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BCRP protein levels do not differ regionally in adult human livers, but decline in the elderly

Zoe Riches¹, Ngu Abanda^{2,3}, and Abby C. Collier¹

¹Faculty of Pharmaceutical Sciences, University of British Columbia, 2405 Wesbrook Mall, Vancouver, BC V6T1Z3, CANADA.

²Department of Tropical Medicine, Medical Microbiology and Pharmacology, University of Hawaii, Honolulu, Hawaii 96813, USA.

³Biotechnology Center, University of Yaounde I, Yaounde, Cameroon.

Abstract

The aim of this study was to characterize the ontogeny and variability of the BCRP (ABCG2) transporter in healthy human liver. Levels of BCRP mRNA and protein were determined with q-RT-PCR and western blot in a cohort of 87 human livers aged from 7 days to 87 years. A study of the regional expression of BCRP within adult livers was also performed in a nested cohort of 14 individuals with multiple samples per person collected from pre-selected sites. Levels of BCRP mRNA were not significantly different at any age, but protein levels for BCRP were lower in the elderly compared with adults ($p < 0.001$) and children ($p < 0.05$). The intra-liver levels of BCRP protein ranged approximately 6.5-fold and inter-liver BCRP protein varied 8.5-fold in the cohort. No differences in BCRP mRNA or protein were observed with sex or ethnicity, although higher levels of BCRP mRNA were observed in livers from overweight individuals (Body Mass Index 25-29.9) as compared to underweight or ideal weight individuals. There were no differences in the levels of BCRP mRNA or protein in different regions of the large lobe ($n = 3$ regions), small lobe ($n = 3$ regions), directly adjacent to the portal vein or directly adjacent to the common bile duct. This indicates that BCRP researchers can source tissue from all parts of the adult liver without artificial bias in their results. Lower BCRP protein expression in the elderly may be associated with compromised xeno- and endobiotic transport.

Keywords

ABC transporters; development; geriatrics; obesity

*Corresponding author: Abby C. Collier, Ph.D., Faculty of Pharmaceutical Sciences, University of British Columbia, 2405 Wesbrook Mall, Vancouver, BC V6T 1Z3, CANADA, Phone: +1-604-827-2380, Fax: +1-604-822-3035, abby.collier@ubc.ca.

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1. Introduction

Active transport, both into and out-of cells, is necessary for xeno- and endobiotic disposition and for maintaining homeostasis. There are many families of active transport proteins but amongst these, the ATP-binding Cassette (ABC) proteins have come to prominence recently, particularly in the fields of pharmacology and toxicology [1]. Because they are relatively newly described, we lack general population data as well as specific sub-population such as developmental or senescence data, in our understanding of these important transporters.

The ABC transporters are a very large family of trans-membrane proteins that have varied and promiscuous substrate affinities [1]. One of the most important ABC proteins in terms of physiological, pharmacological and toxicological processes, is the breast cancer resistance protein (BCRP, ATP Binding Cassette Protein G2, ABCG2) [2-4]. The 75 kDa BCRP protein was identified in 1998 as it has similar properties to P-glycoprotein (P-gp, ABCB1) however unlike P-gp, BCRP is a half-protein as it has only one nucleotide binding domain and one membrane-spanning domain[4]. The protein is membrane bound and thought to function as a homodimer or homotetramer. Expression of human BCRP mRNA has been detected in the brain, liver, kidneys, small intestine, colon, placenta, prostate, spinal cord, adrenal gland, uterus, and testes [5-8]. Comparatively, fewer studies have investigated BCRP protein [6, 9-11]. Although, BCRP protein has been detected at the blood-brain barrier, on the apical membranes of the placental syncytiotrophoblasts and GI tract, and on the canalicular membrane of liver hepatocytes. The organs and sites at which BCRP is expressed provides some insight into the important roles that the BCRP protein plays. For example, BCRP expressed in the placenta, intestine and blood-brain barrier restricts the uptake of substances while, in the liver, BCRP eliminates drugs and bile salts into bile. The BCRP protein is also expressed in stem cells and is involved in porphyrin and heme transport [12].

The BCRP protein has a pharmacological role. In the intestine, BCRP limits uptake of drugs and in the liver it eliminates drugs. As such, BCRP is important in both drug distribution and elimination. BCRP is associated with the transport of drugs such as abacavir, lamivudine, gefitinib and anti-cancer drugs (e.g. mitoxantrone, topotecan, doxorubicin). Increase in the expression of BCRP has been linked to cancer resistance and recent studies has shown its presence in cancer stem cells which suggests it could be involved in chemo-resistance of these cells [13]. Many studies have looked at acute myeloid leukemia and drug resistance due to BCRP [13].

Besides drug transport, a recent study has shown that BCRP can also transport both sulfated and unsulfated bile acids which suggests it could have an important physiological role in bile acid homeostasis [14]. Although bile salt export pump (BSEP) is the most prominent bile salt transporter the BCRP transporter was able to transport bile acids examined by a number of different experimental approaches. It can also transport many sulfated and glucuronidated steroids such as estrone-3-sulfate and 17 β -estradiol-17-(β -D-glucuronide) showing it has involvement in endogenous transport of hormones and steroids [2].

As the BCRP transporter has so many important roles we hypothesize that any changes in expression could have detrimental effects to that individual not only due to increased exposure to drugs and xenobiotics but also due to reduced elimination of bile acids and dysregulation of hormones. Specifically the ontogeny of the transporter could be important such as in the very young during development or in the elderly when there are many physiological changes including reduced liver size that might alter expression [15].

Very few BCRP studies have been carried out in human liver and of these, even fewer determine protein expression, due to difficulties in working with transporter proteins, which are membrane bound and large in size, making even traditional methods such as western blot challenging [6, 16-18]. Most studies also have small sample sizes, primarily due to [lack] of available human samples ($n = 15$) [16-18]. However a recent study by Prasad et al [16] obtained 65 individual donor samples (aged 7 to 70 yrs) and used LC-MS-MS technique to quantify BCRP protein. They detected BCRP protein in 77% of the livers with a mean of 137.9 ± 42 attomol/ μ g of membrane protein. Another study that investigated the expression of BCRP in the central nervous system of neonates showed low expression, as early as 22 weeks of gestation, as compared to adult samples [9]. At present there are very few studies in pediatric liver and there has not been a comprehensive examination of BCRP expression in different hepatic zones.

In this study we have investigated the expression of BCRP in the livers of 87 individuals that did not have chronic illnesses and experienced sudden death. This work encompasses the null hypotheses that 1) Transporter proteins do not change with age 2) Transporter proteins are not affected by obesity, sex or ethnicity and 3) Transporter proteins do not differ in regions of the liver. Although we do not present dynamic transport data, we clearly show differences with BCRP proteins in the elderly, accepting the alternative hypothesis with respect to age. A change in the level of transport proteins must necessarily alter transport dynamics across membranes, meaning that we may have discovered the mechanism (at least in part) for changes in drug and toxin disposition in the elderly. The protein expression showed that BCRP declined in the elderly compared with adults which may have significant implications in this vulnerable group, particularly because elderly patients are more likely to experience polypharmacy.

2. Materials and Methods

All chemicals and reagents were from Sigma-Aldrich, St Louis MO, USA unless otherwise stated.

2.1 Tissue Samples

Livers S9 and total tissue lysate samples were either purchased commercially from Cellzdirect (Durham, NC, USA), Puracyp (Carlsbad, CA, USA), Xenotech (Lenexa, KS, USA), or released from the Hawaii Biorepository, with approval from the Institutional Review Board for Human Ethics at the University of Hawaii CHS#15844 and the Review Ethics Board at the University of British Columbia H14-00092. Table 1 summarizes the demographics of the cohort and Table 2 summarizes the donor demographics for nested adult samples used in hepatic regional expression analysis.

2.2 Extraction of mRNA and cDNA preparation

Total RNA was extracted from 50-100 µL of liver S9 or lysate fractions that had been previously prepared from ~5g tissue pieces. RNA was isolated using the RNeasy Mini kit according to the manufacturer's instruction (Qiagen, Valencia, CA). The RNA purity and concentration were determined by Nanodrop (ThermoFisher Scientific, Wilmington, DE), then RNA was aliquoted stored at -80°C until use when it was thawed and total RNA (100 ng from S9 or 1 µg from lysates) was reverse-transcribed, in a final volume of 20 µL, with an ABI High Capacity Reverse Transcription Kit (Life Technologies, Burlington, ON) as per the manufacturer's instructions.

2.3 SYBR Green q-RT-PCR and primer selection

Real-time PCR was performed using cDNA template (5 ng RNA equivalent for BCRP from either S9 or total liver lysate samples), and 300 nM forward and reverse primers (for BCRP F: 5'-TGA CGG TGA GAG AAA ACT TAC-3', R: 5'-TGC CAC TTT CAG ACC T-3' with a predicted amplicon length of 121, accession number NM_004827 [19]. For 18S F: 5'-CAC GGC CGG TAC AGT GAA A-3' and R: 5'-AGA GGA GCG AGC GAC CAA-3', with a predicted amplicon length of 71 and accession number: NR_003286.2 [20, 21]) on an ABI Step-one-plus real time PCR system (Life Technologies) in triplicate for each sample. Detection was with SYBR Green in a total volume of 20 µl containing 10 µl of PerfeCTa SYBR Green SuperMix for IQ (Quanta BioSciences, Gaithersburg, MD). Cycling conditions were 1 cycle for 30 sec at 95°C, followed by 40 cycles of a 5 sec at 95°C, 15 sec 60°C, 10 sec 70°C followed by a melt curve of 15 sec 95°C, 60 sec 60 °C, 15 sec 95 °C. The threshold value detection (C_T) was set in the exponential phase of amplification. For quantification, values were normalized to the value of 18S ribosomal RNA measured for each sample using primers as previously described [20, 21].

Data for q-RT-PCR was displayed utilizing StepOne™ software version 2.3 (Applied Biosystems, Foster City, CA). Resulting C_T values were analyzed and converted to fold change differences using the 2^{-C_T} method for relative quantitation [22]. Unpaired students t-tests or Mann-Whitney u-tests were performed on C_T values normalized against 18S as the housekeeping gene using GraphPad Prism version 5.0b for Mac OS X (GraphPad Software, San Diego, California).

2.4 Immunoblotting for BCRP protein

To detect BCRP protein, large format 18 × 24 cm Tris-glycine-SDS 10% acrylamide pre-cast gels (Jule Inc, Milford CT, USA) were used to resolve 50 µg of liver S9 or lysate. Each sample was analyzed on at least 2 separate gels. The proteins were transferred onto PVDF membranes at constant 500 mAmps for 2 hours using submarine transfer (Hoefer Inc, Holliston MA, USA) before being blocked with 5 % non-fat milk powder (NFMP) for 1 hour. Primary anti-BCRP mouse monoclonal antibody, 1:2000 (Ab3380, Abcam), was incubated overnight at 4 °C then washed three times for 10 mins in PBS containing 0.1 % Tween-20. Secondary antibody donkey anti-mouse conjugated horseradish peroxidase (HRP, 1:5000, Jackson Immunolabs, Westgrove PA, USA) was added for 1 hour room temperature followed by three times for 10 mins washes and developed using ECL and x-ray film detection. Liver expression was normalized to a pooled S9 liver sample included on

every blot (200 individuals; XT200, Xenotech, Lenexa KS, USA) allowing determination of variability from population average.

The samples were semiquantified with Image J 1.48v (<http://imagej.nih.gov/ij>). Briefly, images were scanned as tiff files, opened in Image J and converted to 32-bit grey images. A single square box was drawn and moved horizontally along the blot to determine mean grey values for each lane. Background (mean of 3 readings) was subtracted from each band.

2.5 Statistical Analyses

For demographics with binary outcomes (e.g. sex, ethnicity), student's t-tests or ANOVA were performed between groups. Correlations were performed using Pearson's correlation or Spearman's correlation after determining the distribution normality of the data using the D'agostino-Pearson Omnibus test. All statistical analyses were performed using Prism 5.0 for Mac OSX (Graph Pad Prism, San Diego, CA).

3. Results

3.1 Gene expression of BCRP

After removing samples that resulted in low quality of RNA (determined by OD260/280 < 0.8) and/or low yield, the mRNA expression of BCRP in n = 75 samples was determined. The variability between runs was determined by including the same sample in 4 independent runs, the mean $C_t \pm SD$ was 25.62 ± 0.25 which is a CV of 0.98 %. The mRNA for BCRP was detected in 89 % of the cohort with mean $C_T \pm SEM$ (BCRP gene C_T minus 18S C_T) value of 22.7 ± 0.3 .

When examining the data set as a population, data were normally distributed and the C_T values ranged from 15.9 to 28.1 cycles. The BCRP mRNA C_T were not related to age (Figure 1). Gene expression for BCRP was further compared with age, grouped into pediatrics (< 18 years), adults (18 - 64 years) and geriatrics (> 65 years) (Figure 1). The lower the C_T value the higher the mRNA expression therefore the mRNA levels of BCRP were highest in geriatrics ($C_T 21.4 \pm 0.6$) followed by adults ($C_T 22.9 \pm 0.4$) and then pediatrics ($C_T 23.2 \pm 1.3$, Figure 1). Using the 2^{-C_T} method there was a 3-fold and 3.5-fold greater expression of BCRP mRNA in geriatrics compared with adults and pediatrics, respectively, although none of these categories were significantly different from each other.

Overweight individuals had a significantly lower C_T value for BCRP mRNA expression than underweight individuals when analyzed by Student's t-test ($p < 0.05$), which corresponds to 10-fold higher mRNA levels (2^{-C_T} method) in overweight individuals than underweight individuals (Figure 2A). There were no significant differences in BCRP mRNA with ethnicity or sex (Figure 2B, 2C).

3.2 Protein expression of BCRP

The BCRP protein was detected at low levels in the S9 liver samples with an example of the western blot obtained shown in Figure 3A. There was a 175-fold population variability for BCRP protein expression (n = 72) which ranged from 0.028 to 4.90 mean pixel density normalized to a pooled liver S9 control. Additionally, population data were not normally

distributed (as determined by the D'agostino-Pearson Omnibus test), likely due to the skew of lower protein levels in older people causing kurtosis. Indeed, BCRP protein levels were significantly negatively correlated with age although the correlation was moderate (Spearman $r = -0.3$, $p = 0.0049$, Figure 3B). When broken into age categories, mean BCRP protein expression was 1.2 ± 0.4 , 0.9 ± 0.1 , and 0.3 ± 0.1 mean pixel density for pediatric, adults and geriatrics, respectively. The elderly liver exhibits a significantly lower BCRP protein expression compared with pediatrics ($p < 0.05$) and adults ($p < 0.001$, ANOVA with Dunn's multiple comparison test, Figure 3C).

There were no significant differences observed with BCRP protein expression and obesity (measured by BMI), ethnicity or gender (Figure 4).

3.3 Differences in regional hepatic BCRP expression

The potential for regional differences in transporter expression was assayed in a cohort of 14 livers where individual samples were known to be taken from 8 distinct pre-defined regions: three from the large lobe (distal, medial, central); three from small lobe (distal, medial, central), one sample adjacent to the bile duct (ABD), and one adjacent to the portal circulation (APC) (Table 2, Figure 5A).

There were no significant differences in BCRP mRNA for any of the regions (Figure 5B). The BCRP mRNA C_T values ranged from 14.3 to 23.6 cycles. There were also no significant differences in BCRP protein levels between the regions for the 17 individuals (Figure 5C). However, an individual can have up to a 6.5-fold variability in BCRP protein expression within the same liver (Figure 5E) while the inter-liver variability in the cohort was 8.5-fold.

The association between mRNA and protein levels was compared and there was a positive relationship between expression levels (Figure 5D). This means that the lower the mRNA levels of BCRP the lower the protein expression of BCRP and tends to indicate that BCRP is primarily transcriptionally regulated. This statement is supported, also, by the similar levels of variability between mRNA and protein both within each liver and between different donors.

4. Discussion

The major outcomes from this study were the determination that BCRP has lower protein expression in the elderly compared with adults and children, and that BCRP proteins do not differ within regions of the human liver. The findings indicate that geriatric patients might have altered hepatic transport and reduced clearance of xenobiotics, drugs and endobiotics. Additionally, researchers receiving pieces of liver from random sampling will not be subject to confounding effects based on region-specific difference in BCRP protein expression.

The BCRP transporter is involved in both physiological and pharmacological transport of hormones and bile acids as well as removal of endogenous and exogenous chemicals therefore any changes in expression could have profound effects on the individual. The elderly population are the biggest consumer of drugs accounting for approximate 40% of all

prescriptions [15]. In the elderly population the decrease in BCRP expression would result in possible over exposure to drugs and chemicals and decreased elimination of bile acids and hormones. A recent study investigated BCRP transporter and acetaminophen (paracetamol) showed changes in PK with age [23]. They compared the PK data in 4 groups (60-70 yrs, 70-80 yrs and 80-90 yrs compared to 20-40 yrs, n = 10) and detected a greater exposure to intravenous acetaminophen with age. Although they could not measure the amount of BCRP they measured a BCRP polymorphism, however this did not correlate with acetaminophen clearance. This suggests the changes were due to age rather than genetics which supports our findings of reduced BCRP protein. There are many examples of drugs where the PK changes with increased age, such as anti-cancer drug Anagrelide [24], and one of the many reasons for this change could be the reduction in BCRP protein. Using the data obtained within our study we can incorporate this into predictive drug disposition models such as physiologically-based pharmacokinetics (PBPK) to improve the accuracy and precision of these models and ultimately provide best treatment for elderly people.

A recent study using LC-MS-MS to quantify BCRP did not show any age related differences (n = 65 livers) [16]. However the youngest sample in their study was a 7 year old and oldest 70 years with very small numbers in the extremes of age (none under 7 years and only two over the age of 65 years) hence these previous authors could not have detected the differences we have identified.

The levels of BCRP mRNA did not differ significantly with regards to age however we detected a slightly higher expression of mRNA in geriatrics compared with adults and children which was opposite to protein levels. Although the mRNA levels did not differ significantly, the protein levels did. Previous studies have shown that protein and mRNA levels for higher-level complex and membrane bound proteins, do not necessarily correlate [25, 26] and this highlights the need for protein determinations that complement mRNA screens. With regards to the q-RT-PCR method there can also be debate regarding the housekeeping gene to use. If 18S has a drawback, it is that there are large distances between cycles of 18S and genes of interest when determining low abundance genes such as transporter proteins or drug metabolizing enzymes. Moreover as 18S mRNA is in high abundance small changes in its C_T value may greatly affect normalization of the target gene, which could result in a magnified error when calculating mRNA abundance.

The mRNA levels was significantly higher in overweight individuals (BMI > 25) as compared to ideal or normal weight. Although it may be logical to infer that being overweight increases BCRP protein levels, the effects of obesity did not follow this pattern because morbidly obese people did not show elevated BCRP mRNA. Whether this pattern is related to pathological or environmental changes in transitioning from overweight to obese is unknown, but the interplay of these processes likely has dynamic effects on BCRP transporter expression. Others have also demonstrated that BCRP responds differentially according to disease state and [presumably] disease-specific signaling. For example, lower expression of BCRP has been correlated with the severity of gout and associated with lower urate removal in peripheral tissues [27, 28]. One plausible reason for this, since gout is associated with obesity [29], would be that increased BCRP expression in the liver might constitute a systemic response to cope with excess urate. However, because not all obese

people have gout, this likely constitutes a selective signaling response rather than a generalized response to obesity itself.

We did not detect any sex differences in human BCRP protein expression. There are known differences in rodents where levels of Bcrp mRNA are higher in male mouse liver compared to females [30]. These same authors also showed that BCRP protein was significantly higher in male than female human livers (n = 4 male and n = 5 female) [30]. Although we were unable to confirm this finding, our results are consistent with the more recent study by Prasad et al (2014) [16] who showed no sex differences in BCRP protein expression (n= 65) using targeted proteomics.

When regional expression of hepatic BCRP was considered from multiple different sites within the same liver, no significant differences in mRNA or protein levels were observed. The intra-organ variability in protein levels was 6.5-fold, which is quite considerable within a single organ. Originally, we hypothesized that BCRP expression might be higher immediately adjacent to the portal circulation and/or the bile duct based on the efflux role of the transporter, but this was not the case. Despite this, because the liver is made up of lobules containing many portal triads and central veins, it is not surprising to find BCRP expression throughout the liver adjacent to these smaller ducts and providing global efflux. These data demonstrate that researchers can receive tissues from any part of the adult liver to compare between individuals and their results will not be confounded by regional differences in liver BCRP expression. This is particularly valuable information for researchers who source their tissues from commercial vendors, where for the most part regionality is not available. These are also useful data for researchers who receive their tissues from resections or biopsies taken from throughout the liver, reassuring them that there is no regional confounding in their comparisons.

There was no correlation between mRNA and protein levels in the population demographic and variability study using S9 proteins (n = 87). This is likely a function of the S9 fraction not being optimal for studying absolute levels of transport proteins, although absolute levels of mRNA would be present. This is because BCRP in S9 fractions is representative of BCRP being “made”, i.e. in transit from ribosome to ER to cell surface, or BCRP being destroyed at the end of its functional life. In contrast, BCRP mRNA levels correlated with protein when multiple samples from the same liver were tested (n = 14 individuals) using total liver lysates. This is the correct matrix for these comparisons because total lysates contain plasma membranes, where S9 does not. Also, the 6.5-fold inter-individual variation in the total lysates, is comparable to the 3-3.5-fold inter-individual variability observed in previous studies [6, 16]. We also suggest that the higher variation than previously reported is partly due to the ethnically diverse makeup of the livers used in the regional study, consisting of 21% Pacific Islanders, 36% Asians and 43% Caucasian and reflective of genetic variability. Protein-based studies are usually critical for confirming RNA screening efforts, but it would appear based on the lysate studies that transcription (mRNA levels) may be a good marker of BCRP protein presence in the adult human liver. This is not unprecedented, as other studies have observed correlations between mRNA and protein with, for example, some cytochromes P450 [18, 31, 32].

As noted above, using S9 fractions to investigate transporters is not ideal, lysates for the demographic studies would have been preferable. For the population demographic studies, we had a greater number of donors where S9 fractions were available. Although levels of BCRP proteins were understandably lower in S9 fractions, we believe that when comparing individual S9 fractions, the relative levels between donors would be the same because all samples have been depleted of plasma membranes (i.e. the same amount of transporter proteins was removed proportionally) in the production of the S9.

In summary our research has shown for the first time that BCRP protein is expressed at lower levels in the elderly and could be a mechanism for increased exposure of drugs detected in this population. Improved understanding of transport protein dynamics is critical for determining drug and chemical efficacy and toxicity. Because BCRP protein levels are lower in the elderly, this might indicate that transport is compromised and has implications for drug and chemical safety. More research in this area, including functional studies may reveal mechanisms of xenobiotic toxicity through lower elimination in the elderly.

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Highlights

- The BCRP transporter has lowest protein expression in the elderly (≥ 65 years).
- Overweight individuals had higher BCRP mRNA levels compared with underweight or ideal weight.
- There are no regional expression differences in BCRP protein levels in the adult liver.

Figure 1. Gene expression of BCRP in a cohort of 75 liver samples

Gene expression was normalized to 18S to give ΔC_T value compared to age for BCRP. **A:** There was no obvious relationship between mRNA levels and age. **B:** Dot blots compared gene expression levels with age grouped into pediatric (< 18 years), adult (19 – 64 years) and geriatric (> 65 years) and data were analyzed by Student's t-test.

Figure 2. The mRNA expression of BCRP compared with obesity, ethnicity and gender
 The mRNA expression was measured using specific primers for each transporter and SYBRGreen detection and transporter gene expression was normalized to 18S to give ΔC_T value. Data were analyzed by Student's t-test and overweight individuals had significantly higher gene expression compared with underweight individuals (* $p < 0.05$).

Figure 3. Protein expression of BCRP in 72 liver samples compared with age

The BCRP protein was detected by Western blotting and normalized to the BCRP levels detected in a pooled S9 liver sample ($n = 200$). **A:** Example of Western blot of 22 individuals with 50 μg liver S9 loaded compared with pooled S9 sample (Xenotech, $n = 200$), BCRP = BCRP-expressed in baculosome (50 ng), and blank = baculosome only (50 ng). **B:** Population correlation between age and BCRP protein expression (Spearman's correlation $r = -0.3$, $**p < 0.01$). Graph shows linear regression with 95 % confidence intervals, (dotted line). **C:** Protein expression compared to individuals grouped by age and analyzed by non-parametric ANOVA and BCRP expression in geriatrics was significantly lower compared with pediatrics and adults (Dunn's multiple comparison test $*p < 0.05$, $***p < 0.001$).

Figure 4. Protein expression of BCRP compared with BMI, ethnicity and gender

Western blots were carried out to detect BCRP protein in 72 individual liver S9 samples (n 2). **A:** Expression of BCRP protein in obese individuals, measured by BMI. **B:** Differences between ethnicity and BCRP protein expression. **C:** The expression of BCRP proteins compared with sex.

Figure 5. Regionality in BCRP expression in the human liver

A: Diagram of the different regions where samples taken within the same liver. D = distal (large lobe n = 8; small lobe n = 6), M = medial (large lobe n = 7; small lobe n = 6), C = central (large lobe n = 10, small lobe n = 5), APC = adjacent to portal circulation (n = 6) and ABD = adjacent to bile duct (n = 11). The total number of livers used for this sub-study was 14 and these were designated A-N. **B:** Levels of mRNA expression for BCRP did not differ across different liver regions. **C:** The levels of BCRP protein do not differ across different human liver regions. **D:** There is a positive relationship between mRNA and protein for BCRP. **E:** The fold-variability within 10 individual's livers for which we had two or more samples (n = 8 regions for A, D, E, J, n = 7 regions for B and C, n = 4 regions for I and n = 2 regions for F, G and H). The range of BCRP protein levels within each liver was between 0.7 and 6-fold.

Table 1

Demographic information of the 87 donors

Age Mean N	Ethnicity	Gender	Body Mass Index (BMI)	
Population N = 87 Age range 0.018 – 87yrs	Caucasian		Underweight (BMI < 18.9)	7 %
	Asian		Ideal weight (19 - 24.9)	30 %
	Pacific Islander	Female	Overweight (25 - 29.9)	16 %
	African American	Male	Obese (30 - 39.9)	24 %
	Hispanic	Unknown	Morbidly obese (≥ 40.1)	9 %
	Other/unknown		Unknown/not included	14 %
Pediatrics 18yrs	African-American		Underweight (BMI < 18.9)	N = 4
	Caucasian	Female	Ideal weight (19 - 24.9)	N = 2
	Pacific Islander	Male	Overweight (25 - 29.9)	N = 2
	Hispanic	Unknown	Obese (30 - 39.9)	N = 4
	Other/unknown		Morbidly obese (≥ 40.1)	N = 4
Adult 19 - 64yrs	Caucasian		Underweight (BMI < 18.9)	N = 2
	Asian		Ideal weight (19 - 24.9)	N = 19
	Pacific Islander	Female	Overweight (25 - 29.9)	N = 10
	African American	Male	Obese (30 - 39.9)	N = 19
	Hispanic		Morbidly obese (≥ 40.1)	N = 5
	Other/unknown		Unknown/not included	N = 5
Geriatrics 65yrs	Caucasian		Underweight (BMI < 18.9)	N = 5
	Pacific Islander	Female	Ideal weight (19 - 24.9)	N = 2
		Male	Overweight (25 - 29.9)	N = 2
			Obese (30 - 39.9)	N = 3
			Morbidly obese (≥ 40.1)	N = 3
			Unknown/not included	

Table 2

Demographic information of the donors used to investigate regional expression in the liver

ID	Number of liver samples taken	Age	BMI	Gender	Ethnicity
A	8	44	38.8	Male	Hawaiian or other pacific Islander
B	7	20	43.7	Male	Hawaiian or other pacific Islander
C	7	37	38.0	Male	Caucasian
D	8	48	31.6	Female	Asian
E	8	46	31.7	Female	Caucasian
F	2	57	22.7	Male	Asian
G	2	69	27.8	Male	Caucasian
H	2	62	35.5	Male	Caucasian
I	4	18	29.9	Male	Hawaiian or other pacific Islander
J	8	52	24.5	Male	Caucasian
K	1	61	21.1	Female	Japanese
L	1	51	29.3	Male	Asian
M	1	47	20.3	Male	Asian
N	1	32	25.5	Male	Caucasian