

# DISCUSSION

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Acute myeloid leukemia (AML) is a heterogeneous group of genetically diverse hematopoietic malignancies<sup>(54)</sup>. Current chemotherapy enables a high percentage of patients with AML to enter complete remission (CR), but many remain refractory to therapy or experience relapse with resistant disease. Because of the wide heterogeneity of this disease, predicting a patient's risk for treatment failure or relapse at the time of diagnosis is important for the optimum selection of treatment strategies<sup>(174)</sup>. The karyotype at the time of diagnosis provides the most important prognostic information in adults with AML, but 40 to 50% of patients do not have clonal chromosomal aberrations<sup>(66,175)</sup>. All such cases of cytogenetically normal AML are currently categorized in the intermediate-risk group, yet this group is quite heterogeneous<sup>(84,176)</sup>.

Constitutive genetic characteristics of the patients may play an important role in the prognosis of AML. In this regard, it is well recognized that most drugs exhibit wide inter-patient variability in their efficacy and toxicity<sup>(177,178)</sup>. For many drugs, such differences are due, in part, to polymorphisms in genes encoding drug-metabolizing enzymes, drug transport proteins, and genes that recognize and repair DNA lesions<sup>(179)</sup>. These polymorphisms represent common variations in a DNA sequence that may lead to either reduced or increased activity of the encoded gene<sup>(177)</sup>. The most frequent type of polymorphisms is the so-called single nucleotide polymorphism (SNP), which accounts for over 90% of genetic variation in the human genome<sup>(180)</sup>. DNA is at constant risk of damage from both endogenous and exogenous sources. Cells have highly complex pathways to accomplish repair of DNA damage and maintain genomic integrity<sup>(181,182)</sup>. The capacity to respond to and repair DNA damage in an accurate manner varies among individuals. Genetic polymorphisms have been described for multiple genes associated with DNA repair and may contribute to this inter-individual variation<sup>(183,184)</sup>.

The anticancer activity of most chemotherapy drugs relies on the induction of DNA damage in rapidly cycling tumor cells. Daunorubicin induce DNA damage by inhibiting DNA topoisomerases and cytarabine accomplishes this by nonproductive incorporation of Ara-CTP into nascent DNA or RNA<sup>(159,185)</sup>. Consequently, the regulation of DNA repair pathways in tumor cells is a critical factor for their response to chemotherapy drugs and may influence drug toxicity and remission<sup>(160,186)</sup>. NER is the repair pathway that eliminates the widest variety of damage to the human genome, including UV-induced photoproducts, bulky monoadducts, cross-links, and oxidative damage<sup>(187,188)</sup>. There is evidence that NER also removes DNA lesions induced by chemotherapy<sup>(189,190)</sup>. Xeroderma pigmentosum group D (*XPD*) gene, also called excision repair cross-complementing group 2 (*ERCC2*), codes for a superfamily 2 (SF2) helicase with a 5'→3' polarity. *XPD* is a component of the ten-subunit complex TFIIH, which plays a role in both transcription initiation from RNA polymerase II promoters and in the NER pathway. *XPD* is the dominant helicase activity within TFIIH, and is essential for the structure and function of the complex<sup>(151,191)</sup>. Consequently, functional DNA repair capacity is thought to differ significantly between the polymorphic variants of *XPD*<sup>(192,193,194)</sup>.

In the present study, our main aim was the evaluation of two *XPD* polymorphisms in relation to the response to induction chemotherapy as well as the chemotherapy-induced toxicities in 51 adult cytogenetically normal de novo AML patients. The two *XPD* polymorphisms investigated in our study are Asp312Asn (G→A) and Lys751Gln (A→C). Both polymorphisms cause amino acid substitution in the protein product but they do not reside in known or hypothesized helicase/ATPase domains. However, *XPD* Lys751Gln is a

conserved substitution, and while Asp312Asn is a nonconservative substitution, it is found at the same amino acid residue of mouse, hamster<sup>(195)</sup>, and fish XPD<sup>(196)</sup>. This sequence conservation is indicative of a functional role for these residues<sup>(197)</sup>.

In the current work, we investigated the relation between *XPD* polymorphisms and response to chemotherapy on one hand and a number of clinical and laboratory parameters on the other. As was expected, age, gender and FAB subtypes had no significant association with any of the *XPD* polymorphisms or with the response to chemotherapy. Similarly, CD34 expression and lymphoid marker co-expression were not associated with response or toxicities, this result was in agreement with Lauria et al. in (1995)<sup>(198)</sup> and Sperling et al. in (1995)<sup>(199)</sup>. CNS infiltration was associated with the response to chemotherapy but not with the *XPD* polymorphisms. In line with the work of Chang et al. in (2004)<sup>(200)</sup> and Kobayashi et al. in 2007<sup>(201)</sup>, we found that CNS infiltration was associated with resistant AML disease, all patients who suffered CNS disease, did not achieve remission.

Association between *XPD* polymorphisms and response to chemotherapy was evaluated in our study, no relation was found between *XPD* Asp312Asn polymorphism and the response to chemotherapy. On the other hand *XPD* Lys751Gln had an impact on the outcome of chemotherapy. Patients with the common homozygous genotype (AA) of *XPD*751 polymorphism were more likely to achieve complete remission. Few researchers investigated the association between the *XPD* variants and the response to chemotherapy in AML patients; some of them support our findings. One of these groups are Allan et al. in (2004)<sup>(202)</sup> who investigated 341 AML patients, and concluded that the glutamine variant was associated with a poorer prognosis relative to the lysine variant. Similarly, Monzo et al. in (2006)<sup>(159)</sup> found an association between *XPD*751 Lys/Lys variant and lower risk of relapse in intermediate-risk AML patients. Moreover, Strom et al. in (2010)<sup>(203)</sup> observed statistically significant lower OS in intermediate risk AML patients with the mutant genotype of *XPD* Lys751Gln.

The reason for the association of *XPD*751 polymorphism with response to chemotherapy can be attributed to the subtle alterations in the DNA repair proficiency that is caused by that polymorphism. Of particular interest in the literature, is the *XPD* glutamine variant at codon 751, which is predicted to affect protein function and has been shown to modulate outcome in patients treated with chemotherapy<sup>(204,205,206)</sup>. Previous research<sup>(159,160,202,203)</sup> concluded that the less proficient DNA repair variant of *XPD*751Gln (C) is associated with lower CR rates and more resistant disease in AML patients. The association of this variant with poorer outcome might be explained by the fact that inefficient DNA repair leads to additional instability and more aggressive tumors<sup>(159,207,208)</sup>.

A number of researchers support the association between the mutant *XPD*751 genotype (Gln (C)) and sub-optimal DNA repair proficiency. An example of these groups are Spitz et al. in (2001)<sup>(188)</sup> who studied the functional consequences of the *XPD* Lys751Gln polymorphism among 341 white lung cancer cases and 360 age-, sex-, ethnicity-, and smoking-matched controls. They reported that the variant Gln751Gln genotype was consistently associated with the suboptimal DNA repair capacity (DRC). Similarly, Matullo et al. in (2001)<sup>(209)</sup> observed DNA adduct levels significantly elevated for *XPD*751Gln never-smoking homozygotes. In addition, Hou et al. in (2002)<sup>(210)</sup> suggested that the *XPD* mutant alleles may be associated with reduced repair of aromatic DNA adducts in general and increases lung cancer risk among never-smokers. In line of these findings, Au et al. in (2003)<sup>(211)</sup> used ultraviolet light to irradiated blood lymphocytes from 80 nonsmoking donors to challenge the cells to repair the induced DNA damage, and analyzed expression of chromosomal aberrations (CA) specific to the inducing agent. *XPD* 751Gln was associated with a significant increase in chromatid breaks compared with wild type. The data indicated

that *XPD* 751Gln is significantly defective in NER. In concordance, Hu et al. in (2000)<sup>(212)</sup> correlated DRC with *XPD* genotypes in a group of 66 prostate cancer cases and 54 controls. Both cases and controls homozygous for the mutant variant had lower DRC than those with the wild-type genotype. Moreover, Hemminiki et al. in (2001)<sup>(213)</sup> found *XPD* exon 23 C allele to be associated with depressed repair among melanoma and basal cell carcinoma and healthy controls aged 50 years or older.

Not all researchers agree with the association between *XPD* 751Gln and resistant disease, a study by Mehta et al. in (2006)<sup>(214)</sup> did not demonstrate any differences in outcome of AML therapy in children with various *XPD*751 genotypes. Their contradicting results can be explained by the inclusion of various cytogenetic categories in their study; while all the patients enrolled in our own study were cytogenetically normal. Moreover, children with AML differ from adults in terms of the biology of their disease, for example, some studies show that older adults have increased frequency of adverse cytogenetic features compared to children<sup>(215,216)</sup>. Over time, adult patients have more opportunity to accumulate additional genetic insults (secondary hits), with perhaps increased susceptibility to develop cancers that are more resistant to therapy. Consequently, Outcomes for treatment of adult AML are commonly inferior to those reported in pediatric series<sup>(217,218)</sup>. Another unexpected finding was reported by Kuptsova-Clarkson et al. in (2010)<sup>(219)</sup> who observed that, among secondary but not de novo AML patients, the odds of achieving complete remission (CR) were higher for the *XPD* 751Gln/Gln genotype. Their results can be attributed to the additional cytogenetic aberrations sustained by the genome of secondary AML patients. In addition, secondary AML is more prevalent in old patients<sup>(92)</sup> and is usually more resistant to standard treatment than de-novo AML<sup>(30)</sup>.

Moreover, some researchers deny the association of variant *XPD*751Gln allele (C) with deficient DNA repair. One group who reported contradicting findings are Lunn et al. in (2000)<sup>(192)</sup> who assessed the DNA repair proficiency of 31 women with a family history of breast cancer, using a cytogenetic assay that detects X-ray induced chromatid aberrations (breaks and gaps). They found the common *XPD* Lys/Lys751 genotype, not the mutant variant, was associated with sub-optimal repair of DNA damage induced by X-irradiation. Their results may be attributed to the small study population, inter-relationships among *XPD* genotype, familial risk status and the in vitro model of the study. By reviewing the literature, more conflicting data emerged regarding the relation between *XPD*751 polymorphism and proficiency of DNA repair<sup>(219,220)</sup>. These contradicting data have led to suggestions that functionality of the codon 751 polymorphism may be exposure- and pathway-specific, affecting both DNA repair and cell death<sup>(187)</sup>. Reduced DNA repair capacity might cause resistance to chemotherapy, by interrupting cell signals that lead to apoptosis. As an example, it has been reported that reduced mismatch repair efficiency may cause resistance to cytotoxic drugs, by an inability to detect DNA damage, thus preventing cells from undergoing DNA damage-induced apoptosis<sup>(221,222,223)</sup>. Further support of the notion that DNA repair and apoptosis are functionally linked comes from the experiment in which p53-dependent apoptosis is compromised in fibroblasts from patients with *XPD* variant alleles. This phenotype could be restored by expression of *XPD*751 wild type variant (Lys (A))<sup>(224,225)</sup>.

In explanation of the functional link between *XPD*751 polymorphism and apoptosis, Allan et al. in (2004)<sup>(202)</sup> postulated two general mechanisms by which the *XPD* codon 751 polymorphism may modulate myeloid cell death in response to chemotherapy: either via a direct role for *XPD* in signaling cell death, or indirectly via *XPD* repair of protoxic DNA lesions. Directly, codon 751 polymorphism may exert an effect on the myeloid cell death machinery via interaction between *XPD*, through its carboxy terminus domain, and p53<sup>(187)</sup>.

Thus codon 751 polymorphism can modulate the ability of XPD to signal P53-dependent cell death, possibly following stalled transcription or repair<sup>(202,225)</sup>. Indirectly, codon 751 variant may modulate myeloid cell death by affecting the efficiency of NER to repair chemotherapy induced protoxic DNA lesions. Like P53, P44 also interacts with the carboxy terminus of XPD, stimulating its helicase activity<sup>(226)</sup>. The 751Gln variant of the *XPD* gene leads to a conformational change in the coded protein at the domain of interaction between the XPD protein and p44 protein, inside the TFIIH complex<sup>(227)</sup>. Thus, codon 751 polymorphism can weaken but does not abolish P44 binding, which in turn compromises NER by attenuating the helicase activity of XPD. Because XPD is a component of both transcription-coupled and global genomic repair, a generic effect on NER capacity is likely to affect the repair of both protoxic and promutagenic chemotherapy-induced DNA lesions<sup>(202)</sup>.

In our work, we examined the relation between the two *XPD* polymorphisms and chemotherapy-induced toxicities. *XPD* Asp312Asn polymorphism was not associated with any of the studied chemotherapy-induced toxicities. On the other hand *XPD* Lys751Gln polymorphism, was associated with cardiotoxicity but not with hepatotoxicity, nephrotoxicity or metabolic toxicity. Patients with *XPD*751 CC variant (Gln/Gln) were more likely to suffer chemotherapy-induced cardiotoxicity.

One previous research investigated the association between the *XPD* polymorphisms and chemotherapy-induced toxicities in AML patients. Kuptsova et al. in (2007)<sup>(160)</sup> found that patients with at least one Gln751C/Asp312G haplotype, as compared to all other haplotypes, had a 2-fold increase in risk of liver toxicity. Other researchers support these findings. Le Morvan et al. in (2006)<sup>(228)</sup> showed that ERCC2, in the NCI-60 tumor cell line panel, was associated with variations in DNA NER activity, and drug cytotoxicity. Half maximal inhibitory concentration (IC<sub>50</sub>) values of anticancer agents (including topoisomerase inhibitors and antimetabolites) were lower in mutant homozygous lines than in common homozygous or heterozygous lines. Furthermore, Kuptsova-Clarkson et al. in (2010)<sup>(219)</sup> suggested that compromised repair activity may lead to accumulation of more DNA damage, leading to more profound treatment-related toxicities in normal tissues, and may predispose towards secondary cancers.

The reason for which, cardiotoxicity was significantly associated with the *XPD* Lys751Gln polymorphism is not clear. However, recent attention was focused on the association between chemotherapy-induced cardiotoxicity and DNA polymorphisms in order to help selecting the chemotherapeutic regimen most likely to benefit each individual patient<sup>(111)</sup>. A report by Guven et al. in (2007)<sup>(229)</sup> studied the *XPD* Lys751Gln polymorphism in patients with coronary artery disease. They found an association between the mutant allele C and the evidence of genotoxicity in their patients. It has been suggested that oxidative stress and the generation of reactive oxygen species may play an important role in the induction of DNA injury<sup>(230)</sup>. NER together with base excision repair is responsible for repair of the oxidative DNA damage<sup>(231)</sup>. Accordingly, genetic polymorphisms in the *XPD* DNA repair gene may influence individual variation in DNA repair capacity in response to chemotherapy, leading to increased risk of developing cardiotoxicity in those having the inefficient C allele.

In the present work, we identified *XPD* codon 751 polymorphism as a prognostic marker in de novo cytogenetically normal AML patients treated with chemotherapy. Although it is still early to establish a firm conclusion based on our work, yet this data might represent a small step towards better understanding of the role played by the DNA repair genes polymorphisms in the outcome of acute myeloid leukemia therapy.