Importance of xeroderma pigmentosum group D polymorphisms in susceptibility to ovarian cancer

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Abstract

The purpose of this study was to evaluate the role of *XPD* genotypes as genetic indicator of susceptibility to ovarian cancer. We have used a case–control study. We analysed DNA samples from 141 ovarian cancer patients and 202 control subjects, for three *XPD* polymorphisms using PCR-RFLP. We observed that *Asn312Asn XPD* genotype carriers have increased susceptibility of ovarian cancer (OR = 2.46 95% CI 1.20–5.06; $P = 0.015$). Furthermore, we found that carriers of *Gln751Gln XPD* genotype have an increased susceptibility of ovarian cancer (OR = 3.40 95% CI 1.61–7.15; $P = 0.001$). *Asn312Asn* and *Gln751Gln* are particularly associated with an early-stage of disease. Our results suggest an important role for *Asn312Asn* and *Gln751Gln XPD* polymorphisms in the susceptibility to ovarian cancer.
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Keywords: XPD; NER; DNA repair; Ovarian cancer; Polymorphisms

1. Introduction

Ovarian cancer has the highest mortality rate of all gynaecologic cancers because it is seldom diagnosed at an early stage, when the likelihood of remission is greatest [1]. In this way, it is very important to define the factors concerned in the carcinogenesis of ovarian cancer, trying to help to an early detection of this disease.

Nucleotide excision repair (NER) is the most important and versatile pathway by which mammalian cells removes DNA lesions caused by physical and chemical carcinogens. A wide spectrum of structurally unrelated lesions such as ultra violet (UV) induced photoproducts, bulky chemical DNA adducts, and particularly distorting interstrand cross links, induced by chemotherapeutic agents, are efficiently removed by the NER pathway [2,3]. A considerable inter-individual variation in DNA repair capacity has been observed in the general population, and it has been reported that individuals with variable NER capacity are at increased risk of developing cancer [4,5]. Therefore, polymorphisms in DNA repair genes have the potential to be cancer risk factors in the population.

In NER pathway, the products of more than a dozen genes are involved in the process of restoring the normal structure [2]. *Xeroderma pigmentosum Group D* (*XPD*), also known as *ERCC2*, is an essential member

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of NER pathway. The protein XPD is a part of the basal transcription factor TFIIH with ATPase-driven $5'$–$3'$ helicase activity [6], being responsible for the separation of the DNA strands during DNA repair [7]. Several polymorphisms in the *XPD* gene have been identified [8]. Recent studies have shown that two common polymorphisms in the *XPD* gene may be associated with differential DNA repair capacity [9–12]. The *Asp312Asn XPD* polymorphism is characterized by a G–A change, being responsible for aspartic acid (Asp) to asparagine (Asn) amino acid substitution in the coding region of the *XPD* gene [8]. The other common polymorphism is located in codon 751 and gives rise to a A→C change, resulting in lysine (Lys) to glutamine (Gln) amino acid substitution in coding region of *XPD* gene [8], being designed as *Lys751Gln XPD* polymorphism. The frequencies of these polymorphisms are present in 29–46% of Caucasian population [5,8,9]. Another frequent polymorphism in *XPD* gene is located in exon 6, codon 156, with a nearly frequency of 25% in health Caucasian population (8). The *C156A XPD* polymorphism results in a C→A change, being a silent alteration [8]. Given, the importance of the *XPD* gene in multiple tasks such as RNA transcription and NER pathway, the *XPD* polymorphisms may operate as an important candidate in genetic susceptibility factors in commonly occurring forms of cancer.

In human ovarian cancer, it has been reported a frequent loss of heterozygoty on chromosome 19q [13,14]. The DNA repair gene *XPD/ERCC2* is located on 19q13.2–13.3. Dabholkar et al. [15] reported abnormalities of mRNA expression of *XPD* that may be characteristic of epithelial ovarian carcinoma.

The aim of this study is to evaluate the influence of the *C156A*, *Asp312Asn* and *Lys751Gln XPD* polymorphisms in the individual susceptibility to ovarian cancer.

2. Materials and methods

2.1. Patients

We evaluated the association between *XPD* exon 6, exon 10 and exon 23 polymorphisms and risk of ovarian cancer using a case–control study. One hundred and twenty-six patients with histologically confirmed epithelial ovarian carcinoma and sequentially admitted at the Portuguese Oncology Institute-Porto, in the Northern area of Portugal, since 1999–2001, were included in this study. They were evaluated according to the staging system of the International Federation of Gynaecology and Obstetrics (FIGO). Information on ovarian cancer patients was available from clinical archive files. All cases were from Caucasian ethnicity and the

mean age of the patients was 54 years. The control group consisted of 202 healthy women, with a median age of 54.3 years, who did not present a clinical history of cancer, and were residents in the same geographic area of the cancer group. Ethnicity information was available for all of control subjects (100% Caucasian). Informed consent was obtained from each patient and healthy individuals.

2.2. Polymerase chain reaction/restriction fragment length polymorphisms (PCR-RFLP) analysis

DNA was extracted from leukocytes of peripherical blood by proteinase K/cloroform/isopropanol treatment [16].

Genotyping for *C156A*, *Asp312Asn* and *Lys751Gln XPD* polymorphisms was carried out using previously described PCR-RFLP methods (Hemminki et al., 2001, *C156A* polymorphism; Matullo et al., 2001, *Asp312Asn* polymorphism; Hemminki et al., 2001, *Lys751Gln* polymorphism) [17,18]. PCR products were digested with specific restriction endonuclease enzymes *C156A* polymorphism PCR product was digested *i*th 10U *Hinf* I (Fermentas®) for more than 18 h at 37 °C, followed by electrophoresis in 3% agarose gel. The C allele was represented by 82 plus a 206 base pair (bp) fragments and the A variant allele by a 288 bp fragment (Fig. 1). *Asp312Asn* polymorphism PCR products were digested with 10 U *Taq* I (Fermentas®) for more than 18 h at 65 °C. The G allele was cut into two fragments (166 and 22 bp), while the A allele was represented by a 188 bp fragment (Fig. 2). *Lys751Gln* polymorphism PCR products were digested with 10U *Pst* I (Fermentas®) for more than 16 h at 37 °C. A and C allele were visualized as fragments of 161 and 41 bp plus 120 bp, respectively, after separated by electrophoresis in a 3% agarose gel (Fig. 3).
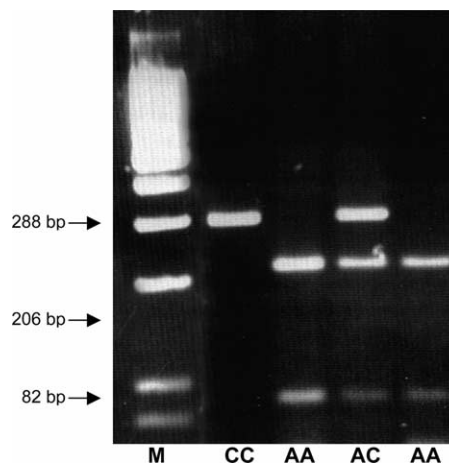

Fig. 1. Example of RFLP analysis of *XPD* exon 6 genotypes. AA genotype—fragment of 288 bp; *CC* genotype—fragments of 82 bp plus 206 bp. (M—100 bp ladder).
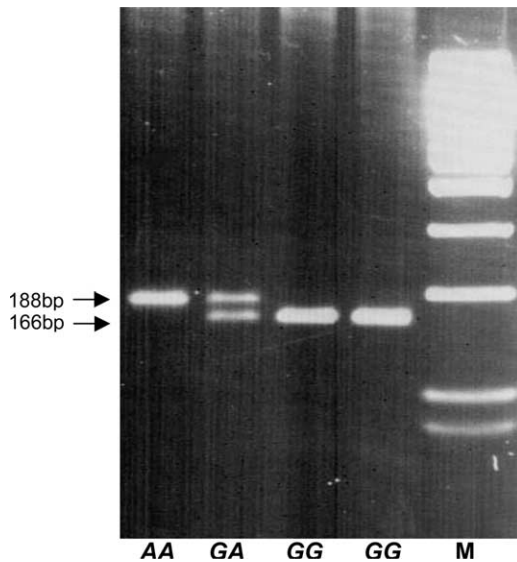
Fig. 2. Example of RFLP analysis of *XPD* exon 10 genotypes. AA (*Asn312Asn*) genotype—fragment of 188 bp; GG (*Asp312Asp*) genotype—fragments of 22 bp (not visualized) plus 166 bp. (M—100 bp ladder).
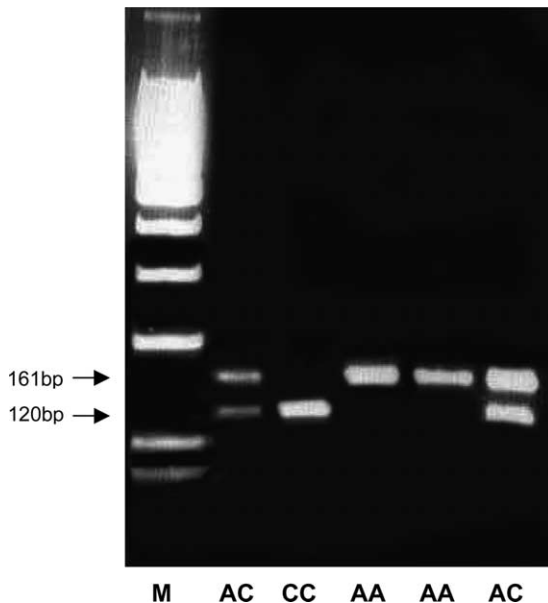

Fig. 3. Example of RFLP analysis of *XPD* exon 23 genotypes. AA (*Lys751Lys*) genotype—fragment of 161 bp; CC (*Gln751Gln*) genotype—fragments of 41 bp (not visualized) plus 120 bp. (M—100 bp ladder).

2.3. Statistical analysis

Chi-square (χ^2-test) analysis was used to compare categorical variables. The odds ratio (OR) and its 95% confidence interval (CI) were calculated to measure the association between *XPD* allelic variants and ovarian cancer.

Logistic regression analysis was used to calculate the adjusted OR and 95% CI for the influence of *XPD* genotypes in the risk of ovarian cancer, adjusted for age. A stratification of both patient and control groups was made according to mean age of these groups (women older than 54 years vs women younger than 54 years).

We calculated the attributable proportion (AP) [19], as the fraction of disease attributable to the risk factor.

Whenever appropriate, the observed number of each genotype was compared with that expected for a population in the Hardy–Weinberg Equilibrium by using a goodness of fit χ^2-test.

3. Results

The distribution of *C156A*, *Asp312Asn* and *Lys751Gln XPD* polymorphisms among ovarian cancer cases and controls is shown in Table 1. The frequency of the *C156A XPD* genotypes was 0.20, 0.58 and 0.22 to *CC*, *CA* and *AA*, respectively, in control group and 0.30, 0.52, 0.18 in cases. No significant differences were found in the distribution of *C156A* genotypes in ovarian cancer cases and controls. The *Asp312Asp* genotype frequencies are 0.55 and 0.54 in control group and in cases group, respectively. We found that patients with ovarian cancer had a higher frequency of *Asn312Asn* genotype than the control group (16.7 vs 7.5%). When we considered *Asp312Asp* plus *Asp312Asn* genotypes as a reference group, we observed that women carriers *Asn312Asn* genotype were at increased risk of developing ovarian cancer (OR=2.45 95%CI 1.19–5.04; $P=0.013$). Logistic regression analysis confirmed this association of *Asn312Asn* genotype with a statistically significant increase of ovarian cancer risk

Table 1
Frequencies and odds ratio (OR) of *C156A*, *Asp312Asn* and *Lys751Gln XPD* polymorphisms among ovarian cancer cases and control individuals

Genotypes	Controls n (%)	Cases n (%)	OR (95% CI)	P-value
Exon 6 (C156A)				
CC/CA	147 (78.4)	97 (82.2)	Reference	0.418
AA	40 (21.6)	21 (17.8)	0.79 (0.44–1.41)	
Exon 10 (Asp312Asn)				
Asp/Asp vs Asp/Asn	184 (92.5)	95 (83.3)	Reference	0.013
Asn/Asn	15 (7.5)	19 (16.7)	2.45 (1.19–5.04)	
Exon 23 (Lys751Gln)				
Lys/Lys vs Lys/Gln	190 (94.1)	104 (82.5)	Reference	0.001
Gln/Gln	12 (5.9)	22 (17.5)	3.35 (1.59–7.04)	

Table 2
Multivariate analysis for the presence of risk genotypes and susceptibility to ovarian cancer and the onset of ovarian cancer under the age of 54 years

Increased risk for	Genotypes	OR	95% CI	P-value
Cancer				
	Asn/Asn (exon 10)	2.46	1.20–5.06	0.015
	Gln/Gln (exon 23)	3.40	1.61–7.15	0.001
Cancer onset under age of 54 years				
	Asn/Asn (exon 10)	3.63	1.34–9.83	0.011
	Gln/Gln (exon 23)	5.15	1.89–14.05	0.001

(Table 2). We observed that women Asn312Asn genotype carriers have a significant increase of ovarian cancer risk under the age of 54 years, being this association confirmed by logistic regression analysis (Table 2; OR = 3.63; 95% CI 1.34–9.83; P = 0.011). The frequency of the Lys751Lys genotype in our control group was 0.47 and 0.44 in cases group. We found that Gln751Gln genotype was more frequent in the ovarian cancer group (17.5%) than in the control group (5.9%) (Table 1). We demonstrate that carriers of this genotype have an increased risk of developing ovarian cancer (OR = 3.35 95% CI 1.59–7.04; P = 0.001). This association was confirmed by logistic regression analysis, performing an adjustment of OR for median age (Table 2). Furthermore, this association was stronger for the onset of ovarian cancer under the age of 54 years confirmed by logistic regression analysis (Table 2; OR = 5.15 95% CI 1.89–14.05; P = 0.001).

For the entire case group, the proportion of ovarian cancer cases attributable to the Asn312Asn genotype and Gln751Gln genotype was 9.9 and 12.4%, respectively. When considering the age of onset younger of ovarian cancer of 54 years the proportion of ovarian cancer cases attributable to the Asn312Asn genotype and Gln751Gln genotype was 14.5 and 21.0%, respectively.

Subjects were cross-classified by Asn312Asn and Gln751Gln genotypes in Table 3. We found that the

carriers of both of these genotypes have a threefold increase in ovarian cancer risk (OR = 3.10; 95% CI 1.24–7.75; P = 0.016). The risk was further increased in the age of onset of 54 years (OR = 5.24 95% CI 1.56–17.55; P = 0.007).

The observed genotype distributions for C156A, Asp312Asn and Lys751Gln XPD are in agreement with Hardy–Weinberg Equilibrium.

The clinicopathological features in ovarian cancer patients according to XPD genotypes are shown in Table 4. We did not find significant statistical differences between groups of patients with A156A homozygote variant and others genotypes, regarding age at diagnosis, clinical stage and histologicaltype and grade. We observed a higher frequency of Asn312Asn and Gln751Gln genotypes in ovarian cancer patients presenting I/II stage than patients with III/IV stage, being these differences statistically significant (P = 0.005; P = 0.014, respectively). Another statistical significant difference was observed when we compared the group of patients with I histological grade and II/III presenting Gln751Gln homozygote variant (P = 0.018).

4. Discussion

In this study, we examined the association between C156A, Asp312Asn and Lys751Gln XPD polymorphisms and ovarian cancer susceptibility in a Portuguese population. This is the first study assessing the relationship between XPD polymorphisms and ovarian cancer risk.

Ovarian cancer is an aggressive disease with an increasing mortality. It is elementary to elucidate the aetiology of this form of cancer to be able to an early detection and to achieve a possible prevention.

The XPD protein is involved in different cellular processes. It is integrated in TFIIH complex, which is involved in multiple tasks, as transcription and phosphorylation of numerous substrates [20,21]. Also it is essential to NER pathway, where it has a helicase

Table 3
Combination between the Asn312Asn and Gln751Gln genotypes in controls and ovarian cancer cases

	Genotypes	Controls	Cases	OR (95% CI)	P-value
All cases					
	Asn/Asn and Gln/Gln	8 (4.1)	13 (11.6)	3.04 (1.22–7.57)	0.013**
	Others*	185 (95.9)	99 (88.4)		
Onset under age of 54 years					
	Asn/Asn and Gln/Gln	4 (3.8)	10 (16.9)	5.20 (1.55–17.43)	0.004***
	Others*	102 (96.2)	49 (83.1)		

*No Asn/Asn and Gln/Gln genotypes; **P = 0.016; OR = 3.10 95% CI 1.24–7.75, using logistic regression analysis adjusting for age; ***P = 0.007; OR = 5.24 95% CI 1.56–17.55, using logistic regression analysis adjusting for age.

Table 4
Relation between *C156A*, *Asp312Asn* and *Lys751Gln XPD* genotypes and clinicopathological parameters in ovarian cancer

Parameter	*C156A* genotypes		P-value	*Asp312Asn* genotypes		P-value	*Lys751Gln* genotypes		P-value
	Homozy-gote variant	Others		Homozy-gote variant	Others		Homozy-gote variant	Others	
Age (years)*	51.3 ± 16.1	54.6 ± 13.6	0.426**	52.8 ± 13.9	54.3 ± 14.2	0.574**	51.0 ± 14.5	54.6 ± 14.0	0.307**
FIGO stage									
I/II	6 (16.2)	31 (83.8)	0.846***	11 (30.6)	25 (69.4)	0.005***	13 (29.5)	31 (70.5)	0.014***
III/IV	11 (17.7)	51 (82.3)		5 (8.5)	54 (91.5)		7 (10.9)	57 (89.1)	
Histological grade									
I	2 (9.5)	19 (90.5)	0.304***	6 (31.6)	13 (68.4)	0.018***	6 (26.1)	17 (73.9)	0.214***
II/III	11 (19.3)	46 (80.7)		5 (9.1)	50 (90.9)		9 (14.5)	53 (85.5)	
Histological type									
Serous	9 (45.0)	49 (59.0)	0.256***	10 (17.9)	46 (82.1)	0.837***	11 (17.7)	51 (82.3)	0.722***
Others	11 (55.0)	34 (41.0)		7 (16.3)	36 (83.7)		10 (20.4)	39 (79.6)	

*Mean $\pm$ SD; **Mann–Whitney test; ***χ^2 test.

activity [7]. Mutations in the *XPD* gene can affect the activity of these processes, giving rise to repair and transcription defects, abnormal responses to apoptosis and, probably, hormonal dysfunctions.

In the present study, the variant allelic frequencies of *C156A*, *Asp312Gln* and *Lys751Gln XPD* polymorphism in control group are in agreement with previous findings in other populations [5,9].

We did not observe any association between *C156A XPD* polymorphism and genetic susceptibility to ovarian cancer risk. We found that women that are carrier of the *Asn312Asn* genotype have a near threefold risk of ovarian cancer in comparison with women carriers *Asp312Asp* or *Asp312Asn* combined. This suggests that the *Asn312* allele may be a risk factor to ovarian cancer.

Furthermore, when we look to subgroups of cases and control according to the mean onset age of disease, we found that the risk of developing ovarian cancer is almost a fourfold increase in women younger than 54 years that presented the *Asn312Asn* genotype. These results are consistent with reports that suggest a lower DNA repair capacity associated to *Asn312Asn* genotype [9,10]. The *XPD* exon 10 polymorphism is characterized by a G→A nucleotide substitution, causing an Asp→Asn amino acid change at codon 312 of *XPD* gene [8]. The biological function of this amino acid substitution has not yet been elucidated.

However, the fact that this residue has been highly conserved through evolution [8,22] and give rise to an amino acid substitution, suggests a strong effect in the enzymatic activity of XPD protein. We also reported that women with *Gln751Gln XPD* genotype have a 3.4-fold-risk increased of ovarian cancer. The fact that this genotype has been associated with a lower DNA repair capacity [9,10] comes in agreement with our results.

This association is even more noteworthy according to age onset of cancer in women younger than 54 years. The *Gln751Gln XPD* polymorphism results from a A→C variation in codon 751 giving rise to the amino acid substitution Lys to Gln [8], which is a change from a basic to a polar amino acid. This completely changes the electronic configuration of the amino acid, and is a major change, located in the important domain of interaction between XPD protein and its helicase activator, inside the TFIIH complex [21]. In theory, the consequence should be the most important in terms of XPD activity. Furthermore, the *Asp312Asn* and *Lys751Gln XPD* polymorphisms have been linked to susceptibility to other cancers, namely in head and neck [23], bladder [24], lung [25–28], melanoma [29,30], oesophageal [31], breast [32,33] and prostate [34]. We reported a stronger association of the *Asp312Asn* and *Lys751Gln XPD* polymorphisms with ovarian cancer risk in women in early-stage of disease. This could suggest the involvement of this gene merely in initiation rather than in progression of the disease.

The association between *Asn312Asn* and *Gln751Gln XPD* polymorphisms and ovarian cancer is biologically plausible, because the XPD protein functions as a $5'$–$3'$ helicase in the NER mechanism, which is responsible for the repair of many DNA lesions [2] and plays a role in activating apoptosis through interaction between p53 and TFIIH to remove damaged cells [35,36].

Furthermore, the *Gln751Gln XPD* polymorphism is located in an important domain responsible for the interaction between XPD protein and p44 protein, its helicase activator, inside the TFIIH complex [21]. There is strong evidence suggesting estrogens as the relevant hormone in hormonal etiology of ovarian cancer [37–39]. Some experimental evidences have

shown that catechol estrogens are oxidized to activated species that react with DNA to form depurinating adducts and thereby initiate cancer, namely ovarian cancer [40,41]. In this way, XPD protein, as a member of NER, could represent an important molecule in carcinogenesis of ovarian cancer. Another support of the importance of XPD in ovarian carcinogenesis is it localization in chromosome 19q13.3 region, region with frequent loss of heterozygosity in epithelial ovarian carcinoma [13,14]. This region encodes several others genes involved in DNA repair, for example, *ERCC1* and *XRCC1*, and linkage disequilibrium between these polymorphisms and other DNA repair genes may occur leading to a possible explanation to a low DNA repair capacity in ovarian cancer patients.

Our study is the first to associate *XPD* polymorphisms with ovarian cancer susceptibility. Our results suggest that the *Asn312Asn* and *Gln751Gln XPD* polymorphisms may be a genetic risk factor to ovarian cancer and particularly associated with an earlier onset of ovarian cancer. However, this study has some limitation, being the most important the sample size and the lack of a replicating study. A recent report suggests that studies regarding the association between genetic variants and cancer must have in account not only the statistical significance (*P*-value) but also the false positive report probability (FPRP) [42]. Future studies in other populations using a large sample of cases and controls and the FPRP criterion will be helpful to confirm our results.

Genetic polymorphisms in DNA repair genes or other genes, contributing to low to-moderate cancer risk, may be important for a better understanding of the molecular epidemiology of ovarian cancer [19,43–47]. As well, this definition could help to outline chemoprevention strategies. Furthermore, since XPD proteins are essential to the NER pathway responsible for the removal of DNA adducts produced by platinum agents such as cisplatin, which is commonly used in chemotherapy of ovarian cancer [48], it would be also important to study the influence of these polymorphisms in the response to chemotherapy and overall survival.

Acknowledgements

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