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Pharmacogenomics with red cells: a model to study protein variants of drug transporter genes

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Abstract

The PharmacoScan pharmacogenomics platform screens for variation in genes that affect drug absorption, distribution, metabolism, elimination, immune adverse reactions and targets. Among the 1,191 genes tested on the platform, 12 genes are expressed in the red cell membrane: *ABCC1, ABCC4, ABCC5, ABCG2, CFTR, SLC16A1, SLC19A1, SLC29A1, ATP7A, CYP4F3, EPHX1* and *FLOT1*. These genes represent 5 ATP-binding cassette proteins, 3 solute carrier proteins, 1 ATP transport protein and 3 genes associated with drug metabolism and adverse drug reactions. Only *ABCG2* and *SLC29A1* encode blood group systems, JR and AUG, respectively. We propose red cells as an *ex vivo* model system to study the effect of heritable variants in genes encoding the transport proteins on the pharmacokinetics of drugs. Altered pharmacodynamics in red cells could also cause adverse reactions, such as haemolysis, hitherto unexplained by other mechanisms.

Keywords

pharmacogenomics; pharmacogenetics; drug transporters; erythrocytes; DMET; PharmacoScan

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Author contribution

WAF asked the relevance of red cells for pharmacogenomics studies; KS and TMS screened the literature and compiled the tables; all authors researched and discussed the data. WAF, KS and TMS edited the drafts and wrote the manuscript.

Conflict of interest

The authors declared having no competing financial interest relevant to this article.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Background

Many proteins of the red cell membrane have been recognized as blood groups. The currently established 36 blood group systems are encoded by 41 genes [1]. They are involved in various cellular functions: transport of substrates (*ABCG2, ABCG6, SLC29A1, AQP1, AQP3, SLC14A1, SLC4A1 and XK*); cellular adhesion (*ACKR1, BCAM, BSG, CD151, CD44, ERMAP, ICAM4, MIC2 and SEMA7A*); enzymatic activity (*ABO, ACHE, ART4, GBGT1, GCNT2, KEL, FUT1 and FUT3*); red cell stability (*GYPC, RHAG, RHCE, RHD, SLC4A1 and SMIM1*); viral and bacterial attachment (*A4GALT, B3GALNT1, FUT3, GYPA, GYPB and GYPE*); complement interaction (*C4A, C4B, CD55, CD59 and CR1*); and unknown function (*XG*) [1–4]. Several of these membrane proteins serve as transporters that contribute to the absorption, tissue distribution and elimination of various drugs [1, 2]. Moreover, drug transporters often influence homeostatic expression of a variety of genes that regulate drug metabolism and disposition [5, 6]. The potential for these membrane proteins to influence pharmacology has been poorly studied.

Transporters are classified into 2 superfamilies: ATP-binding cassette (ABC) proteins and solute carrier (SLC) proteins. ABC transporters are involved in the translocation of a wide variety of substrates including amino acids, sugars, vitamins, inorganic ions, peptides, hormones, large polypeptides (>100 kD) and therapeutics [7, 8]. In eukaryotes, ABC proteins contribute only to the ATP-dependent efflux of substrates from cells against a concentration gradient [9, 10]. SLC proteins mediate the cellular uptake of drugs through facilitated diffusion or secondary active transport [11].

Similar to ABC and SLC transporters, the ion pumps (ATPases) [12] and ion channels [13] transport ions, such as Na^+, K^+, H^+, Cl^- and Ca^{2+}, across the cell membrane, utilizing energy from ATP hydrolysis or electrochemical gradients, respectively. Aquaporins are a special class of bidirectional channel proteins that are involved in the transfer of water across the membrane driven by the osmotic gradient [14].

Inter-individual variation in the human genome due to single-nucleotide variations (SNVs), small-scale insertions and deletions (InDels) and copy number variations (CNVs) may result in altered pharmacokinetic and pharmacodynamic characteristics of drugs leading to a lack of therapeutic efficacy or a risk for drug-induced toxicity [15, 16]. Variations in genes encoding drug transporters have been documented to affect responsiveness to chemotherapeutic agents [15, 17]. Rarely, sensitivity of red cells to the direct toxicity of the drugs can lead to drug-induced haemolytic anaemia [18–21]. Some medications bind to the RBC cell surface or alter RBC surface antigens resulting in immune attack [22]. Drug-induced immune complexes can bind to RBCs [22], and alloantibody therapies that react with RBC antigens also cause haemolysis [23]. Lastly, oxidative injury to RBCs results from peroxide formation and subsequent haemolysis, particularly in populations who harbour deleterious variants in G6PD or haemoglobin H [24]. Drug metabolism can alter drug-induced haemolytic anaemia [25, 26]. And drugs bound to red cell proteins, including blood group proteins, can bind drug-dependent antibodies [22, 27]. Such antibodies can cause drug-induced immune haemolytic anaemia [28]. Therefore, the potential for inter-individual

variation in drug binding and transport resulting from novel genetic variations should be explored and eventually considered to guide indications and dose recommendations.

The DMET Plus array, launched in 2012, scans 1936 variations (1931 SNVs and 5 CNVs) in 231 absorption, distribution, metabolism and elimination (ADME)-related genes [29, 30]. The PharmacoScan Solution array, an updated version of DMET Plus launched in 2016, scans 4627 variations in 1191 genes of known or suspected pharmacogenomic consequences. PharmacoScan incorporated all 231 genes from DMET Plus and nearly all of its variations but scans many additional variations and genes not present on DMET Plus.

The NIH Clinical Center has implemented a clinical decision support (CDS) for patients who are on medications where SNVs may assist with optimal dose or prediction of adverse events [31, 32]. In this pharmacogenomics approach, we have been screening HLA antigens by nucleotide sequencing to avoid exposure of patient with distinct HLA alleles to drugs associated with severe allergic reactions (e.g. allopurinol and carbamazepine) [31]. Nucleotide variations affecting proteins with transporter and metabolic functions have been determined by the DMET Plus microarray platform to adjust drug dose in patients with variants of high, intermediate or low activity [32].

We review the involvement of blood group proteins and other red cell membrane proteins and their potential applications to provide mechanistic insights in pharmacogenomics. As red cells are easily accessible, we propose an approach of using human red cells with variants of drug transport proteins, naturally occurring among blood donors and other healthy individuals. They can serve as an *ex vivo* model systems to study the kinetics of drug transport, as it may be affected by the protein variants.

Data search criterion and Methods

The exact number of genes expressed in the red cell membrane with drug transport function is unknown. We examined the blood group genes with drug transport function in red cells and represented on a commercial genotyping platform: the PharmacoScan array (Thermo-Fisher Scientific). The Clinical Pharmacogenetics Implementation Consortium (CPIC) is an international consortium that provides genotype-based drug guidelines to optimize drug therapy [33]. The CPIC drug–gene pairs table includes a total of 363 drug–gene interactions (DGIs), representing 214 unique drugs and 127 unique genes [33]. Among the 1,191 genes present on the 2 arrays, only 113 gene–drug pairs are covered by the CPIC guidelines. One CPIC gene *FCGR3A* is present on red cell membrane (with low confidence [34]) but not present on any of the arrays. The remaining 13 genes in CPIC (*ABL2, ASL, HPRT1, NAGS, SERPINC1, CYP2A7P1, CYB5R1, CYB5R2, CYB5R4, MT-RNR1, PROS1, TMEM43 and YEATS4*) are not present on red cell membrane and thus irrelevant for the current approach [34]. By searching the published literature and public databases [34], we retrieved the genes that are present on the PharmacoScan array and also expressed on red cell membranes.

Red cell membrane genes among the PharmacoScan and CPIC drug–gene pairs

We found 12 red cell membrane genes that met our search criterion (Table 1). Apart from the ABC (*ABCC1, ABCC4, ABCC5, ABCG2 and CFTR*), SLC (*SLC16A1, SLC19A1 and SLC29A1*) and ATP transporters (*ATP7A*), 3 additional genes associated with drug metabolism (*CYP4F3* and *EPHX1*) and adverse drug reactions (ADRs; rs3909184 in *FLOT1*) were identified. Hegedus et al. [34] associated each gene with a confidence level to evaluate the potential validity of its protein's presence in the red cell membrane: high level if the protein was present in at least two mass spectrometry studies or was an established blood group or CD marker; medium level if the protein was present in at least 1 mass spectrometry study; and low level if the protein was identified only semi-automatically from reviews [34]. We summarized the clinical interpretation of drug-gene pairs, based on the PharmGKB Clinical Annotations tables.

Only 2 of the 12 genes define blood group systems

Variations in the proteins of the red cell membrane are the hallmark and requirement for defining blood group systems. However, only 2 of the 12 genes from the present search are defined as blood group systems. The *ABCG2* gene encodes the JR (ISBT 032) [35, 36], and the *SLC29A1* gene encodes the AUG blood group system (ISBT 036; Table 2) [37].

JR blood group system

The high prevalence Jr^a antigen was first reported in 1970. JR was defined as a blood group system in 2012 [38]. The dbSNP database lists 341 non-synonymous or frame shift variants in the *ABCG2* gene. Until today, however, all individuals who developed anti-Jr^a lack the whole JR protein from their red cell membranes. The antibody can cause haemolytic transfusion reactions and severe haemolytic disease of the foetus and newborn (HDFN) [35, 36, 39].

AUG blood group system

The high prevalence At^a antigen was first identified in 1967. AUG was defined as a blood group system in 2015 [37]. The dbSNP database lists 351 non-synonymous or frame shift variants in the *SLC29A1* gene. Only 3 variants encoding 4 antigens in the AUG system are known. Individuals carrying these variants developed alloantibodies, which can cause haemolytic transfusion reactions and mild HDFN [40, 41].

Other blood group systems

In addition to *ABCG2* and *SLC29A1*, the 4 blood group system genes *ABO* (ABO; ISBT 001), *BCAM* (LU; ISBT 005), *ACKR1* (FY; ISBT 008) and *CR1* (KN; ISBT 022) are also represented on the PharmacoScan array. Some resources consider them having impact in pharmacogenomics [42]. We do not review these 4 blood groups because CPIC did not identify a drug-gene pair for them.

Protein structural feature of the 12 genes

As expected for membrane transporters, 9 proteins are multi-pass transmembrane proteins (Table 3). Another 2 proteins, EPHX1 and LTB4H, are single-pass transmembrane proteins. Only 1 protein, FLOT1, is inserted in the inner leaflet of the plasma membrane of the red cell but does not traverse it. None of the 12 proteins identified were GPI-anchored [43–45]. The 12 proteins are involved in the transport of a wide variety of drugs in humans (Table 4).

Disease association of the 12 genes

Gene variants (alleles) of any of the 12 genes have been associated with various diseases. Variations can occur at the genetic level, involve changes of the mRNA and protein expression, and affect the localization of the proteins in cellular compartments. The number of such variants is growing, and their tabulation is basic for pharmacogenomics (Table S1).

ABCC1

ABCC1 is the first identified member of the ABCC subgroup and is ubiquitously expressed in almost all human tissues [46]. Increased MRP1 protein or mRNA concentrations or both were found in many haematologic and solid malignancies as predictor of poor chemotherapy response [47]. A number of variations in *ABCC1* were associated with therapeutic response, cancer prognosis, drug toxicity and disease susceptibility [48, 49].

ABCC4

Increased MRP4 membrane localization and retention were associated with drug resistance in acute myeloid leukaemia [50]. Expression changes caused by an intronic CNV in *ABCC4* correlated with an increased risk for oesophageal squamous cell carcinoma in the Chinese Han population [51]. A large number of SNVs in *ABCC4* altered the affinity for the protein's substrate drugs [49, 52, 53].

ABCC5

ABCC5 variants were associated with tumour response to gemcitabine-based chemoradiotherapy and survival in patients with pancreatic cancer [54]. Increased *ABCC5* mRNA concentrations were reported in lung, colon, pancreatic and breast cancer [49].

ABCG2

Increased ABCG2 protein concentrations were associated with poor outcome in large B-cell lymphoma [55] and acute myeloid leukaemia [56]. Increased ABCG2 protein expression correlated with reduced survival of patients with small cell and non-small cell lung cancers [57]. A genome-wide association study (GWAS)-associated *ABCG2* alleles with hyperuricaemia and gout [58–60]. *ABCG2* variations were associated with various malignancies including colorectal cancer, lymphoma and leukaemia [61]. The *ABCG2* variant (rs2231142, Gln141Ly) causes reduction of transport activity [62] and increased drug concentrations leading to drug-induced toxicity [63]. Alloimmunizations occurred, complicated transfusions and caused HDFN disease (see JR blood group).

SLC16A1

MCT1 protein was overexpressed in cancer cells and involved in pH regulation [64]. The *SLC16A1* variant (rs1049434, Asp490Glu) correlated with survival rates in patients with non-small cell lung [65] and colorectal cancers [66]. *SLC16A1* promoter mutations were implicated in hereditary exercise-induced hyperinsulinism and hypoglycaemia [67] and ketoacidosis [68].

SLC19A1

SLC19A1 variants affected methotrexate toxicity and outcome in leukaemia [69]. A recent meta-analysis suggested a role of *SLC19A1* rs1051266 variant in haematopoietic malignancies [70].

SLC29A1

Decreased ENT1 protein expression correlated with recurrence and poor outcome in patients with hepatocellular carcinoma after surgery [71]. Expression of *SLC29A1* mRNA and ENT1 protein in tumour tissues was a predictive marker of outcome in cancer patients receiving gemcitabine [72]. *SLC29A1* promoter region variants altered gene expression and gemcitabine chemosensitivity [73]. The *SLC29A1* variant (rs45573936, Ile216Thr) may increase the risk for seizures during alcohol withdrawal [74]. Alloimmunizations occurred, complicated transfusions and caused HDFN disease (see AUG blood group).

CYP4F3

CYP4F3 variants were associated with the risk of ulcerative colitis [75] and lung cancer [76].

CFTR

Absence, reduced concentration, or malfunction of the CFTR protein resulted in cystic fibrosis [77, 78] and cystic fibrosis-associated diseases, including bronchiectasis [79], chronic pancreatitis [80] and congenital bilateral absence of the vas deferens [81].

FLOT1

The *FLOT1* gene is located 620 kb upstream of the HLA-B gene on the short arm of chromosome 6. A *FLOT1* variant (rs3909184) was identified as a tagging SNV for the *HLA-B* 15:02* allele, associated with carbamazepine-induced Stevens–Jonson syndrome and toxic epidermal necrolysis in the Asian population [31, 82, 83]. A recent study identified *FLOT1* variants affecting *FLOT1* mRNA expression as susceptibility risk factor for major depressive disorder [84]. Upregulation of *FLOT1* mRNA or FLOT1 protein expression may promote oesophageal squamous cell [85], colorectal [86], breast [87] and hepatocellular cancer [88].

ATP7A

ATP7A variants caused various copper transport disorders, such as Menkes disease [89], occipital horn syndrome [90] and the ATP7A-related distal motor neuropathy [91].

EPHX1

The low-activity genotype of the *EPHX1* exon 3 variant (rs1051740, Tyr113His) was associated with a decreased risk for lung cancer in Caucasians [92]. Functional variants were also associated with susceptibility to various cancers, such as lung [93], upper aerodigestive tract [94–96], colorectal [97], bladder [98] and breast cancer [99].

Advantages of red cells in pharmacologic studies

Previous studies, using site-directed mutagenesis, have been applied in cell cultures, such as human embryonic kidney-293 [100] and Madin–Darby canine kidney cells [101] or oocytes from *Xenopus laevis* [102]. However, these methods and cellular assays can be artificial, expensive, laborious and time-consuming. Proteomic analysis of the red cells, the most abundant cells in human body [103], has identified multiple transporter proteins in their membrane. Several of these proteins are known to be involved in the influx or efflux of clinically important drugs [34].

The membrane structure of the red cell is arguably the best studied of all human cell types [104], which enables us to draw worthwhile conclusions [105]. Red cells can be haemolysed and later resealed to regain limited permeability [106]. This technical feature is rather unique for red cells. No wonder that several studies utilized resealed human erythrocyte membranes, dubbed ghosts, as model system for drug transport studies [107, 108]. Use of ghosts circumvented the interference from proteins and enzymes present in the erythrocyte cytoplasm [109].

Study topics for pharmacogenomics with red cells

Clinical syndromes: haemolysis

The *SLC28A3*, a drug transporter gene not expressed on the red cell membrane, is tested on both the DMET and PharmacoScan arrays. A *SLC28A3* variant (rs10838138) was associated with a lower incidence of severe haemolytic anaemia in patients with chronic hepatitis C receiving pegylated interferon and ribavirin [110]. Haemolytic events may however remain undetected until the haemolysis becomes rather severe. Haemolysis by drugs can be caused by 2 mechanisms: (1) non-immune mediated, and (2) immune mediated.

Haemolysis, non-immune mediated

Non-immune-mediated drug-induced haemolytic anaemia is due to direct toxicity through irreversible damage of red cells [18–21, 25, 26, 28]. Various other factors such as red cell enzymopathy, infections, uraemia, diabetic ketoacidosis, deficient of vitamin E and low levels of glucose can increase the haemolytic effect of a drug [28]. Drugs, such as phenylhydrazine [111] cause haemolysis in all subjects in relatively low concentrations; while primaquine, acetanilid, nitrofurantoin, p-aminosalicylic acid, naphthalene, phenylsemicarbazide, sulphonamides and sulphones cause haemolysis in normal subjects only in high concentrations [28, 112, 113]. Genetic variants in drug

transport or drug metabolism genes may determine the intracellular concentration of the drug and its impact on haemolysis.

Haemolysis, immune mediated

Although underdiagnosed, an incidence of approximately 1 per million per year [114, 115] has been proposed for drug-induced immune haemolytic anaemia, a rare but severe hypersensitivity reaction to drug administration [116, 117]. It is caused by warm autoantibodies against red cells induced by many antibiotic, anti-inflammatory and chemotherapy drugs [118, 119]. A large and growing list of drugs have been associated with drug-induced immune haemolytic anaemia, and the most common are piperacillin, cefotetan and ceftriaxone [118]. Platinum-based chemotherapeutic agents such as oxaliplatin, cisplatin and carboplatin are also known to induce drug-induced immune haemolytic anaemia in rare cases [25, 118, 120, 121]. While drug-induced immune haemolytic anaemia is often diagnosed by excluding alternative causes rather than by direct evidence, genetic variants of red cell membrane proteins, other than blood group proteins, are not routinely considered.

We wonder how many clinical haemolytic events are not properly attributed to be caused by variants of membrane proteins? Each protein variant is rare, but a large fraction of patients may carry one of the host of such variants.

Reservoir or sink for a drug

Red cells may function as a reservoir or sink. Their effectiveness can vary if protein variants are involved. Drug transporter proteins can bind drugs to the red cell surface or transport the drug into the red cell cytoplasm. Either way, the drug's plasma concentration may be reduced, delaying or preventing efficient delivery of therapeutics to target tissues. The role of red cell membrane proteins has been studied extensively in drug transport or drug binding [122]. The effect of these proteins' variants has not been systematically evaluated so far.

Drug delivery

Resealed red cells have been manufactured for in vivo drug delivery [123]. They have a long life span, excellent biocompatibility, complete biodegradability and low immunogenicity [124]. Protein variants may be a lesser concern when allogeneic red cells are manufactured. In an autologous setting, the variant of a red cell membrane protein in the patient would matter.

Drugs can be targeted to red cells in two ways, such as encapsulation and conjugation. The drugs are encapsulated inside the ghosts, which reduces the possibility of an immune reaction and protects the drug from inactivation [125]. Molecular variants of transport proteins may alter the entrapment and eventual release of the drug. By chemical or genetic means, drugs can be physically conjugated to lectins and other ligands that bind to distinct red cell membrane proteins [126]. For example, single-chain variable region fragment (scFv) of TER-119, a monoclonal antibody to the mouse analogue of human glycophorin A (GPA), was genetically attached to complement-regulating proteins including decay-accelerating factor (DAF) which protected the mouse red cells against lysis by complement [127]. Of

course, molecular variants of red cell surface proteins can alter the binding affinity of the drug-ligand conjugates and affect the bioavailability of the drug.

Limitations

Red cells recapitulate the *in vivo* condition where the expression of a transporter protein and presence of multiple transporters for same drug are accounted for. Studying the kinetics of drug transport using red cells harbouring naturally occurring variants of drug transport proteins may allow direct insight in pharmacokinetics for red cells. Such results may be carefully extrapolated to other cell types that express any of the 12 genes in their cell membranes. However, using ghosts as model systems has its limitations: the protein isoforms and the amount of protein expressed may differ between red cells and other tissues; also, the membrane lipid composition, cytoskeleton proteins and interacting proteins differ among cell types.

Transplant and iatrogenic chimeras

Peripheral blood, routinely used for pharmacogenetic analysis, would reflect the genotype of the donor after a hematopoietic stem cell transplantation. Chronic transfused patients and patients with solid organ transplants are known to accept donor granulocytes and lymphocytes even with leucoreduced donor blood [128, 129]. Being an emerging field, there is a dearth of information on the relevance of donor or recipient genotype to pharmacologic outcome, and both the donor and recipient genetic backgrounds and their discrepancies should be taken into account.

Therapeutics with potentially important RBC pharmacogenomics relationships

Methotrexate

Methotrexate polyglutamates accumulate within erythrocytes in a dose-dependent fashion, significantly influencing long-term methotrexate plasma concentrations [130]. One study evaluated the relationship between *ABCC1* variants and methotrexate concentrations in erythrocytes, finding that rs35592 was associated with lower methotrexate polyglutamate concentrations and rs3784862 was associated with higher concentrations [131]. Other studies have identified genetic variants in the folate transporter (*SLC19A1, FOLT* and *RFC1*) that are associated with erythrocyte folate concentrations [132,133]. Although controversial [134], RBC methotrexate polyglutamate concentrations are associated with genetic variants in *SLC19A1* [135]. *SLC19A1* loss results in reduced methotrexate uptake and methotrexate resistance in erythroleukaemia cells [136]. Variants in RBC transporters have also been associated with methotrexate plasma concentrations [137], and RBC folate concentrations have been associated with methotrexate outcomes [138]. Although methotrexate likely targets white cells, methotrexate polyglutamates in circulating RBCs may be associated with clinical efficacy of methotrexate, determining both dose and therapeutic selection [139]. Such relationships may underlie the association between variants in *ABCC1, SLC19A1* and other polymorphisms with methotrexate efficacy. Thus, understanding how allelic variants in RBC transporters influence this relationship may

increase the likelihood of developing precision use of methotrexate. This field remains in its infancy.

Mercaptopurines

Located in a variety of tissues, including erythrocytes, thiopurine methyl transferase (TPMT) is the major metabolic detoxification route for mercaptopurines. Red blood cells may act as a reservoir for mercaptopurine metabolites, and low erythrocyte TPMT activity is a marker for mercaptopurine toxicity [140] and lower risk of relapse [141]. Both MRP4 and MRP5 transport mercaptopurine out of red blood cells, whereas ENT1 is a mercaptopurine uptake transporter associated with mercaptopurine sensitivity [142–144]. The rs3765534 polymorphism in *ABCC4* impairs membrane localization and is associated with significant mercaptopurine sensitivity [145, 146]. One study determined that variants in *SLC29A1* were associated with erythrocyte concentrations of thiopurines in patients receiving azathioprine for neuromyelitis optica spectrum disorders [147]; however, the genetic influences of erythrocyte transport and its implications on the pharmacology of mercaptopurines are rather poorly studied.

Antiretrovirals

Low erythrocyte inosine triphosphatase (ITPA) activity is associated with the development of adverse events during antiretroviral therapy [148, 149] and metabolizes purine analogues used in HIV treatment [149]. Since ITPA activity is decreased in individuals infected with HIV [150], factors influencing ITPA metabolism in erythrocytes may be of significant importance. Several studies have identified variants in transporters that are associated with the pharmacokinetics or clinical outcome of antiretrovirals [151–158]. However, to our knowledge, no study has yet determined whether these variants are associated with intra-erythrocyte concentration of these medications, and therefore, the availability of antiretroviral substrates to erythrocyte ITPA.

Nucleoside analogues

SLC29A1 (encoding ENT1) is involved in the pharmacology of many nucleoside analogues (e.g. cytarabine, gemcitabine, 5FU, pentostatin, zidovudine, ribavirin, dipyridamole and draflazine) [159]. Interestingly, we did not find a single study that has evaluated whether RBC ENT1 uptake effects the pharmacology of these medications. Since ribavirin is known to cause dose-limiting haemolytic anaemia [160], variants in this transporter should be studied to determine whether a population of individuals is at particular risk of haemolytic anaemia during ribavirin therapy.

ABCG2 substrates

ABCG2 transports a very wide variety of medications from different classes, and genetic variants in *ABCG2* have been associated with the pharmacokinetics and outcomes of numerous therapeutics (Table S1). The implications of erythrocyte ABCG2 expression remain poorly characterized. Yet, changes in the expression of ABCG2 resulting from genetic variation are reflected in the red cell membrane [161]. One study discovered a novel ABCG2 variant (ABCG2-M71V; rs148475733) after noting that certain patients had very

low (50% of average) ABCG2 erythrocyte membrane expression levels [162]. Thus, RBC transporter expression can be used to identify potentially important variants affecting the expression or function of transporters. Further study is warranted on ABCG2 expression in red cell membranes and the implications of such expression in pharmacology.

CFTR potentiators

Erythrocytes are representative of the CFTR status of patients [163]. Membrane preparations from erythrocytes are already used to study CFTR structure, function and density [164–166]. Numerous genetic variants are associated with CFTR potentiators (Table S1). Thus, erythrocyte membrane preparations may be useful for non-invasive diagnostic purposes, developing novel CFTR potentiators, or understanding unusual clinical outcomes [167, 168]. Such approaches do not appear to be prevalent in the literature.

Summary

Red cells are easily accessible for pharmacologic studies. The DMET and more recently the PharmacoScan arrays are increasingly used worldwide for clinical pharmacogenetic decision-making. A thorough search of literature identified 12 genes that are scanned by the arrays and also expressed in the red cell membrane. We propose red cells as an *ex vivo* model system to study the effect of variants of these 12 membrane proteins on the pharmacokinetics of drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genes present in the red cell membrane and routinely tested in pharmacogenomics.

		Pharmacogenomics platform	
Gene	**Red cell membrane confidence threshold***	**DMET**	**PharmacoScan**
ABCC1	High	Yes	Yes
ABCC4	High	Yes	Yes
ABCC5	High	Yes	Yes
ABCG2	High	Yes	Yes
SLC16A1	High	Yes	Yes
SLC19A1	Medium	Yes	Yes
SLC29A1	High	Yes	Yes
CYP4F3	Medium	Yes	Yes
CFTR	High	No	Yes
FLOT1	High	No	Yes
ATP7A	High	Yes	Yes
EPHX1	High	Yes	Yes

* High = identified in at least 2 mass spectrometry-based studies, an established blood group, or a CD marker for red cells; Medium = identified in only 1 mass spectrometry-based study [34].

Table 2

Genomic characteristics of the 12 genes[‡].

Gene	Chromosome location	Genomic size	GenBank number	Exons	Length of cDNA (nucleotides)	Length of coding sequence (CDS) (nucleotides)
ABCC1	16p13.11	200498 bp	NG_028268.1	31	6504	4596
ABCC4	13q32.1	288618 bp	NG_050651.1	31	5871	3978
ABCC5	3q27.1	98027 bp	NG_047115.1	30	5790	4314
ABCG2 [*]	4q22.1	68596 bp	NG_032067.2	16	4206	1968
SLC16A1	1p13.2	44507 bp	NG_015880.2	5	3927	1503
SLC19A1	21q22.3	29905 bp	NG_028278.2	6	4982	1776
SLC29A1 [†]	6p21.1	10648 bp	NG_042893.1	14	2201	1497
CYP4F3	19p13.12	19864 bp	NG_007964.1	13	5053	1563
CFTR	7q31.2	188703 bp	NG_016465.4	27	6132	4443
FLOT1	6p21.33	15143 bp	NC_000006.12	13	1866	1284
ATP7A	Xq21.1	139740 bp	NG_013224.2	23	8492	4503
EPHX1	1q42.12	35489 bp	NG_009776.1	9	1847	1368

[*] *ABCG2* - Junior blood group system (JR; ISBT 032) [35, 36].

[†] *SLC29A1* - Augustine blood group system (AUG; ISBT 036) [37].

[‡] For a list of variants in the 12 genes and associated clinical outcomes, see Table S1).

Table 3

Protein characteristics of the 12 genes.

Gene	Protein	Length (amino acids)	Topology*		Transmembrane segments
			Amino-terminal	Carboxy-terminal	
ABCC1	Multidrug resistance-associated protein 1 (MRP1)	1531	Extracellular	Cytoplasm	17
ABCC4	Multidrug resistance-associated protein 4 (MRP4)	1325	Cytoplasm	Cytoplasm	12
ABCC5	Multidrug resistance-associated protein 5 (MRP5)	1437	Cytoplasm	Cytoplasm	12
ABCG2	ATP-binding cassette sub-family G member 2 (ABCG2)	655	Cytoplasm	Cytoplasm	6
SLC16A1	Monocarboxylate transporter 1 (MCT1)	500	Cytoplasm	Cytoplasm	12
SLC19A1	Folate transporter 1 (RFC1)	591	Cytoplasm	Cytoplasm	12
SLC29A1	Equilibrative nucleoside transporter 1 (ENT1)	498	Cytoplasm	Extracellular	11
CYP4F3	Docosahexaenoic acid omega-hydroxylase (LTB4H)	520	Extracellular	Cytoplasm	1
CFTR	Cystic fibrosis transmembrane conductance regulator (CFTR)	1480	Cytoplasm	Cytoplasm	12
FLOT1	Flotillin-1 (FLOT1)	427	Cytoplasm	Cytoplasm	0
ATP7A	Copper-transporting ATPase 1 (MNK)	1500	Cytoplasm	Cytoplasm	8
EPHX1	Epoxide hydrolase 1 (EPHX1)	455	Cytoplasm	Extracellular	1

*Predicted or experimentally proven location of the amino- or carboxy-terminal protein ends at the cytoplasmic or extracellular side of the plasma membrane.

Table 4

Common substrate drugs of the 12 genes.

Gene	Substrate drugs
ABCC1	Doxorubicin, methotrexate
ABCC4	Antivirals: acyclovir, ritonavir, adefovir, tenofovir; Diuretics: furosemide, hydrochlorothiazide; Cephalosporins: ceftizoxime, cefazolin; Cytotoxic drugs: methotrexate, 6-mercaptopurine, 6-thioguanine, topotecan; olmesartan, para-methoxy-N-ethylamphetamine. renal excretion of a wide variety of antiviral, cytostatic, antibiotic and cardiovascular drugs
ABCC5	Methotrexate, 6-thioguanine (anticancer drug), PMEA (anti-HIV drug), 5-fluorouracil, rosuvastatin, atorvastatin, kainic acid, domoic acid, ZJ43
ABCG2	Anthracyclines, daunorubicin, doxorubicin, topotecan, SN-38, irinotecan, methotrexate, imatinib, irinotecan, Mitoxantrone, nucleoside analogs, prazosin, pantoprazole, statins, topotecan, rosuvastatin, teriflunomide, chlorothiazide
SLC16A1	3-bromopyruvate (3-BrPA), a cancer drug candidate that inhibits glycolysis
SLC19A1	5-methyl-tetrahydrofolate, methotrexate
SLC29A1	Cladribine, cytarabine (for AML, ALL etc), fludarabine, gemcitabine capecitabine fialuridine, ribavirin
CYP4F3	Metabolize numerous drug substrates
CFTR	Ivacaftor
FLOT1	Carbamazepine
ATP7A	Cisplatin, oxaliplatin, carboplatin (overexpression causes sequestration of the drugs into intracellular vesicles)
EPHX1	Carbamazepine