Expression Levels of the ABCG2 Multidrug Transporter in Human Erythrocytes Correspond to Pharmacologically Relevant Genetic Variations

Ildikó Kasza1,2, György Várady1,2,5, Hajnalka Andrikovics3, Magdalena Koszarska3, Attila Tordai3, George L. Scheffer4, Adrienn Németh2, Gergely Szákacs5, Balázs Sarkadi1,3,5,*

1 Membrane Research Group of the Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary, 2 CellPharma Kft, Budapest, Hungary, 3 Hungarian National Blood Transfusion Service, Budapest, Hungary, 4 Free University Medical Center, Amsterdam, The Netherlands, 5 Institute of Molecular Pharmacology and Institute of Enzymology, Research Center for Natural Sciences, Hungarian Academy of Sciences (HAS), Budapest, Hungary

Abstract

We have developed a rapid, simple and reliable, antibody-based flow cytometry assay for the quantitative determination of membrane proteins in human erythrocytes. Our method reveals significant differences between the expression levels of the wild-type ABCG2 protein and the heterozygous Q141K polymorphic variant. Moreover, we find that nonsense mutations on one allele result in a 50% reduction in the erythrocyte expression of this protein. Since ABCG2 polymorphisms are known to modify essential pharmacokinetic parameters, uric acid metabolism and cancer drug resistance, a direct determination of the erythrocyte membrane ABCG2 protein expression may provide valuable information for assessing these conditions or for devising drug treatments. Our findings suggest that erythrocyte membrane protein levels may reflect genotype-dependent tissue expression patterns. Extension of this methodology to other disease-related or pharmacologically important membrane proteins may yield new protein biomarkers for personalized diagnostics.

Citation: Kasza I, Várady G, Andrikovics H, Koszarska M, Tordai A, et al. (2012) Expression Levels of the ABCG2 Multidrug Transporter in Human Erythrocytes Correspond to Pharmacologically Relevant Genetic Variations. PLOS ONE 7(11): e48423. doi:10.1371/journal.pone.0048423

Editor: Jian-Ting Zhang, Indiana University School of Medicine, United States of America

Received June 22, 2012; Accepted September 25, 2012; Published November 15, 2012

Copyright: © 2012 Kasza et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work has been supported by grants from OTKA (Hungarian: National Scientific Research Fund) NK83533 and KMOP (The Central Hungary Region Operational Programme) –1.1.2–07/1–2008–0003. Gergely Szákacs is supported by the Lendület grant from the Hungarian Academy of Sciences. Hajnalka Andrikovics is a recipient of the Janos Bolyai Research Scholarship from the Hungarian Academy of Sciences. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors working in research institutions have no conflicting financial interest to declare. Ildikó Kasza and György Várady have been employed by the CellPharma Kft, Hungary. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: sarkadi@biomembrane.hu

Introduction

Personalized medicine requires the development of biomarker diagnostic assays, reflecting individual variations and thus allowing tailored therapeutic interventions. Membrane proteins, contributing to about 30% of the total number of human proteins, play a key role in numerous human pathological conditions, while currently no simple assays are available for the determination of their tissue levels. Although genomic studies have established the pharmacological relevance of a large number of single nucleotide polymorphisms (SNP) and mutations, the direct correlation between genetic variations and membrane protein expression levels remains to be established. Clearly, as membrane proteins undergo complex processing, trafficking, and elimination, in many cases mRNA levels do not correspond to the ultimate protein expression in the relevant membrane.

Human erythrocytes express numerous integral membrane proteins (currently estimated at about 350 different proteins), including transporters, receptors, blood group antigens and proteins with confirmed involvement in human diseases [1,2,3,4]. Although the expression of membrane proteins involved in erythropoiesis may not directly correspond to that observed in other specific tissues, the straightforward availability of blood samples and a simple and rapid, quantitative membrane protein assay platform could make the erythrocyte membrane widely applicable for biomarker analysis.

Based on this concept, we have developed an antibody-based quantitative assay for the determination of erythrocyte membrane proteins. As a pharmacologically relevant example, in this report we describe flow cytometry studies for measuring the expression of the ABCG2 multidrug transporter in human erythrocytes. The ABCG2 multidrug transporter is preferentially expressed in pharmacological barriers, in the liver, kidney and stem cells. This protein modulates the absorption, metabolism and toxicity of numerous drugs and xenobiotics, and causes multidrug resistance in cancer [5,6,7,8,9,10,11,12]. Polymorphic variants or nonsense mutations of ABCG2 were found to be associated with interindividual variability in drug response to anticancer chemotherapy and the outcome of psoriasis or multiple sclerosis treatments [13,14,15,16,17,18,19,20,21,22,23,24]. Recently, a significant disease-association for a polymorphic ABCG2 variant (resulting in ABCG2-Q141K) has been observed in gout [24,25,26,27,28].

It is well documented that mutations and polymorphisms of the ABCG2 gene may cause mis-trafficking and early degradation that may contribute to decreased protein expression. A common variant of ABCG2 (c.421C>A; Q141K), with a variable allele frequency between 5–30% in various ethnic groups (see ref. [29]), was shown to decrease membrane protein expression in model
cells, despite unchanged mRNA levels [30,31,32,33,34]. Still, a lower expression level of the ABCG2-Q141K variant has not been confirmed at physiologically relevant sites, given the difficulties in obtaining and processing human tissues.

It has been shown earlier that the erythrocyte membrane contains functional ABCG2 protein [35,36,37,38]. Recently, two papers have been published, linking the rare blood group Jun to the ABCG2 protein, showing that Jun- individuals have no ABCG2 expression in their red cell membranes. These individuals had mutations in their ABCG2 gene on both alleles, resulting in early termination of transcription, while had no apparent disease conditions [39,40].

In this report we show that individuals heterozygous for the potentially miss-processed ABCG2 variant (Q141K) have significantly lower ABCG2 protein expression in their red cells than individuals carrying the wild-type ABCG2 gene. Moreover, heterozygous individuals with an ABCG2 nonsense mutation on one allele, have about 50% reduction in their red cell ABCG2 protein expression. These data suggest that determination of the ABCG2 protein expression in the erythrocyte membrane may provide clinically valuable information for assessing the role of this protein in relevant diseases, metabolic conditions, or the efficiency and/or toxicity of drug treatments.

**Methods**

Anticoagulated blood samples of healthy volunteers (47 unrelated individuals and 14 family members of two probands selected from the donor cohort) were fixed in paraformaldehyde (PFA), stained with monoclonal antibodies specifically recognizing membrane proteins, and subjected to flow cytometry (FACS). In parallel, genomic DNA was isolated from the blood samples; common SNPs of the ABCG2 gene were screened by LightCycler real time PCR amplification; all probands had mutations in their ABCG2 gene on both alleles, resulting in the absence of ABCG2 protein expression controls, we used K562 cells retrovirally transduced to express ABCG2, as described previously [44]. As described earlier [44], PFA fixation of the membrane ABCG2 protein eliminates the conformation-sensitivity of its interaction with the 5D3 antibody, and provides maximum labeling.

**Determination of Expression Levels**

In order to use a combination of all relevant anti-ABCG2 antibodies for quantifying ABCG2 expression in the red cell membranes, we calculated an average binding factor, based on the relative staining efficiencies observed. In all experiments BXP34 gave 3 times greater relative staining (as measured by the Geo Mean values of the gated populations) than either the BXP21 or the 5D3 antibodies (the exact epitopes of the mAbs are unknown), therefore in the calculated ABCG2 factor we used the following equation: (BXP34/3)*BXP21+5D3/3. As documented in Figure 2, a linear correlation between the calculated average of BXP34 and BXP21 binding (BXP34/3)*BXP21/2 in the red cell ghosts and the values measured for the cell-surface reactive, human-specific 5D3 binding in whole red cells was observed. The calculated intra- and inter assay variations are presented in the Results section and in the Supplementary Materials.

**Additional Methods**

In order to compare the relative expression of ABCG2 in the red cell membrane to those in known expression systems, we performed Western blot experiments using isolated red cell membranes, isolated S9 insect cell membranes expressing the human ABCG2 protein, and A431 cells overexpressing ABCG2 (see Supplementary Materials and refs. [45,46,47]. In accordance with previous data in the literature [35,36,37,38,39], we detected both the monomeric and dimeric forms of ABCG2 in the red cell membrane but found that this assay is not suitable for the proper quantitation of small changes in ABCG2 expression (see Supplementary Materials).
ABCG2 in the Erythrocyte Membrane

A

FSC vs. SSC

B

ghosts

cells

ABCG2(BXP34)+
GAM-PE(IgG1)

C

ABCG2(BXP21)+
GAM-PE(IgG2a)

D

ABCG2(SD3)+
GAM-PE(IgG2b)

E

Glycophorin-A-
FITC
ABCG2 in the Erythrocyte Membrane

Results and Discussion

We have used a flow cytometry based assay for the quantitative determination of ABCG2 expression in erythrocytes. We found that the forward/side scatter plot delineates two major populations, corresponding to PFA-fixed intact erythrocytes and erythrocyte membrane “ghosts”, respectively (Fig. 1A). Antibodies recognizing intracellular epitopes of ABCG2, that is BXP21 and BXP34 (see refs. [41,42]), bind to ghosts that are accessible from both sides of the membrane (Fig. 1B-C), but not to the fixed, intact erythrocytes. Conversely, the 5D3 monoclonal antibody, recognizing an extracellular epitope of the ABCG2 protein (see ref. [46]) shows binding to both PFA-fixed whole red cells and the ghost fraction (Fig. 1D). The different membrane accessibility in the two erythrocyte fractions was also confirmed using antibodies recognizing an extracellular Glycophorin A epitope (Fig. 1E), or the intracellular epitopes of the human plasma membrane calcium ATPase protein ([43], plasma membrane calcium ATPase, PMCA, see below). Moreover, retention of the viability dye, calcein was observed in the fixed whole cell fraction, while not in the ghost fraction (data not shown).

As documented in Fig. 1, all three ABCG2-specific antibodies detected significant expression of ABCG2 in erythrocytes. In order to allow quantitative protein determination, we titrated the antibodies to obtain maximum binding. After antibody titration all the relevant monoclonal antibodies were applied in concentrations exceeding maximum binding levels (for the PMCA protein see the Supplementary Materials). Since the exact epitopes of the ABCG2 mAbs are unknown, and BXP34 labeling consistently gave three times greater relative staining than either the BXP21 or the 5D3 antibodies, for ABCG2 expression we used a weighed average, named “RBC-G2 factor” (see Methods). As shown in Figure 2, a linear correlation between the weighed average binding of the two mAbs, BXP34 and BXP21, recognizing intracellular epitopes in the ghost fraction, and the binding of the cell-surface reactive 5D3 mAb (in whole red cells) was observed.

We have performed detailed intra-assay and interassay analyses which gave acceptable reproducibility. The performance of the assays with different antibody stainings is shown in the Supplementary Materials, Table S1. The intraassay imprecision (coefficient of variation [CV%] expressed as SD/mean %) for the ABCG2 factor was 5.3%, as measured from 8 peripheral blood aliquots from the same healthy volunteer on the same day. Intersay imprecision was calculated from samples taken from the same individual on different days. We obtained an interassay CV% for ABCG2 factor of 10.1%, when using results obtained from 29 individuals with replicate measurements (using 2–4 replicates in each case).

In additional control experiments we used intact or lysed rat and pig red cells to analyze the specificity of the anti-ABCG2 antibodies applied. We found that the three human-specific anti-ABCG2 antibodies and the anti-Glycophorin A monoclonal antibody did not label rat or pig red cells.

Next, we examined if the red cell membrane ABCG2 protein levels correlated with pharmacologically relevant polymorphisms that are known to influence protein expression in model cells. For this purpose we quantified the expression of the erythrocyte ABCG2 in 47 unrelated, healthy individuals that were also screened for the presence of two most prevalent ABCG2 polymorphic variants found in the Caucasian population (V12M and Q141K) [29]. Significant ABCG2 levels, encompassing a wide range of expression were detected in the red blood cells of all individuals. Differences of the erythrocyte ABCG2 expression
could not be attributed to age or sex. However, when the samples were grouped according to their genotypes, we found that the red blood cells of individuals carrying the heterozygous Q141K variant exhibited significantly lower expression of ABCG2 (3.27±1.19), as compared to homozygous wild-type individuals (6.13±0.61, p = 0.011) (Fig. 3). There was no significant difference between homozygous wild-type individuals and heterozygous V12M carriers, although the number of the carriers of this variant was relatively low. As a summary of the ABCG2 polymorphism analysis data, among the 47 donors we found 11 individuals with the heterozygous presence of the DNA sequence coding for the Q141K variant (carrier frequency: 23.4%, allele frequency: 11.7±6.6%), and 3 individuals with the heterozygous presence of the V12M variant (carrier frequency: 6.4%; allele frequency: 3.2±3.6%). These results correspond to the general polymorphism distributions in the European populations (see [29]).

Interestingly, we found two unrelated individuals showing much lower than the average (about 50%) erythrocyte ABCG2 expression (2.65±0.29) (Fig. 3). Sequencing of the entire coding region of the ABCG2 gene revealed that these individuals carry heterozygous mutations resulting in premature termination (without further polymorphic variations). A nonsense mutation, causing an arginine to stop codon change at codon 236 in exon 7 (c.706C>T, p.R236X, rs140207606 described previously [39,40]) was found in heterozygous form in proband 1. A small deletion (c.791_792delTT, L264HfsX14) described in a recent paper by [43] causing frameshift and the truncation of the protein was found in proband 2.

In order to clarify if a direct relationship exists between the heterozygous stop mutations and the erythrocyte ABCG2 expression levels, we obtained blood samples from the family members of the two probands carrying these premature termination mutations. As shown in Fig. 4, we found a co-segregation of the reduced erythrocyte ABCG2 expression levels (about 50% reduction) and the respective mutations in the two families. These findings show a direct correlation between ABCG2 variants and erythrocyte membrane expression, and indicate a general bi-allelic expression pattern for ABCG2, as has been suggested, based on mRNA data [47,48,49].

In order to examine the specificity of the lower ABCG2 expression related to the genotype changes, we have also analyzed the relative quantitative expression of other erythrocyte membrane proteins. Here we document the compared quantitative expression patterns of the calcium pump protein, PMCA, the Glycophorin A protein, and the ABCG2 protein within a family, in which we found individuals with low ABCG2 expression, due to premature termination of ABCG2 transcription on one allele (Figure 5). The 5F10 monoclonal antibody applied here specifically recognizes all four PMCA isoforms, containing a common epitope [43]. As shown, while the pattern of ABCG2 expression showed significant differences corresponding to the presence of a heterozygous mutation (labeled as+/−), PMCA or GlyA expression levels, although with some variations, were independent from the ABCG2 expression levels.

As a summary, we have developed a simple and reliable flow cytometry assay to quantitate the expression of the human ABCG2 protein in erythrocytes, and found a close correlation between protein expression and the ABCG2 genotype. This technology has major advantages as compared to other available methods.

As documented in the Supplementary materials, we have performed detailed Western blotting studies of the ABCG2 in the isolated membranes or the whole red cells of donors with variable expression levels. However, although the ABCG2 bands in the red cells can be well detected, this laborious and time consuming technology cannot be properly used to evaluate quantitative differences in the ABCG2 expression levels between various blood samples.

Another possible strategy for the absolute quantification of a membrane protein like ABCG2 is to use quantitative LC-MS/MS technology, e.g. described in reference [50]. However, the LC-MS/MS measurements require highly specialized, expensive equipment and detailed standardization of the protein fragmentation, internal standards, etc. This was not the goal of the present work, as the relative red cell membrane expression levels were correlated with the respective genetic backgrounds. We suggest that the flow-cytometry method presented here is more suitable for a rapid, widely applicable clinical laboratory diagnostics.

In this study we show that the erythrocyte ABCG2 levels correspond to the genetic background, thus may reflect the overall tissue expression patterns of this protein. We suggest that the method presented here may provide the basis for the development of generally applicable membrane protein biomarkers. It has been documented that ABCG2 expression is regulated by various xenobiotics, drugs, or stress conditions, e.g. hypoxia – see [10,11,12,29]. In a larger cohort of donors and hematological patients we are currently studying the role of drug treatment and environmental factors in the erythrocyte expression levels of the ABCG2 protein. These may provide clinically valuable data in the context of drug absorption, distribution, and toxicity, as well as predicting treatment efficiency and potential multidrug resistance. A quantitative measure of the expression of ABCG2, shaping ADME-Tox properties and drug sensitivity, should significantly promote a personalized approach in pharmacology. Although the ABCG2 expression in the cancerous tissues does not uniquely depend on the overall genetic background (special regulatory changes or gene rearrangements may occur), the red cell ABCG2 protein levels may reflect the physiological tissue expression patterns, which modify the efficiency or toxicity of the cancer drug treatment. These questions are still to be explored in larger scale studies.

Figure 3. ABCG2 is differentially expressed in the red blood cells of individuals carrying homozygous wild-type, heterozygous polymorphic or premature stop codon mutant ABCG2 alleles. Boxplot presentation showing the median and the 25–75th percentiles, whiskers represent 10–90th percentiles. ABCG2 expression is calculated based on the combined reactivity of anti-ABCG2 mAbs (RBC-G2 factor – see Methods). Labels: individuals carrying wild-type ABCG2 (WT), polymorphic (Q141K, V12M) ABCG2 alleles, or a heterozygous stop mutation (STOP). doi:10.1371/journal.pone.0048423.g003
In addition, the above simple methodology may be applicable for a wide variety of clinically relevant membrane proteins, expressed in the erythrocytes. As shown in Figure 5 and in the Supplementary Materials, the plasma membrane calcium pump (PMCA) or Glycophorin A can be quantitatively detectable in the human red cells by this method. In our current experiments, not
presented here in detail, we have found that the above described technology can be applied to quantitatively assess the expression of several other membrane proteins (including ABCA1, ABCB6, ABCC1, ABCC3, and ABCG4). We are currently performing population studies to evaluate the significance of SNPs and mutations in the expression levels of these membrane proteins.

Supporting Information

Figure S1 Western blot analysis of isolated red cell membrane preparations, compared to ABCG2-expressing Sf9 cell membrane preparations or A431 tumor cells, expressing ABCG2 [1].

Figure S2 Comparison of ABCG2 expression on Western blot – detection by BXP21 antibody.

References

1. Alexandre BM (2010) Proteomic mining of the red blood cell: focus on the membrane proteome. Expert Rev Proteomics 7: 163–168.
2. Goodman SR, Kudla A, Ammann L, Kaldunashvili D, Dassen O (2007) The human red blood cell proteome and interactome. Exp Biol Med (Maywood) 232: 1391–1408.
3. Pasini EM, Kirkegaard M, Mortensen P, Lutz HU, Thomas AW, et al. (2006) In-depth analysis of the membrane and cytosolic proteome of red blood cells. Blood 108: 791–801.
4. Pasini EM, Lutz HU, Mann M, Thomas AW (2010) Red blood cell (RBC) membrane proteomics–Part I. Proteomics and RBC physiology. J Proteomics 73: 401–420.
5. Allen JD, Schinkel AH (2002) Multidrug resistance and pharmacological protection mediated by the breast cancer resistance protein (BCRP/ABCG2). Mol Cancer Ther 1: 427–434.
6. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, et al. (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 95: 15665–15670.
7. Junker JW, Bueltelar M, Wagenaur E, Van Der Valk MA, Scheffer GL, et al. (2002) The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. Proc Natl Acad Sci U S A 99: 15649–15654.
8. Krishnamurthy P, Ross DD, Nakashima T, Bailey-Dell K, Zhou S, et al. (2004) The stem cell marker Brgp/ABCG2 enhances hypoxic cell survival through interactions with heme. J Biol Chem 279: 24218–24225.
9. Krishnamurthy P, Schrutz JD (2006) Role of ABCG2/BCRP in biology and medicine. Annu Rev Pharmacol Toxicol 46: 381–410.
10. Robey RW, Polgar O, Deeken J, To KW, Bates SE (2007) ABCG2: determining its relevance in clinical drug resistance. Cancer Metastasis Rev 26: 39–57.
11. Sarkadi B, Homolya L, Szakacs G, Varadi A (2006) Human multidrug resistance ABCB and ABCG transporters: participation in a chemoinnate immune defense system. Physiol Rev 86: 1179–1236.
12. Szakacs G, Varadi A, Ozvegy-Laczka C, Tordai A, Nemet K, et al. (2006) The role of the human ABCG2 multidrug transporter and its variants in cancer therapy and toxicology. Cancer Lett 254: 62–72.
13. Kondo G, Suzuki H, Itoha M, Otawa S, Sasaki J, et al. (2004) Functional analysis of SNPs variants of BCRP/ABCG2. Pharm Res 21: 1059–1068.
14. Leimanis ML, Georges E (2007) ABCG2 membrane transporter in mature human erythrocytes. Biochem Biophys Res Commun 354: 345–350.
15. Maliepaard M, Scheffer GL, Faneley IF, van Gastelen MA, Pijnenborg AG, et al. (2001) Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 61: 3450–3454.
16. Zhou S, Zeng Y, Nye PA, Nair G, Stewart CF, et al. (2005) Increased expression of the Aβ2/g2 protein during erythroid maturation plays a role in decreasing cellular protoporphyrin IX levels. Blood 105: 2571–2576.

Figure S3 Calibration of 5F10 antibody binding and saturation.

(TIF)

Materials S1

(DOC)

Acknowledgments

We appreciate the help in these experiments of Dr. Kristina Truta-Feles and Édith Szabó.

Author Contributions

Conceived and designed the experiments: IK AT GS BS. Performed the experiments: IK GV HA BS. Analyzed the data: HA AT GV GLS BS. Contributed reagents/materials/analysis tools: GLS HA. Wrote the paper: IK GS HA BS.

1. Alexandre BM (2010) Proteomic mining of the red blood cell: focus on the membrane proteome. Expert Rev Proteomics 7: 163–168.
2. Goodman SR, Kudla A, Ammann L, Kaldunashvili D, Dassen O (2007) The human red blood cell proteome and interactome. Exp Biol Med (Maywood) 232: 1391–1408.
3. Pasini EM, Kirkegaard M, Mortensen P, Lutz HU, Thomas AW, et al. (2006) In-depth analysis of the membrane and cytosolic proteome of red blood cells. Blood 108: 791–801.
4. Pasini EM, Lutz HU, Mann M, Thomas AW (2010) Red blood cell (RBC) membrane proteomics–Part I. Proteomics and RBC physiology. J Proteomics 73: 401–420.
5. Allen JD, Schinkel AH (2002) Multidrug resistance and pharmacological protection mediated by the breast cancer resistance protein (BCRP/ABCG2). Mol Cancer Ther 1: 427–434.
6. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, et al. (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 95: 15665–15670.
7. Junker JW, Bueltelar M, Wagenaur E, Van Der Valk MA, Scheffer GL, et al. (2002) The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. Proc Natl Acad Sci U S A 99: 15649–15654.
8. Krishnamurthy P, Ross DD, Nakashima T, Bailey-Dell K, Zhou S, et al. (2004) The stem cell marker Brgp/ABCG2 enhances hypoxic cell survival through interactions with heme. J Biol Chem 279: 24218–24225.
9. Krishnamurthy P, Schrutz JD (2006) Role of ABCG2/BCRP in biology and medicine. Annu Rev Pharmacol Toxicol 46: 381–410.
10. Robey RW, Polgar O, Deeken J, To KW, Bates SE (2007) ABCG2: determining its relevance in clinical drug resistance. Cancer Metastasis Rev 26: 39–57.
11. Sarkadi B, Homolya L, Szakacs G, Varadi A (2006) Human multidrug resistance ABCB and ABCG transporters: participation in a chemoinnate immune defense system. Physiol Rev 86: 1179–1236.
12. Szakacs G, Varadi A, Ozvegy-Laczka C, Tordai A, Nemet K, et al. (2006) The role of the human ABCG2 multidrug transporter and its variants in cancer therapy and toxicology. Cancer Lett 254: 62–72.
13. Kondo G, Suzuki H, Itoha M, Otawa S, Sasaki J, et al. (2004) Functional analysis of SNPs variants of BCRP/ABCG2. Pharm Res 21: 1059–1068.
14. Leimanis ML, Georges E (2007) ABCG2 membrane transporter in mature human erythrocytes. Biochem Biophys Res Commun 354: 345–350.
15. Maliepaard M, Scheffer GL, Faneley IF, van Gastelen MA, Pijnenborg AG, et al. (2001) Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 61: 3450–3454.
16. Zhou S, Zeng Y, Nye PA, Nair G, Stewart CF, et al. (2005) Increased expression of the Aβ2/g2 protein during erythroid maturation plays a role in decreasing cellular protoporphyrin IX levels. Blood 105: 2571–2576.
39. Saison C, Helias V, Ballif BA, Peyrard T, Puy H, et al. (2012) Null alleles of ABCG2 encoding the breast cancer resistance protein define the new blood group system Junior. Nat Genet 44: 174–177.

40. Zelinski T, Coghlan G, Liu XQ, Reid ME (2012) ABCG2 null alleles define the Jr(a-) blood group phenotype. Nat Genet 44: 131–132.

41. Scheffer GL, Maliepaard M, Pijnenborg AG, van Gastelen MA, de Jong MC, et al. (2000) Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines. Cancer Res 60: 2589–2593.

42. Diestra JE, Scheffer GL, Catala I, Maliepaard M, Schillens JH, et al. (2002) Frequent expression of the multi-drug resistance-associated protein BCRP/ MXR/ABCP/ABCG2 in human tumours detected by the EXP-21 monoclonal antibody in paraffin-embedded material. J Pathol 198: 213–219.

43. Caride AJ, Filoteo AG, Enyedi A, Verma AK, Penniston JT (1996) Detection of isoform 4 of the plasma membrane calcium pump in human tissues by using isoform-specific monoclonal antibodies. Biochem J 316 (Pt 1): 353–359.

44. Hegedus C, Ozvegy-Laczka C, Apati A, Magocs M, Nemet K, et al. (2009) Interaction of nilotinib, dasatinib and bosutinib with ABCB1 and ABCG2: implications for altered anti-cancer effects and pharmacological properties. Br J Pharmacol 158: 1153–1164.

45. Fischer S, Lakatos PI, Lakatos L, Kovacs A, Molnar T, et al. (2007) ATP-binding cassette transporter ABCG2 (BCRP) and ABCB1 (MDR1) variants are not associated with disease susceptibility, disease phenotype response to medical therapy or need for surgery in Hungarian patients with inflammatory bowel diseases. Scand J Gastroenterol 42: 726–733.

46. Ozvegy-Laczka C, Laczko R, Hegedus C, Litman T, Varady G, et al. (2008) Interaction with the 5D3 monoclonal antibody is regulated by intramolecular rearrangements but not by covalent dimer formation of the human ABCG2 multidrug transporter. J Biol Chem 283: 26059–26070.

47. Cusatis G, Sparreboom A (2008) Pharmacogenomic importance of ABCG2. Pharmacogenomics 9: 1005–1009.

48. Kobayashi D, Ieiri I, Hirota T, Takane H, Maegawa S, et al. (2005) Functional assessment of ABCG2 (BCRP) gene polymorphism to protein expression in human placenta. Drug Metab Dispos 33: 94–101.

49. Sisuung TM, Baum CE, Kirkland CT, Gao R, Gardner ER, et al. (2010) Pharmacogenetics of membrane transporters: an update on current approaches. Mol Biotechnol 44: 152–167.

50. Li N, Palandra J, Nemirovskiy OV, Lai Y (2009) LC-MS/MS mediated absolute quantification and comparison of bile salt export pump and breast cancer resistance protein in livers and hepatocytes across species. Anal Chem 81: 2251–2259.