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Original article

# Decoding variants in drug-metabolizing enzymes and transporters in solid tumor patients by whole-exome sequencing

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ABSTRACT

*Background:* Pharmacogenetics is involved in customizing therapy according to the genetic makeup of an individual, and is applicable for chemotherapy, radiotherapy as well as targeted therapy. Drug metabolizing enzymes (DMEs) involving both phase I, and phase II reactions are widely studied. Our study was involved in whole exome sequencing (WES) of cancer patients, followed by analysis for identifying key variations in DMEs, and associated transporters that have a potential impact on treatment outcome.
*Methodology:* A total of 181 solid tumor patients at stage >/= III were subjected to WES by the SureSelect$^{XT}$ Human All Exon V6 + UTR library preparation kit, and sequencing in the Illumina NextSeq 550 system. Bioinformatics analysis involved use of GATK pipeline, and the variants were further assessed for population frequency, functional impact with annovar insilico algorithms. Further variant information from significant DMEs, and transporters were extracted and analyzed with PharmGKB to assess level of evidence and infer their impact on the pathways involved in drug response.
*Results:* The total study cohort of 181 solid tumor patients included 60 males, and 121 females respectively. Among DMEs, deleterious mutation in dihydropyrimidine dehydrogenase (*DPYD;* rs67376798), solute carrier organic anion transporter family member 1B1 (*SLCO1B1*5*), and cytochrome P450 2D6 (*CYP2D6*10*) associated with metabolism of anticancer drugs was detected to be in high frequency of 26%, 21% and 25% respectively.
*Conclusion:* Our analysis detected variations in both phase I and phase II DMEs, as well as associated transporter genes which has been documented to reduce drug efficacy, as well as cause grade 3 and 4 toxicity. Our study reiterates the significance of pharmacogenomics in stratifying patients for appropriate therapy regimen focused at better treatment outcome and quality of life.


## 1. Introduction

Genetic makeup is a unique factor that influences physical, physiological, and psychological factors of an individual. Personal genomics has earned a key position in the clinical domain, wherein genetic variations are being studied to assess disease susceptibility, predict treatment, and management outcome, as well as identify risk of relapse, and transmission. Pharmacogenomics is a science that studies inter-individual variations in drug response to determine the best suited drug of choice for treatment, and today is widely used in cancer management. Genomic differences have been detected to be present every 300–1000 nucleotides throughout the human genome, and identifying these variations that contribute majorly to the population variation is the fundamental of a genetic study (Roden and George, 2002; Sachidanandam et al., 2001). The pharmacology of drugs involve pathways for activation, and metabolism, and the dosage is highly influenced by the genotype, and the corresponding phenotype in many factors, including the drug metabolizing enzymes (DMEs), solute carrier transporters, etc. Beyond treatment efficacy, handling treatment failure, and adverse drug reactions (ADRs) is critical, which can be influenced

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by stable-inherited genetic factors as well as variables like lifestyle factors, gender, age, and status of organ function (Pirmohamed, 2006; Meyer et al., 2013).

Pharmacogenetics which is focused on identifying genetic variants for drug efficacy or toxicity was put to use from the year 2000s (Kocal and Baskin, 2017). The P4 concept best describes the purpose of personalized medicine, as it includes; predictive, personalized, preventive, and participatory medicine and it differentiates individual patients into groups based on the molecular profile to determine the most effective, and safe chemotherapy agent (Tian et al., 2012). Genome sequencing, and mutation analysis, are the backbone of pharmacogenetics, and there are many FDA approved biomarkers for anti-cancer drugs including; dihydropyrimidine dehydrogenase (*DPYD*/DPD) for capecitabine and fluorouracil; thiopurine S-methyltransferase (*TPMT*) for cisplatin, mercaptopurine, thioguanine; cytochrome P450 2D6 (CYP2D6) for tamoxifen, and rucaparib, to name a few. The clinical indications for testing include, adverse reaction, and toxicity monitoring, as well as for dose determination, and administration (US FDA, 2020). Pharmacogenomics involves studying both the patient and tumor genome as variations in both have been seen to impact transport, efflux, retention, and penetration of the anti-cancer drug.

In DMEs, the cytochrome P450 (*CYPs*) has been widely studied as they are the major family of enzymes involved in oxidative biotransformation of drugs, and xenobiotic compounds, and are phase I metabolizing enzymes (Guengerich, 2008; Zanger et al., 2008). Most of the *CYP*-family enzymes have a multifactorial control involving polymorphisms in *trans*-genes, and other host factors like age, gender, diurnal variation, except for CYP2D6, wherein monogenic polymorphisms cause variability in effect. Based on the impact of polymorphisms on enzyme activity, individuals are classified as either poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), or ultrarapid metabolizers (UM) for that particular drug, due to lack-of-function or gain-of-function mutation. For a prodrug which undergoes metabolic activation within the system, the pharmacological activity as well as toxicity of the metabolites are considered; eg: formation of morphine from codeine by CYP2D6 (Zanger and Schwab, 2013). Polymorphisms in *CYP2D6* have been noted in 10% of the general population causing the PM phenotype (Kocal and Baskin, 2017). The uridinediphosphate glucuronosyl transferase 1A1 (*UGT1A1*) is a phase II metabolizing enzyme, involved in glucuronidation of exogenous and endogenous substrates. *UGT1A1* has been widely studied for its effect on anti-cancer drugs, including topoisomerase inhibitors I and II, irinotecan, and etoposide (Bosma et al., 1994).

DPD is a rate-limiting enzyme involved in metabolism of 5-fluorouracil (5-FU), and mutations in *DPYD* have been documented to increase the half-life of drugs causing significant toxicity including myelosuppression, mucositis, hand-foot syndrome, etc. Population studied have identified the prevalence of complete DPD deficiency to be 5% (Amstutz et al., 2011). Thymidylate synthase (TS/*TYMS*), which is involved in transformation of dUMP, is essential for DNA replication. Fluorodeoxyuridylate (5-FdUMP) which is activated from 5-FU forms a complex with TS and folate, leading to inhibition in DNA synthesis, and thus, reduced expression of the same has been associated with better drug response, and longer survival among colorectal patients when treated with 5-FU (Lecomte et al., 2004). The methylene tetrahydrofolate reductase (*MTHFR*) regulatory enzyme which is involved in folate metabolism has been documented to affect metabolism of methotrexate causing toxicity, when variations cause reduction in its activity (Ulrich et al., 2003). The *TPMT* gene is involved in S-methylation of thiopurine drugs causing inactivation, anddeficiency in the same has been associated with accumulation of cytotoxic thiopurine nucleotides causing severe toxicity. The frequency of intermediate and low activity frequency for *TPMT* has been detected to be between 6–11% and 0.3% respectively (Stanulla et al., 2005). Glucose-6-phosphate dehydrogenase (G6PD) is a highly polymorphic gene with over 300 variants recorded till date, and is involved in the pentose phosphate pathway, crucial for red blood cell (RBC) metabolism. The kinase inhibitor dabrafenib, has been documented to increases risk of hemolytic anemia among G6PD deficient patients, and thus carries an FDA label warning for continuous monitoring when used (Kocal and Baskin, 2017).

The aim of the present study was to identify the key pharmacogenetic factors in DMEs of a cohort of solid tumor patients in stages III and above by whole exome sequencing (WES). WES has been proven to be powerful for assessing protein-coding regions of a genome. WES data has been associated with the ability to provide secondary findings, or findings of convenience, which are different to the primary variants identified in disease-causing genes. Information on coding variants becomes easily detectable by WES, as it provides comprehensive pharmacogenomic profiling. The data will aid in assessing efficacy of ongoing therapy as well as predict ADR, and treatment outcome.

## 2. Materials & methods

### 2.1. Study cohort

The study cohort consisted of 181 solid tumor patients including 60 males, and 121 females, respectively. The patients were recruited from the University hospital and all subjects provided written consent to participate in the study. The study was approved by the University hospital ethics review board.

### 2.2. Sample collection and genomic DNA extraction

Genomic DNA extraction for the WES was done from EDTA-peripheral blood, using the QIAamp® DNA Blood Mini QIAcube Kit (QIAGEN, Germantown, MD) as per manufacturer instructions. The extracted genomic DNA was quantified using the NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific Inc., USA).

### 2.3. Library preparation and next-generation sequencing

Library preparation was done using the SureSelect$^{XT}$ Human All Exon V6 + UTR (Agilent Technologies Inc., USA). This protocol targets 75 Mb of genomic region representing 99% of coding regions in addition to 5′ and 3′-untranslated region sequences. Pre-enrichment libraries were constructed with SureSelect Low Input reagent kit, and exome enrichment of the DNA library was done in a hybridization reaction with biotinylated baits from the SureSelect Human All Exon V6 + UTR Enrichment Kit. Sequencing of the prepared DNA libraries was done in the NextSeq 550 sequencer (Illumina Inc., USA) in an average coverage of 100× with 2 × 150 bps paired-end reads.

### 2.4. Data analysis and annotation

Raw sequencing reads were demultiplexed using Illumina bcl2-fastq, and were further assessed with the quality control tool, FastQC (Fig. S1). Adapter sequences were removed with cutadapt, and the trimmed reads were further mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner (BWA-mem). The polymerase chain reaction (PCR) duplicates were removed by SAMtools. Variants were called using GATK, and visually inspected by the Integrative Genomics Viewer (IGV, Broad Institute), (Fig. S2) and further annotated with ANNOVAR. Insilico impact of coding variants on protein function was predicted using Polyphen 2, SIFT scores, GADD, and with default settings, and vari-

ant frequency among different populations was determined utilizing public genomic databases. Variants were categorized as 1) nonsynonymous, 2) synonymous, 3) frameshift deletion or insertion, 4) splicing, 5) stop gain or loss, or 6) functional intronic or promoter variants. Post initial bioinformatics analysis, all relevant absorption, distribution, metabolism, and excretion (ADME) related genes including publicly available gene lists from pharmGKB and PharmaADME.org were extracted. These genes were classified into different groups based on functions/pathways and included, the ABC family, SLC family, members of Phase I and II family, nuclear receptors/transcription regulators, and genes which are potentially related to ADME.

## 3. Results

### 3.1. Patient information

The study cohort included 181 solid tumor patients including 60 males, and 121 females. All cases were in stage >/= III and included both primary and metastatic tumors. Cancer of stage IV was found to be at a maximum among both males and females at 82% and 88% respectively. Between primary and metastatic cancer types, the latter was found to be at a maximum frequency of 55%, and 60% among males, and females respectively. Among cancer type, the pancreatic adenocarcinoma among males, and colorectal adenocarcinoma among females was found to be maximum at 17% each respectively. The patient characteristics of the study cohort have been highlighted in Figs. 1a and 1b.

### 3.2. Variations in genes of clinical relevance

The WES raw data was analyzed by the GATK, and the variants specific to DMEs were extracted. These included a total of 1093 variants (Table S1) including those from the *ABC* family of transporters, phase I and II metabolizing enzymes, nuclear receptors, the *SLC* family, and other rate-limiting enzyme-coding genes. A total of 23 variants from 14 genes were found to have PharmGKB level 1 and 2 evidence in this study, and to have an impact on drug response or efficacy.

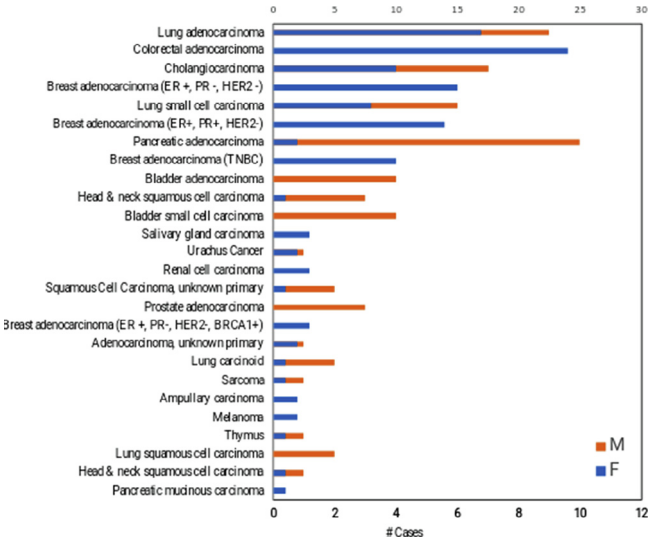


**Fig. 1a.** Distribution of cancer indications studied in the cohort. A - Represents the spectrum of cancers studied for in the cohort among males and females. Note: M - Males; F - Females; All cases of colorectal adenocarcinoma, salivary gland carcinoma, ampullary carcinoma, melanoma, and pancreatic mucinous carcinoma were females in the cohort.

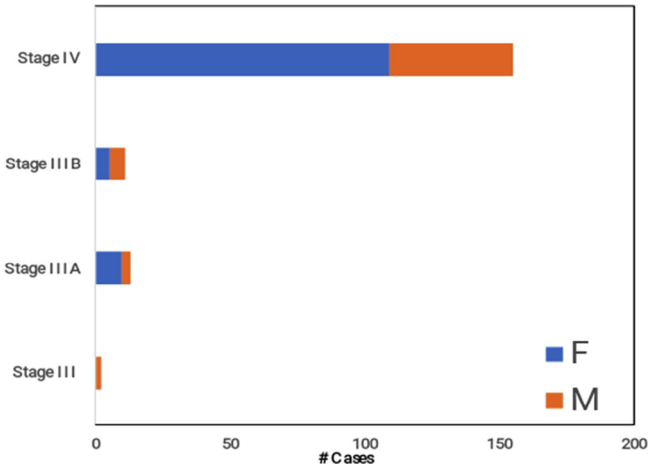


**Fig. 1b.** Distribution of tumor stages included in study cohort: Tumor stages analyzed for pharmacogenomics marker in the study, Note: M - Males; F- Females.

### 3.3. In silico assessment of variants in pharmacogenes

The extracted DME variants were further annotated using public databases, including the Exome Aggregation Consortium (ExAC), the 1000 Genomes, and the functional effect predicting tools including, PolyPhen (Polymorphism Phenotyping), SIFT (Sorting Intolerant Form Tolerant) and CADD (Combined Annotation Dependent Depletion) which scores the deleteriousness of the variants detected.

The functional impact have been highlighted in Figs. 2a–2g for different variants types detected in each category of DME including phase I (Fig. 2a) and phase II (Fig. 2b) DME, the *CYP* family (Fig. 2c), the *ABC* (Fig. 2d) and *SLC* transporters (Fig. 2e), nuclear receptors (Fig. 2f) and other transporters (Fig. 2g).

### 3.4. Analysis of variants in pharmacogenes

All the DMEs annotated variants were further annotated for level of evidence (LoE) and relevance using the PharmGKB. A total of 23 variants were annotated with level of evidence 1A, 2A and 2B. The summary of findings has been highlighted in Table 1. All the PharmGKB variants were studied for their role in affecting anti-

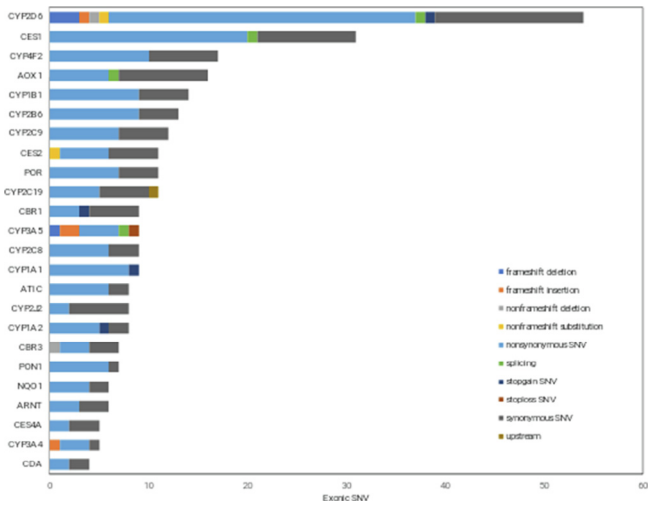


**Fig. 2a.** Variants detected in Phase I DME categorized by functional effect: Spectrum of variant types detected by WES in each of the phase I DME, Non-synonymous SNVs dominated the category of variants detected,

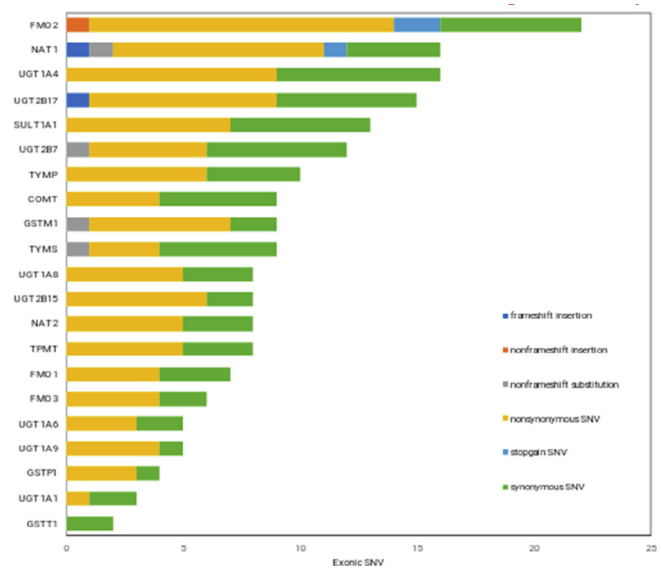


**Fig. 2b.** Variants detected in Phase II DME categorized by functional effect: Spectrum of variants detected by WES in the phase II DMEs, Synonymous and non-synonymous SNVs dominated the category.

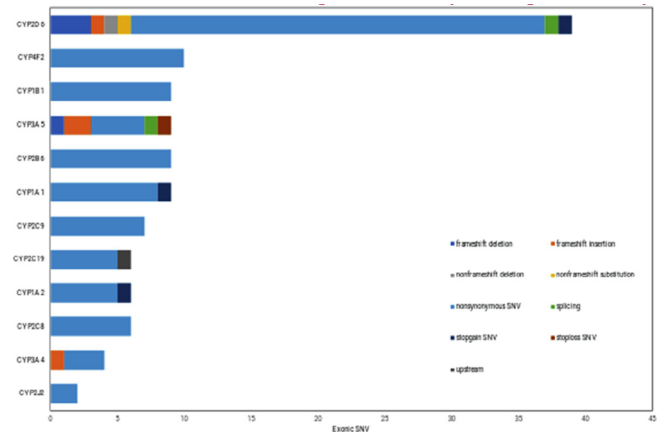


**Fig. 2c.** Variants detected in the *CYP* gene family categorized by functional effect: Spectrum of variant types detected in the *CYP* family by WES Non-synonymous SNVs dominated the findings, though frameshift insertion were detected in *CYP2D6*, *CYP3A5*, and *CYP3A4*.

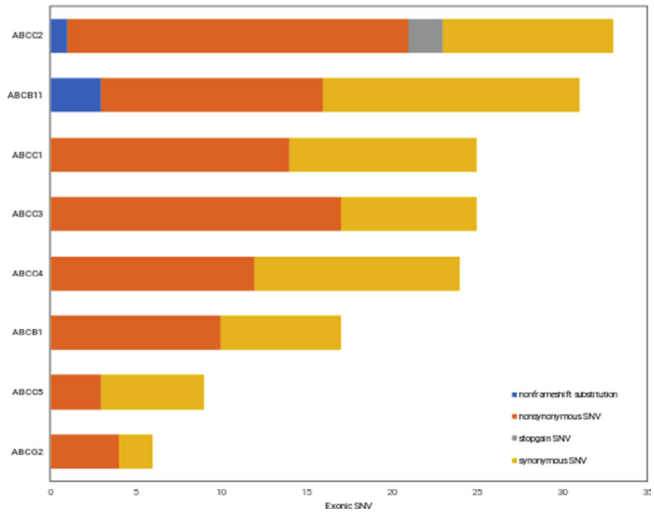


**Fig. 2d.** Variants detected in *ABC* family of transporters categorized by functional effect: Spectrum of variant types detected by WES in *ABC* family. Majority exhibited both synonymous and non-synonymous SNVs, while non-frameshift substitution and stopgain SNV was detected in *ABCC2*.

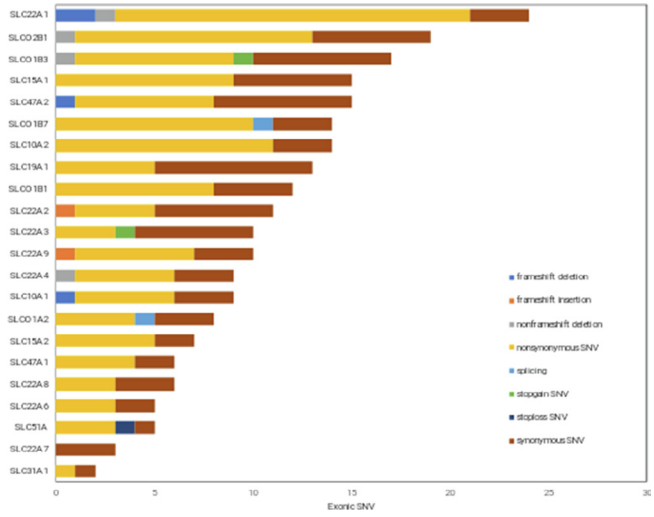


**Fig. 2e.** Variants detected in *SLC* family of transporters categorized by functional effect: Spectrum of variations detected by WES in *SLC* family. Non-synonymous and synonymous variants were detected across all genes.

cancer drug activity, and the variants of *CYP2D6*, *DPYD*, *SLCO1B1*5* and *TPMT* were detected. The *DPYD* p.D949V was detected to at maximum frequency of 26%, and *SLCO1B1*5* at 21%. The *TPMT* variants of *2, *3B and *3C were detected at frequencies of 0.4%, 3%, and 5% respectively. In case of the *CYP2D6* family, the non-functioning and widely studied variants of *3A, *4 and *10 alleles were detected at frequency of 5%, 15%, and 25% respectively.

In case of *CYP2C9*, the *2 allele which has been associated with neoadjuvant chemotherapy resistance in nodular breast cancer cases, and a cancers with hereditary load, both in homozygous and heterozygous form, was also detected at a frequency of 19% (Seredina et al., 2012).

## 4. Discussion

Variability in drug response is the fundamental for pharmacogenetics, which is impacted by many extrinsic, as well as intrinsic factors including ethnicity. In order to clinically implement the drug-polymorphism outcome knowledge, it is primarily important to identify prevalence of the same, among both cases and controls. Our study was aimed at identifying variations in DMEs, and associated transporters from a cohort of solid tumor patients in stages III and above using WES. The information thus extracted we believe will give insight on disease progression, current therapy outcome, as well as determining future course of action.

A total of 181 solid tumor patients including 60 males, and 121 females were included in the study, and WES was done using the Illumina Nextseq 500 sequencer. The males in the cohort represented a total of 16 cancer types, while the females represented a total of 21 cancer types. The cancer type with highest frequency among males was the pancreatic adenocarcinoma at 17%, while among females it was detected to be the colorectal adenocarcinoma at 17%. The raw data was analyzed and polymorphism information pertaining specifically to the DMEs and the associated transporters was extracted. Function prediction tools were used to assess impact of the polymorphism as deleterious or benign, and PharmGKB was used to detect the LoE. A total of 23 variants (Table 1) were detected with PharmGKB LoE of 1A, 2A and 2B.

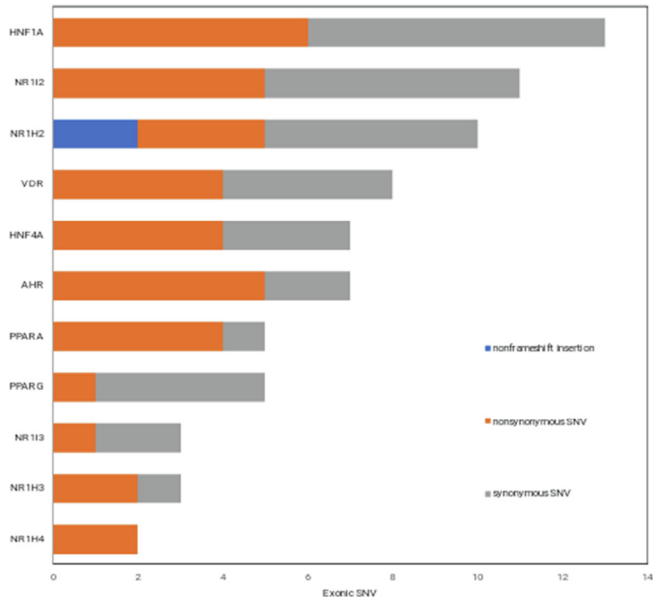


**Fig. 2f.** Variants detected in Nuclear receptors categorized by functional effect: Spectrum of variants detected by WES among the nuclear receptors. Non-synonymous and synonymous SNVs were detected in majority.

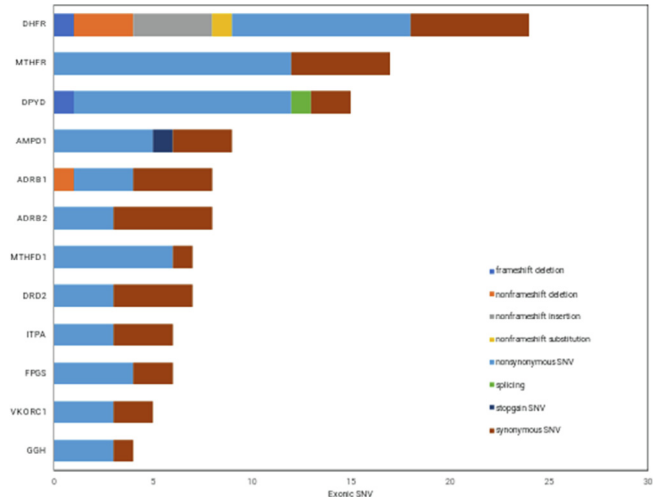


**Fig. 2g.** Variants detected in other transporter genes categorized by functional effect: Spectrum of variants detected by WES across other transporter genes. Non-synonymous and synonymous SNVs were detected across all genes studied.

Our analysis found the *CYP3A5*3* splice variant at the highest frequency of 91%. This has been reported to be a very common cryptic splice site mutation which leads to a premature stop codon at the junction between exons 3 and 4. The resulting mRNA has been documented to undergo rapid degradation, and this polymorphism in both *3/*3 and *1/*3+*3/*3 form has been found to be significantly associated with lung cancer patients at p = 0.0004 and p = 0.0001 (Busi and Cresteil, 2005; Subhani et al., 2015). In case of the *CYP2D6* family, the poor metabolizer alleles of *3, *4 and *10 which cause a frame shift, a splicing defect, and an unstable enzyme, were detected at frequencies of 5%, 15%, and 25% respectively (Bertilsson et al., 2002). The *CYP2D6*10T/T* has been associated with tamoxifen metabolism, and in such cases the efficacy of toremifene has been detected to be better (Lan et al., 2018).

In case of *DPYD,* a total of 450 missense variations have been reported in the dbSNP database, of which only twenty have been detected to cause a functional impact. Of the four which have been considered to be of relevance in the clinical scenario because of their toxic effects, the c.1905G > A (rs3918290; IVS14 + 1 G > A), c.1679 T > G (rs55886062), and c.2846 A > T (rs67376798) were detected at frequencies of 3%, 0.3%, and 26% respectively. The variants have been associated with grade 3, and 4 toxicities on treatment with 5-FU (Sherry et al., 2001; Amstutz et al., 2018).

**Table 1**
PharmGKB output for DMEs and Transporter variants detected in the study.

| Gene | Allele | dbSNP | Current Study AF | PharmGHB LoE | 1000 Genomes AF |
|---|---|---|---|---|---|
| *CYP3A5* | *3 | rs776746 | 91% | 1A | 0.05 |
| *VKORC1* | | rs9923231 | 39% | 1A | 0.40 |
| *UGT1A1* | *28 | rs3064744 | 35% | 1A | 0.29 |
| *CYP4F2* | *3 | rs2108622 | 32% | 1A | 0.27 |
| *CYP2C19* | *17 | rs12248560 | 26% | 1A | 0.23 |
| *DPYD* | | rs67376798 | 26% | 1A | 0.01 |
| *CYP2D6* | *10 | rs1065852 | 25% | 1A | 0.20 |
| *SLCO1B1* | *5 | rs4149056 | 21% | 1A | 0.17 |
| *CYP2C9* | *2 | rs1799853 | 19% | 1A | 0.12 |
| *CYP2C19* | *2 | rs4244285 | 16% | 1A | 0.15 |
| *CYP2D6* | *4 | rs3892097 | 15% | 1A | 0.19 |
| *CYP2C9* | *3 | rs1057910 | 9% | 1A | 0.06 |
| *CYP2D6* | *3A | rs35742686 | 5% | 1A | 0.02 |
| *TPMT* | *3C | rs1142345 | 5% | 1A | 0.03 |
| *DPYD* | *2A | rs3918290 | 3% | 1A | 0.01 |
| *TPMT* | *3B | rs1800460 | 3% | 1A | 0.03 |
| *TPMT* | *2 | rs1800462 | 0.40% | 1A | 0.004 |
| *DPYD* | *13 | rs55886062 | 0.30% | 1A | 0.001 |
| *ABCB1* | | rs1045642 | 40% | 2A | 0.47 |
| *SLCO1B1* | *1B | rs2306283 | 35% | 2A | 0.40 |
| *CYP2B6* | *9 | rs3745274 | 29% | 2A | 0.23 |
| *CYP2D6* | *9 | rs5030656 | 3% | 2A | 0.02 |
| *ABCG2* | | rs2231142 | 21% | 2B | 0.10 |

Note – AF: Allele frequency, LoE: Level of Evidence.

The solute carrier transporters mediate the influx of cytotoxic drugs into the cell, and the *SLCO1B1* mediates hepatic uptake of different drugs (Niemi et al., 2011). The common variant *SLCO1B1*5* (rs4149056; p.V174A) decreases the activity of the organic anion-transporting polypeptide OATP1B1, and was detected at a frequency of 21% in our study. This variant has been associated with development of chemotherapy-induced amenorrhea (CIA) among breast cancer patients treated with cyclophosphamide-based regimen (Reimer et al., 2016). This variant has also been associated with tegafur-uracil (UFT)-induced liver dysfunction with an elevation in levels of aspartate aminotransferase and alanine transaminase. UFT which is a combination of tegafur, a prodrug of 5-FU, and uracil is widely used for treatment of breast cancer, and other solid tumors like lung, gastric and rectal cancers (Kamio et al., 2019).

TPMT metabolizes all thiopurines, and is involved is the inactivation of mercaptopurine, which is a combination maintenance therapy among children with acute lymphoblastic leukemia (ALL). The activity is inherited as a monogenic co-dominant trait and the alleles *2, *3A, *3B and *3C account for ~95% of the inactivating alleles. The most common in the US has been documented to be *TPMT*3A* at ~5%, while along the East Asians, African-Americans and some African populations it is *TPMT*3C* at a frequency of ~2% (Azimi et al., 2014). Our study detected the frequency of *TPMT* alleles *2, *3B and *3C as 0.4%, 3% and 5% respectively. The CPIC dosing recommendations for *TPMT*-genotype based mercaptopurine dosing include starting with reduced or drastically reduced dosage, depending in presence of one or two non-functional alleles, and adjusting dose based on myelosuppression, and disease-specific guidelines (Dean, 2017).

The *UGT1A1*28* has been recommended to be used as a monitoring index among cancer patients receiving irinotecan-based

chemotherapy because of its significant association with toxicity, including severe diarrhea and neutropenia (Liu et al., 2017). Our study detected the frequency of the same to be 35%. The hepatic artery infusion (HAI) of irinotecan, oxaliplatin and 5-FU with intravenous cetuximab, has been found to be highly effective among previously treated patients with unresectable liver metastases from colorectal cancer. The *VKORC1* variant rs9923231 has been detected to be associated with early and objective response to HAI, as well as survival, wherein the T/T genotype was found to exhibit early response, and greatest 4-year survival (Levi et al., 2017). Our study detected this variant at a frequency of 39%. The *CYP2B6*9* has been detected to affect chemotherapy involving cyclophosphamide, propofol, and bupropion, and our study detected the frequency of this variant to be 29% (Ohnami et al., 2017).

The *ABC* family of transporters play a key role in the efflux of over 80% of the chemotherapy drugs. The *ABCG2* has been implicated in drug resistance of anticancer drugs including adriamycin, and platinum drugs. Its expression has been significantly associated with short survival. In case of the *ABCG2* variant rs2231142, studies have found this to be associated with better response to anthracycline-based chemotherapy (Wu et al., 2015; Hu et al., 2019). Our study detected the frequency of this variant to be 21%.

## 5. Conclusion

The application of WES and the information regarding polymorphisms in DMEs, and transporters aids in developing knowledge regarding disease progression, and the various intrinsic factors that can affect disease treatment. Development of personalized treatment strategies with pharmacogenetics is no longer an option with the advent of targeted agents and immunotherapy. Our WES study on cancer cases detected a number of DME variants which significantly affect therapy outcome. This reiterates the need for inclusion of broad-panel pharmacogenetics test in the clinical space to facilitate informed disease management.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.10.052.

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