


Letter regarding Zhao et al. entitled “*DPYD* gene polymorphisms are associated with risk and chemotherapy prognosis in pediatric patients with acute lymphoblastic leukemia”

Tumor Biology
June 2017: 1–3
© The Author(s) 2017
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/1010428317701629
journals.sagepub.com/home/tub


Maarten J Deenen^{1,2}, Linda M Henricks³, Gabe S Sonke⁴,
Jan HM Schellens^{3,5} and Didier Meulendijks⁶

Abstract

Zhao et al. investigated the association between germline genetic polymorphisms in *DPYD*, the gene encoding dihydropyrimidine dehydrogenase, and (1) the risk of developing pediatric acute lymphoblastic leukemia and (2) outcome of acute lymphoblastic leukemia following the treatment with 5-fluorouracil plus oxaliplatin (FOLFOX). The authors found that the common *DPYD* variant c.85T>C (rs1801265, *DPYD**9A) was significantly associated with (1) risk of developing pediatric acute lymphoblastic leukemia, (2) complete response rate, (3) event-free survival, and (4) treatment-related toxicity. The authors conclude that patients carrying the c.85T>C C allele have an increased risk of developing acute lymphoblastic leukemia and have inferior outcome, and that *DPYD* c.85T>C can be used as a guide for individualized treatment and the decision to utilize 5-fluorouracil in acute lymphoblastic leukemia patients. In our view, the published article gives rise to multiple critical issues regarding the study's rationale and the methodology used, which strongly question the validity of the authors' conclusions.

Keywords

DPD-deficiency, acute lymphoblastic leukemia, pharmacogenetics

Date received: 2 September 2016; accepted: 29 December 2016

Zhao et al.¹ performed a case-control analysis in 147 pediatric acute lymphoblastic leukemia (ALL) patients and in 102 age-matched healthy volunteers to study the association between *DPYD* variants and the risk of developing ALL. They found a higher frequency of *DPYD* c.85T>C in ALL cases versus controls and concluded that c.85T>C increases susceptibility to ALL with an odds ratio (OR) for risk of ALL for the TT genotype versus the CC genotype of 0.115 ($p=0.015$); this corresponds to an OR of 8.7 for the CC genotype versus TT, implying a major effect size. Unfortunately, the authors omitted any discussion on other studies investigating the functional and clinical relevance of *DPYD* c.85T>C nor provided a rationale for conducting their study.

DPYD c.85T>C is not recognized as a functionally relevant *DPYD* variant.² It was shown that patients carrying

¹Department of Clinical Pharmacy, Catharina Hospital, Eindhoven, The Netherlands

²Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands

³Department of Clinical Pharmacology, Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

⁴Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

⁵Faculty of Science, Utrecht Institute of Pharmaceutical Sciences (UIPS), Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, Utrecht, The Netherlands

⁶Dutch Medicines Evaluation Board (CBG-MEB), Utrecht, The Netherlands

Corresponding author:

Maarten J Deenen, Department of Clinical Pharmacy, Catharina Hospital, Michelangelolaan 2, 5623 EJ Eindhoven, The Netherlands.
Email: maarten.deenen@catharinaziekenhuis.nl



the homozygous c.85T>C CC genotype had normal dihydropyrimidine dehydrogenase (DPD) enzyme activity: 0.13 nmol/min/mg versus the population average of 0.14.³ In line with this finding, multiple large studies, including one meta-analysis, showed no association between *DPYD* c.85T>C and fluoropyrimidine-associated toxicity.^{4–7} Given the questionable functional and lack of clinical effect, we ask the authors to provide the rationale for their study as well as to discuss on potential explanations for the strong effect size observed.

Methodological shortcomings

In our view, the article by Zhao et al. has multiple methodological shortcomings. One shortcoming is that cases and controls were matched solely by age, and not by sex and race, which is also associated with ALL.⁸ In addition, hospital-based controls were used instead of population-based controls which would be more appropriate, and would have allowed more controls per case to be included. Furthermore, the preferred statistical analysis would be conditional logistic regression, and not chi-square tests.

A major shortcoming is that in the association analysis between *DPYD* polymorphisms and treatment outcome, no adjustment for the strongest general prognostic factors in pediatric ALL, that is, immunophenotype, age, sex, race, initial white blood cell count, and genomic/cytogenetic features, was made.⁹ An imbalance in these prognostic factors could have affected the results. In our view, even if the association between *DPYD* c.85T>C and treatment outcome would exist, it is unlikely that its effect size would outweigh that of the known prognostic factors, strongly questioning the clinical value of *DPYD* c.85T>C as a prognostic and/or predictive factor as claimed by the authors. In view of these methodological issues, we question the internal validity of the results and ask the authors to report baseline prognostic factors according to c.85T>C genotype (TT, TC, and CC) and report a multivariable analysis.

In addition, it is noted that the observed association between c.85T>C and risk of ALL seems to result solely from a higher frequency of the homozygous c.85T>C CC genotype among ALL cases (7.5% vs 1.0% in controls), while frequencies of TT and CT genotypes were comparable between groups. Similarly, only the 11 patients carrying the CC genotype had a lower complete response (CR) rate (64%), while patients carrying TT or CT had a CR rate similar to the population (88%). Based on these findings, it would be logical to analyze event-free survival (EFS) of CC versus TT+CT genotypes. Yet, the authors compared TT with CT+CC genotypes, without further justification. We would be interested to see EFS for each individual genotype (TT, TC, and CC).

Furthermore, it is noted that the range of follow-up is reported to be 0.5–36 months. Surprisingly, however, EFS data upto 60 months are provided. Moreover, the 36-month EFS times presented in Table 6 are not the same as depicted

in Figure 2(a)–(d). Also, there is a discordance between the reported frequencies of the c.85T>C CC genotype in the text and Table 3 (1 vs 11 homozygous CC carriers among the cases, respectively).

Finally, the authors did not adjust *p* values for multiple testing. If correction would have been applied, the results might not have remained significant.

Selected treatment regimen

Zhao et al. reported that patients were treated with surgery and FOLFOX. First, it is unclear that what type of surgery patients underwent for their hematological disease. Second, FOLFOX is not a standard treatment for pediatric ALL according to Asian, US, or European clinical practice guidelines.^{9,10} Moreover, we are not aware of any data supporting efficacy of FOLFOX in ALL. Standard treatment agents of pediatric ALL include glucocorticoids, vincristine, asparaginase, doxorubicin, cytarabine, and cyclophosphamide.⁹

Since FOLFOX was used by Zhao et al. the external validity (extrapolability) can be strongly questioned in view of current clinical practice.

Toxicity associations

Besides the alleged association between *DPYD* c.85T>C and susceptibility to ALL and effectiveness of FOLFOX, the authors also conclude that *DPYD* c.85T>C is associated with toxicity.

Common 5-fluorouracil (5-FU)-associated toxicities are hematological toxicity, mucositis, stomatitis, diarrhea, and hand-foot syndrome and are typically associated with DPD-deficiency.^{11,12} Nonetheless, no differences in the incidences of these toxicities according to *DPYD* genotypes were found. The authors did, however, find associations between *DPYD* c.85T>C and both “renal function damage” and “liver function damage” (not further specified). These terms appear to refer to reduced creatinine clearance and elevated liver function tests (e.g. aspartate transaminase/alanine transaminase (AST/ALT)) according to the reference provided for toxicity scoring (which, besides, is not standard, as the common terminology criteria for adverse events [CTC-AE] criteria are typically used for scoring toxicity in cancer).^{13,14} Reduced renal function and elevated liver function tests are unlikely results of 5-FU treatment.^{11,12} Importantly, these adverse events could be related to the disease state of the respective patients rather than to treatment, since renal failure and elevated liver function tests can be complications of ALL and/or concomitant pharmacotherapy.¹⁵ Therefore, it could be hypothesized that the 11 patients carrying the *DPYD* c.85T>C CC genotype presented more often with renal and liver-function related adverse events as a result of poorer disease state, which could in turn explain their inferior CR rate and EFS. This makes an association between *DPYD* c.85T>C and treatment-related toxicity highly unlikely.

Conclusion

In summary, we have major concerns regarding the internal and external validity of the findings by Zhao et al., and in view of the described methodological shortcomings, question whether *DPYD* c.85T>C is associated with risk of ALL and treatment outcome. We feel that the results from this study should be interpreted with great caution.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Zhao X-Q, Cao WJ, Yang HP, et al. *DPYD* gene polymorphisms are associated with risk and chemotherapy prognosis in pediatric patients with acute lymphoblastic leukemia. *Tumour Biol* 2016; 37(8):10393–10402.
2. Caudle KE, Thorn CF, Klein TE, et al. Clinical pharmacogenetics implementation consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. *Clin Pharmacol Ther* 2013; 94: 640–645.
3. Seck K, Riemer S, Kates R, et al. Analysis of the *DPYD* gene implicated in 5-fluorouracil catabolism in a cohort of Caucasian individuals. *Clin Cancer Res* 2005; 11: 5886–5892.
4. Amstutz U, Farese S, Aebi S, et al. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics* 2009; 10: 931–944.
5. Gross E, Busse B, Riemenschneider M, et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. *PLoS One* 2008; 3: e4003.
6. Rosmarin D, Palles C, Church D, et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. *J Clin Oncol* 2014; 32: 1031–1039.
7. Afzal S, Gusella M, Vainer B, et al. Combinations of polymorphisms in genes involved in the 5-Fluorouracil metabolism pathway are associated with gastrointestinal toxicity in chemotherapy-treated colorectal cancer patients. *Clin Cancer Res* 2011; 17: 3822–3829.
8. Ward E, DeSantis C, Robbins A, et al. Childhood and adolescent cancer statistics. *CA Cancer J Clin* 2014; 64: 83–103.
9. Hunger SP and Mullighan CG. Acute lymphoblastic leukemia in children. *N Engl J Med* 2015; 373: 1541–1552.
10. Yeoh AEJ, Tan D, Li CK, et al. Management of adult and paediatric acute lymphoblastic leukaemia in Asia: resource-stratified guidelines from the Asian Oncology Summit 2013. *Lancet Oncol* 2013; 14: e508–e523.
11. Hoff PM, Ansari R, Batist G, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. *J Clin Oncol* 2001; 19: 2282–2292.
12. Van Cutsem E, Twelves C, Cassidy J, et al. Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. *J Clin Oncol* 2001; 19: 4097–4106.
13. Miller AB, Hoogstraten B, Staquet M, et al. Reporting results of cancer treatment. *Cancer* 1981; 47: 207–214.
14. National Cancer Institute. *Common terminology criteria for adverse events (CTCAE) v4.03*. Rockville, MD: National Cancer Institute, 2010.
15. Segal I, Rassekh SR, Bond MC, et al. Abnormal liver transaminases and conjugated hyperbilirubinemia at presentation of acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2010; 55: 434–439.