150/150	140	160	112	188	40	60	50	24	64	62	0.280	PB	PCR-RFLP	Berhane *et al.* (2012)
Australian (Caucasian)	115/130	125	105	134	126	38	49	28	37	60	33	0.385	HB	PCR-RFLP	Dhillon *et al.* (2011)
India (Asian)	195/250	230	160	312	188	84	62	49	105	102	43	0.039	PB	PCR	Mittal *et al.* (2012)
Iran (Caucasian)	180/180	130	230	94	266	23	84	73	15	64	101	0.292	HB	PCR-RFLP	Present study

NS, not significant.
[a] HWE equilibrium in the control group (groups with $p < 0.05$ did not satisfy the HWE).

TABLE 4 Association results in the meta-analysis

Group	G versus A		GG versus AA		AG versus AA		AG + GG versus AA		GG versus AA + AG	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Total	1.19 (1.070–1.32)	0.001	1.07 (0.87–1.30)	0.530	0.93 (0.76–1.13)	0.447	0.94 (0.69–1.26)	0.663	1.15 (1.04–1.28)	0.007
Asian	1.13 (0.96–1.34)	0.135	1.01 (0.67–1.53)	0.957	0.78 (0.52–1.18)	0.241	0.91 (0.64–1.30)	0.620	1.19 (0.99–1.43)	0.064
Caucasian	1.22 (1.01–1.48)	0.039	1.17 (0.94–1.46)	0.158	1.12 (0.92–1.37)	0.268	0.95 (0.53–1.69)	0.849	1.12 (0.98–1.28)	0.094
African-American	1.12 (0.78–1.60)	0.551	0.95 (0.29–3.11)	0.934	0.96 (0.55–1.69)	0.894	0.98 (0.569–1.69)	0.945	1.31 (0.76–2.27)	0.326
Sample size<400	1.12 (0.98–1.28)	0.111	1.03 (0.76–1.40)	0.830	0.83 (0.63–1.11)	0.203	0.95 (0.74–1.21)	0.653	1.22 (1.04–1.45)	0.017
Sample size>400	1.32 (1.13–1.55)	< 0.001	1.10 (0.84–1.44)	0.496	1.08 (0.84–1.38)	0.540	0.97 (0.54–1.72)	0.903	1.11 (0.97–1.27)	0.117
PB	1.13 (0.99–1.29)	0.083	0.97 (0.71–1.31)	0.831	0.82 (0.59–1.12)	0.208	0.83 (0.52–1.33)	0.443	1.14 (1.01–1.29)	0.028
HB	1.29 (1.13–1.48)	< 0.001	1.17 (0.91–1.50)	0.227	1.04 (0.80–1.35)	0.773	1.10 (0.87–1.39)	0.444	1.19 (0.97–1.47)	0.101
$P_{HWE} > 0.05$	1.19 (1.06–1.32)	0.003	1.11 (0.91–1.36)	0.316	0.97 (0.79–1.18)	0.733	0.96 (0.70–1.32)	0.806	1.16 (1.05–1.30)	0.006

PB, population-based; HB, hospital-based.

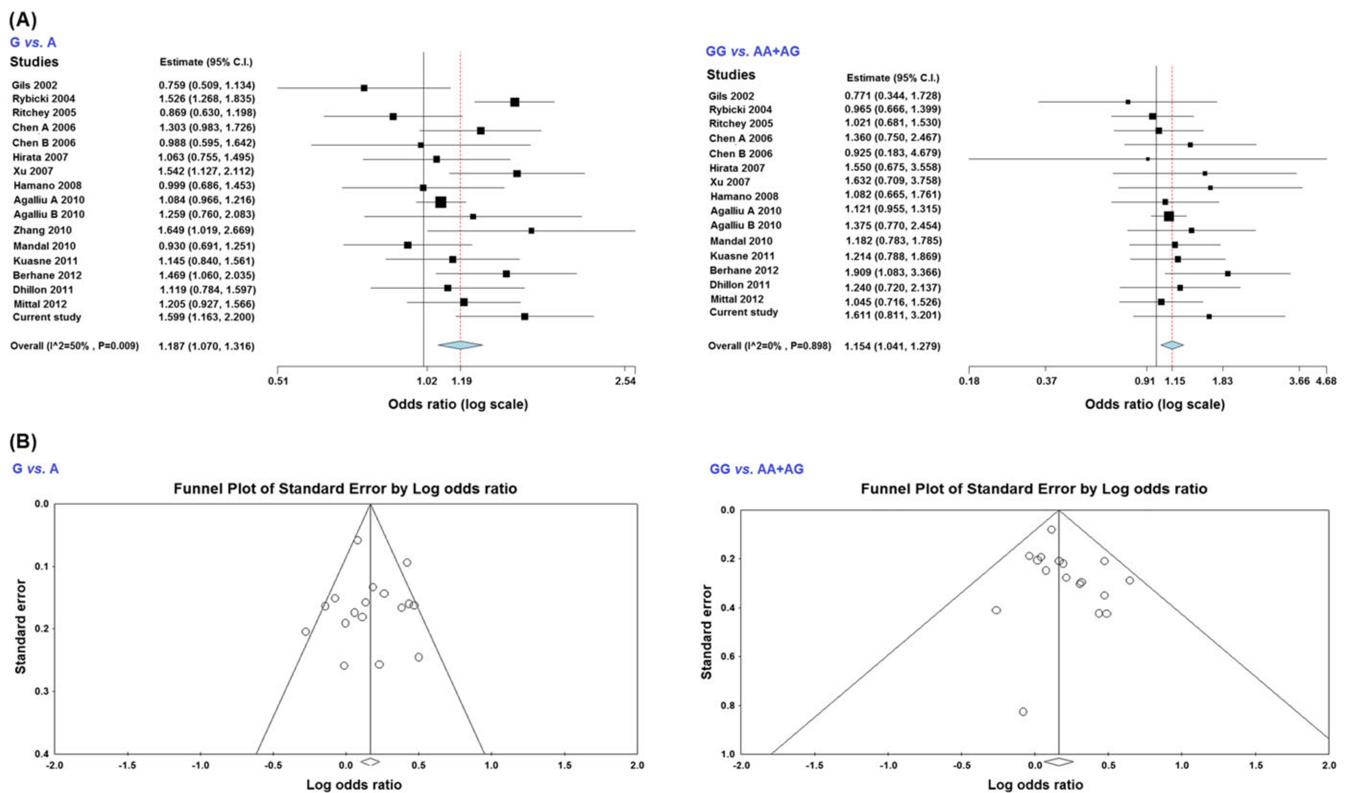

FIGURE 2 Forest and funnel plots. A, Forest plot for the association of the Arg399Gln polymorphism of the *XRCC1* gene with PCa in allelic (left) and recessive (right) models. B, Funnel plot for the association of the Arg399Gln polymorphism of the *XRCC1* gene with PCa in allelic (left) and recessive (right)

alkylation and methylation.[5,25] Mutations in some genes that cause a deficiency or an absence of DNA repair protein functions can result in a DNA repair deficit.[25] Among the mutations that increase the risk of PCa, polymorphisms comprise a large proportion.[3] Many studies confirm that SNPs in DNA repair genes can increase the risk of DNA damage and cancer.[6,8,12,29] The four most important DNA repair mechanisms are base excision repair, nucleotide excision repair, double-strand break repair and mismatched repair.[13] Base excision repair eliminates small lesions containing oxidized or reduced bases, fragmented and non-bulky adducts or those created by methylation. Nucleotide excision repair eliminates larger lesions that are usually caused by exogenous or environmental damage. Double-strand break repair, which is more difficult compared to other repairs, corrects replication errors or exogenous causes such as ionizing radiation. The last DNA repair mechanism is mismatched repair, which adjusts replication errors (base–base or insertion–deletion mismatch) resulting in DNA polymerase mistakes.[12,13] The X-ray repair cross-complementing group 1 gene as a DNA repairing gene can interact with numerous important repair proteins, playing an essential role in base excision repair and the repair of single-strand breaks of DNA, and also exhibits polymorphic variations.[15] Among several polymorphisms that are known in the *XRCC1* gene, Arg399Gln has been associated with

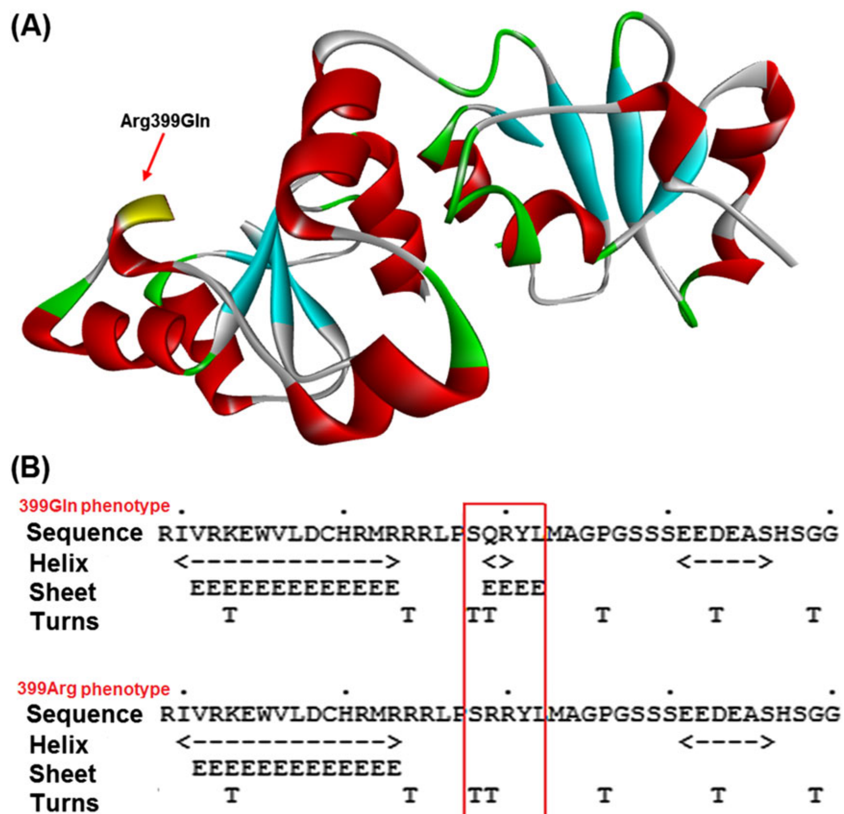

FIGURE 3 Three-dimensional and secondary structures of XRCC1 around the Arg399Gln substitution. A, Arg399Gln substitution is located on a helix region. B, Partial secondary structure of XRCC1 for 399Gln (up) and 399Arg (down) phenotypes. Changes in secondary structure are indicated by a red box

reduced DNA repair capacity.[1] Indeed, the special effects of this polymorphism in *XRCC1* are not well recognized, although it is probable that Arg399Gln may be associated with unregulated cell growth and carcinogenesis.[13] There are two C-terminal BRCT domains that interact with DNA ligase III alpha. The Arg399Gln SNP is located on the first BRCT domain.[30,31] Therefore, this SNP may affect heterodimerization of the protein with DNA ligase III.

According to our previous studies and to clarify the molecular aspects of Arg399Gln substitution on the XRCC1 protein, we used bioinformatics tools.[32,33] In silico analysis provides a time- and cost-consuming way of analyzing coding and non-coding SNPs.[34-36] Our bioinformatics analysis indicated that the Arg399Gln polymorphism is a damaging variation. This SNP could change the instability index of the protein, which would have an influence on protein activity.[37] This result was consistent with the Polyphen2 output that reported Arg399Gln to be a damaging SNP. The PolyPhen-2 webserver indicates the likely influence of coding SNPs on the stability and function of proteins. It assesses the potential damaging impacts of the non-synonymous SNPs based on a functional interpretation of SNPs and maps coding SNPs, as well as some other outlines. This server is also related to the UCSC database (https://genome.ucsc.edu).[22]

In conclusion, the results of the present study suggest that the *XRCC1*-Arg399Gln polymorphism could be as a prognostic and predictive biomarker for Pca, especially in a Caucasian population. There are some limitations to the present study that should be mentioned. In our case–control study, we did not consider gene–gene and gene-environmental interactions. Also, it would be too better to analyze the case–control study in a larger sample size with different ethnicities. In our meta-analysis, we did not access original data such as the clinical characteristics of participants to adjust our calculations. Because tumor features have not been taken into account, it is probable that some gene variations impact upon the tumor features in a manner that may pose a genetic risk factor for other disorder features. Also, there were no studies relating to an African population that would improve our meta-analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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