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Identification of ADME genes polymorphic variants linked to trastuzumab-induced cardiotoxicity in breast cancer patients: Case series of mono-institutional experience

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ABSTRACT

Background: Long-term survival induced by anticancer treatments discloses emerging frailty among breast cancer (BC) survivors. Trastuzumab-induced cardiotoxicity (TIC) is reported in at least 5% of HER2+BC patients. However, TIC mechanism remains unclear and predictive genetic biomarkers are still lacking. Interaction between systemic inflammation, cytokine release and ADME genes in cancer patients might contribute to explain mechanisms underlying individual susceptibility to TIC and drug response variability. We present a single institution case series to investigate the potential role of genetic variants in ADME genes in HER2+BC patients TIC experienced.

Methods: We selected data related to 40 HER2+ BC patients undergone to DMET genotyping of ADME constitutive variant profiling, with the aim to prospectively explore their potential role in developing TIC. Only 3 patients ("case series"), who experienced TIC, were compared to 37 "control group" matched patients cardiotoxicity-sparing. All patients underwent to left ventricular ejection fraction (LVEF) evaluation at diagnosis and during anti-HER2 therapy. Each single probe was clustered to detect SNPs related to cardiotoxicity.

Abbreviations: BC, Breast Cancer; TIC, Trastuzumab-Induced Cardiotoxicity; LVEF, Left Ventricular Ejection Fraction; ADME, Absorption, Distribution, Metabolism, and Elimination; SNP, Single Nucleotide polymorphism; OS, Overall Survival; CVD, Cardiovascular Disease; ROS, Reactive Oxygen Species; TK, Tyrosine Kinase; ADCC, Antibody-Dependent Cellular Cytotoxicity; NK cell,, Natural Killer; PK, Pharmacokinetic; PD,, Pharmacodynamic; PGx, Pharmacogenomics; CPT, Cardioprotective Therapy; UM, Ultra-rapid Metabolizers; EM, Extensive Metabolizers; IM, Intermediate Metabolizers; PM, Poor Metabolizers; NYHA, New York Heart Association Functional classification; PS, Performance Status; T-dxd, trastuzumab-deruxtecan; UGTs, Uridine 5´-diphospho-glucuronosyltransferase enzymes; *UGT1A1*, UDP Glucuronosyltransferase family 1 member A1; CYP2D6, Cytochrome P450 2D6; EGF, Epidermal Growth Factor; NRG-1, Neuregulin; GDC TCGA-BRCA, The Cancer Genome Atlas Breast Invasive Carcinoma in Genomic Data Commons Data Portal.

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Results: In this retrospective analysis, our 3 cases were homogeneous in terms of clinical-pathological characteristics, trastuzumab-based treatment and LVEF decline. We identified 9 polymorphic variants in 8 ADME genes (UGT1A1, UGT1A6, UGT1A7, UGT2B15, SLC22A1, CYP3A5, ABCC4, CYP2D6) potentially associated with TIC. *Conclusion:* Real-world TIC incidence is higher compared to randomized clinical trials and biomarkers with potential predictive value aren't available. Our preliminary data, as proof of concept, could suggest a predictive role of pharmacogenomic approach in the identification of cardiotoxicity risk biomarkers for anti-HER2 treatment.

1. Background

Drug-induced heart failure represents an important cause of mortality in cancer patients. Indeed, the new anti-HER2 drugs significantly improve overall survival (OS), although cardiovascular disease (CVD) is the first cause of morbidity and mortality for breast cancer (BC) long survivors [1].

In HER2 breast cancer (HER2+ BC), anti-HER2 target therapy represented a strong paradigm-change able to modify the natural history of this BC subtype.

Trastuzumab, a monoclonal antibody direct to HER2 receptor, was the first biological agent approved for HER2+ BC treatment and still remains the cornerstone of HER2+BC strategy for the dramatical improvement on survival outcome. Trastuzumab has a demonstrated role in reducing relapses and death in all HER2+ BC both in adjuvant and metastatic setting and can be used in combination with other drugs, such as pertuzumab or lapatinib and tucatinib as tyrosine kinase inhibitors (TKIs) [2–4]. Moreover, every new antibody drug-conjugated anti-HER2 (ADC) is based on trastuzumab [5–7].

However, both trastuzumab and other anti-HER2 therapies may lead to symptomatic heart failure and loss of left ventricular contractile function characterized by decreased left ventricular ejection fraction (LVEF). In registrational and non registrational clinical trials, Trastuzumab is administered at a loading dose of 8 mg/kg, followed by maintenance doses of 6 mg/kg every 3 weeks, while the recommended starting dose of Trastuzumab Emtansine is 3.6 mg/kg given as an intravenous infusion every 3 weeks (21-day cycle) and 5.4 mg/kg for Trastuzumab Deruxtecan, with the same administration schedule. However, considering the different dosage and concentration of the drug, heart failure associated with HER2-targeted ADCs is extremely low compared to trastuzumab, as recently demonstrated in a published meta-analysis [8].

Several risk factors seem to correlate with the development of anti-HER2 related cardiotoxicity, including age, comorbidity as hypertension, diabetes, smoking, obesity and previous anthracycline exposure that, still, represents the major risk factor [9].

Indeed, trastuzumab-induced cardiotoxicity (TIC) risk is about 3.2% and 0.5%, respectively, in terms of LVEF decline and symptomatic heart failure in the absence of previous anthracycline therapy, while the incidence, in patients with previous anthracycline exposure, rises in a range from 4.0% to 18.6% and 0.4–4.1%, with regards to decreased LVEF and severe heart failure [10]. Moreover, drug toxicity could cause myocardium damage with consequent Type I irreversible myocardial dysfunction, while the inhibition of physiological myocardial functioning leads to the type II reversible cardio-toxicity [11]. Particularly, mechanisms underlying TIC are different compared to anthracyclines. Indeed, anthracycline could cause myocardial necrosis/fibrosis (type I cardiotoxicity) involving lipid peroxidation and DNA damage, generation of mitochondrial reactive oxygen species (ROS), disruption of mitochondrial biogenesis, and induction of ferroptosis; conversely, TIC occurs via reversible disruption of the protective functions of HER2 in the cardiomyocyte without apparent ultra-structural changes (type II cardiotoxicity), after treatment interruption [12]. However, pathophysiology of TIC is unclear and seems to involve several signal transduction pathways, DNA-repair mechanisms, and neo-angiogenesis. Trastuzumab has effect on tyrosine kinase (TK) activity of HER2 and phosphorylation of ErbB2-Y1248, inducing antibody-dependent cellular cytotoxicity and inhibiting HER2-mediated mitogenic signaling [13]. Moreover, the status of phosphorylated ErbB2-Y1248 could correlate with trastuzumab sensitivity and the interaction of HER2 with non-receptor Csk-homologous kinase (CHK) was negatively associated to the activity of HER2 signaling, leading to inhibition of BC cell proliferation. Moreover, trastuzumab causes downregulation of BCL-XL antiapoptotic protein and promotes the upregulation of several proapoptotic mechanisms [14]. Trastuzumab effect is not dose-dependent, differently from anthracycline related irreversible cardiotoxicity [15].

Trastuzumab solubility might be enhanced through the combination with compounds able to impact on pH. This might result in increased bioavailability which could actually improve its global effectiveness. Several works investigated the role of conformational stability modification through catalytic enzyme activation which could act on cytotoxicity. Interestingly, the natural derivate could influence ADME metabolism and the drug activity. Moreover, piperin, as other natural agents, reverses multi-drug resistance in cancer cells and enhances bioavailability of chemotherapeutic agents [16].

Another approach to overcome the limitations related to poor PK and tumor penetration related to mAb size could be the trastuzumab derivatization or encapsulation strategies, as drug nanocrystal technology, to improve its cytotoxic activity, bioavailability, accumulation at tumor site and allows escape the immune system, reduced in vivo systemic degradation, longer formulation shelf life and improved patient compliance [17,18].

Next generation of ADC targeting HER2 can improve therapeutic efficacy in breast cancer also promoting a bystander effect, due to more efficient release from the linker [19].

Moreover, the binding to carrier protein could contribute to impact on bioavailability and efficacy inducing conformational changes which promote a steric barrier and avoid kinase activation or modify the interaction between HER2 and other proteins and overcoming resistance [20].

Tumour response to trastuzumab containing regimen induces also local and systemic immunomodulation through both inhibition of oncogenic signalling and stimulation of antibody-dependent cellular cytotoxicity (ADCC) with the involvement of different immune cells such as natural killer (NK) cell, macrophages, neutrophils and eosinophils which furtherly activate adaptive immune response through antigen presentation, cytokine production and chemotaxis [21,22]. The interindividual variability in therapy efficacy and toxicity is multifactorial and may be due to micro-environment and disease related factors, but also to inherited genetic makeup; while somatic genomic variants of several genes (*TP53*, *PIK3CA*, AKT1, PTEN deletion, HER2, ATM, CDH1, *APC*, *KRAS, NRAS)* may account for variability of the efficacy of target therapy [23,24]. Single nucleotide polymorphisms (SNPs) tagged polymorphic variants or copy number variations (CNVs) in genes involved in the absorption, distribution, metabolism, and elimination (ADME) of drugs may contribute to affect drug response, in terms of pharmacokinetic (PK) and pharmacodynamic (PD) [25,26]. A better understanding of the effects of inflammation on drug metabolism and PK could aid to predict the individual metabolic function. Considering these differences, pharmacogenomics (PGx) allows to tail treatments to minimize the risk of toxicity and increase chances of therapeutic success [27]. Moreover, experimental evidence highlights the role of immune cell metabolism

and cytokine- and inflammatory disease-states related to the activity and expression of CYP450 enzymes and transporters involved in the PK/PD of drugs, including immunotherapy, may contribute to interindividual variability without interferences on activity of related therapeutics [21,28]. Pro-inflammatory cytokines, released during systemic inflammation, could mediate regulation of ADME genes expression, influencing immunotherapy response and toxicity through xenobiotics and drugs metabolism or transport. Thus, ADME genes function could be regulated by co-medication, inflammation disease status and comorbidities which significantly modulate drug exposure variability and its clinical impact. In a small number of patients, chemotherapy induces short- and long-term cardiotoxicity by unknown biological mechanism, in addition to their more common gastrointestinal, hematologic, or neurological toxicities. Advancements in genomic knowledges have highlighted the role of polymorphic variants and biological pathways correlated to the risk of cardiotoxicity from different anticancer drugs. ADME genes may have a role not only in drug-drug interactions but also in susceptibility to diseases and other important adverse events like cardiotoxicity during target therapy [29,30]. The opportunity to allow the identification of new biomarkers in order to stratify patients at the greatest risk of cardiotoxicity due to HER2-targeted therapy might improve the safety of tailored drugs opening a new scenario in the understanding of the mechanisms underlying the cytokine mediated modulation of ADME gene [31].

Biomarkers used in clinical practice as indicators of cardiac damage aren't able to predict the risk of cardiotoxicity of cancer chemotherapy. An effective prevention strategy could include the use of fewer cardiotoxic chemotherapeutical regimens, cardiovascular risk factor management and cardioprotective therapy (CPT), guided by monitoring of cardiac function.

The aim of this work is to present our experience focused on understanding the potential role of genetic variants in ADME genes in the development of TIC in HER2+ BC patients, considering the involvement of inflammation and immune system and the potential impact of ADME genes

We consider a "case series" of 3 HER2+ BC patients, who underwent trastuzumab-containing regimens and experienced cardiotoxicity, indirectly compared to 37 matched "control group" cardiotoxicity-spared, to explore whether a particular combination of ADME polymorphic variants can characterize the genome of cardiotoxicity-experiencing patients for better stratification of cardiotoxicity risk.

2. Methods

In 2022, we analyzed our single Centre database containing data related to 40 patients undergone to DMET profiling ADME gene constitutive variant prospective profiling after providing informed consent based on the Institutional bioethical standards and treated with anti-HER2 target therapy (prot. N. 2012.73). All patients were followed at Teaching Hospital R. Dulbecco (Mater Domini facility) of Catanzaro, Oncology Unit. Particularly, only 3 patients, here reported as "case series", experienced TIC and were compared to 37 "control group" matched cardiotoxicity-spared patients considering some homogeneous inclusion criteria: all patients experienced an HER2+ BC and all patients were treated with the same approach in the context of natural history of disease. By DMET Plus array platform enrolled patients were genotyped for 1931 single nucleotide polymorphisms (SNPs) and 5 Copy Number Variations (CNVs) in order to explore the potential role of ADME genes in causing cardiotoxicity, taking into account the potential impact of ADME modulated inflammation effects on ventricular electrophysiology alteration [32].

All patients regularly underwent echocardiography (GE Ultrasound System, GE Healthcare, Milwaukee, Wisconsin) with quarterly evaluation of selected parameters such as ventricular ejection fraction (LVEF).

To identify the possible correlation between specific ADME genetic variants and cardiotoxicity we indirectly compared all genotyped DMET markers and clustered each probe for SNPs frequency, filtering out all the probes for which there was failure resulting from lack of a call (no call) or for "possible rare allele"/"zero copy number variants".

2.1. Pharmacogenomic Analysis

Genomic DNA was extracted from peripheral whole blood by using (GoldMag Co. Ltd., Xi'an City, China) according to the manufacturer's instructions. The samples were genotyped by the DMET Plus assay (Thermo Fisher Scientific Inc., Waltham, MA, USA) as previously described [33,34]. Genotyping calls were extracted by DMET™ Console software. Call rate less than 95% was used as exclusion criteria from further analysis [35]. By the comprehensive DMET™ plus microarray platform we have simultaneously genotyped 1936 genetic variants for 231 genes encoding drug-metabolizing enzymes and transporters in 40 HER2+ BC patients treated with trastuzumab to investigate genetic determinants of cardiotoxicity [33,34,36]. The final dataset was based on 1495 genetic markers in 231 genes, after the exclusion from further analysis of 100% of genotype concordance and high rate of "possible rare allele"/"no call"/"zero copy number variants". Using the DMET™ Console Software, genotypes were translated in gene-level diplotypes using star allele nomenclature and then into a metabolizer status describing the relative level of metabolic activity, thus ultra-rapid metabolizers (UM), extensive metabolizers (EM), intermediate metabolizers (IM), and poor metabolizers (PM). To study potential genotype differences between the 3 HER2+ BC patients (case series) who underwent trastuzumab regimens and experienced cardiotoxicity and the other 37 matched control patients, we indirectly compared all genotyped DMET markers to identify differences in the association of polymorphic variants in ADME genes which could characterize the genomic phenotype of patients developing trastuzumab cardiotoxicity. To support this association in case series patients we performed cluster analysis. For each probe on SNP microarray we created clusters of SNPs to detect group of SNPs in our population, applying Hamming distance method for comparing categorical data and quantifying differences between SNP alleles. After the phase of normalization and quality control to enhance the data's accuracy and uniformity were retained only the probes in which all genotypes were assigned by DMET Console software. So we grouped SNPs into clusters, where SNPs with smaller Hamming distances are interpreted as more genetically similar and thus grouped, revealing underlying genetic relationships in the data [37].

3. Results

3.1. Case series' presentation and literature review

We describe the characteristics and cancer history of 3 HER2+ BC patients who experienced TIC, retrospectively compared to 37 HER2 + BC cardiotoxicity-spared patients described in Table 1.

Data obtained by single Centre institutional database of HER2 + BC patients followed at our Teaching Hospital. Median age was 60 years; all patients received LVEF evaluation quarterly during trastuzumab exposure. The median time of TIC onset was > 40 months. Considering our case series: one patient showed an irreversible toxicity with a New York Heart Association Functional classification (NYHA) class 3; one patient discontinued anti-HER2-treatment for metastatic BC, due to decline in performance status (PS ECOG 3) after 10 years of anti-cancer treatments; one patient resumed treatment after complete cardiotoxicity recovery, and she is still in trastuzumab therapy.

Timeline of each "case series" patient history was reported in Fig. 1.

3.1.1. Case 1

The first "case series" concerns a 61-year-old female, with diagnosis of invasive ductal carcinoma, triple positive BC phenotype, de novo IV stage for bone, brain and lymph node metastasis, without any comorbidities. The patient underwent a docetaxel/trastuzumab first-line

Table 1
Patient's characteristics: for a total of 40 patients.

Characteristics	Number of Patients (%)
Median age (60 years)	
-Age between 18–60 years	**23**(5,57)
-Age >60 years	**17**(5,42)
Performance stastus (PS ECOG)	
-0	**32(80)**
-1	**8** (20)
-2	**0 (0)**
Hormone receptor status (HR)	
-Positive	**35**(5,87)
-Negative	**5** (5,12)
Stage at diagnosi	
-I-II	**16**(40)
-III-IV	**24 (60)**
Gradin	
-G1	**3**(5,7)
-G2	**13**(5,32)
-G3	**24 (60)**
Chemotherap	
-Adjuvant chemotherapy	**23**(5,57)
-Chemotherapy in metastatic disease	**17**(5,42)

treatment for 6 cycles followed by further 10 months of trastuzumab maintenance, and a Whole-Brain Radiation therapy for brain involvement. After 13 months from diagnosis, with 7 months of progression free survival, she experienced a lymph node disease progression treated with lapatinib and capecitabine for II line treatment, for six cycles. Further two lines of chemotherapy (vinorelbine and paclitaxel) associated with trastuzumab and maintenance with trastuzumab for 3 years in total, associated with hormonal therapy (letrozole in the maintenance setting and then fulvestrant for further lymph node progression of disease) had been administered. Hormonal therapy was discontinued due to brain progression. The patient underwent neurosurgery for brain lesion followed by T-DM1 treatment for 10 months. Last treatment with paclitaxel and trastuzumab for 12 cycles occurred for brain and lymph node progression. Only this last treatment, after 11 years by diagnosis was discontinued for impaired systolic function with ejection fraction 49% Simpson (vs 62% of the previous control). This condition has been irreversible for clinical deterioration.

3.1.2. Case 2

A 59-year-old female, with diagnosis of invasive ductal carcinoma, triple positive BC phenotype, was treated with left quadrantectomy and lymphadenectomy, then received adjuvant chemotherapy anthracycline-free, radiotherapy and hormonal therapy with tamoxifen for 5 years. After 9 years, for lung relapse, she underwent right middle pulmonary lobectomy and subsequent trastuzumab/ docetaxel treatment for 6 cycles followed by trastuzumab maintenance for 32 months. The anti-HER2 treatment was discontinued for cardiotoxicity with reduction of ejection fraction of 11% (59 vs 70% of the previous control). Thus, the patient received an aromatase inhibitor maintenance. After one year, for CT-scan node progression, considering the recovery of normal ejection fraction, two further trastuzumab-schedules were performed: vinorelbine for 6 cycles and then docetaxel. The patient continued maintenance treatment for 3 years. For lung progression the patient experienced atypical pulmonary resection followed by T-DM1. This treatment was continued for 43 cycles interrupted for thrombocytopenia. After 3 months, because of pleural effusion, the patient started trastuzumab-deruxtecan (T-dxd) with CT-scan complete response after 4 months. No further anti-HER2 discontinuation was needed. At present, the patient is still alive.

3.1.3. Case 3

A 74-year-old patient with history of ischemic heart disease and mitral valve plastic, had diagnosis of invasive lobular carcinoma right BC, triple positive BC phenotype, IIIB stage. The patient underwent radical mastectomy and lymphadenectomy and subsequent adjuvant chemotherapy for 6 cycles associated with anti-HER2 target therapy and radiotherapy on the right thoracic wall and right lateral cervical node stations. After adjuvant treatment, the patient received trastuzumab maintenance therapy for 1 year and aromatase inhibitor for 5 years. For hilar and subcarinal lymph nodes relapse she underwent treatment with exemestane 25 mg/die and trastuzumab after 7 years from diagnosis. After one year, the patient underwent several clinical and

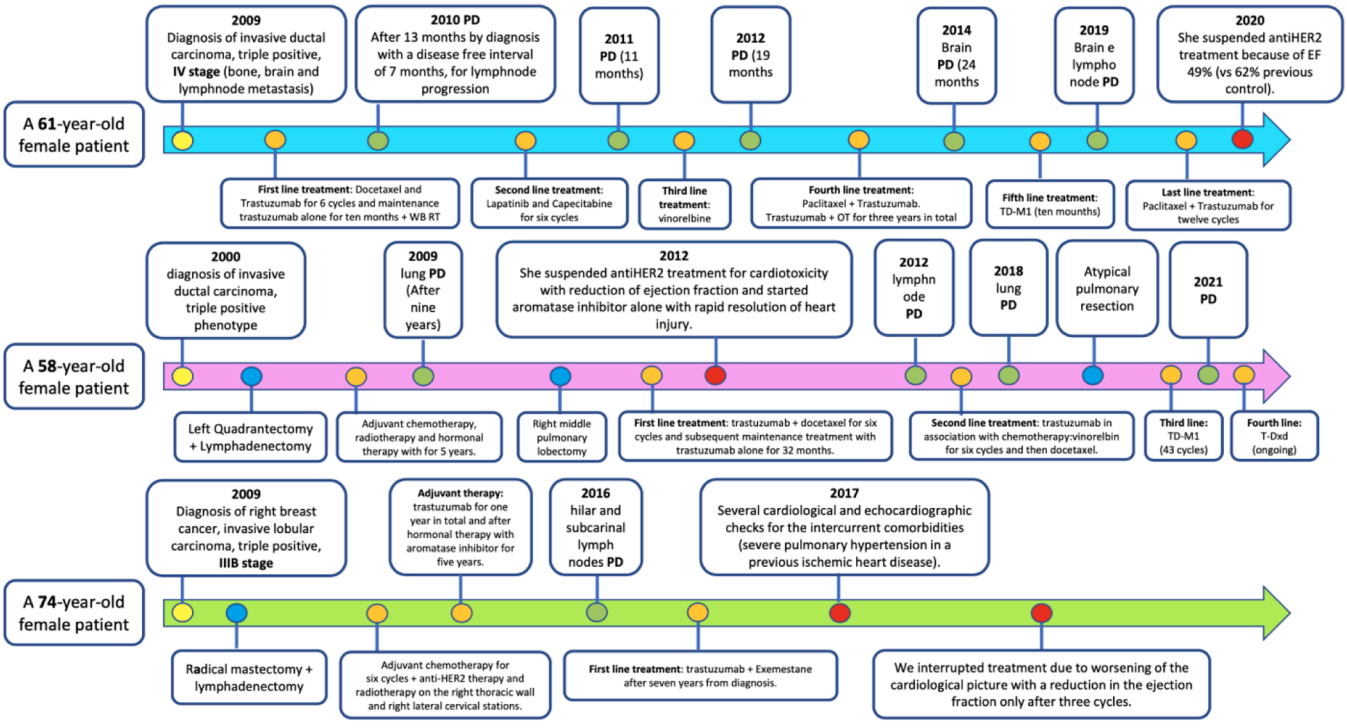

Fig. 1. Timeline patient history: each color reflects the single patient history of case series.

echocardiographic checks for intercurrent comorbidities (severe pulmonary hypertension in a previous ischemic heart disease). Treatment was discontinued due to reduction in the ejection fraction of 10% (56% vs 66% of the previous control) after 3 courses. This condition has been irreversible for clinical deterioration.

3.2. DMET analysis results

Analyzing the genomic profile of the 3 cardiotoxicity-experienced cases compared to 37 consecutive series of control cardiotoxicity-spared matched in our database, we identified a particular genetic association in 8 ADME genes with 9 SNPs only in patients developing trastuzumab-induced cardiotoxicity, as shown in Table 2.

All SNPs were in heterozygosity with the exception for the rs1751034, rs887829 and rs776746 in homozygosity. A cluster analysis confirmed this correlation with cardiotoxicity (Fig. 2).

3.2.1. SNPs linked to cardiotoxicity

Among these SNPs, the rs6759892 in *UGT1A6* and rs776746 in *CYP3A5* are already known for their correlation to cardiotoxicity induced by doxorubicin and cyclophosphamide.

The *UGT1A6* and *UGT1A7* encode uridine 5´-diphospho-glucuronosyltransferase enzymes (UGTs) are involved in Phase II reaction of various compounds and thus, in inactivation of toxic substrates. The rs6759892 in *UGT1A6* is a missense variant that leads to an aminoacidic substitution in the position 7 of protein with altered enzyme activity, reduction (30–100%) of glucuronidation activity *in vitro* and consequent impaired drug metabolism [38]. This genetic variant has been previously reported to carry a predictive role in assessing risk for anthracycline cardiotoxicity, whose slower metabolism may accumulate reactive oxygen species and toxic alcohol metabolites. The rs7586110 in *UGT1A7* is a regulatory variant located at −57 kb upstream the gene. This SNP leads to a reduced promoter activity.

The *UGT1A1* (UDP Glucuronosyltransferase family 1 member A1) enzyme is functionally linked to SN-38 inactivation and detoxification. The most frequent genetic variant that affects *UGT1A1* function is a dinucleotide TA_n repeat polymorphism. In particular, the TA_7 allele (*UGT1A1*28*) is related to reduced enzyme activity and increased blood levels of irinotecan active SN-38 metabolite and toxicity. According to the CPIC Atazanavir guideline, the rs887829 is a surrogate marker to analyze the TA_7 allele, since the rs887829 T allele is in very strong linkage disequilibrium (LD) with the TA7 allele (*UGT1A1**80) [39]. The rs887829 in *UGT1A1* is also associated with morphine toxicity. Patient who was TT homozygous developed sleep apnea and respiratory depression after IV morphine intake [40]. Moreover, genotype CC is associated with decreased response to deferasirox in beta-Thalassemia affected individuals and with decrease of warfarin dose in individuals with heart valve replacement as compared to genotypes CT + TT [41,42].

The variant *UGT1A1* g.3664 A > C (c.1352 A > C, rs3755319) is a noncoding transcript variant. In the ClinVar database, this variant is responsible for transient familial neonatal hyperbilirubinemia (OMIM: 237900) [43,44].

Table 2
Association of genetic variants identified in patients with cardiotoxicity.

SNP	Gene	Genotype
rs7586110	*UGT1A7*	G/T
rs6759892	*UGT1A6*	G/T
rs3755319	*UGT1A1*	A/C
rs887829	*UGT1A1*	C/T
rs1902023	*UGT2B15*	G/T
rs628031	*SLC22A1*	A/G
rs776746	*CYP3A5*	G/G
rs1751034	*ABCC4*	A/G
rs3892097	*CYP2D6*	A/G

The U*GT2B15* rs1902023 polymorphism (D85Y) has been associated with an increased risk of prostate cancer and with a decreased response to acetaminophen (paracetamol) [45,46]. Moreover, in urine samples from 66 healthy white and Asian volunteers was reported that the UGT2B15*2 (rs1902023) polymorphism was correlated to reduction in acetaminophen glucuronide concentrations and clearance as well as with higher circulating acetaminophen protein-adduct concentrations in both European-American and African-American individuals [46].

The risk allele A of the Met408Val (rs628031) polymorphism in the *SLC22A1* gene has been associated with decreased therapeutic efficacy of metformin in several populations worldwide [47].

The *CYP3A5* *3 (rs776746) was associated with cardiotoxicity in many studies [48–50]; it has been postulated that change in CYP3A5 activity could have functional consequences and increase the accumulation of chemotherapeutics, such as anthracyclines (doxorubicin) and cyclophosphamide, and the production of ROS in cardiomyocytes.

The *ABCC4* rs1751034 missense variant has been associated with the metabolism of antiretroviral drug tenofovir [51]. Patients with the rs1751034 CC/CT genotype carriers may have decreased concentrations of tenofovir as compared to patients with the TT genotype.

The cytochrome P450 2D6 (CYP2D6) is highly polymorphic and involved in the metabolism of up to 25% of the drugs that are commonly used. The rs3892097 in *CYP2D6* is a splice site variant (1846 G > A) with functional consequent at protein level (null variant). The *CYP2D6*4* allele (rs3892097) is responsible for a PM phenotype and is associated with low CYP2D6 enzyme activity while the UM phenotype is associated with high hydroxy-metabolite plasma concentrations and increased risk of cardiotoxicity [52].

4. Discussion

Exploring our database regarding the HER2+ BC population followed at our center, we identified a characteristic association of 9 germline polymorphic variants in 8 ADME genes, found only in 3 HER2+ BC cardiotoxicity-experiencing patients compared with 37 matched patients HER2+ BC cardiotoxicity-free, supported by performed cluster analysis, which could open new scenarios in improving prevention strategies for risk stratification of cardiotoxicity in HER2+ BC. Taking into account the small sample size and the limited role of this retrospective evaluation, we can afford some considerations on the role that ADME genes could have in the context of mAb therapy highlighting the involvement of the immune system and inflammation in the regulation of therapeutic response.

It is still unclear the mechanisms underlying TIC; *in vivo* and *in vitro* studies demonstrate the critical role of the epidermal growth factor (EGF) signaling system and of ErbB2 during heart development, suggesting that TIC is directly related to inhibitory activity on HER2 signaling [53–55]. In tumor samples and cardiomyocytes, trastuzumab binding to an extracellular segment of the HER2 protein prevents heterodimerization of surrounding HER3/4 receptors in response to neuregulin (NRG-1) signals, thereby blocking any further downstream signaling. Also, downregulation of HER signaling causes inhibition of autophagy while the compromised cardiomyocyte's ability to adapt the increased oxidative stress impairs regulation of intracellular ROS levels [14]. Pericardial and serum oxidative stress markers are indeed correlated with heart failure. Moreover, both genetic and non-genetic factors greatly affect drugs PK, PD, and it is known the role of ROS, inflammation, inflammatory mediators and immune system in the modulation of metabolism and transport of drugs also in cancer patients. Some authors investigated *in vivo* like human 3D liver spheroid model, but not in 2D liver model, the role of pro-inflammatory cytokines on the expression of key ADME genes, involved in the metabolism of several clinically used drugs [56]. The exposure of spheroids to relevant concentrations of common pro-inflammatory cytokines as IL-1β, IL-6, or TNFα developed an increment in CYP2E1, and UGT1A3 mRNA expression while a pronounced decrement in mRNA expression of CYP3A4 and UGT2B10 and a

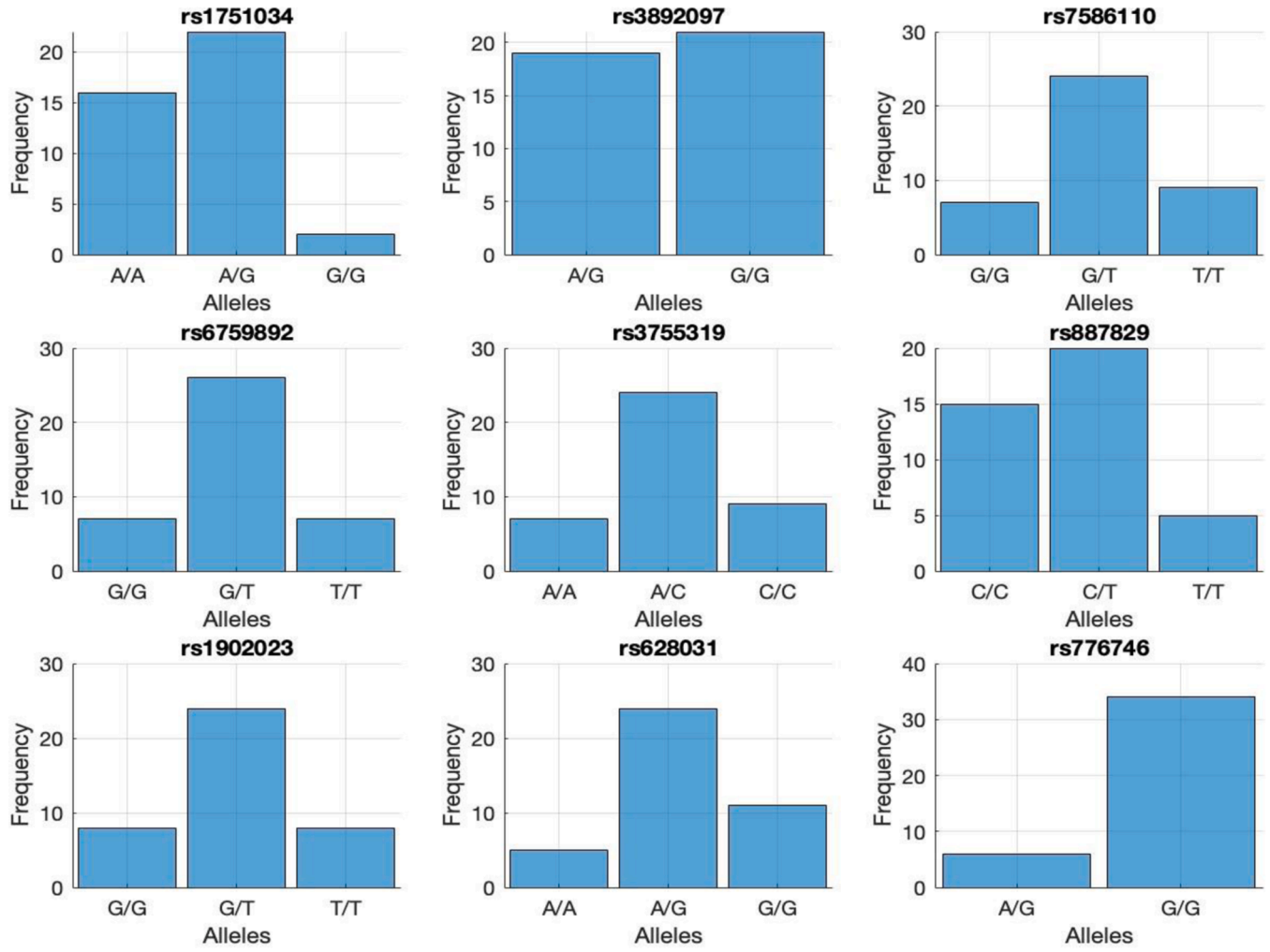

Fig. 2. The cluster analysis on each probe genotyped by DMET Plus, after filtering out all the probes for "no call" or for "possible rare allele"/"zero copy number variants", reveals the association of 9 polymorphic variants with cardiotoxicity.

less pronounced reduction of CYP1A2, CYP2C9, CYP2C19, and CYP2D6 mRNA expression [56].

Therefore, impact of cytokine modulation and inflammation can modulate the patient's response with potential enhanced efficacy or toxicity correlated to changes in drug pharmacokinetics, as well as the co-interaction of several factors, affecting drug metabolism, are potentially able to cause the genotype-phenotype mismatches altering the individual's genotype based-prediction of drug metabolism and the real capacity to metabolize drugs [42,43]. Recently, other authors reported the role of *ABCB1* in regulating immune genes in breast cancer [57]. *ABCB1* together with *ABCC1*, and *ABCG2* is correlated to chemo-resistant breast cancer and patients with ABCB1-positive cancer fail to respond to chemotherapy. In 1217 breast cancer samples, RNA from The Cancer Genome Atlas Breast Invasive Carcinoma in Genomic Data Commons Data Portal (GDC TCGA-BRCA), *ABCB1* expression was correlated with lymph node metastasis and worst prognosis, as well as its high expression of *ABCB1* was also associated with higher expression of *IL6, CSF1, CSF3*, and *PTGS2* and decreased expression of *VEGFA,* in a dataset from the GEO database. Thus, *ABCB1* involvement seems to predict a poor prognosis in BC patients through regulation of cytokine release. At our knowledge, this is the first study including a unique population genotyped for 231 ADME genes in the context of trastuzumab-induced cardiotoxicity. We highlight in 3 HER2+ BC cardiotoxicity-experiencing patients a specific association of some polymorphic variants in ADME genes unknown for their correlation to cardiotoxicity and others like *UGT1A6* rs6759892, *CYP3A5* rs776746 and rs3892097 *CYP2D6* whose correlation is already known. We hypothesized that the 9 genetic variants described and here recognized overall, may have a determining role in the development of cardiotoxicity.

5. Conclusion

These preliminary findings, although need validation in an independent dataset of HER2+ BC patients to assess the real impact of a genetic association of variants in ADME genes, could contribute to the identification of patients experiencing TIC for a preemptive prevention. The retrospective design of this work, a limited sample size, and the heterogeneity of patient treatment lines were the major limitations of this analysis. By identifying potential predictive risk biomarkers involved in this kind of toxicity, although as proof of concept, might open the way for the development of new strategies to prevent these toxicities and for the treatment selection. However, these findings need to be validated in a prospective cohort, matching information to specific cardiac biomarkers (troponines and ProBNP) and LVEF evaluation.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Regione Calabria, Sezione Area Centro (Approval Code: prot. N. 2012.73).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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CRediT authorship contribution statement

Maria Renne: Investigation. **Pierosandro Tagliaferri:** Writing – review & editing, Supervision, Resources, Data curation. **Maria Teresa Di Martino:** Validation. **Mariamena Arbitrio:** Writing – review & editing, Writing – original draft, Validation, Project administration, Formal analysis, Conceptualization. **Federica Falcone:** Investigation. **Mario Cannataro:** Software. **Francesco Luciano:** Investigation. **Pierfrancesco Tassone:** Writing – review & editing, Supervision, Resources, Data curation. **Domenico Ciliberto:** Writing – review & editing, Methodology. **Ludovica Tedesco:** Investigation. **Antonella Crispino:** Investigation. **Stefania Esposito:** Formal analysis. **Francesca Scionti:** Validation, Formal analysis, Conceptualization. **Valentina Farenza:** Investigation. **Giuseppe Agapito:** Software, Formal analysis. **Caterina Labanca:** Investigation. **Maria Cucè:** Investigation. **Nicoletta Staropoli:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Conceptualization.

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.

Data availability

Data will be made available on request.

References

[1] G. Jerusalem, P. Lancellotti, S.B. Kim, HER2+ breast cancer treatment and cardiotoxicity: monitoring and management, Breast Cancer Res Treat. 177 (2) (2019) 237–250.
[2] D. Cameron, M.J. Piccart-Gebhart, R.D. Gelber, M. Procter, A. Goldhirsch, E. de Azambuja, et al., 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial, Lancet 389 (10075) (2017) 1195–1205.
[3] A. Fabi, P. Malaguti, S. Vari, F. Cognetti, First-line therapy in HER2 positive metastatic breast cancer: is the mosaic fully completed or are we missing additional pieces? J. Exp. Clin. Cancer Res 35 (2016) 104.
[4] D.J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, et al., Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N. Engl. J. Med 344 (11) (2001) 783–792.
[5] H.S. Rugo, G. Bianchini, J. Cortes, J.W. Henning, M. Untch, Optimizing treatment management of trastuzumab deruxtecan in clinical practice of breast cancer, ESMO Open 7 (4) (2022) 100553.
[6] S.M. Swain, M. Shastry, E. Hamilton, Targeting HER2-positive breast cancer: advances and future directions, Nat. Rev. Drug Discov. 22 (2) (2023) 101–126.
[7] C.F. Waller, J. Mobius, A. Fuentes-Alburo, Intravenous and subcutaneous formulations of trastuzumab, and trastuzumab biosimilars: implications for clinical practice, Br. J. Cancer 124 (8) (2021) 1346–1352.
[8] F. Liu, H. Li, G. Yin, Y. Pan, Incidence of HER2-targeted antibody-drug conjugates-related cardiac events: a meta-analysis, J. Cancer 15 (1) (2024) 90–102.
[9] N. Dempsey, A. Rosenthal, N. Dabas, Y. Kropotova, M. Lippman, N.H. Bishopric, Trastuzumab-induced cardiotoxicity: a review of clinical risk factors, pharmacologic prevention, and cardiotoxicity of other HER2-directed therapies, Breast Cancer Res. Treat. 188 (1) (2021) 21–36.
[10] R.S. Copeland-Halperin, J.E. Liu, A.F. Yu, Cardiotoxicity of HER2-targeted therapies, Curr. Opin. Cardiol. 34 (4) (2019) 451–458.
[11] A. Koutsoukis, A. Ntalianis, E. Repasos, E. Kastritis, M.A. Dimopoulos, I. Paraskevaidis, Cardio-oncology: a focus on cardiotoxicity, Eur. Cardiol. 13 (1) (2018) 64–69.
[12] K.T. Sawicki, V. Sala, L. Prever, E. Hirsch, H. Ardehali, A. Ghigo, Preventing and treating anthracycline cardiotoxicity: new insights, Annu Rev. Pharm. Toxicol. 61 (2021) 309–332.
[13] V.S. Hahn, D.J. Lenihan, B. Ky, Cancer therapy-induced cardiotoxicity: basic mechanisms and potential cardioprotective therapies, J. Am. Heart Assoc. 3 (2) (2014) e000665.
[14] N. Mohan, J. Jiang, M. Dokmanovic, W.J. Wu, Trastuzumab-mediated cardiotoxicity: current understanding, challenges, and frontiers, Antib. Ther. 1 (1) (2018) 13–17.
[15] M.C. Todaro, L. Oreto, R. Qamar, T.E. Paterick, S. Carerj, B.K. Khandheria, Cardioncology: state of the heart, Int J. Cardiol. 168 (2) (2013) 680–687.
[16] S. Rezaei, H.S. Meftah, Y. Ebtehajpour, H.R. Rahimi, J. Chamani, Investigation on the Effect of Fluorescence Quenching of Calf Thymus DNA by Piperine: caspase Activation in the Human Breast Cancer Cell Line Studies, DNA Cell Biol. 43 (1) (2024) 26–38.
[17] L. Lu, Q. Xu, J. Wang, S. Wu, Z. Luo, W. Lu, Drug nanocrystals for active tumor-targeted drug delivery, Pharmaceutics 14 (4) (2022).
[18] T.M. Caputo, G. Barisciano, C. Mule, A.M. Cusano, A. Aliberti, L. Muccillo, et al., Development of High-Loading Trastuzumab PLGA Nanoparticles: a Powerful Tool Against HER2 Positive Breast Cancer Cells, Int J. Nanomed. 18 (2023) 6999–7020.
[19] S. Duro-Sanchez, M. Nadal-Serrano, M. Lalinde-Gutierrez, E.J. Arenas, C. Bernado Morales, B. Morancho, et al., Therapy-Induced Senescence Enhances the Efficacy of HER2-Targeted Antibody-Drug Conjugates in Breast Cancer, Cancer Res 82 (24) (2022) 4670–4679.
[20] X. Wu, H. Yang, X. Yu, J.J. Qin, Drug-resistant HER2-positive breast cancer: molecular mechanisms and overcoming strategies, Front Pharm. 13 (2022) 1012552.
[21] F. Stanke-Labesque, E. Gautier-Veyret, S. Chhun, R. Guilhaumou, French Society of P, Therapeutics. Inflammation is a major regulator of drug metabolizing enzymes and transporters: consequences for the personalization of drug treatment, Pharm. Ther. 215 (2020) 107627.
[22] R. Abes, J.L. Teillaud, Modulation of tumor immunity by therapeutic monoclonal antibodies, Cancer Metastas. Rev. 30 (1) (2011) 111–124.
[23] S. Nik-Zainal, H. Davies, J. Staaf, M. Ramakrishna, D. Glodzik, X. Zou, et al., Landscape of somatic mutations in 560 breast cancer whole-genome sequences, Nature 534 (7605) (2016) 47–54.
[24] N. Cancer Genome Atlas, Comprehensive molecular portraits of human breast tumours, Nature 490 (7418) (2012) 61–70.
[25] F. Scionti, M.T. Di Martino, S. Sestito, A. Nicoletti, F. Falvo, K. Roppa, et al., Genetic variants associated with Fabry disease progression despite enzyme replacement therapy, Oncotarget 8 (64) (2017) 107558–107564.
[26] M. Arbitrio, F. Scionti, M.T. Di Martino, L. Pensabene, P. Tassone, P. Tagliaferri, Pharmacogenetics/Pharmacogenomics of Drug-Metabolizing Enzymes and Transporters, ISBN 9780128208762, in: Comprehensive Pharmacology, 1, Elsevier, 2022, pp. 657–697. ISBN 9780128208762.
[27] M. Arbitrio, F. Scionti, M.T. Di Martino, D. Caracciolo, L. Pensabene, P. Tassone, et al., Pharmacogenomics Biomarker Discovery and Validation for Translation in Clinical Practice, Clin. Transl. Sci. 14 (1) (2021) 113–119.
[28] R.D. Harvey, E.T. Morgan, Cancer, inflammation, and therapy: effects on cytochrome p450-mediated drug metabolism and implications for novel immunotherapeutic agents, Clin. Pharm. Ther. 96 (4) (2014) 449–457.
[29] F. Scionti, G. Agapito, D. Caracciolo, C. Riillo, K. Grillone, M. Cannataro, et al., Risk Alleles for Multiple Myeloma Susceptibility in ADME Genes, Cells 11 (2) (2022).
[30] N. Staropoli, M. Arbitrio, A. Salvino, F. Scionti, D. Ciliberto, R. Ingargiola, et al., A Prognostic and Carboplatin Response Predictive Model in Ovarian Cancer: a Mono-Institutional Retrospective Study Based on Clinics and Pharmacogenomics, Biomedicines 10 (5) (2022).
[31] A. Grela-Wojewoda, M. Puskulluoglu, B. Sas-Korczynska, T. Zemelka, R. Pacholczak-Madej, W.M. Wysocki, et al., Biomarkers of Trastuzumab-Induced Cardiac Toxicity in HER2- Positive Breast Cancer Patient Population, Cancers (Basel) 14 (14) (2022).
[32] C. Campana, R. Dariolli, M. Boutjdir, E.A. Sobie, Inflammation as a Risk Factor in Cardiotoxicity: an Important Consideration for Screening During Drug Development, Front Pharm. 12 (2021) 598549.
[33] M. Arbitrio, M.T. Di Martino, F. Scionti, G. Agapito, P.H. Guzzi, M. Cannataro, et al., DMET (Drug Metabolism Enzymes and Transporters): a pharmacogenomic platform for precision medicine, Oncotarget 7 (33) (2016) 54028–54050.
[34] G. Agapito, M. Settino, F. Scionti, E. Altomare, P.H. Guzzi, P. Tassone, et al., DMET (TM) Genotyping: tools for Biomarkers Discovery in the Era of Precision Medicine. High. Throughput 9 (2) (2020).
[35] P.H. Guzzi, G. Agapito, M.T. Di Martino, M. Arbitrio, P. Tassone, P. Tagliaferri, et al., DMET-analyzer: automatic analysis of Affymetrix DMET data, BMC Bioinforma. 13 (2012) 258.
[36] F. Scionti, M.T. Di Martino, D. Caracciolo, L. Pensabene, P. Tagliaferri, M. Arbitrio, Tools in Pharmacogenomics Biomarker Identification for Cancer Patients, Methods Mol. Biol. 2401 (2022) 1–12.
[37] C. Wang, W.H. Kao, C.K. Hsiao, Using hamming distance as information for SNP-Sets Clustering and Testing in Disease Association Studies, PLoS One 10 (8) (2015) e0135918.
[38] F. Aminkeng, C.J.D. Ross, S.R. Rassekh, M.J. Rieder, A.P. Bhavsar, S. Sanatani, et al., Pharmacogenomic screening for anthracycline-induced cardiotoxicity in childhood cancer, Br. J. Clin. Pharm. 83 (5) (2017) 1143–1145.
[39] R.S. Gammal, M.H. Court, C.E. Haidar, O.F. Iwuchukwu, A.H. Gaur, M. Alvarellos, et al., Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for UGT1A1 and Atazanavir Prescribing, Clin. Pharm. Ther. 99 (4) (2016) 363–369.

[40] M.S. Toce, H. Kim, S. Chung, B.S. Krauss, Prolonged central apnoea after intravenous morphine administration in a 12-year-old male with a UGT1A1 loss-of-function polymorphism, Br. J. Clin. Pharm. 85 (1) (2019) 258–262.
[41] S.H. An, B.C. Chang, K.E. Lee, H.S. Gwak, Influence of UDP-Glucuronosyltransferase Polymorphisms on Stable Warfarin Doses in Patients with Mechanical Cardiac Valves, Cardiovasc Ther. 33 (6) (2015) 324–328.
[42] S. Allegra, J. Cusato, S. De Francia, F. Longo, E. Pirro, D. Massano, et al., The effect of vitamin D pathway genes and deferasirox pharmacogenetics on liver iron in thalassaemia major patients, Pharm. J. 19 (5) (2019) 417–427.
[43] L. Gailite, A. Valenzuela-Palomo, L. Sanoguera-Miralles, D. Rots, M. Kreile, E. A. Velasco, UGT1A1 Variants c.864+5G>T and c.996+2_996+5del of a Crigler-Najjar Patient Induce Aberrant Splicing in Minigene Assays, Front Genet 11 (2020) 169.
[44] L. Gailite, D. Rots, I. Pukite, G. Cernevska, M. Kreile, Case report: multiple UGT1A1 gene variants in a patient with Crigler-Najjar syndrome, BMC Pedia 18 (1) (2018) 317.
[45] T. Hajdinjak, B. Zagradisnik, Prostate cancer and polymorphism D85Y in gene for dihydrotestosterone degrading enzyme UGT2B15: frequency of DD homozygotes increases with Gleason Score, Prostate 59 (4) (2004) 436–439.
[46] M.H. Court, Z. Zhu, G. Masse, S.X. Duan, L.P. James, J.S. Harmatz, et al., Race, Gender, and Genetic Polymorphism Contribute to Variability in Acetaminophen Pharmacokinetics, Metabolism, and Protein-Adduct Concentrations in Healthy African-American and European-American Volunteers, J. Pharm. Exp. Ther. 362 (3) (2017) 431–440.
[47] E.P. Mofo Mato, M. Guewo-Fokeng, M.F. Essop, P.M.O. Owira, Genetic polymorphisms of organic cation transporter 1 (OCT1) and responses to metformin therapy in individuals with type 2 diabetes: a systematic review, Med. (Baltim.) 97 (27) (2018) e11349.
[48] M. Linschoten, A.J. Teske, M.J. Cramer, E. van der Wall, F.W. Asselbergs, Chemotherapy-Related Cardiac Dysfunction: a Systematic Review of Genetic Variants Modulating Individual Risk, Circ. Genom. Precis Med 11 (1) (2018) e001753.
[49] X. Yang, G. Li, M. Guan, A. Bapat, Q. Dai, C. Zhong, et al., Potential Gene Association Studies of Chemotherapy-Induced Cardiotoxicity: a Systematic Review and Meta-Analysis, Front Cardiovasc Med 8 (2021) 651269.
[50] Z. Huang, J. Wang, J. Qian, Y. Li, Z. Xu, M. Chen, et al., Effects of cytochrome P450 family 3 subfamily A member 5 gene polymorphisms on daunorubicin metabolism and adverse reactions in patients with acute leukemia, Mol. Med Rep. 15 (6) (2017) 3493–3498.
[51] S. Cheli, S. Baldelli, A. De Silvestri, M. Fusi, D. Minisci, C. Gervasoni, et al., ABCC4 single-nucleotide polymorphisms as markers of tenofovir disoproxil fumarate-induced kidney impairment, Pharm. J. 21 (5) (2021) 586–593.
[52] B. Carvalho Henriques, E.H. Yang, D. Lapetina, M.S. Carr, V. Yavorskyy, J. Hague, et al., How Can Drug Metabolism and Transporter Genetics Inform Psychotropic Prescribing? Front Genet 11 (2020) 491895.
[53] K.F. Lee, H. Simon, H. Chen, B. Bates, M.C. Hung, C. Hauser, Requirement for neuregulin receptor erbB2 in neural and cardiac development, Nature 378 (6555) (1995) 394–398.
[54] G.M. Cote, D.B. Sawyer, B.A. Chabner, ERBB2 inhibition and heart failure, N. Engl. J. Med 367 (22) (2012) 2150–2153.
[55] R. Florido, K.L. Smith, K.K. Cuomo, S.D. Russell, Cardiotoxicity From Human Epidermal Growth Factor Receptor-2 (HER2) Targeted Therapies, J. Am. Heart Assoc. 6 (9) (2017).
[56] K. Kloditz, E. Tewolde, A. Nordling, M. Ingelman-Sundberg, Mechanistic, Functional, and Clinical Aspects of Pro-inflammatory Cytokine Mediated Regulation of ADME Gene Expression in 3D Human Liver Spheroids, Clin. Pharm. Ther. 114 (3) (2023) 673–685.
[57] H.K. Chen, Y.L. Chen, C.Y. Wang, W.P. Chung, J.H. Fang, M.D. Lai, et al., ABCB1 Regulates Immune Genes in Breast Cancer, Breast Cancer 15 (2023) 801–811.