Systems Biology in Reproductive Medicine

ISSN: 1939-6368 (Print) 1939-6376 (Online) Journal homepage: http://www.tandfonline.com/loi/iaan20

Polymorphisms of DNA repair genes XRCC1 and LIG4 and idiopathic male infertility

Hadi Ghasemi, Iraj Khodadadi, Amir Fattahi, Abbas Moghimbeigi & Heidar Tavilani

To cite this article: Hadi Ghasemi, Iraj Khodadadi, Amir Fattahi, Abbas Moghimbeigi & Heidar Tavilani (2017): Polymorphisms of DNA repair genes XRCC1 and LIG4 and idiopathic male infertility, Systems Biology in Reproductive Medicine, DOI: 10.1080/19396368.2017.1374488

To link to this article: http://dx.doi.org/10.1080/19396368.2017.1374488

View supplementary material

Published online: 09 Oct 2017.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=iaan20

Download by: [University of Sherbrooke] **Date:** 10 October 2017, At: 09:58

SYSTEMS BIOLOGY IN REPRODUCTIVE MEDICINE
https://doi.org/10.1080/19396368.2017.1374488

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Check for updates

Polymorphisms of DNA repair genes *XRCC1* and *LIG4* and idiopathic male infertility

Hadi Ghasemi[a], Iraj Khodadadi[a], Amir Fattahi[b], Abbas Moghimbeigi [c], and Heidar Tavilani[d]

[a]Department of Biochemistry, Hamadan University of Medical Sciences, Hamadan, Iran; [b]Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; [c]Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran; [d]Urology & Nephrology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

ABSTRACT

Sperm DNA damage is one of the associated factors of idiopathic male infertility and abnormal spermatogenesis. This study was conducted to assess possible association between risk of male infertility with *X-ray repair cross complementing group 1* (*XRCC1*) Arg399Gln (G to A) and *DNA ligase 4* (*LIG4*) Thr9Ile (C to T) gene polymorphisms which are involved in different DNA repair pathways. In this case-control study 191 fertile and 191 infertile men (29-40 years old) were enrolled. The single-nucleotide polymorphism genotypes and alleles of *XRCC1* Arg399Gln and *LIG4* Thr9Ile were assessed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. There was no significant association between *XRCC1* Arg399Gln polymorphism and risk of male infertility. The frequency of *LIG4* Thr9Ile genotypes and alleles were statistically different between fertile and infertile men ($p<0.001$). We found that the CT genotype increased infertility risk more than threefold (OR, 3.12; 95% CI, 1.803-5.407). The *LIG4* TT genotype carriers had decreased progressive motile sperm ($p<0.05$) and increased non-progressive motile sperm ($p<0.001$) compared with the CC genotype. Moreover, sperm concentration in subjects carrying the CT genotype was lower than that observed in CC carriers ($p<0.05$). The results revealed that the GG/CT and GA/CT combinations of genotypes increase the risk of infertility 3.5 and fourfold, respectively ($p=0.021$ and 0.004, respectively). This study demonstrated that there was an association between *LIG4* Thr9Ile polymorphism and male infertility and suggests CT genotype as a risk factor for male infertility.

ARTICLE HISTORY
Received 31 January 2017
Revised 17 June 2017
Accepted 13 July 2017

KEYWORDS
DNA ligase; DNA repair; gene polymorphism; male infertility; X-ray repair cross complementing protein 1

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

Introduction

About 50% of all infertility cases are due to male reproductive system disorders and different studies have shown that male reproductive function is declining [Andersson et al. 2008]. Despite developments in diagnostic methods, more than half of the causes of male infertility remain unknown [Dohle et al. 2005]. In idiopathic infertile males, the semen analysis is abnormal and there is no clear diagnosis for infertility problems [Ferlin and Foresta 2014].

Owing to the high levels of reactive oxygen species (ROS) production during spermatogenesis [Fisher and Aitken 1997], diseases such as varicocele [Koksal et al. 2003] and the effects of environmental contaminants and drugs, the risk of genomic integrity damage is high [Morris 2002]. Therefore, it has been suggested that one of the major causes of male infertility and abnormal spermatogenesis, especially in idiopathic cases, might be the damage to the sperm DNA [Jahantigh and Hosseinzadeh Colagar 2017; Wright et al. 2014]. Interestingly, high sperm DNA damage and chromosomal fragility have been found in infertile men in comparison with fertile men [Papachristou et al. 2006]. Thus, maintaining sperm DNA integrity and repairing the damage appear essential for male fertility. Among the different types of DNA damage, single strand breaks (SSBs) and double-strand breaks (DSBs) are the most important types of damage which are caused by different factors such as radiation, oxidative stress, chemotherapeutic drugs, and alkylation [Lieber 2010].

The human body has developed a DNA repair mechanism, which includes several repair pathways. Homologous recombination (HR) and non-homologous end-joining (NHEJ) are two of the main repair pathways, which have critical roles in repairing DNA throughout the cell cycle including mitosis and meiosis. These pathways are believed to function in germ cell formation [Rothkamm et al. 2003]. One of the mechanisms that could possibly reduce DNA repair capacity is polymorphic variants of genes, which are

CONTACT Heidar Tavilani tayebinia@umsha.ac.ir Urology & Nephrology Research Center, Hamadan University of Medical Sciences, Shaheed Fahmideh Ave., Hamadan, Iran.
Supplemental data for this article can be accessed on the publisher's website.
© 2017 Taylor & Francis

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

involved in the HR and NHEJ pathways. Several functional genetic polymorphisms have been identified in these genes, altering repair capacity [Ji et al. 2010a; Wang et al. 2003]. Association of male infertility with the polymorphisms in genes involved in the DNA repair system have recently been demonstrated [Anifandis et al. 2017; Jahantigh and Hosseinzadeh Colagar 2017; Ji et al. 2012; Markandona et al. 2015]. X-ray repair cross complementing group 1 (XRCC1) is a multi-domain protein which acts as a 'scaffold' to draw other parts of the DNA base damage repair pathway [Thompson and West 2000]. This 70 kDa protein interacts with various enzymes such as DNA polymerase-b (Pol-b), poly-ADP ribose polymerase (PARP), AP endonuclease-1 (APE1), human DNA glycosylase (hOGG1), and DNA ligase III (LIG3) to facilitate HR and DNA SSB repair [Schreiber et al. 2002; Vidal et al. 2001]. High expression of the *XRCC1* gene has been reported in rodent and primate testes [Walter et al. 1994]. It has also been found that the XRCC1 protein is abundant in pachytene spermatocytes as well as in round spermatids [Walter et al. 1996]. The *XRCC1* gene is located on chromosome 19q13.2 and many single nucleotide polymorphisms have been identified [Hung et al. 2005]. Arg399Gln (G to A; rs25487) polymorphism on exon 10 is one of polymorphisms which is likely to modify the function of the XRCC1 protein [Hung et al. 2005]. Some studies using human lymphocytes have reported the association of this polymorphism with DNA repair impairment and high DNA adducts (reviewed in [Ginsberg et al. 2011]). Also, in a Chinese population study, the association of the Arg399Gln polymorphism with increased risk of idiopathic azoospermia has been reported [Gu et al. 2007a]. In addition, high levels of sperm DNA damage have been reported in men with GG and GA genotypes, who were exposed to genotoxic and carcinogenic substances [Rubes et al. 2010].

DNA ligase 4 (LIG4) is an ATP-dependent DNA ligase and in complex with XRCC4 carries out end-joining in the NHEJ pathway [Lieber et al. 2003]. It was found that dysfunction of this complex reduces genomic integrity and increases radiation sensitivity [Riballo et al. 1999]. The *LIG4* Thr9Ile (C to T; rs1805388) polymorphism changes the N-terminal region of the LIG4 protein and reduces adenylation and ligation activities of this protein. Therefore, it can affect the NHEJ pathway function [Girard et al. 2004]. Considering the high expression of the *LIG4* gene (13q33-34) in the testes and its important roles in spermatogenesis, this polymorphism might be involved in male infertility. In supporting this notion, a relationship between the *LIG4* gene (13q33-34) polymorphism with low DNA repair capacity and high risk of male infertility has been reported in a Chinese population [Ji et al. 2013].

Owing to the necessity of finding potential polymorphic markers which are involved in idiopathic male infertility and the existence of limited studies about the role of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms in male infertility, we have conducted a case-control study to assess the possible association between these polymorphisms and the risk of male infertility. Also, we have evaluated the effects of genetic interactions between these two polymorphisms, which are involved in different repair pathways in male fertility.

Results

Basic information about locus, subjects, and phenotypes are presented in Supplementary Table 1. Semen parameters of infertile groups are shown in Table 1 and the genotypes of DNA repair geneswere assessed by RFLP analysis shown in Figure 1. For example, in the XRCC1 Arg399Gln polymorphism, the absence of restriction site resulted in a 242bp single fragment which was referred to GG genotype whereas its breaking down into two bands (148 and 94 bp) represented AA genotype.

In contrast, the existence of three bands (242, 148, and 94 bp) was assumed as heterozygous GA genotype (Figure 1A). For LIG4 Thr9Ile polymorphism, the absence of restriction site resulted in a 121 bp fragment indicating homozygous TT genotype, while its breaking down into two bands (65 and 56 bp) represented CC genotype and observing three bands (121, 65, and 56 bp) was considered as heterozygous CT genotype (Figure 1B). Data summaries are presented in Tables 2 and 3. The data were presented according to the "standardization of reporting genetics of male infertility" suggested by Traven et al. [2017]. The distribution of both polymorphisms was not in Hardy-Weinberg equilibrium. There were no significant differences in frequencies of *XRCC1* Arg399Gln genotypes and alleles between fertile and infertile groups ($p>0.05$), as shown in Table 2. Moreover, logistic regression analysis revealed the lack of association between this polymorphism and the risk of male infertility. However, the distribution of *LIG4* Thr9Ile genotypes was significantly different between the fertile and infertile groups (Table 3). The frequency of the CC genotype was statistically higher in the fertile men than in infertile men

Table 1. Semen profile of infertile males.

Semen Parameters	Infertile (n=191)
Sperm concentration (million/ml)	41.96±29.44
Sperm normal morphology (%)	19.98±14.09
Progressively motile sperm[1] (%)	15.35±11.71
Non-progressively motile sperm[1] (%)	16.78±12.52
Immotile sperm[1] (%)	55.38±27.37

Results are presented as mean ± SD. [1]Grade of sperm movement according to WHO [2010] criteria.

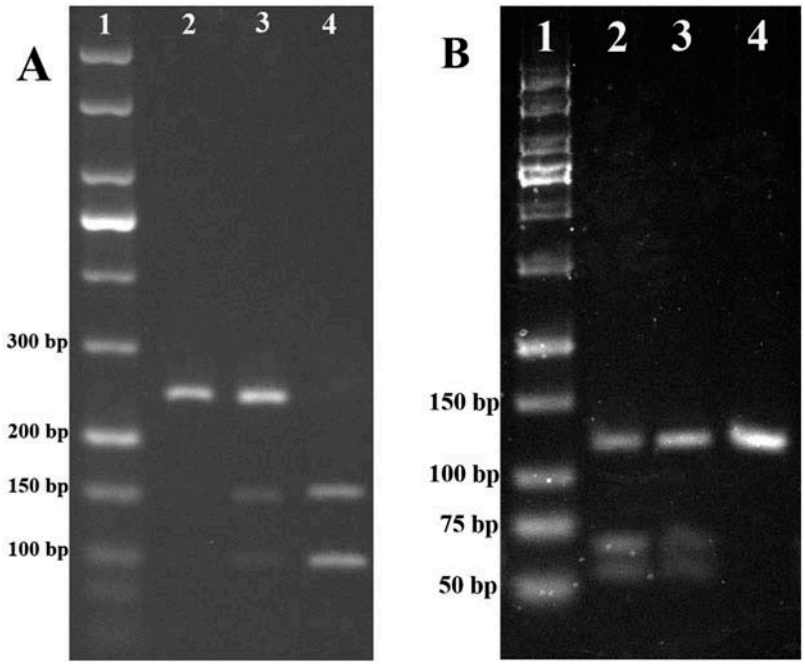

Figure 1. RFLP analysis of *XRCC1* and *LIG4*. Polymorphisms were investigated by RFLP technique using MspI and HpyCH4 III (Thermo Scientific, USA) as restriction enzymes for ARCC1 and *LIG4*, respectively. Digested products have been checked by electrophoresis on 3.0% agarose gel and then visualized under UV light. (A) *XRCC1* Arg399Gln (G to A). Lane 1 is 50 bp DNA ladder (Jena Bioscience, Germany); Lane 2 is GG genotype (242bp); Lane 3 is GA genotype (242, 148 and 94 bp); Lane 4 is AA genotype (148 and 94 bp); (B) *LIG4* Thr9Ile (C to T). Lane 1 is 50 bp DNA ladder (Jena Bioscience); Lane 2 is CC genotype (65 and 56 bp); Lane 3 is CT genotype (121, 65 and 56 bp); Lane 4 is TT genotype (121 bp).

Table 2. The frequency of *XRCC1* Arg399Gln (G to A) genotypes, alleles, and odds ratios in fertile (*n* = 191) and infertile (*n* = 191) males using χ2 test and regression logistics analysis.

Gene and polymorphism information		Genotypes frequencies			*p*-Value (χ^2, df)	Odd ratio, 95% CI (Lower-Upper, *p*)
Gene name/symbol	*X-ray repair cross complementing 1/XRCC1*		**Fertile** n (%)	**Infertile** n (%)		
Entrez ID/location	7515/19q13.31	GG	92 (48.16%)	78 (40.84%)	0.149 (χ^2=2.077, df=1)	Reference group
Race/Number of participants (infertile-controls)	Iranian/191-191	GA	91(47.64%)	106 (55.5%)	0.125 (χ^2=2.358, df=1)	1.347 (0.910-2.074, *p*=0.130)
Study approach	PCR-RFLP	AA	8(4.2%)	7 (3.66%)	0.792 (χ^2=0.069, df=1)	1.032 (0.358-2.974, *p*=0.953)
Disease/Ontology ID	Male infertility/12336	GA+AA	99 (51.84%)	113 (59.16%)	0.149 (χ^2=2.077, df=1)	1.346 (0.898-2.018, *p*=0.150)
Polymorphism ID/location	rs25487/exon 10		**Alleles frequencies**			
Genomic coordinate of the polymorphism (start-end)	43551524-43551624	G	275 (71.99%)	262 (68.59%)	0.303 (χ^2=1.059, df=1)	Reference group
Polymorphism biotype/Residue change	Missense/Arg399Gln	A	107 (28.01%)	120 (31.41%)		1.177 (0.863-1.606, *p*=0.304)

(p<0.001). A significantly higher frequency of the CT genotype was also observed in the infertile group compared to the fertile men. Our results revealed that the CT genotype could increase infertility risk by more than three-fold (OR, 3.122; 95% CI, 1.803-5.407). This negative effect on male fertility was also observed in T allele carriers which could increase the risk of male infertility more than 2.5-fold (OR, 2.506; 95% CI, 1.641-3.826). Association between various genotypes and semen parameters in the infertile group are presented in Table 4. The results demonstrated the lack of association between *XRCC1* Arg399Gln polymorphism with sperm motility and morphology. However, sperm concentration in AA carriers was significantly lower than that of subjects carrying GG and GA genotypes (p<0.05). The results revealed that *LIG4* Thr9Ile homozygous TT genotype carriers had decreased progressive motile sperm (p<0.05) and increased non-progressive motile sperm (p<0.001) compared with the wild type CC genotype. Also in subjects with the CT genotype, the percentage of non-progressively motile sperm was higher than that of the CC genotype (p<0.001).

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

Table 3. The frequency of *LIG4* Thr9Ile (C to T) genotypes, alleles, and odds ratios in fertile (n = 191) and infertile (n = 191) males using χ2 test and regression logistics analysis.

Gene and polymorphism information		Genotypes frequencies			*p*-Value (χ^2, df)	Odd ratio, 95% CI (Lower-Upper, *p*)
Gene name/symbol	*DNA ligase 4/LIG4*		**Fertile** *n* (%)	**Infertile** *n* (%)		
Entrez ID/location	3981/13q33-34	CC	162 (84.82%)	125 (65.45%)	<0.001 (χ^2=19.181, df=1)	Reference group
Race/Number of participants (infertile-controls)	Iranian/191-191	CT	22 (11.52%)	53 (27.75%)	<0.001 (χ^2=15.944, df=1)	3.122 (1.803-5.407, p<0.001)
Study approach	PCR-RFLP	TT	7 (3.66%)	13 (6.8%)	0.168 (χ^2=1.899, df=1)	2.407 (0.933-6.211, p=0.069)
Disease/Ontology ID	Male infertility/ 12336	CT+TT	29 (15.18%)	66 (34.55%)	<0.001 (χ^2=19.181, df=1)	2.95 (1.798-4.839, p<0.001)
Polymorphism ID/location	rs1805388/exon 2		**Alleles frequencies**			
Genomic coordinate of the polymorphism (start-end)	108211193-108211293	C	346 (90.58%)	303 (79.32%)	<0.001 (χ^2=18.927, df=1)	Reference group
Polymorphism biotype/Residue change	Missense/Thr9Ile	T	36 (9.42%)	79 (20.68%)		2.506 (1.641-3.826, p<0.001)

Table 4. Semen parameters according to the various genotypes of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms in infertile group (n=191).

Genotypes	Sperm motility [1] (%)			Sperm concentration (million/ml)	Sperm normal morphology (%)
	Progressively motile sperm	Non-progressively motile sperm	Immotile sperm		
XRCC1 Arg399Gln					
GG	15.65 ± 12.86	16.70 ± 11.66	53.61 ± 28.46	40.66 ± 27.51	19.43 ± 13.88
GA	14.94 ± 10.43	17.12 ± 13.27	57.66 ± 26.02	44.63 ± 30.67	20.63 ± 14.47
AA	18.31 ± 17.51	12.45 ± 10.69	40.65 ± 33.14	18.21 ± 16.00[a*,b*]	16.42 ± 11.07
LIG4 Thr9Ile					
CC	16.46 ± 10.13	9.56 ± 8.52	52.59 ± 28.58	50.73 ± 28.32	19.56 ± 14.18
CT	15.46 ± 12.36	15.72 ± 13.01[a**]	59.56 ± 21.86	40.96 ± 31.82[a*]	21.47 ± 14.07
TT	9.79 ± 10.55[a*]	19.69 ± 11.65[a**]	65.25 ± 32.99	38.37 ± 29.12	13.92 ± 11.80

Results are presented as mean ± SD; [1]Grade of sperm movement according to WHO [2010] criteria. Statistical comparison: [a]Variant vs. Wild type; [b]Heterozygous variant vs. Homozygous variant; Statistical significance: **p<0.001; *p<0.05.

Moreover, sperm concentration in subjects carrying the CT genotype was lower than that observed in the CC carriers (p<0.05).

We have also investigated the distribution of different genotype combinations of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms in the studied population (Table 5). In total, 7.85% fertile men and 18.33% of infertile men had variant genotypes at both loci (*XRCC1* Arg399Gln and *LIG4* Thr9Ile). A statistically higher frequency of the GG/CC combination was observed in fertile men compared to infertile individuals (p=0.001) whereas any genotype combinations of *XRCC1* Arg399Gln polymorphism with heterozygous CT genotype from *LIG4* Thr9Ile polymorphism (i.e., GG/CT and GA/CT) were found markedly higher in infertile men (p=0.021 and p=0.004, respectively), as shown in Table 5. Accordingly, logistic regression analysis confirmed the likelihood of interaction of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms due to the increased risk of male infertility observed in GG/CT and GA/CT carriers compared with those that have the GG/CC homozygous wild type genotype.

Discussion

Idiopathic male infertility accounts for a large proportion of all infertility cases with unknown mechanisms [Dohle et al. 2005; Kothandaraman et al. 2016]. Previous studies have suggested that genetic factors and DNA defects could be involved in idiopathic male

Table 5. Interaction of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms for male infertility risk.

	Fertile *n* (%)	Infertile *n* (%)	*p*-Value (χ^2, df)	Odd ratio, 95% CI (Lower-Upper, *p*)
GG/CC	78 (40.84%)	48 (25.13%)	0.001 (χ^2=10.658, df=1)	Reference group
GG/CT	11 (5.76%)	24 (12.57%)	0.021 (χ^2=5.316, df=1)	3.545 (1.595-7.883, p=0.002)
GG/TT	3 (1.57%)	6 (3.14%)	0.312 (χ^2=1.024, df=1)	3.250 (0.776-13.605, p=0.107)
GA/CC	76 (39.79%)	71 (37.17%)	0.599 (χ^2=0.276, df=1)	1.518 (0.936-2.462, p=0.091)
GA/CT	11 (5.76%)	28 (14.66%)	0.004 (χ^2=8.253, df=1)	4.136 (1.887-9.066, p<0.001)
GA/TT	4 (2.09%)	7 (3.67%)	0.359 (χ^2=0.824, df=1)	2.844 (0.791-10.228, p=0.110)
AA/CC	8 (4.19%)	6 (3.14%)	0.586 (χ^2=0.297, df=1)	1.219 (0.398-3.727, p=0.729)

Seven combinations were analyzed. AA/CT and AA/TT genotype combinations were not included in the table due to the statistical limitations and the low number of cases. df: degrees of freedom, CI: confidence interval, χ^2: Chi-square.

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

infertility [Ferlin and Foresta 2014]. Single nucleotide mutations or polymorphisms could be abundantly found in different genes and studies have shown that the mutation in a gene, sometimes could alter activity of the gene product [Fung et al. 2014]. A very recent study has shown that polymorphisms in genes including *XRCC5* VNTR, *XRCC6*, and *XRCC7* 6721G>T are associated with male infertility [Jahantigh and Hosseinzadeh Colagar 2017]. Polymorphisms in genes such as *XRCC1* and *LIG4* could be important, because the products of these genes play crucial roles in major pathways of DNA repair [Caldecott 2014; Park et al. 2014]. These genes are highly expressed in testis [Ji et al. 2013; Walter et al. 1994], so any decrease in activity of these proteins could disrupt the DNA repair system and lead to the accumulation of mutations and breaks in sperm DNA. According to the above description, polymorphisms in these genes could affect the quality of sperm and decrease infertility. This study aimed to investigate the association of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms with male infertility.

Some studies have shown that the *XRCC1* Arg399Gln polymorphism alters XRCC1 protein function and reduces the ability of DNA damage removal after irradiation and exposure to genotoxic compounds more than three-fold [Girard et al. 2004; Li et al. 2006]. However, our results indicated that genotype and allele distribution of *XRCC1* Arg399Gln polymorphism did not significantly differ between fertile and infertile men, and therefore, no association was found between this polymorphism and male infertility. Our findings are consistent with the results observed by Ji et al. [2010b], who have reported the lack of relationship between this polymorphism and the risk of male infertility and sperm DNA damage. In the other study, Zheng and colleagues investigated the association between Arg399Gln polymorphism and infertility with idiopathic azoospermia and their results revealed that the SNP Arg399Gln of *XRCC1* could be a marker of genetic susceptibility to idiopathic azoospermia [Zheng et al. 2012]. In a similar study on idiopathic azoospermia subjects, no differences were found in allele and genotype distribution of the Arg399Gln polymorphism between patients and healthy men [Gu et al. 2007b]. In this study, however, it has been noted that the AA genotype may reduce the risk of idiopathic azoospermia. It was reported that the repair efficiency of the wild type genotype is higher than that of the AA genotype which could be due to decreased XRCC1 activity [Li et al. 2006]. Rubes et al. [2010] have also shown higher sperm chromatin damage and repressed spermatogenesis in men carrying the GG and GA genotypes compared with AA genotype carriers. While this polymorphism is located in the structural region of the gene and most likely could affect protein activity, it has recently been claimed that the A allele increases *XRCC1* gene expression [Zipprich et al. 2010]. The Arg399Gln polymorphism occurs in the BRCT1 binding domain and the role of this domain in *XRCC1* gene structure is unclear, but according to the previous studies and the results of this study, it seems that this polymorphism has little effect on the protein activity and cannot endanger male fertility under normal conditions [Ginsberg et al. 2011]. There is compelling evidence for increased sperm DNA damage in A allele carriers compared with the G allele [Ji et al. 2010b]. Therefore, it is plausible that A allele carriers require higher activity of DNA repair pathway and *XRCC1* gene expression to prevent reducing sperm quality and male infertility. Thus, any alteration in XRCC1 activity due to the Arg399Gln polymorphism may disrupt the function of DNA repair machinery and impact male fertility.

Results of the present study showed that there were significant differences in genotype and allele distributions of *LIG4* Thr9Ile polymorphism between fertile and infertile men. There was a higher frequency of CC genotype in the fertile group compared to infertile subjects, whereas fertile men showed a lower frequency of CT genotype than the infertile group ($p<0.001$). Also, results revealed a higher frequency of T allele in infertile men compared with fertile men. Regression analysis showed that the CT genotype could be a risk factor for infertility and increases the risk of male infertility by more than three-fold. Similar to our results Ji et al. [2013] have demonstrated that the CT and TT genotypes are associated with increasing the risk of male infertility more than 1.5-fold and 2.5-fold, respectively. Due to the importance of the NHEJ pathway in the removal of DNA damage and the key role of LIG4 in this pathway, any disruption in the activity of LIG4 can cause male infertility. Since *LIG4* Thr9Ile polymorphism alters the N-terminal region of the LIG4 protein, which is essential for its ligase activity, and based on previous studies confirming a two- to three-fold reduction in LIG4 ligase activity induced by this polymorphism [Girard et al. 2004], it seems that our results are in accordance with previous observations.

It should be mentioned that the observed distribution for *XRCC1* and *LIG4* polymorphisms were not in Hardy-Weinberg equilibrium. Such deviation from the equilibrium could reflect different reasons including biological selection effects, small sample size, or genotyping errors [Nielsen et al. 1998]. In order to confirm the accuracy of data collection and genotyping method

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

we rechecked the data and re-genotyped at least 20% of the samples. Also the existence of several studies on similar polymorphism of *XRCC1* among the Iranian population of even lower sample sizes have consistently reported a genotype distribution in equilibrium [Fard-Esfahani et al. 2011; Jahantigh and Hosseinzadeh Colagar 2017; Saadat et al. 2008]. This made us consider ethnic bias as a possible underlying reason. In support of this explanation, previous studies on a population of the same region (Hamadan city) with a different sample size showed inconstancy with the Hardy-Weinberg equilibrium [Hassanzadeh et al. 2013; Khosropour et al. 2017] which could be due to inter-ethnic marriage. However, this does not exclude a possible effect of approximately small sample size in the present study, and thus further studies with larger sample sizes are required. Investigation of the association between XRCC1 Arg399Gln polymorphism and semen characteristics in infertile men revealed that the AA genotype carriers have significantly lower sperm concentration compared to men with GG or GA genotypes. Such a difference could be due to the role of XRCC1 in spermatogenesis as a high level expression has been seen in the testes, pachytene spermatocytes, and round spermatids [Walter et al. 1994; Walter et al. 1996]. Since the XRCC1 Arg399Gln polymorphism decreases XRCC1 activity and functionality [Hung et al. 2005], it can reduce spermatogenesis and consequently sperm concentration. However, owing to fewer infertile men with AA genotype in our study (seven patients), it is difficult to draw a proper conclusion.

In the *LIG4* Thr9Ile polymorphism, we observed that infertile men with the TT genotype had lower sperm motility than the wild type carriers. An increased percentage of non-progressive motile sperm and decreased sperm concentration was observed in infertile patients with CT genotype in comparison with CC genotype carriers. LIG4 has a role in spermatogenesis and DNA repair machinery in the sperm [Ji et al. 2013]. Reducing the activity of LIG4 due to the polymorphism could disrupt cell cycle and meiosis and lead to sperm reduction. It has also been observed that this polymorphism is associated with increased sperm DNA damage [Ji et al. 2013] and previous studies have shown negative correlations between sperm DNA damage and semen parameters such as motility [Zini et al. 2001]. Results of the present study are consistent with the results of a previous study showing negative association between *LIG4* Thr9Ile polymorphism and sperm concentration in infertile men [Ji et al. 2013].

Further investigation assessed the likelihood of association of different genotype combinations of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms with male infertility. The results revealed that the GG/CT and GA/CT combinations of genotypes can increase the risk of infertility more than 3.5- and four-fold, respectively. Since XRCC1 and LIG4 are members of different DNA repair pathways, at the moment the mechanism underlying likelihood interactions of *XRCC1* Arg399Gln and *LIG4* Thr9Ile polymorphisms is not well understood. Considering that the AA genotype increases *XRCC1* expression [Zipprich et al. 2010] and has protective effects against idiopathic azoospermia [Gu et al. 2007b], there is a possibility that in the condition of reduced sperm quality due to the *LIG4* Thr9Ile polymorphism, carrying GG or GA genotypes of *XRCC1* paves the way for male infertility.

There were some limitations in the present study such as: (1) we did not have access to fertile men's semen samples, (2) we did not evaluate sperm DNA damage and its association with these polymorphisms, and (3) the number of homologous variant carriers was low. Therefore, large scale studies together with DNA damage assessment are needed to overcome these limitations.

In conclusion, this study demonstrated that there was an association between the *LIG4* Thr9Ile polymorphism and male infertility. Thus, the existence of CT genotype could be a risk factor for male infertility. Moreover, an association between *LIG4* Thr9Ile genotype distribution and sperm motility and concentration was revealed. However, there was no association between *XRCC1* Arg399Gln polymorphism and male infertility and a lower sperm concentration in AA genotype carriers compared to that of subjects with GG and GA genotypes was observed.

Materials and methods

Specimen collection and processing

In this case-control study, a total of 382 Iranian men (191 fertile and 191 infertile) were included. Infertile men (aged 29-40 years old) with abnormal semen analysis according to World Health Organization (WHO) metrics were selected from patients referred to the Fatemieh Fertility Clinic of Hamadan University of Medical Sciences. Infertile individuals had no children after one year (or more) of unprotected sexual intercourse. According to WHO [2010] guidelines for semen analysis, sperm motility was categorized as: progressive motility (PR), non-progressive motility (NP), and immotility (IM). The lower reference limits (5th centiles and their 95% confidence intervals) for semen parameters were: sperm concentration 15 million/ml, PR 32%, and normal sperm morphology 4%. In addition, asthenozoospermia was defined by the percentage

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

of PR spermatozoa below the lower reference limit [WHO 2010]. In our study, the infertile group consisted of asthenozoospermic individuals. Patients with certain infertility causes (e.g., abnormal karyotype, infectious diseases, and varicocele) were excluded from our study. Fertile individuals had children (one to three children and less than five years had passed since they had children) without benefit from assisted reproductive technologies and did not differ in age from the patients. All patients gave written informed consent before sample collection and this study was approved by the Ethical Committee of Hamadan University of Medical Sciences. After three d of sexual abstinence, semen samples were collected from infertile men and semen parameters were analyzed according to the WHO procedures by CASA [WHO 2010]. Blood samples were obtained from all participants by venous puncture and collected in heparin containing tubes. After centrifugation, the cell containing part was stored at -80°C for DNA extraction.

Genotyping

Genomic DNA was extracted from leukocyte pellets using phenol/chloroform extraction methods. A ratio of A260/A280 absorbance was used to qualify extracted DNA. PCR was performed (PCR PreMix; BIONEER kit, Korea) to amplify 242 bp and 121 bp fragments which contained *XRCC1* Arg399Gln (G to A) and *LIG4* Thr9Ile (C to T) polymorphismic sites, respectively (Supplementary Table 1). Previously described primers were used as the following: *XRCC1* Arg399Gln: forward, 5′-CCCCAAGTACAGCCAGGTC-3′ and reverse, 5′-TGTCCCGCTCCTCTCAGTAG-3′ [Duell et al. 2000]; *LIG4* Thr9Ile: forward, 5′-TCTGTATTC GTTCTAAAGTTGAACA-3′ and reverse5′-TGCTT TACTAGTTAAACGAGAAGAT-3′ [Yin et al. 2012]. The size of all amplified PCR products was confirmed on a 1% agarose gel. *XRCC1* and *LIG4* genes were genotyped by restriction fragment length polymorphism technique using MspI and HpyCH4 III enzymes (Thermo Scientific, USA), respectively. Digested samples were checked by electrophoresis on 3.0% agarose gel and then visualized under UV light.

Statistical analysis

Data were analyzed using SPSS V.16 software and the results were expressed as mean ± standard deviation. The genotypes distribution was checked for Hardy-Weinberg equilibrium. To confirm normal distribution of data One-Sample Kolmogorov-Smirnov test was applied. The allele and genotype frequencies were compared between the groups using Chi-Square test. Also, for further evaluation of the polymorphisms, the relative risk on male infertility logistic regression analysis was used. Semen parameters among three genotypes were compared by One-way ANOVA analysis and differences between two genotypes were analysed using Tukey post-hoc test. Statistical significance was assumed at the $p<0.05$ level.

Acknowledgments

This research was supported by Hamadan University of Medical Sciences. This work was done in the Department of Biochemistry, Hamadan University of Medical Sciences, Hamadan, Iran.

Declaration of interests

The authors are academic members or postgraduate students of Hamadan University of Medical Sciences and are not directly funded by the Government of Iran.

Notes on contributors

Designed the study, wrote the article, and contributed in the critical revision: HT, IK; Contributed in sample collection and performing experiments: HG, AF; Statistical analysis: AM, AF.

ORCID

Abbas Moghimbeigi http://orcid.org/0000-0002-3803-3663

References

Andersson, A.M., Jørgensen, N., Main, K., Toppari, J., Meyts, E., Leffers, H., et al. (2008) Adverse trends in male reproductive health: we may have reached a crucial 'tipping point'. Int J Androl **31**:74–80.

Anifandis, G., Markandona, O., Dafopoulos, K., Messini, C., Tsezou, A., Dimitraki, M., et al. (2017) Embryological Results of Couples Undergoing ICSI-ET Treatments with Males Carrying the Single Nucleotide Polymorphism rs175080 of the MLH3 Gene. Int J Mol Sci **18**:pii: E314.

Caldecott, K.W. (2014) DNA single-strand break repair. Exp Cell Res **329**:2–8.

Dohle, G.R., Colpi, G., Hargreave, T., Papp, G., Jungwirth, A., Weidner, W., et al. (2005) EAU guidelines on male infertility. Eur Urol **48**:703–711.

Duell, E.J., Wiencke, J.K., Cheng, T.-J., Varkonyi, A., Zuo, Z.F., Ashok, T.D.S., et al. (2000) Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis **21**:965–971.

Fard-Esfahani, P., Fard-Esfahani, A., Fayaz, S., Ghanbarzadeh, B., Saidi, P., Mohabati, R., et al. (2011) Association of Arg194Trp, Arg280His and Arg399Gln polymorphisms in X-ray repair cross-complementing group 1 gene and risk of differentiated thyroid carcinoma in Iran. Iran Biomed J **15**:73.

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017

Ferlin, A. and Foresta, C. (2014) New genetic markers for male infertility. Curr Opin Obstet Gynecol **26**:193–198.

Fisher, H.M. and Aitken, R.J. (1997) Comparative analysis of the ability of precursor germ cells and epididymal spermatozoa to generate reactive oxygen metabolites. J Exp Zool **277**:390–400.

Fung, K.L., Pan, J., Ohnuma, S., Lund, P.E., Pixley, J.N., Kimchi-Sarfaty, C., et al. (2014) MDR1 synonymous polymorphisms alter transporter specificity and protein stability in a stable epithelial monolayer. Cancer Res **74**:598–608.

Ginsberg, G., Angle, K., Guyton, K. and Sonawane, B. (2011) Polymorphism in the DNA repair enzyme XRCC1: utility of current database and implications for human health risk assessment. Mutat Res **727**:1–15.

Girard, P.-M., Kysela, B., Härer, C.J., Doherty, A.J. and Jeggo, P.A. (2004) Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. Hum Mol Genet **13**:2369–2376.

Gu, A., Ji, G., Liang, J., Xia, Y., Lu, N., Wu, B., et al. (2007a) DNA repair gene XRCC1 and XPD polymorphisms and the risk of idiopathic azoospermia in a Chinese population. Int J Mol Med **20**:743–747.

Gu, A.H., Liang, J., Lu, N.X., Wu, B., Xia, Y.K., Lu, C.C., et al. (2007b) Association of XRCC1 gene polymorphisms with idiopathic azoospermia in a Chinese population. Asian J Androl **9**:781–786.

Hassanzadeh, T., Maleki, M., Saidijam, M. and Paoli, M. (2013) Association between leptin gene G-2548A polymorphism with metabolic syndrome. J Res Med Sci **18**:668–673.

Hung, R.J., Hall, J., Brennan, P. and Boffetta, P. (2005) Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. Am J Epidemiol **162**:925–942.

Jahantigh, D. and Hosseinzadeh Colagar, A. (2017) XRCC5 VNTR, XRCC6-61C> G, and XRCC7 6721G> T Gene Polymorphisms Associated with Male Infertility Risk: Evidences from Case-Control and In Silico Studies. Int J Endocrinol **2017**:4795076.

Ji, G., Gu, A., Zhou, Y., Shi, X., Xia, Y., Long, Y., et al. (2010a) Interactions between exposure to environmental polycyclic aromatic hydrocarbons and DNA repair gene polymorphisms on bulky DNA adducts in human sperm. PLoS One **5**:e13145.

Ji, G., Gu, A., Zhu, P., Xia, Y., Zhou, Y., Hu, F., et al. (2010b) Joint effects of XRCC1 polymorphisms and polycyclic aromatic hydrocarbons exposure on sperm DNA damage and male infertility. Toxicol Sci **116**:92–98.

Ji, G., Long, Y., Zhou, Y., Huang, C., Gu, A. and Wang, X. (2012) Common variants in mismatch repair genes associated with increased risk of sperm DNA damage and male infertility. BMC Med **10**:49.

Ji, G., Yan, L., Liu, W., Huang, C., Gu, A. and Wang, X. (2013) Polymorphisms in double-strand breaks repair genes are associated with impaired fertility in Chinese population. Reproduction **145**:463–470.

Khosropour, S., Hamidi, M., Fattahi, A., Khodadadi, I., Karami, M., Fazilati, M., et al. (2017) Leptin and leptin-receptor polymorphisms in fertile and infertile men. Syst Biol Reprod Med **63**:7–14.

Koksal, I., Usta, M., Orhan, I., Abbasoglu, S. and Kadioglu, A. (2003) Potential role of reactive oxygen species on testicular pathology associated with infertility. Asian J Androl **5**:95–100.

Kothandaraman, N., Agarwal, A., Abu-Elmagd, M. and Al-Qahtani, M.H. (2016) Pathogenic landscape of idiopathic male infertility: new insight towards its regulatory networks. npj Genomic Medicine **1**:16023.

Li, Y., Marion, M.-J., Zipprich, J., Freyer, G., Santella, R., Kanki, C., et al. (2006) The role of XRCC1 polymorphisms in base excision repair of etheno-DNA adducts in French vinyl chloride workers. Int J Occup Med Environ Health **19**:45–52.

Lieber, M.R. (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end joining pathway. Annu Rev Biochem **79**:181.

Lieber, M.R., Ma, Y., Pannicke, U. and Schwarz, K. (2003) Mechanism and regulation of human non-homologous DNA end-joining. Nat Rev Mol Cell Biol **4**:712–720.

Markandona, O., Dafopoulos, K., Anifandis, G., Messini, C.I., Dimitraki, M., Tsezou, A., et al. (2015) Single-nucleotide polymorphism rs 175080 in the MLH3 gene and its relation to male infertility. J Assist Reprod Genet **32**:1795–1799.

Morris, I.D. (2002) Sperm DNA damage and cancer treatment. Int J Androl **25**:255–261.

Nielsen, D.M., Ehm, M.G. and Weir, B.S. (1998) Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. Am J Hum Genet **63**:1531–1540.

Papachristou, F., Lialiaris, T., Touloupidis, S., Kalaitzis, C., Simopoulos, C. and Sofikitis, N. (2006) Evidence of increased chromosomal instability in infertile males after exposure to mitomycin C and caffeine. Asian J Androl **8**:199–204.

Park, J.H., Welner, R.S., Tenen, D.G. and Yan, C.T. (2014) Lig4 Is Essential for Maintaining HSC Homeostasis. Blood **124**:606.

Riballo, E., Critchlow, S., Teo, S., Doherty, A., Priestley, A., Broughton, B., et al. (1999) Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. Curr Biol **9**:699–S692.

Rothkamm, K., Krüger, I., Thompson, L.H. and Löbrich, M. (2003) Pathways of DNA double-strand break repair during the mammalian cell cycle. Mol Cell Biol **23**:5706–5715.

Rubes, J., Rybar, R., Prinosilova, P., Veznik, Z., Chvatalova, I., Solansky, I., et al. (2010) Genetic polymorphisms influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res **683**:9–15.

Saadat, M., Kohan, L. and Omidvari, S. (2008) Genetic polymorphisms of XRCC1 (codon 399) and susceptibility to breast cancer in Iranian women, a case–control study. Breast Cancer Res Treat **111**:549–553.

Schreiber, V., Amé, J.-C., Dollé, P., Schultz, I., Rinaldi, B., Fraulob, V., et al. (2002) Poly (ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. J Biol Chem **277**:23028–23036.

Thompson, L.H. and West, M.G. (2000) XRCC1 keeps DNA from getting stranded. Mutat Res **459**:1–18.

Traven, E., Ogrinc, A. and Kunej, T. (2017) Initiative for standardization of reporting genetics of male infertility. Syst Biol Reprod Med **63**:58–66.

Vidal, A.E., Boiteux, S., Hickson, I.D. and Radicella, J.P. (2001) XRCC1 coordinates the initial and late stages of DNA a basic site repair through protein–protein interactions. EMBO J **20**:6530–6539.

Walter, C.A., Lu, J., Bhakta, M., Zhou, Z.-Q., Thompson, L.H. and Mccarrey, J.R. (1994) Testis and somaticXrcc-1 DNA repair gene expression. Somat Cell Mol Genet **20**:451–461.

Walter, C.A., Trolian, D.A., Mcfarland, M.B., Street, K.A., Gurram, G.R. and Mccarrey, J.R. (1996) Xrcc-1 expression during male meiosis in the mouse. Biol Reprod **55**:630–635.

Wang, Y., Spitz, M.R., Zhu, Y., Dong, Q., Shete, S. and Wu, X. (2003) From genotype to phenotype: correlating XRCC1 polymorphisms with mutagen sensitivity. DNA Repair **2**:901–908.

WHO. (2010) Laboratory manual for the examination and processing of human semen. Fifth Edition. World Health Organization, Geneva 7–113.

Wright, C., Milne, S. and Leeson, H. (2014) Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. Reprod Biomed Online **28**:684–703.

Yin, M., Liao, Z., Liu, Z., Wang, L.E., O'reilly, M., Gomez, D., et al. (2012) Genetic variants of the nonhomologous end joining gene LIG4 and severe radiation pneumonitis in nonsmall cell lung cancer patients treated with definitive radiotherapy. Cancer **118**:528–535.

Zheng, L.-R., Wang, X.-F., Zhou, D.-X., Zhang, J., Huo, Y.-W. and Tian, H. (2012) Association between XRCC1 single-nucleotide polymorphisms and infertility with idiopathic azoospermia in northern Chinese Han males. Reprod Biomed Online **25**:402–407.

Zini, A., Bielecki, R., Phang, D. and Zenzes, M.T. (2001) Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril **75**:674–677.

Zipprich, J., Terry, M.B., Brandt-Rauf, P., Freyer, G.A., Liao, Y., Agrawal, M., et al. (2010) XRCC1 polymorphisms and breast cancer risk from the New York Site of the Breast Cancer Family Registry: A family-based case-control study. J Carcinog **9**:4.

Downloaded by [University of Sherbrooke] at 09:58 10 October 2017