IMPACT OF DPYD VARIANTS IN FLUOROPYRIMIDINE BASED-THERAPY: THE STATE OF THE ART

O. CATAPANO1,2, O. BARLETTA2, M. DI PAOLO3, D. FAIOLI2, R. DI FRANCIA4,5

1Merigen Molecular Diagnostic Lab, Naples, Italy
2Italian Association of Pharmacogenomics and Molecular Diagnostics, Caserta, Italy
3CETAC Research center, Caserta, Italy
4Gruppo Oncologi Ricercatori Italiani (GORI ONLUS), Pordenone, Italy.
5Hematology-Oncology and Stem Cell Transplantation Unit, National Cancer Institute, Fondazione “G. Pascale” IRCCS, Naples, Italy

Abstract – Background: The 5-Fluorouracil (5-FU) is the backbone of different regimens for the treatment of several solid tumours. Unlikely, some patients develop gastrointestinal and hematologic toxicities when treated by the 5-FU, leading to the suspension of therapy. Current evidences of pharmacogenomics, have reported several dihydropyrimidines dehydrogenase (DPYD) polymorphisms associated to genes involved with fluoropyrimidine catabolism.

Methods: Some adverse drug response due to the administration of 5-FU can be predicted through pharmacogenomics testing tools. This report reviews the recent findings on the polymorphism for DPYD genes that are involved in the metabolic degradation of 5-FU and its association with the toxic effect in patients. Literature searching in the web were based on the English language restrictions. We used the following keywords and MeSH terms in conjunction with a highly sensitive search strategy: (“genetic polymorphism” or “single nucleotide polymorphism” or “SNP” or “variation” or “variant”) and (“dihydropyrimidines dehydrogenase” or “DPYD” or “DPD”) and (“colorectal cancer” or “gastrointestinal carcinogenesis” or “colorectal tumor” or “colorectal carcinoma” or “large intestine cancer” or “large intestine carcinoma” or “large colon cancer”). Also, we will take in considerations the recent methods used to identify these genetic alterations.

Conclusions: The present review suggests that DPYD IVS14+1, 496A>G and 2194G>A polymorphisms were correlated with the incidence of marrow suppression, gastrointestinal reaction and hand-foot syndrome in colorectal cancer (CRC) patients However, many clinicians acknowledge the importance of genetics in drug response and are favourable about using genetic tests to guide therapy, and to make treatment decisions for their patients maximizing benefit and minimizing toxicity.

Keywords: Pharmacogenomics, DPYD polymorphisms, DPD enzyme, 5-FU, Genotyping cost, colorectal cancer (CRC).

INTRODUCTION

For decades, 5-fluorouracil (5-FU) was the sole active agent in the treatment of several solid tumours including gastrointestinal malignances1. This has changed markedly since the year 2000, with the approval of irinotecan, oxaliplatin, and several humanized monoclonal antibodies (MoAbs) that target growth factor receptors as the epidermal growth factor receptor (EGFR). The best way to combine and sequence these agents is still undergoing validation. Despite its clinical benefit, 5-FU and its pro-
drugs (capecitabine and tegafur-Uracile) are associated with frequent gastrointestinal and hematologic toxicities\(^2\), which often lead to treatment discontinuation. It is known that, fluoropyrimidine drugs undergo complex metabolic biotransformation. The metabolic way of Fluoropyrimidine starts by conversion of 5-FU into active metabolite, 5-fluoro-2-deoxyuridine monophosphate (FdUMP) that leads to inhibition of Thymidylate Synthase (TYMS) and 5-fluoro-uridine monophosphate (FUMP). Both pathways cause inhibition of DNA “de novo” synthesized. 5-fluorouracil is converted to FdUMP and FUMP through two pathways: 1. Orotate Phosphoribosyltransferase (OPRT); and 2. Thymidine Phosphorylase (TP). The vast majority (about 80%) of administered 5-FU is metabolized by the enzyme Dihydropyrimidine Dehydrogenase (DPYD) into the inactive form, dihydrofluorouracil (FUH2), and excreted as a fluoro-β-alanine (FBAL)\(^3\).

Gene products involved in this biotransformation and related to tolerance and response to 5-FU-based chemotherapy have been well documented\(^4,5\); they include several enzymes carrying well-known mutations, as DPYD and TYMS; additional genetic variation in 5-FU-metabolizing genes includes enzyme which adds the thiol group for elimination Glutathione S-Transferase (GSTP1) and those drug targets such as Methyleneetetrahydrofolate Reductase (MTHFR). However, if the detection of these genetic variants on DPYD gene is routinely incorporated either into clinical practice or large clinical trials, knowledge concerning the predictive value of Pharmacogenomics and Pharmacogenetics (PGx) which will eventually enable the individualization of optimized therapy could be gained\(^6\). However, we still need a precise demonstration that PGx tests offer an added value, in terms of relative cost and benefit.

Furthermore, trials evaluating the pharmacoeconomic impact of genotyping testing in Fluoropyrimidine based-therapy will likely provide answers for policy making in the incorporation of PGx testing into clinical practice. The primary aim of a cost-effectiveness analysis is to provide sufficiently robust information for decision-makers to allocate resources to healthcare interventions. Overviews of cost-effectiveness studies on PGx technologies are now available\(^7\). A relevant example is the National Institute for Health and Clinical Excellence (NICE). NICE forms a Diagnostic Advisory committee, which is willing to stimulate Pharma and Academic communities to produce a robust set of data, including the design and data source in economic models of healthcare\(^8\).

We review here the clinical use of 5-FU and the correlation of the DPYD genetic variations related to therapy. In addition, an overview of the cost of genotyping specific germline polymorphisms in drug-metabolizing gene (DPYD), associated to 5-FU treatment, were evaluated. Variation in the activity of this enzymes illustrates the proof of principle of PGx in the design of appropriate therapeutic interventions. The secondary endpoint of this review is to provide information for the oncologist on the advantage and limitations, in terms of suitability, of the most common available methods for molecular detection of the SNPs of DPYD. Moreover, we think that this way could have a key role for the treatment choice in so called frail patients (i.e. elderly and HIV-positive patients) for whom the efficacy and especially the toxicity profile are important aspects\(^9,10,11\).

However, one should keep in consideration that it will not be feasible to conduct randomized trials on each and every diagnostic test, and the economic value of such tests can be modelled using decision analysis techniques\(^12\).

**LITERATURE SEARCH AND SELECTION CRITERIA**

The MEDLINE (1966-2013), the Cochrane Library Database (Issue 12, 2013), EMBASE (1980-2013), Web of Science (1970-2013) were searched with English language restrictions. We used the following keywords and MeSH terms in conjunction with a highly sensitive search strategy: (“genetic polymorphism” or “single nucleotide polymorphism” or “SNP” or “variation” or “variant”) and (“dihydropyrimidines dehydrogenase” or “DPYD” or “DPD”) and (“colorectal cancer” or “gastrointestinal carcinogenesis” or “colorectal tumor” or “colorectal carcinoma” or “large intestine cancer” or “large intestine carcinoma” or “large colon cancer”).

A manual search on the basis of references identified in the included articles was performed to obtain other potential articles. The following criteria were utilized to identify the eligibility of included studies: (1) the study must concern the correlations between DPYD genetic polymorphisms and toxicity of 5-FU in CRC patients; (2) All patients involved in the meta-analysis received 5-FU chemotherapy regimen for the first time, and they did not develop chronic liver disease or any liver dysfunction that may have an impact on the metabolism of 5-FU; (3) Sufficient information about the frequency of DPYD genetic polymorphisms should be provided in the article. The articles that were not in accordance with our inclusion criteria must be excluded. If authors published several studies of the same subjects, either the most recent or largest sample size publication was included.
GENETIC VARIATION IN DPYD GENE

Applied researches based on candidate gene screening have demonstrated an (the) associations between Fluoropyrimidine treatment outcomes and polymorphisms in DPYD.

The human DPYD gene consists of 23 exons, and includes 3 kb in length of coding sequences. To date, more than 30 SNPs and deletion mutations have been identified within DPYD gene, although the majority of these variants have no functional consequences on enzymatic activity.

Expression of DPYD enzyme has been related to tolerance and response to 5-FU-based chemotherapy. Specifically, low expression of DPYD has been associated with accumulation of 5-FU, thereby exposing patients to increased risk of severe or lethal toxicities, while high expression of DPYD has been associated with poor response to 5-FU (Table 1).

Nevertheless, the precise mechanism by which DPYD genetic polymorphisms lead to enhanced toxicity of 5-FU in CRC patients is still largely unknown. It is well established that 5-FU is an important component of many standard treatments in the multimodal therapy of CRC, which always induces side effects and toxicity-related death unfortunately.

It should be noted that DPYD acts as a rate-limiting enzyme in the catabolism of 5-FU, converting 5-FU to 5-fluorodihydrouracil (FDHU), which is further metabolized to its final metabolite 5-fluoro-betalaanine excreted in the urine. In particular, the deficiency of DPYD enzyme activity is closely related to a delay in the clearance of 5-FU, which may inevitably enhance the toxic side effects of 5-FU. We therefore hypothesized that DPYD genetic polymorphisms might alter the expression and function of DPYD, and may decrease its ability in clearance of 5-FU.

The frequency of low DPYD enzymatic activity has also been shown to vary significantly among different ethnic populations. A prospective study conducted by Schwab et al. evaluated all these potential genetic predictors of 5-FU treatment-related toxicity. The most known DPYD SNPs associated with grade 3 and 4 toxicities are intronic variant IVS14 + 1 G > A (also named DPYD*2A), and mutation A1627G. Several clinical assays have been developed assessing DPYD enzyme activity, mRNA expression, and metabolite formation, as well as SNPs within DPYD: as important results previously demonstrated that a homozygote DPYD*2A genotype results in complete deficiency (high-risk patients) while the heterozygous DPYD*2A genotype results in partial deficiency of DPYD enzyme. Several genotyping methods to screen the known DPYD gene germline mutations have been developed, without defining better platforms for their use in the daily diagnostic routine. It includes: conventional Polymerase Chain Reaction (PCR) followed by sequencing, Single-Strand Conformational Polymorphism (SSCP), Pyrosequencing, Fluorescent Resonance Energy Transfer (FRET) probes.

GENOTYPING OF 5-FU PHARMACOGENOMICS VARIANTS

Generally, genotyping is performed either by custom service laboratories or academic referenced laboratories, as well as by using commercial kits (when available). In the USA, diagnostics products are regulated by the Food and Drug Administration (FDA), whereas diagnostic services are under the rules of the Clinical Laboratory Improvement Act (CLIA). In Europe this field is covered by in vitro Diagnostic (IVD) directive, without a distinction between commercial products (used by laboratories) and diagnostics service. For information on assay technical performance, the grey literature was searched, in particular websites of commercial laboratories offering DPYD and/or TYMS genotyping. In both circumstance, a voluntary list of international laboratories (with CLIA certification in the US and CE mark in Europe) that perform genetic tests can be found on a National Institute of Health-funded website named GeneTests [http://www.ncbi.nlm.nih.gov/sites/GeneTests/lab?db=GeneTests], although only a small minority of genetic tests listed on this site are PGx tests. At the moment of writing, to our knowledge, there are no available tests approved by the FDA or the CE for genotyping detection of all variants listed in Table 1. Clinical laboratories may develop and validate tests in-house (“homebrew”) and perform them as a laboratory service; which may further reduce the cost of analysis.

A panel of Diagnostic kits being actively manufactured and marketed for distribution have been developed to detect genomic profile of the patients receiving 5-FU (Table 2). Currently, of note there are no assay kits approved by the FDA or marked IVD for genetic testing for DPYD genotypes. These commercial tests were searched (via google) using the following two strategies: 1. “Fluorouracil” [MeSH] AND OR “dihydropyrimidine dehydrogenase” [MeSH], limited to, diagnostics test, and the English language; 2. “Fluorouracil” [MeSH] AND OR “dihydropyrimidine dehydrogenase” [MeSH], limited to, gene AND toxicity, limited to human subjects and the English language. In addition, bibliographies from recent review articles and clinical studies were hand-searched for relevant studies, excluding letters and editorials.
2194G>A polymorphisms were associated with the occurrence of gastrointestinal reaction. Among different ethnicity, DPYD genetic polymorphisms showed a closely relationship with the development of marrow suppression and gastrointestinal reaction in the Asians, revealing that there was ethnic difference in the effects of DPYD polymorphisms on clinical response of 5-FU based-therapy. Although the potential mechanism of ethnicity differences is still not fully understood, we supposed that ethnicity may result in differences in alleles and genotypes among different ethnic populations.

The findings are in accordance with a previous study which demonstrated an allele-dose dependent association of the non-synonymous sequence aberration c.496A>G, and indicated that the methionine-valine exchange caused by the c.496A>G polymorphisms were associated with the occurrence of gastrointestinal reaction. Among different ethnicity, DPYD genetic polymorphisms showed a closely relationship with the development of marrow suppression and gastrointestinal reaction in the Asians, revealing that there was ethnic difference in the effects of DPYD polymorphisms on clinical response of 5-FU based-therapy. Although the potential mechanism of ethnicity differences is still not fully understood, we supposed that ethnicity may result in differences in alleles and genotypes among different ethnic populations.

DISCUSSION

The results of our reviewing searching showed clearly deep implication of the DPYD genetic variants to be significantly related with marrow suppression, gastrointestinal reaction and hand-foot syndrome toxicity after 5-FU based-therapy.

To investigate the influence of potential factors on the specific marrow suppression and gastrointestinal reaction of CRC patients receiving 5-FU chemotherapy, many authors carried out stratified analysis based on SNP and ethnicity. In the subgroup stratified by SNP, recent results indicated that there were significant association of IVS14+1, 464T>A, and 2194G>A polymorphisms with the incidence of marrow suppression in CRC patients receiving 5-FU chemotherapy. In addition, many authors were found that IVS14+1, 496A>G and 2194G>A polymorphisms were associated with the occurrence of gastrointestinal reaction. Among different ethnicity, DPYD genetic polymorphisms showed a closely relationship with the development of marrow suppression and gastrointestinal reaction in the Asians, revealing that there was ethnic difference in the effects of DPYD polymorphisms on clinical response of 5-FU based-therapy. Although the potential mechanism of ethnicity differences is still not fully understood, we supposed that ethnicity may result in differences in alleles and genotypes among different ethnic populations.

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Table 1. Major described variants of DPYD gene correlated to toxicity.

<table>
<thead>
<tr>
<th>Genetic variants</th>
<th>Enzyme activities</th>
<th>Annotation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS14+1G&gt;A</td>
<td>Decreased DPD Enzyme not active in A/A homozygous</td>
<td>Heterozygosity for the A allele correlated with marrow suppression and gastrointestinal toxicity. A/A homozygote were observed rarely; it could be fatal Leukopenia.</td>
<td>17-19</td>
</tr>
<tr>
<td>496A&gt;G</td>
<td>Decreased, Changing in aminoacid (Met&gt;Val)</td>
<td>Homozigous for Methionine may influence the breakdown of the 5-FU and provoke severe drug-adverse effects in CRC patients</td>
<td>14</td>
</tr>
<tr>
<td>2194G&gt;A</td>
<td>Decreased</td>
<td>High incidence of marrow suppression in CRC patients</td>
<td>14</td>
</tr>
<tr>
<td>1627A&gt;G</td>
<td>Severe nausea vomiting</td>
<td>The elimination constant (Ke) for 5-FU was significantly lower in patients homozygous for the G allele.</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 2. Widely used commercial kits for genotyping of most common known genetic abnormalities in the DPYD gene for Fluoropyridines pharmacotherapy.

<table>
<thead>
<tr>
<th>GENE (SNP)</th>
<th>Commercially available kit (Vendor)</th>
<th>Method-based test</th>
<th>Website</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPYD (IVS14+1G&gt;A)</td>
<td>*SALSA MLPA p103DPYD RUO (MRC-Holland)</td>
<td>Multiplex Ligation-dependent Probe Amplification</td>
<td><a href="http://www.mlpa.com/WebForms/WebFormProductDetails.aspx?Tag=znAPLaupKgMjaDFE30bmuqXhL/LugfK8">www.mlpa.com/WebForms/WebFormProductDetails.aspx?Tag=znAPLaupKgMjaDFE30bmuqXhL/LugfK8</a></td>
</tr>
<tr>
<td>DPYD*2A Genotyping kit-Fluorouracil Toxicity RUO CE (EntroGen)</td>
<td>DNA microarray</td>
<td><a href="http://www.entrogen.com/web/dpyd-genotyping-reagents-fluorouracil-toxicity.php">www.entrogen.com/web/dpyd-genotyping-reagents-fluorouracil-toxicity.php</a></td>
<td></td>
</tr>
<tr>
<td>AmpliDPYD*2° RUO CE (Diachem-srl)</td>
<td>PCR+ sequencing</td>
<td><a href="http://www.diachem-srl.it/home.php">www.diachem-srl.it/home.php</a></td>
<td></td>
</tr>
</tbody>
</table>

*We systematically searched the English literature using google. Combination of Bio-Medical Subject Headings terms (5-FU genetic variants, 5-FU pharmacogenomics) combined with manufacturing tests, DPYD were used. Each vendor’s website was screened by visualizing the home page and list of product for sale.

*No FDA or CE (Conformité Européenne) marked

§RUO: Research Use Only

All websites was accessed until July 2014
transition has posed a deleterious effect on DPYD deficient patients\textsuperscript{25}. Moreover, Kristensen et al also revealed that sequence variations in the DPYD gene may influence the breakdown of the common anti-cancer drug 5-FU and provoke severe drug-adverse effects in CRC patients receiving 5-FU therapy\textsuperscript{14}.

Highly considerable, our web-research also has a number of potential limitations: i) due to the small number of clinical studies, did not include all the data from all trials to assess the correlations between DPYD polymorphisms and toxicity of 5-FU in Gastrointestinal cancer patients, which may have a negative effect on the general applicability of our findings. Consequently, the function of our report should be considered elementary; ii) in a retrospective study, there are no guidelines as to how much information a meta-analysis should include to be reliable, which may explain why many controversies occur when the results of meta-analysis and large trials were not consistent; iii) another potential limitation is that our report was unable to acquire original data from the included studies.

More importantly, our report has a clear selection criterion in literature search strategy. In order to achieve strong objectivity, all the research methods were based on strict inclusion and exclusion criteria. Besides, meta-analysis undertaken according to these rigorous statistical analyses will lead to a more reliable conclusion.

The usefulness of the described genetic variants for clinical practice will depend on their improving diagnostic prediction or fostering changes in prevention or treatment strategies. Particularly, the molecular testing for mutation in DPYD gene, allows the identification of patients who are most likely to respond to 5-FU. To meet this need, scientists and clinicians must collect information, informed consent, and tissue samples in the expectation of future studies addressing potential future questions\textsuperscript{1}.

Even though, he clinical utility of described polymorphisms involved in 5-FU based-therapy is in part limited by: 1. less wide diffusion of genotyping methods in routine clinical diagnostics; 2. the evidence that PGx testing improves clinical outcomes is still an open question; and 3. the cost-effectiveness of the testing being unknown.

The technology platform needed for DPYD traits are different; it does depend from the type of mutation (insertion, deletion or SNP). For inherited SNPs there is a copious of suitable methods for genotyping able to detect mutant allele either in heterozygosis or homozygosis. Rational selection of the best method to detect them is dependent from the specifics aims of different laboratories\textsuperscript{22}. Only few studies have addressed the cost-effectiveness of pharmacogenomics testing implication in clinical practice\textsuperscript{6}. For example, van den Akker et al\textsuperscript{26} included thiopurine S-methyltransferase (TPMT) genotyping prior to 6-mercaptopurine treatment in paediatric Acute Lymphoblastic Leukemia (ALL); the mean calculated cost from 4 European countries was € 2100.00 per life-year considering low myelosuppression-related hospitalization; the cost for genotyping of TMPT mutation averaged around € 150.00\textsuperscript{26}. Early outline of genotyping cost for “home brew” pharmacogenomic tests averaged about € 20.00 per SNP\textsuperscript{21}.

Furthermore, the major issues to consider for the clinical laboratories (who are responsible for providing PGx services), are: i) the availability of FDA-cleared tests; ii) the current absence of public reimbursement; iii) the need for genotyping accuracy; and iv) the need to find clinical expertise to interpret laboratory results\textsuperscript{7,28}. In addition to the issues related to assay validation, additional equipment requirements, and time consumption, one main issue preventing the clinical application of these assays is their limited sensitivity and specificity. For example, Morel et al\textsuperscript{19} have shown that the sensitivity, specificity, and positive and negative predictive values of the detection of the 3 major SNPs (IVS14 + 1 G>A, 2846A>T, and 1679T>G) in DPYD, as factors predictive of 5-FU toxicity, were 0.31, 0.98, and 0.62 and 0.94, respectively. They found that only about 60\% of patients who carry genetic variations in DPYD develop severe 5-FU toxicity.

**CONCLUSIONS AND FUTURE OUTLOOK**

Hopefully, the future implementation of the methods for genotyping of variants influencing fluoropyrimidine-based therapy will result in personalized treatments and eventually, in shifting the clinical benefit from toxicity related dosing towards toxicity prevention. Therefore, it is fundamental that pharmaceutical and biotechnology companies join together, in order to develop an extensive study on the standardization method to validated tests suitable for routine diagnostics in pharmacogenomics of 5-FU.

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The authors stated that there are no conflicts of interest regarding the publication of this article.
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