100,000 Genomes Project: Validation and Reporting Guidance for DPYD Variants

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1. Document Version History

Version	Summary of main changes and reasons
V1	NHS provided finalised version of DPYD Validation and Reporting Guidance document to Genomics England for sign off

Note: The document version history detailed above is effective for the last live version of this document only. Details of changes for prior versions are available in EQMS.

2. Introduction

Germline genetic variants in the dihydropyrimidine dehydrogenase (*DPYD*) gene can confer an increased risk of severe and even fatal toxicity, when patients with one or more copies of these variants are treated with the fluoropyrimidines, capecitabine or 5-fluorouracil. The gene encodes an enzyme which plays a role in the rate-limiting catabolism step of 5-fluoropyrimidine metabolism and specific sequence variants are known to impact on the activity of the enzyme. If treating clinicians are aware that a patient is heterozygous or homozygous for one of these variants, in many cases they will be able to assess the need to adjust therapy regimens to reduce the risk of toxicity.

From Spring 2019, the whole genome sequence (WGS) data will be analysed for the presence of four *DPYD* gene variants (table 1) prospectively on all cancer participants in the 100,000 Genomes Project as a pilot exercise. This pilot will help to determine the clinical effectiveness of analysing these variants within the NHS.

This document provides technical guidance to NHS Genomic Medicine Centres (GMCs) on the validation and clinical reporting of returned toxicity-related *DPYD* gene variants. This technical guidance should always be used in conjunction with the 100,000 Genomes Project Cancer Whole Genome Sequencing Validation and Reporting guidance

3. DPYD Variants

The *DPYD* variants being analysed and reported as part of the 100,000 Genomes Project have been selected because they have strong evidence for no function or decreased function. These variants form the basis of genotype-guided therapeutic guidelines for capecitabine and 5-fluorouracil prescribing, published by the Clinical Pharmacogenetics Implementation Consortium (CPIC)[1] and members of the Dutch Pharmacogenetics Working Group in the Netherlands. Other *DPYD* variants are not currently being analysed and reported because there is insufficient evidence at this time for implication in fluoropyrimidine-induced toxicity.

NHS GMCs will be notified of the presence of the variants listed in table 1 prospectively for all cancer participants regardless of their cancer type. It is recognised that fluoropyrimidines, capecitabine or 5-fluorouracil, are not used as therapy for all cancer types and consequently *DPYD* variant information may not be relevant to all cancer participant's current condition. However, results are being reported on all participants' as they may be beneficial to a small subset if the participant develops other cancers where *DPYD* status is relevant.

DPYD variant results will be returned to the NHS GMCs from Genomics England in part 3 (analysis of germline variants) of the "Cancer Whole Genome Sequence Analysis" document (figure 1). Further information regarding the return of germline findings can be found in section 4 of the 100,000 Genomes Project Cancer Whole Genome Sequencing Validation and Reporting guidance document.

Table 1: DPYD gene variants analysed [2,3]

Nucleotide change[i]	Protein change[ii]	rsID	Haplotype name	Genomic coordinates (GRCh38)	Predicted Functional Status
c.1905+1G>A	N/A	rs3918290	**2A*	1:97450058C>T	No function
c.1679T>G	p.(Ile560Ser)	rs55886062	**13*	1:97515787A>C	No function
c.2846A>T	p.(Asp949Val)	rs67376798		1:97082391T>A	Decreased function
c.1129-5923C>G c.1236G>A[iii]	N/A[iv] p.(Glu412Glu)	rs75017182 rs56038477	*Part of the HapB3 haplotype*	1:97579893G>C 1:97573863C>T	Decreased function

Figure 1*:* Example of how DPYD results will be presented in part 3 of the Cancer WGS analysis

Germline analysis: Pharmacogenomics

Polymorphic variants in the DPYD gene are associated with severe, sometimes fatal, toxicity to fluoropyrimidine therapy. Analysis was undertaken for 4 established major variants/haplotypes in DPYD (see Technical Information v1.11.main). The following toxicity-related DPYD variant(s) were detected.

Gene	GRCh38 coordinates ref/alt allele	Transcript	CDS change and protein change	Predicted consequences	Population germline allele frequency (1KG | gnomAD)	Genomics England germline allele frequency	Alt allele/total read depth	PharmGKB_ID	Genotype
DPYD	1:97450058C>T	ENST00000370192	c.1905+1G>A	splice_donor_variant	0.0063	0.0041	17/40	PA166153760	0/1

‡ Variants c.1129-5923C>G (rs75017182) and c.1236G>A (rs56038477) are part of a haplotype and should not be considered two independent variants for interpretation.

Additional rarer DPYD variants are associated with toxicity on fluoropyrimidine therapy. Additional DPYD haplotypes are associated with toxicity on fluoropyrimidine therapy that is less severe. Other genetic, clinical and environmental factors may affect a patient's response to fluoropyrimidines and their risk for adverse drug reactions. Chromosomal phasing has not been performed. Interpretation recommendations are based on the assumption that when two variants relating to reduced function are detected, they are in trans (on different chromosomes).

This is a research result. We are returning this to help clinical teams reduce risk of harm from medications. If the result is intended for use in informing clinical management it should be confirmed using a test accredited for clinical use. It remains the responsibility of the health-care provider to determine the best course of treatment for a patient.

4. Technical Validation

Technical validation of the presence of the *DPYD* variant(s) will be undertaken in a laboratory with ISO 15189;2012 accreditation for the appropriate test[4]. Where the NHS GMC laboratory is unable to deliver the required test, the laboratory should outsource the validation testing to an appropriately accredited laboratory. A list of laboratories currently offering fully validated and accredited *DPYD* testing can be found in appendix 1.

All participant results in which toxicity related *DPYD* variants have been identified will be validated regardless of whether a patient is on an active management pathway. Validation of *DPYD* variants is not required for deceased participants. Participant results with no toxicity related *DPYD* variants identified do not require validation to confirm absence of these variants. However, this does not exclude the possibility that the participant is at increased risk for fluoropyrimidine-related toxicity.

The *DPYD* c.1129-5923C>G (rs75017182) and c.1236G>A (rs56038477) variants are considered part of the same haplotype and should not be interpreted as two separate variants. As the intronic c.1129-5923C>G variant (rs75017182) is the likely causative variant, this should be validated. If validation of this variant is not available, c.1236G>A (rs56038477) can act as a proxy/tag SNP for validation of the causative variant. If only the c.1236G>A (rs56038477) is reported, the presence of the c.1129-5923C>G variant (rs75017182) should be checked in the sequencing read file or by another method.

5. Interpretation

Patients identified as having one or more copies of the toxicity related *DPYD* variants listed in table 1 should be considered for dose modification of fluoropyrimidines or alternative drug. The degree of dose reduction required will depend on the overall reduction in the enzyme dihydropyrimidine dehydrogenase (DPD) produced by the *DPYD* genotype. This will be influenced by the specific variants present and whether they are heterozygous, homozygous or compound heterozygous. Clinicians should review the literature for current information on the interpretation of *DPYD* genotypes and predicting the effects on DPD production.

6. Clinical Reporting

Once results have been validated a clinical report which is compliant with ISO15189:2012 standard should be produced and issued. Reports should include details of the *DPYD* genotype, the predicted effect on DPD production and recommendations regarding the adjustment of 5-fluorouracil or capecitabine dosing to be considered by treating oncologists. Information regarding the *DPYD* genotype interpretation and dosing must be compliant with current literature findings. Example clinical reports and suggested phrases for *DPYD* reporting are provided in appendix 2.

Clinical reports should be sent to the most appropriate individuals as identified by the GTAB and must be stored in the participants' Electronic Patient Records (EPR) (or paper clinical patient records if there is no EPR). This will ensure other healthcare professionals e.g. pharmacists, have access to these results if appropriate.

7. Roles and Responsibilities for the DPYD Pilot

Genomics England is responsible for returning *DPYD* variant results relating to 100,000 Genome Project participants to NHS GMCs.

NHS England is responsible for producing guidance and ensuring a standardised approach to the validation and reporting of *DPYD* variants across all NHS GMCs. As this is a pilot exercise to determine the clinical utility of *DPYD* testing of cancer patients then NHS England is responsible for reviewing the outcome data and generating a pilot outcome summary report.

NHS GMCs are responsible for the validation and reporting of toxicity related *DPYD* variants and notification to NHS England of outcomes upon request as follows (but not limited to):

- Were the results reported?
- Were the results acted upon?
- Did the results change clinical management?
- Where are the results stored?

8. Abbreviations/Definitions

DPD	dihydropyrimidine dehydrogenase
DPYD	Gene encoding dihydropyrimidine dehydrogenase
WGS	Whole Genome Sequencing
GMC	Genomic Medicine Centre
UKAS	United Kingdom Accreditation Service
GTAB	Genomic Tumour Advisory Board
CPIC	Clinical Pharmacogenetics Implementation Consortium

9. References and Supporting Documents

[1] Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for *DPYD* Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017

[2] Clinicians should review the literature for current information on the interpretation of *DPYD* genotypes and predicting the effects on DPD production.

[3] *Table 1 legend*

[i] Nucleotide changes according to reference sequence NM_000110.3 (maps to ENST00000370192).

[ii] Protein changes according to reference sequence NP_000101.2.

[iii] The c.1129-5923C>G variant (rs75017182) is the likely causative variant underlying the HapB3 haplotype; this variant is in intron 10 and introduces a cryptic splice site and the partial production of

a nonfunctional transcript[1]. c.1236G>A (rs56038477) is a synonymous variant in LD with the likely causative variant in Europeans, and thus can act as a proxy/tag Single Nucleotide Polymorphism (SNP) – for example, the *DPYD* prospective testing in the Netherlands screened for the exonic c.1236G>A variant rather than c.1129-5923C>G. The genome analysis includes both of these variants, both are likely to be identified (*in cis*) and should be considered as one haplotype variant in the interpretation.
[iv]Not applicable

[4]As set out in section 3 of the 100,000 Genomes Project Cancer Whole Genome Sequencing Validation and Reporting guidance document

PharmGKB summary: fluoropyrimidine pathways. Thorn CF, Marsh S, Carrillo MW, McLeod HL, Klein TE, Altman RB. Pharmacogenet Genomics. 2011 Apr;21(4):237-42.

CPIC https://cpicpgx.org/ (accessed 13 February 2019).

Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, Klein TE, McLeod HL, Caudle KE, Diasio RB, Schwab M. Clin Pharmacol Ther. 2018 Feb;103(2):210-216. For an interactive version of the guidelines table for fluorouracil therapeutic guidelines see https://www.pharmgkb.org/guideline/PA166122686, and for capecitabine see https://www.pharmgkb.org/guideline/PA166109594.

PharmGKB, DYPD reference materials https://www.pharmgkb.org/page/dpydRefMaterials (accessed 6 August 2018).

DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Henricks LM et al. Lancet Oncol. 2018 Oct 18 pii: S1470-2045(18)30686-7. doi: 10.1016/S1470-2045(18)30686-7.

10. Appendices

Appendix 1

Table 2: Information regarding laboratories offering ISO15189:2012 accredited services

Laboratory Name	Contact Details
St Thomas Purine Laboratory	http://www.viapath.co.uk/departments-and-laboratories/purine-research-laboratory-at-st-thomas
South West Genomic Laboratory Hub	https://www.exeterlaboratory.com/genetics/prediction-of-5-fluorouracil-toxicity/
Sheffield Diagnostic Genetics Service	https://www.sheffieldchildrens.nhs.uk/sdgs/

Appendix 2

Example heterozygous and homozygous *DPYD* genomic laboratory reports and suggested phrases for *DPYD* genotypes

Example Report 1 (This example is of a dummy patient)

GENOMIC LABORATORY REPORT

Report to:
Consultant Oncologist
A Hospital
Somewhere in England

Patient Name: Jean HELIX
Date of Birth: 02/11/1984
Sex: Female
NHS No.: 012 345 6789
Hospital number:

Reason for testing
Predictive: To determine whether Jean has dihydropyrimidine dehydrogenase (DPD) deficiency that increases her risk of developing severe 5-fluorouracil or capecitabine associated toxicity.

Result summary
Increased risk of 5-fluorouracil or capecitabine toxicity

Result
Jean is homozygous for the *DPYD* variant c.1905+1G>A.

Implications when treated with 5-fluorouracil or capecitabine
The intracellular concentration of active metabolites ***may be*** increased due to complete DPD deficiency, resulting in an increased risk for fatal drug toxicity (grade ≥3).

Dosing recommendations for 5-fluorouracil or capecitabine
- Do not use 5-fluorouracil or 5-fluorouracil prodrug-based regimens.

Date issued: 18/03/2019

TECHNICAL INFORMATION

Variant details

Gene	Zygosity	HGVS description	Location: GRCh38	Predicted DPD enzyme activity
DPYD	Homozygous	NM_000110.3:c.1905+1G>A	Chr1:g.97450058	0%

Test methodology
Sanger sequence analysis of four *DPYD* variants (c.1905+1G>A, c.1679T>G, c.2846A>T and c.1129-5923C>G) that are associated with partial DPD deficiency and severe toxicity to 5-fluorouracil or capecitabine therapy.

Patient phenotype
Colorectal cancer

Sample details
Laboratory No: 150001
Sample type: DNA from peripheral blood
Sample collected: 10/02/2019
Sample received: 11/02/2019

Example Report 2 (This example is of a dummy patient)

GENOMIC LABORATORY REPORT

Report to:
Consultant Oncologist
A Hospital
Somewhere in England

Patient Name: Jean HELIX
Date of Birth: 02/11/1984
Sex: Female
NHS No.: 012 345 6789
Hospital number:

Reason for testing
Predictive: To determine whether Jean has dihydropyrimidine dehydrogenase (DPD) deficiency that increases her risk of developing severe 5-fluorouracil or capecitabine associated toxicity.

Result summary
Increased risk of 5-fluorouracil or capecitabine toxicity

Result
Jean is heterozygous for the *DPYD* variant c.2846A>T p.(Asp949Val).

Implications when treated with 5-fluorouracil or capecitabine
The intracellular concentration of active metabolites ***may be*** increased, resulting in an increased risk for severe or even fatal drug toxicity (grade ≥3).

Dosing recommendations for 5-fluorouracil or capecitabine
- Reduce the starting dose to 50% of the standard dose.
- Follow this by titration of dose based on toxicity.
- In patients experiencing no or clinically tolerable toxicity in the first two cycles: increase the dose to maintain efficacy; In patients who do not tolerate the starting dose: decrease the dose to minimise toxicities.

Date issued: 18/03/2019

TECHNICAL INFORMATION

Variant details

Gene	Zygosity	HGVS description	Location: GRCh38	Predicted DPD enzyme activity
DPYD	Heterozygous	NM_000110.3:c.2846A>T p.(Asp949Val)	Chr1:g.97082391	75%

Test methodology
Sanger sequence analysis of four *DPYD* variants (c.1905+1G>A, c.1679T>G, c.2846A>T and c.1129-5923C>G) that are associated with partial DPD deficiency and severe toxicity to 5-fluorouracil or capecitabine therapy.

Patient phenotype
Colorectal cancer

Sample details
Laboratory No: 150001
Sample type: DNA from peripheral blood
Sample collected: 10/02/2019
Sample received: 11/02/2019

Table 3: Suggested phrases for DPYD reporting

Suggested Phrases for *DPYD* genotypes	
Heterozygous Genotypes	The patient is heterozygous for the *DPYD* variant [*insert variant]*. A heterozygous genotype is associated with partial DPD deficiency and severe toxicity to fluoropyrimidine therapy. A [*insert %]* dose reduction or alternate therapy should be considered. The patient was genotyped for the *DPYD* variants c.1905+1G>A, c.2846A>T p.(Asp949Val), c.1679T>G p.(Ile560Ser) and c.1129-5923C>G
Homozygote Genotypes	The patient is homozygous for the variant *DPYD* [*insert variant]*. A homozygous genotype is associated with partial DPD deficiency and early, severe toxicity to fluoropyrimidine therapy. The effect of these alleles is additive, the X variant is associated with a [*insert %]* dose reduction. As two alleles are present in a homozygous genotype, consider a [*insert %]* dose reduction or alternate therapy. The patient was genotyped for the *DPYD* variants c.1905+1G>A, c.2846A>T p.(Asp949Val), c.1679T>G p.(Ile560Ser) and c.1129-5923C>G
Compound heterozygous genotypes	The patient is compound heterozygous for the variants *DPYD* [*insert variant]*. and [*insert variant]*. This compound heterozygous genotype is associated with partial DPD deficiency and early, severe toxicity to fluoropyrimidine therapy. Consider a [*insert %]* dose reduction or alternate therapy. The patient was genotyped for the *DPYD* variants c.1905+1G>A, c.2846A>T p.(Asp949Val), c.1679T>G p.(Ile560Ser) and c.1129-5923C>G

100,000 Genomes Project Validation and Reporting Guidance for DPYD variants v1.0

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