

ROLE OF GENETIC VARIATIONS IN DETERMINING TREATMENT OUTCOME IN HEAD AND NECK CANCER

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Disclosure: The author has declared no conflicts of interest.

Received: 20.06.17 **Accepted:** 27.09.17

Citation: EMJ. 2017;2[4]:114-121.

ABSTRACT

Worldwide, head and neck squamous cell carcinoma (HNSCC) is responsible for >550,000 diagnoses and 380,000 deaths annually. It originates in the upper aerodigestive tract and has a multifactorial origin involving both genetic and lifestyle risk factors. The clinical management of HNSCC involves surgery, radiotherapy, and chemotherapy. Several studies point to the role of genetic variations in predicting drug efficacy and toxicity. Cancer pharmacogenomics has fast emerged as a new and promising field for the early identification of genetic markers that can predict drug response or toxicity, with the number of studies of genetic polymorphisms as prognostic factors of HNSCC treatment outcomes growing. The number of studies evaluating the association of candidate polymorphisms in drug-metabolising Phase I and II enzymes with treatment outcome far exceed the studies involving other candidate genes, such as those involved in drug metabolism, DNA repair, and cell cycle regulation. This review focusses on the relevance of genetic variations in genes, where the corresponding gene products play an important role in drug metabolism (*TPMT*, *DPD*), DNA repair (X-ray repair cross complementing 1), cell cycle (tumour protein P53), and carcinogenesis (matrix metalloproteinase 3 and 7), thereby contributing to the treatment outcome for HNSCC. This could greatly help clinicians in identifying genetic markers useful for the selection of optimal drugs, dose, and treatment duration on an individual basis, resulting in improved drug efficacy and decreased toxicity. However, further studies are needed in well characterised and larger HNSCC populations with proper validation of pharmacogenetic markers in experimental settings before application in clinical routine diagnostics.

Keywords: DNA repair, drug metabolism, genetic variations, matrix metalloproteinases (MMP), p53.

INTRODUCTION

It has generally been observed amongst patients that a medication proven efficacious for the majority of patients often fails to work in some other patients. In those cases where it works, it may cause serious side effects, even death, in a small number of patients. It is now well established that a large variability of drug efficacy and adverse drug reactions in patients decides the clinical use, regulation, and market withdrawal of clinical drugs.¹ Similar observations have also been made for cancer medication as significant heterogeneity in the efficacy and toxicity of chemotherapeutic agents has been found to exist across the human population.²

Recent studies from our laboratory have shown that treatment response in head and neck squamous cell carcinoma (HNSCC) patients treated with a

combination of chemotherapy and radiotherapy regimen of combination drugs, such as cisplatin and 5-fluorouracil (5-FU), was poor in patients with variant genotypes of *CYP2C9*, *CYP2C19*, and *CYP2D6*.^{3,4} Furthermore, an association with poor treatment response to chemotherapy was also observed in HNSCC cases for genotypes of *CYP2A6*, *GSTM1*, *GSTT1*, and *GSTP1*.^{5,6} Apart from the Phase I and Phase II drug metabolising enzymes, studies were also conducted to evaluate the role of genetic variants in other drug metabolising enzymes, such as thiopurine S-methyltransferase (*TPMT*) and dihydropyrimidine dehydrogenase (*DPD*), in determining the treatment outcome in HNSCC patients.⁷ This review will focus on some of the major genetic variations in *TPMT*, *DPD*, and some of the other key target genes, such as those involved in DNA repair (X-ray repair cross complementing 1 [*XRCC1*]), cell cycle (tumour protein P53 [*TP53*]),

and carcinogenesis (matrix metalloproteinases, [MMP]), which might play an important role in determining the treatment outcome in HNSCC cases. The genomic structures of *TPMT*, *DPD*, and *XRCC1* are shown in Figure 1, whilst Table 1 lists the single nucleotide variants associated with drug response.⁸

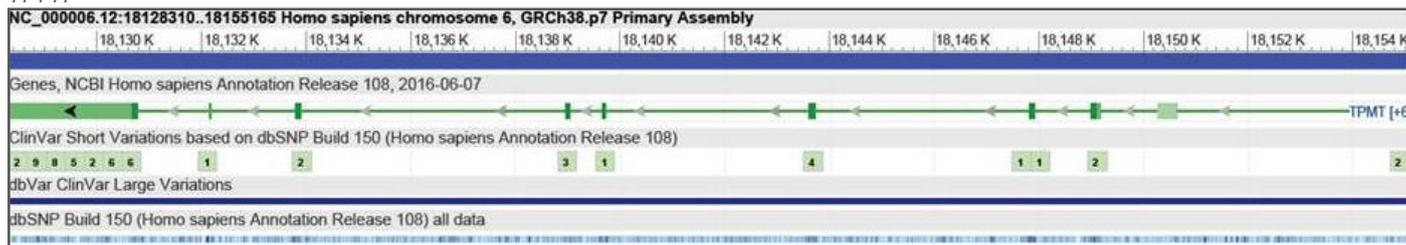
into thioguanine nucleotides, which is the cytotoxic form of the drug. Approximately 10% of patients have an intermediate enzyme activity and have a greater incidence of thiopurine toxicity, while 0.3% are deficient of TPMT activity, having severe or fatal toxicity from mercaptopurine therapy.¹³

DRUG METABOLISM POLYMORPHISMS: THIOPURINE-S-METHYLTRANSFERASE AND DIHYDROPYRIMIDINE DEHYDROGENASE

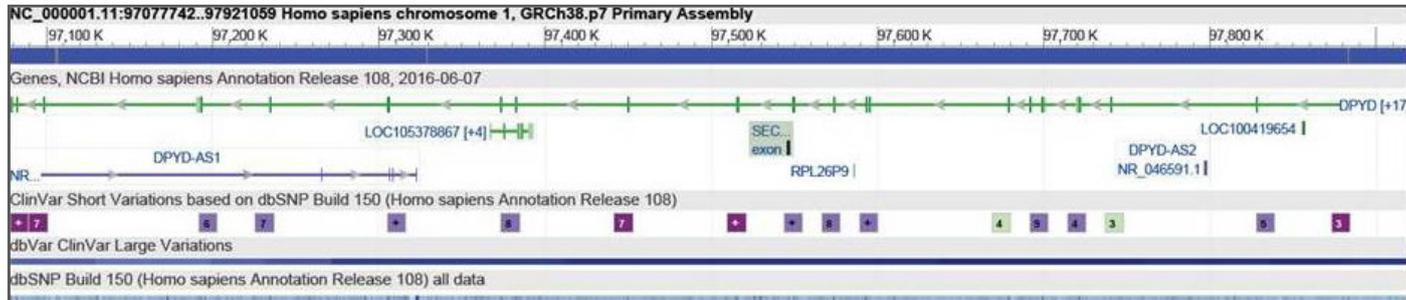
TPMT and DPD are enzymes involved in the metabolism and disposition of drugs, such as 6-mercaptopurine, azathioprine, cisplatin, 5-FU, and 6-thioguanine, that are used for the treatment of different cancers, autoimmune disorders, dermatological conditions, and as an immunosuppressant in graft transplantations.⁹⁻¹² TPMT functions by methylating mercaptopurine and thereby reducing its bioavailability for conversion

Genetic variants present in *TPMT* may alter the treatment response in cases receiving chemotherapeutic drugs. The four major variant alleles that lead to 80-95% intermediate and low enzyme activity of the *TPMT* gene are *TPMT*2* (238G>C), **3A* (460G>A and 719A>G), **3B* (460G>A), and **3C* (719A>G).¹⁴ *TPMT*3A* mutant allele contains two nucleotide transitions, 460G>A and 719A>G in the open reading frame, leading to the substitution of amino acids Ala154Thr and Tyr240Cys, respectively. Our study has shown that the treatment response after receiving cisplatin and 5-FU was modified in patients who carried variant genotypes of *TPMT*3B* and **3C*.⁷

TPMT



DPD



XRCC1

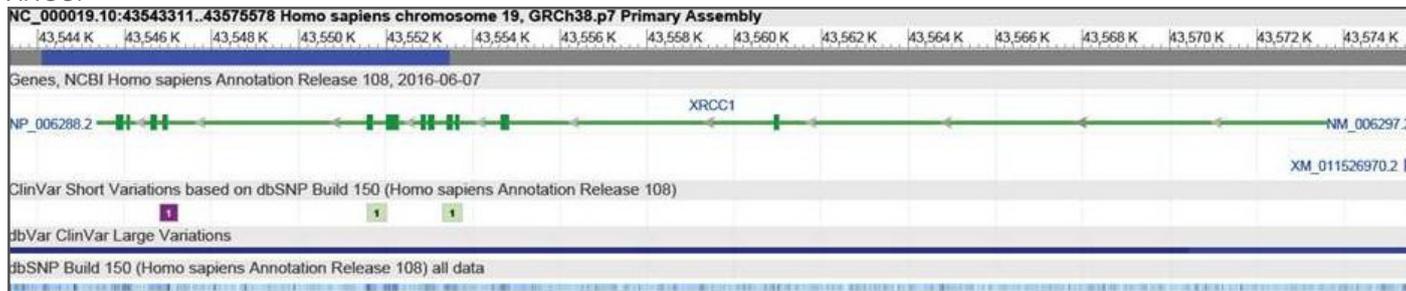


Figure 1: Genomic structures of *TPMT*, *DPD*, and *XRCC1* genes.

DPD: dihydropyrimidine dehydrogenase; *TPMT*: thiopurine S-methyltransferase; *XRCC1*: X-ray repair cross complementing 1.

Adapted from National Center for Biotechnology Information (NCBI).⁸

Table 1: Table showing the single nucleotide variants associated with drug response.

Gene	Variant ID	Variant allele	Transcript change	Protein change	Molecular consequence
<i>TPMT</i>	rs1142345	C	c.719A>G	Tyr240Cys	Missense variant
		C	c.674A>G	Tyr225Cys	Missense variant
	rs56161402	T	c.644G>A	Arg215His	Missense variant
		T	c.599G>A	Arg200His	Missense variant
	rs1800584	T	c.626-1G>A	NA	Splice acceptor variant
		T	c.581-1G>A	NA	Splice acceptor variant
	rs74423290	C	c.500C>G	Ala167Gly	Missense variant
	rs1800460	T	c.460G>A	Ala154Thr	Missense variant
	rs56019966	T	c.420-4G>A	NA	Intron variant
	rs1800462	G	c.238G>C	Ala80Pro	Missense variant
<i>DPD</i>	rs2297595	C	c.496A>G	Met166Val	Missense variant
		C	c.385A>G	Met129Val	Missense variant
		C	c.1A>G	Met1Val	Missense variant
		C	n.735A>G	NA	Non-coding transcript variant
<i>XRCC1</i>	rs25487	C	c.1196A>G	Gln399Arg	Missense variant
<i>TP53</i>	rs1042522	C	c.215C>G	Pro72Arg	Missense variant
		C	c.-939C>G	NA	2KB upstream variant
		C	c.98C>G	Pro33Arg	Missense variant

DPD: dihydropyrimidine dehydrogenase; *NA*: not applicable; *TPMT*: thiopurine 5-methyltransferase; *TP53*: tumour protein 53; *XRCC1*: X-ray repair cross complementing 1.

Since individuals with *TPMT**2, *3A, *3B, and *3C variants are reported to be associated with lower or intermediate enzyme activity,^{15,16} the poor response rate in patients could probably be a result of decreased *TPMT* activity, which may lead to relatively higher intracellular concentration of cisplatin and therefore higher incidence of cisplatin-related toxicity. Liu et al.¹⁷ performed a genome-wide association study of primary erythrocyte *TPMT* activity in children with leukaemia (N=1,026) and found that *TPMT* was the only gene that reached genome-wide significance (top hit rs1142345 or 719A>G; $p=8.6 \times 10^{-61}$). Furthermore, *TPMT* activity in patients behaves as a monogenic trait, further corroborating the importance of *TPMT* genetic testing in clinical settings.

The *DPD* gene also has several reported polymorphisms, out of which consistent data are available for the *DPD**2A allele, which is caused by a G>A transition at a GT splice donor site flanking exon 14 of the *DPD* gene (IVS14+1G>A).¹⁸ This allele is the most common and results in the elimination of a 55-amino-acid-long fluorouracil binding site in the *DPD* protein, leading to the skipping of exon 14 and the production of an inactive protein in 0.9% of the Caucasian population.¹⁹⁻²¹ Both anabolic and catabolic pathways are involved

in the cytotoxic effects of 5-FU;⁹ thus the activity of *DPD* plays a role both in determining the efficacy of the therapy with 5-FU and its toxicity.²²⁻²⁴ Decreased *DPD* activity has been associated with >4-fold risk of severe or fatal toxicity from standard doses of 5-FU.²⁵ *DPD* has another mutation at codon 534, leading to a 1601G>A nucleotide change.²⁶ Our study has also shown that cases carrying the *DPD* genetic variant IVS14+1G>A also showed a poor treatment response.⁷ Other studies also reported that a high prevalence of IVS14+1G>A was associated with increased toxicity and poor treatment response in patients of invasive ductal carcinoma and Grade 3 and 4 toxicity in HNSCC patients.^{27,28} Amongst the reasons for the toxicity of 5-FU are its relatively narrow therapeutic index^{29,30} and low *DPD* activity. Lowered *DPD* activity leads to less effective inactivation of 5-FU, causing an accumulation of excessive levels of fluorodeoxyuridine monophosphate, causing gastrointestinal, haematopoietic, and neurological toxicities that are potentially fatal.²¹ Zhao et al.³¹ investigated the association between germline genetic polymorphisms in *DPD* and outcome of acute lymphoblastic leukaemia following the treatment with 5-FU plus oxaliplatin, and found that the common *DPD* variant c.85T>C (rs1801265, *DPYD**9A) was significantly associated with

complete response rate, event-free survival, and treatment-related toxicity.

DNA REPAIR GENE POLYMORPHISMS: X-RAY REPAIR CROSS-COMPLEMENTING GROUP 1

The DNA damage induced by anticancer agents is repaired by the DNA repair enzymes and, therefore, it can be hypothesised that polymorphisms in DNA repair genes may influence the outcome of treatment. Increased DNA damage may prove beneficial in treatment, as both chemotherapy and radiation rely on DNA damage as part of their mechanisms of tumour cell destruction though the same DNA damage that occurs within the tumour may also take place in normal tissues as a side effect of therapy. *XRCC1* is a major DNA repair gene in the base excision repair pathway and is involved with radiation-related DNA repair. The base excision repair pathway repairs single strand breaks through protein-protein interactions, by interaction of *XRCC1* with PARP-1, PNK, Polb, and Lig3a.³² *XRCC1* interacts with PNK, leading to the stimulation of both the 5'-kinase and 3'-phosphatase activities of PNK; the interaction with Lig3a increases the intracellular stability of the ligase.³² Several single nucleotide polymorphisms (SNP) in *XRCC1* are reported, out of which the most extensively studied are Arg194Trp on exon 6 (dbSNP no. rs1799782), Arg280His on exon 9 (dbSNP no. rs25489), and Arg399Gln on exon 10 (dbSNP no. rs25487).³³

A study conducted by Quintela-Fandino et al.³⁴ tested the ability of *XRCC1*-Arg399Gln gene polymorphism to predict response to cisplatin in peripheral blood mononuclear cells of Stage-IV HNSCC patients. *XRCC1* Gln/Gln was associated with 0% of cases with progressive cancer, 7.7% of cases with stable disease, 30.8% of cases with partial response, and 61.5% of cases with complete response. Duell et al.³⁵ reported that the rate of sister chromatid exchange after exposure to ionising radiation in human lymphocytes was greater in individuals with the *XRCC1*-Gln399Gln genotype than in those with only wild-type genotype. Alsbeih et al.³⁶ tested the association between *XRCC1* G28152A Arg399Gln (rs25487) SNP and late reaction to radiotherapy in 60 nasopharyngeal cancer patients. Significant association was observed between *XRCC1* G28152A genotype ($p \leq 0.05$) and a lower grade of fibrosis (odds ratio: 0.30; 95% confidence interval: 0.10–0.89; $p = 0.02$), and therefore the wild-type was the risk allele.

However, Mahimkar et al.³⁷ did not observe a significant association between polymorphisms of *XRCC1* (Arg194Trp, rs1799782 and Arg399Gln, rs25487) and clinical outcome in advanced oral cancer patients treated with postoperative radiotherapy. Zhai et al.³⁸ investigated the association of *XRCC1* Codon399 polymorphism with treatment efficacy and normal tissue toxicity in Chinese patients with locally advanced nasopharyngeal carcinoma after radiotherapy. It was observed that Codon399 Gln/Gln allele correlated with a higher medium-term tumour regression ratio after radiotherapy for primary nasopharyngeal neoplasm and metastatic lymph nodes (>80% versus 40–60%; $p < 0.01$). Compared with the other two genotypes in this study, patients with *XRCC1* Codon399 Gln/Gln allele were more likely to obtain complete remission of the tumour (100% versus 76% and 67%; $p > 0.05$). However, no correlation between *XRCC1* Codon399 polymorphisms and acute or late radiation-induced injury of normal tissues was observed. Ghazali et al.³⁹ conducted a systematic review of the association of SNP with the occurrence of HNSCC radiotoxicity and evaluated the association of 11 polymorphisms in 8 genes with acute radiotoxicity, and 6 polymorphisms in 4 genes for late radiotoxicity. The risk of severe acute mucositis was associated with the G allele of *XRCC1* (1196A>G) in patients treated with radiotherapy alone or chemotherapy.

CELL CYCLE AND MATRIX METALLOPROTEINASES POLYMORPHISMS: TUMOUR PROTEIN P53 (TP53) AND MMP3, MMP7

The p53 protein, rightly known as the 'guardian of the genome', or the 'cellular gatekeeper', acts by transmitting a variety of stress-inducing signals to different antiproliferative cellular responses. Some major events such as DNA damage, oncogene activation, or hypoxia can lead to p53 activation, thereby regulating processes such as apoptosis, cell-cycle arrest, senescence, or modulation of autophagy.⁴⁰ Somatic mutations of the tumour suppressor gene *p53* occur in around 50% of all human cancers; thus, *p53* is a major player in carcinogenesis.⁴¹ In addition to its role in carcinogenesis, *p53* also plays a major role in deciding the clinical outcome of cancer treatment, because *p53* mutations render tumour cells resistant to chemotherapy.^{42,43} The two *p53* homologues, namely *p63* and *p73*, are also activated by cytostatic

drugs.⁴⁴ p73 also functions in transduction of specific types of DNA damage, as activation of the p73/c-abl pathway by some chemotherapeutic agents causes apoptosis via target genes, such as *AIP1*.^{45,46} p73 exists as several splice variants at both N and C termini, with N-terminal variants lacking the transactivation domain and thereby acting as dominant-negative inhibitors of both p53 and p73.⁴⁷ Out of the polymorphisms encoding either arginine (72R) or proline (72P) at codon 72 of p53, 72R is detected more commonly than 72P in squamous cell carcinomas.^{48,49} There is an over-representation of 72R mutants in carcinomas, which can be explained by the fact that inhibition of p73 is influenced by the polymorphism at codon 72 of p53.

Bergamaschi et al.⁵⁰ reported that the p53-p73 interaction and its regulation by p53 polymorphism is an important determinant of response and survival in head and neck cancer. Patients with specific p53 mutations in the 72R form are least likely to gain complete response to chemotherapy. Upon treatment with cisplatin-based combination therapy, HNSCC cases with the 72R SNP has a worse response than those with the 72P SNP.⁵⁰ Sullivan et al.⁵¹ reported that the response of human H1299 p53 -/- cells to anticancer agents is strongly influenced *in vitro* by a SNP in wild-type p53. The clinical relevance of these observations was presented by the evidence that the superior activity of the 72R wild-type protein is reflected *in vivo* by significantly higher response rates and survival in patients receiving chemo-radiotherapy for advanced head and neck cancer, who express the 72R wild-type variant. Seventy patients with inoperable advanced HNSCC who received cisplatin-based chemo-radiotherapy were involved in the study. All the patients presented with locally advanced, unresectable, tumour, node, and metastasis Stage III/IV HNSCC. In the 43 patients who had a wild-type p53 allele and had an evaluable response to treatment, complete response appeared significantly linked with p53 status (wild-type 72R p53 allele, wild-type 72P p53 allele, or both). Wild-type 72R allele produced the highest complete response rate (27 out of 28 patients [96%]). Similarly, overall survival and progression-free survival were significantly longer in patients with wild-type p53 allele (either 72R or 72P) than in those lacking a wild-type allele. Significant differences for overall survival and progression free survival were observed between cases retaining a wild-type 72R allele, cases retaining a wild-type

72P allele, and cases retaining both wild-type 72R and 72P alleles with the best prognosis in cases retaining a wild-type 72R allele. These clinical results corroborate the *in vitro* data wherein the expression of 72P wild-type p53 resulted in G1 cell cycle arrest with minor induction of apoptosis after challenge with drug doses that cause extensive apoptosis in 72R-expressing variants.⁵⁰

MMP are a family of proteins involved in extracellular matrix degradation, cell proliferation, and angiogenesis, the processes that are involved in carcinogenesis.⁵² Fas ligand (FasL) is a member of the tumour necrosis factor superfamily that induces apoptosis in susceptible cells upon cross-linking of its own receptor, FasL (Apo-1/CD95), which contributes to immune homeostasis and cell-mediated cytotoxicity. FasL is upregulated in cells treated with genotoxic agents such as cisplatin and 5-FU, and thus it can be expected that the downregulation of FasL at the surface of HNSCC tumour cells decreases the activity of anticancer drugs through modulation of the apoptotic pathway. Studies suggest that MMP7 and MMP3 could be implicated in the shedding of FasL from the cell surface, resulting in the generation of soluble FasL with less effective activity for triggering apoptosis by crosslinking with Fas.⁵³⁻⁵⁵ This was experimentally shown by the work of Mitsiades et al.⁵⁶ in Ewing sarcoma and colon carcinoma cells. Blockade of MMP7 activity by MMP inhibitor resulted in sensitisation to treatment with doxorubicin, providing evidence for the role of MMP and FasL in chemotherapy resistance.

Varying expression patterns of *MMP* could be due to the existence of functional genetic polymorphisms in *MMP* promoter regions. The two polymorphisms that result in increased promoter activity of *MMP7* are one at position -181A>G and the other at position -153C>T. For *MMP3*, two alleles have been defined: the deletion (5A) or the insertion (6A) of an adenine at position -1612 (-1612insA), with the 6A allele being associated with low transcription levels.⁵⁷ Amongst other *MMP3* polymorphisms, six are in linkage disequilibrium with -1612insA and one (-A709G) has not been assigned functional importance.⁵⁸ Blons et al.⁵⁹ reported a correlation between *MMP3* promoter polymorphism and response to chemotherapy in HNSCC. It was shown, for the first time, that patients carrying the low transcription level allele 6A had a better response to 5-FU-cisplatin combination therapy than patients carrying the 5A allele. Furthermore, multivariate

analysis, including *TP53* mutational status, showed that *MMP3* was an independent factor in response to treatment. The results were in accordance to a study which showed that the induction of FasL after DNA damage is *p53* independent.⁶⁰ However, *MMP7* promoter polymorphisms did not influence response to treatment.⁵⁹

The MMP have been postulated to interact with the Fas/FasL pathways. Poulaki et al.⁶¹ extensively investigated the involvement of the FasL/Fas pathway in doxorubicin induced apoptosis in a model of Ewing's sarcoma. Clones of the Fas-sensitive, doxorubicin-sensitive Ewing's sarcoma cell line SK-N-MC, which were either Fas-resistant or FasL-deficient, were significantly resistant to Dox. It was also observed that cleavage of FasL by MMP7 (matrilysin) protected the parental SK-N-MC cells from doxorubicin, whereas inhibition of MMP7 activity increased their sensitivity. Gastman et al.⁶² provided a unique observation for the Fas/FasL pathway. The study showed that the pathway can be potentially immunosuppressive and may be involved in the escape of human carcinoma cells from immune destruction. The study found that the expression of FasL on HNSCC cell lines was upregulated by pretreatment of tumour cells with the metalloproteinase inhibitor, BB-94. When PCI-cell lines pretreated with BB-94 were co-incubated with Jurkat cells, they induced

DNA fragmentation in a greater proportion of lymphocytes than did tumour cells not pretreated with the inhibitor.

In conclusion, genetic variations in genes encoding drug metabolising enzymes have explained a great deal of interindividual variation in response and toxicity of anticancer drugs. However, drug metabolism is just one aspect of the interaction of drugs and genes and more efforts should also be made to uncover the role of genetic variations in other important target genes, such as drug transporters, drug receptors, DNA repair, cell cycle, and other pharmacodynamic genes. Reduction of the toxicogenetic and toxicogenomic side effects has been one of the major goals in the search for new anticancer drugs and therapy protocols. Genome-wide linkage analyses may be a more systematic approach to discover genomic regions likely to carry genes, which determine chemotherapy cytotoxicity rather than the candidate gene-based approaches. Efforts should be made to implement SNP genotyping into clinical diagnostics, therefore allowing practitioners to make more accurate decisions for treatment and risk assessment. This will help in clinical decision making regarding treatment strategies, with the goal of avoiding adverse drug reactions while achieving the best drug response.

REFERENCES

1. Evans WE, Relling MV. Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science*. 1999;286(5439):487-97.
2. Watters JW, McLeod HL. Cancer pharmacogenomics: Current and future applications. *Biochim Biophys Acta*. 2003;1603(2):99-111.
3. Yadav SS et al. Association of poor metabolizers of cytochrome P450 2C19 with head and neck cancer and poor treatment response. *Mutat Res*. 2008;644(1-2):31-7.
4. Yadav SS et al. Interaction of drug metabolizing cytochrome P450 2D6 poor metabolizers with cytochrome P450 2C9 and 2C19 genotypes modify the susceptibility to head and neck cancer and treatment response. *Mutat Res*. 2010;684(1-2):49-55.
5. Ruwali M et al. Polymorphism in cytochrome P450 2A6 and glutathione S-transferase P1 modifies head and neck cancer risk and treatment outcome. *Mutat Res*. 2009;669(1-2):36-41.
6. Ruwali M et al. Polymorphism in glutathione S-transferases: Susceptibility and treatment outcome for head and neck cancer. *Xenobiotica*. 2011;41(12):1122-30.
7. Dhawan A et al. Association of genetic variability in enzymes metabolizing chemotherapeutic agents with treatment response in head and neck cancer cases. *Asia Pac J Clin Oncol*. 2017;13(2):e11-e20.
8. National Center for Biotechnology Information (NCBI). Variation Viewer: Homo sapiens:GRCh38.p7 (GCF_000001405.33)Chr 1 (NC_000001.11):196,642,315 - 196,757,066. Available at: <https://www.ncbi.nlm.nih.gov/variation/view/>. Last accessed: 27 September 2017.
9. Wasternack C. Degradation of pyrimidines and pyrimidine analogs - pathways and mutual influences. *Pharmacol Ther*. 1980;8(3):629-51.
10. Heggie GD et al. Clinical pharmacokinetics of 5-fluorouracil and its metabolism in plasma, urine, and bile. *Cancer Res*. 1987;47(8):2203-6.
11. Sahasranaman S et al. Clinical pharmacology and pharmacogenetics of thiopurines. *Eur J Clin Pharmacol*. 2008;64(8):753-67.
12. Fung C et al. Chemotherapy refractory testicular germ cell tumor is associated with a variant in Armadillo Repeat gene deleted in Velco-Cardio-Facial syndrome (ARVCF). *Front Endocrinol (Lausanne)*. 2012;3:163.
13. Marsh S, McLeod HL. Cancer pharmacogenetics. *Br J Cancer*. 2004;90(1):8-11.
14. Umamaheswaran G et al. Distribution of genetic polymorphisms of genes encoding drug metabolizing enzymes and drug Transporters - A review with Indian perspective. *Indian J Med Res*. 2014;139(1):27-65.
15. Yates CR et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: Genetic basis for azathioprine and mercaptopurine intolerance. *Ann*

Intern Med. 1997;126(8):608-14.

16. Black AJ et al. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. *Ann Intern Med.* 1998; 129(9):716-8.

17. Liu et al. Genomewide approach validates thiopurine methyltransferase activity is a monogenic pharmacogenomic trait. *Clin Pharmacol Ther.* 2017;101(3): 373-81.

18. Saif MW. Dihydropyrimidine dehydrogenase gene (DPYD) polymorphism among Caucasian and non-Caucasian patients with 5-FU- and capecitabine-related toxicity using full sequencing of DPYD. *Cancer Genomics Proteomics.* 2013;10(2):89-92.

19. Gross E et al. High-throughput genotyping by DHPLC of the dihydropyrimidine dehydrogenase gene implicated in (fluoro) pyrimidine catabolism. *Int J Oncol.* 2003;22(2): 325-32.

20. Raida M et al. Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (DPD) gene within the 5'-splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)-related toxicity compared with controls. *Clin Cancer Res.* 2001;7(9):2832-9.

21. van Kuilenburg AB et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: Frequency of the common IVS14 + 1G>A mutation causing DPD deficiency. *Clin Cancer Res.* 2001; 7(5):1149-53.

22. Milano G et al. Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity. *Br J Cancer.* 1999;79(2-4):627-30.

23. Ishikawa Y et al. Dihydropyrimidine dehydrogenase activity and messenger RNA level may be related to the antitumor effect of 5-fluorouracil on human tumor xenografts in nude mice. *Clin Cancer Res.* 1999;5(4):883-9.

24. Salonga D et al. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res.* 2000; 6(4):1322-7.

25. Van Kuilenburg AB et al. High prevalence of the IVS14+1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. *Pharmacogenetics.* 2002;12(7):555-8.

26. Ridge SA et al. Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *Br J Clin Pharmacol.* 1998;46(2):151-6.

27. Kumar CK et al. Possible associations of splice site mutation of dihydropyrimidine dehydrogenase (IVS14+1G>A) in adverse drug reactions in some invasive ductal carcinoma patients. *Int J Pharmacol.* 2007;3:130-6.

28. Dhawan D et al. Genetic variability & chemotoxicity of 5-fluorouracil & cisplatin in head & neck cancer patients: A preliminary study. *Indian J Med Res.* 2013;137(1):125-9.

29. Gamelin EC, Boisdron-Celle M. Dose monitoring of 5-fluorouracil in patients with colorectal or head and neck cancer - Status of the art. *Crit Rev Oncol Hematol.* 1999;30(1):71-9.

30. Gamelin EC et al. Relationship between 5-fluorouracil (5-FU) dose intensity and therapeutic response in patients with advanced colorectal cancer receiving infusional therapy containing 5-FU. *Cancer.* 1996;77(3):441-51.

31. Zhao XQ et al. DPYD gene polymorphisms are associated with risk and chemotherapy prognosis in pediatric patients with acute lymphoblastic leukemia. *Tumour Biol.* 2016;37(8): 10393-402.

32. Ramachandran S et al. Single nucleotide polymorphisms of DNA repair genes XRCC1 and XPD and its molecular mapping in Indian oral cancer. *Oral Oncol.* 2006;42(4):350-62.

33. Bao Y et al. XRCC1 gene polymorphisms and the risk of differentiated thyroid carcinoma (DTC): A meta-analysis of case-control studies. *PLoS One.* 2013;8(5):e64851.

34. Quintela-Fandino M et al. DNA-repair gene polymorphisms predict favorable clinical outcome among patients with advanced squamous cell carcinoma of the head and neck treated with cisplatin-based induction chemotherapy. *J Clin Oncol.* 2006;24(26):4333-9.

35. Duell EJ et al. Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis.* 2000;21(5):965-971.

36. Alsbeih G et al. Association between normal tissue complications after radiotherapy and polymorphic variations in TGFB1 and XRCC1 genes. *Radiat Res.* 2010;173(4):505-11.

37. Mahimkar MB et al. Polymorphisms in GSTM1 and XPD genes predict clinical outcome in advanced oral cancer patients treated with postoperative radiotherapy. *Mol Carcinog.* 2012;51 (Suppl 1):E94-103.

38. Zhai XM et al. Significance of XRCC1 Codon399 polymorphisms in Chinese patients with locally advanced nasopharyngeal carcinoma treated with radiation therapy. *Asia Pac J Clin Oncol.* 2016;12(1):e125-32.

39. Ghazali N et al. Genomic determinants of normal tissue toxicity after radiotherapy for head and neck malignancy: A systematic review. *Oral Oncol.* 2012;48(11):1090-100.

40. Green DR, Kroemer G. Cytoplasmic functions of the tumour suppressor p53. *Nature.* 2009;458(7242):1127-30.

41. Hofseth LJ et al. p53: 25 years after its discovery. *Trends Pharmacol Sci.* 2004; 25(4):177-81.

42. Peller S. Clinical implications of p53: Effect on prognosis, tumor progression and chemotherapy response. *Semin Cancer Biol.* 1998;8(5):379-87.

43. Weller M. Predicting response to cancer chemotherapy: The role of p53. *Cell Tissue Res.* 1998; 292(3):435-45.

44. Irwin MS. Family feud in chemosensitivity: p73 and mutant p53. *Cell Cycle.* 2004;3(3):319-23.

45. Gong JG et al. The tyrosine kinase c-Abl regulates p73 apoptotic response to cisplatin-induced DNA damage. *Nature.* 1999;399(6738):806-9.

46. Costanzo A et al. DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic target genes. *Mol Cell.* 2002;9(1):175-86.

47. Stiewe T et al. Transactivation-deficient TA-p73 acts as an oncogene. *Cancer Res.* 2002;62(13):3598-602.

48. Marin MC et al. A common polymorphism acts as intragenic modifier of mutant p53 behaviour. *Nat. Genet.* 2000;25(1):47-54.

49. Brooks LA et al. Preferential retention of codon 72 arginine p53 in squamous cell carcinomas of the vulva occurs in cancers positive and negative for human papillomavirus. *Cancer Res.* 2000; 60(24):6875-7.

50. Bergamaschi D et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. *Cancer Cell.* 2003;3(4):387-402.

51. Sullivan A et al. Polymorphism in wild-type p53 modulates response to chemotherapy in vitro and in vivo. *Oncogene.* 2004;23(19):3328-37.

52. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer.* 2002;2(3):161-74.

53. Powell WC et al. The metalloproteinase Matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr Biol.* 1999;9(24):1441-7.

54. Matsuno H et al. Stromelysin-1 (MMP-3) in synovial fluid of patients with rheumatoid arthritis has potential to cleave membrane bound Fas ligand. *J Rheumatol.* 2001;28(1):22-8.

55. Schneider P et al. Conversion of

membrane bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. *J Exp Med.* 1998;187(8):1205-13.

56. Mitsiades N et al. Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res.* 2001;61(2):577-81.

57. Ye S et al. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in

reduced gene expression. *J Biol Chem.* 1996;271(22):13055-60.

58. Beyzade S et al. Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. *J Am Coll Cardiol.* 2003;41(12):2130-7.

59. Blons H et al. Matrix metalloproteinase 3 polymorphism: A predictive factor of response to neoadjuvant chemotherapy in head and neck squamous cell carcinoma. *Clin Cancer Res.* 2004;10(8):2594-9.

60. Muller M et al. p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. *J Exp Med.* 1998;188(11):2033-45.

61. Poulaki V et al. The role of Fas and FasL as mediators of anticancer chemotherapy. *Drug Resist Updat.* 2001;4(4):233-42.

62. Gastman BR et al. Fas ligand is expressed on human squamous cell carcinomas of the head and neck, and it promotes apoptosis of T lymphocytes. *Cancer Res.* 1999;59(20):5356-64.

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