Pharmacogenetic evaluation of DPYD deficient variants for pre-emptive testing in patients candidate to receive fluoropyrimidine-based chemotherapy: a comprehensive analysis in 1454 patients

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Introduction: 5-fluorouracile (5-FU) is one of the most commonly used chemotherapeutic agent, but its effectiveness is often compromised by toxicity and consequently by treatment discontinuation (Diasio RB et al., 1989). Severe adverse reactions (ADRs), including gastrointestinal, hematologic toxicities and hand-foot syndrome (HFS), are related to an altered 5-FU metabolism (Matsusaka S et al., 2015). The key enzyme of 5-FU matabolism is the dihydropyrimidine dehydrogenase (DPD). Patients carriers of certain allelic variants of DPD gene (DPYD) responsible for reduced enzymatic activity, are poor metabolizer and the related accumulation of 5-FU results in ADRs that can result life-threatening (Offer et al., 2013). Although several single nucleotide polymorphisms (SNPs) in DPYD have been found, the best pharmacogenetic approach to pre-emptive screening of DPD deficiency and improving patient safety and outcomes remains an open issue to be addressed.
Aim: the aim of this study was to evaluate, in a large cohort of patients, which DPD polymorphisms has to be screened in order to reduce the risk of ADRs FL-related.
Material and Methods: The study enrolled a total of 1454 patients. Three ml of whole blood were taken from patients, stored in EDTA, and genomic DNA was extracted by the Biorobot EZ1 (Qiagen, Valencia, CA, USA) for pharmacogenetic tests. Full sequencing of DPD open reading frame by using standard Sanger sequencing platforms was performed in 200 patients suffering from toxicity (Cohort 1), in order to identify the most relevant SNPs. Than, we retrospectively validate the meaning of selected SNP previously identified in 982 patients suffering from FL-toxicity, screening their DNA with a Real Time SNP Genotyping (Applied Biosystems®, Foster City, CA) (Cohort 2). Finally, our selected SNPs were validated in 272 'control patients' that received FL-based treatment, without any toxicity (Cohort 3). Data analysis was performed by SPSS v.22.0 software.
Results: The following 7 SNPs were identified in cohort 1 and were confirmed in cohort 2: c.496A>G, c.1601G>A, c.1627A>G, c.1896T>C, c.1905+1G>A, c.2194G>A and c.2846A>T, all as heterozygous or homozygous condition. Patients of cohort 3, that received a standard treatment dose of a 5-FU-based therapy, without any reduction or interruption of the treatment, showed the following variants: c.496A>G, c.1601G>A, c.1627A>G, c.1896T>C and c.2194G>A. The comparison of cohort 2 and 3 allowed the selection of the following variants to be significantly associated to 5-FU toxicity: c.1905+1G>A, c.2194G>A and c.2846A>T. Interestingly, the SNP c.2194GA/AA strongly correlated with 5-FU toxicity ($p < 0.003$). In particular, it was associated with higher grade of hematological toxicity (anemia and fever) and HFS, expecially when 5-FU was given in combination with other drugs with risk of bone-marrow impairment ($p < 0.003$).
Conclusions: The present study highlights that pharmacogenetic analysis of DPD is part of the optimal use of fluoropyrimidines. Moreover, it suggests the analysis of c.1905+1G>A, c.2194G>A and c.2846A>T as the optimal strategy to reduce the risk of fluoropyrimidine-associated ADRs. Finally, although other mutations of DPYD are associated with a poor-metabolizer phenotype, their extremely low frequency does not suggest a current use at least as pre-emptive screening.

References

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