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# Real-Time Reverse Transcription-PCR Expression Profiling of the Complete Human ATP-Binding Cassette Transporter Superfamily in Various Tissues

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***Background:*** **ATP-binding cassette (ABC) transporters are involved in many physiologic processes, such as lipid transport, sterol homeostasis, immune mechanisms, and drug transport, and cause various human inherited diseases. Thus, the analysis of ABC transporter mRNA expression profiles for basic research, especially in the field of lipid metabolism, for clinical diagnosis, and for monitoring of drug effects is of great interest.**

***Methods:*** **We have developed a rapid, accurate, and highly sensitive real-time reverse transcription-PCR (RT-PCR) method for detection and quantification of all 47 currently known members of the ABC transporter superfamily. Our expression analysis is based on relative quantification using a calibration curve method. With our assay, expression monitoring of a large number of RNA samples in a 384-well format with only 50 ng of total RNA is possible.**

***Results:*** **In contrast to previous expression analyses of single ABC genes, our method allows the rapid and complete analysis of all ABC transporters in given RNA samples. We used our newly established expression panel to study the gene expression of all human ABC transporters in 20 different human tissues. As a result, we identified tissues with high transcriptional activity for ABC transporters. These organs are mainly involved in secretory function (adrenal gland), metabolic function (liver), barrier function (lung, trachea, small intestine), and tropic function (placenta, uterus).**

***Conclusions:*** **Our RT-PCR assay allows rapid, high-throughput transcriptional profiling of the complete ABC transporter superfamily and thus provides a new enabling tool for research, clinical diagnosis of disease, and drug testing and development.**



ATP-binding cassette (ABC)[3] transporters are multispan membrane proteins that promote either the active uptake or efflux of specific substrates across various biological membrane systems (e.g., ABCB1) *(1, 2)* or function as regulatory molecules, such as ABCC7 (CFTR) *(3, 4)*, ABCC8 (SUR1) *(5)*, and ABCA1 *(6)*. On the basis of sequence motifs in the signature region of the ABC, a new nomenclature that divides the ABC proteins into seven subfamilies, ABCA to ABCG, has been proposed *(7)*. Several ABC proteins in the human system are responsible for drug exclusion in compound-treated tumor cells, providing cellular mechanisms for the development of multidrug resistance *(8)*. Recently, several human ABC transporters have been implicated in bile acid, phospholipid, and sterol transport *(9–12)*, in which the expression of these transporters is itself controlled by lipids. Therefore, and because ABC genes are prone to be involved in human genetic disorders, ABC transporters are promising target molecules for the treatment of lipid disorders such as cardiovascular disease *(13)*. These important findings underscore the need for a sensitive, rapid, and accurate

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[3] Nonstandard abbreviations: ABC, ATP-binding cassette; RT-PCR, reverse transcription-PCR; FAM, 6-carboxyfluorescein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; and Ct, threshold cycle.

method for quantification of ABC transporter expression, and a systematic investigation of these molecules in human tissues is thus warranted.

Traditionally, tissue localization and changes in gene expression after stimulation of cells have been monitored by Northern blot analysis, dot-blot analysis, competitive PCR, RNase protection assays, or in situ hybridization. Although these methods are widely accepted and reliable, they require large amounts of RNA and starting material and are usually not amenable to rapid analysis of multiple genes and large sample numbers. In particular, complete analysis of the ABC transporter superfamily in given samples would not be possible.

TaqMan® reverse transcription-PCR (RT-PCR) is a relatively new method that allows real-time measurement of accumulating PCR product based on the 5′ nuclease assay, which uses the 5′-3′exonuclease activity of *Taq* polymerase to cleave a dual-labeled probe, which in turn hybridizes to a target sequence during amplification *(14)*. The TaqMan probe contains a fluorescent reporter dye at the 5′ end and a quencher dye at the 3′ end. As long as the probe is intact, the proximity of the reporter to the quencher suppresses the reporter fluorescence by a Förster-type energy transfer. Disintegration of the probe during TaqMan PCR releases reporter fluorescence, and the intensity of the fluorescence signal measured during the exponential phase of the PCR reaction is proportional to the amount of input target DNA. The resulting fluorescence signal is monitored with an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems), which allows sample processing in a 384-well format and provides the basis for further automation, enabling high-throughput RT-PCR analysis.

Because there are few reports describing the use of TaqMan technology for quantification of ABC transporters, the aim of this study was to establish step by step a complete panel of TaqMan RT-PCR assays for all 47 currently known human ABC molecules. All required information to adapt the method for the individual purposes of researchers and clinical laboratories, including primers and probe sequences as well as complete PCR conditions, are provided. Because only scattered information is available regarding the expression of ABC transporters in human tissue samples, we validated our relative expression method in 20 different human tissues. In the present study, we demonstrate that human tissues vary greatly in ABC transporter expression, with heart and skeletal muscle showing the lowest expression of ABC molecules. In contrast, tissues with high secretion (adrenal gland), metabolic activity (liver), and barrier function (lung, trachea) and organs of the reproductive system (prostate, placenta, uterus) display broad, high expression of ABC transporters. Although most of the ABC transporters are ubiquitously expressed, some members display a very restricted expression pattern, e.g., ABCA3 in the lung, ABCB4 and ABCB11 in the liver and the testis, and ABCG5 and ABCG8 in the liver and the gut.

## Materials and Methods

### TISSUE ACQUISITION AND RNA EXTRACTION

HepG2 cells were obtained from the American Type Culture Collection. The cells were cultured in DMEM (BioWhittaker) supplemented with 100 mL/L heat-inactivated fetal calf serum (Sigma) and incubated in a 5% $CO_2$ atmosphere at 37 °C. Harvesting of cells and RNA extraction were carried out according to the manufacturer's instructions using the RNeasy Midi Kit (Qiagen).

The purity and integrity of the RNA were assessed on the Agilent 2100 bioanalyzer with the RNA 6000 Nano LabChip reagent set (Agilent Technologies). The RNA was quantified spectrophotometrically and then stored at −80 °C.

Human total RNA samples were obtained from Clontech (BD Biosciences Clontech). The RNA panel for the 20 human tissues contained different numbers of individuals between 14 and 70 years of age. The lowest number of individuals was 2 male/female Caucasians for skeletal muscle, and the highest number was 83 male/female Caucasians for bone marrow.

### cDNA SYNTHESIS

First-strand cDNA synthesis was performed with the Reverse Transcription System from Promega. To a master mixture (prepared in house) containing 5 mM $MgCl_2$, 1× reverse transcription buffer, 1 mM deoxynucleotide triphosphate mixture, 1 unit/μL recombinant RNasin® ribonuclease inhibitor, 0.75 U/μL AMV reverse transcriptase, and 1 μg of random hexamer primers, we added 2 μg of total RNA and sterile $H_2O$ to a final volume of 40 μL.

The reaction mixture was incubated at 42 °C for 60 min, followed by heat inactivation of the enzyme at 95 °C for 5 min. After cooling on ice for 5 min, the cDNA was stored at −20 °C.

### PRIMERS AND TaqMan PROBE DESIGN

mRNA sequences for all human ABC transporters were derived from GenBank (for accession numbers, see Table 1), and primers and TaqMan probes were designed with PrimerExpress Software, Ver. 2.0 (Applied Biosystems). Because of the strict primer and probe design criteria for optimal assays, we did not purposefully target the primers and probes to exon/intron boundaries. All primers and 6-carboxyfluorescein (FAM)-labeled probes were obtained from MWG-Biotech. For the normalization of our results, we used a VIC™-labeled glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) TaqMan PDAR endogenous control reagent set (Applied Biosystems). Each of the probes was quenched by 6-carboxytetramethylrhodamine (TAMRA) at its 3′ end.

### PRIMER AND PROBE QUALITY CONTROL

In preliminary experiments, we determined optimum primer and probe concentrations for 12 different ABC transporter genes. We combined three concentrations (50,

**Table 1. Gene-specific primers and probes for TaqMan analysis.**

| Gene | Alternative name | Forward primer, 5′–3′ | Probe, 5′–3′ | Reverse primer, 5′–3′ | GenBank accession no. | Position, bp |
|---|---|---|---|---|---|---|
| *ABCA1* | *ABC1* | TGTCCAGTCCAGTAATGGTTCTGT | TACACCTGGAGAGAAGCTTTCAACGAGACTAACC | CGAGATATGGTCCGGATTGC | NM_005502 | 1359–1439 |
| *ABCA2* | *ABC2* | AAGCCTGTGGAGGATGATGTG | TGGCCAGTGAGCGGCAGCG | GGTCAACGGCCAGGATACG | NM_001606 | 6224–6365 |
| *ABCA3* | *ABC3* | CAAAACCCTGGATCACGTGTT | TGCTGCCCAACCACTGTCTGGG | CCTCCGCGTCTCGTAGTTCT | NM_001089 | 4285–4375 |
| *ABCA4* | *ABCR* | AAAGGTTGCAAACTGGAGTATTAAGAG | CTGGGCCTGACTGTCTACGCCGA | TTGTTGCCCCCACTGTACGT | NM_00350 | 6225–6308 |
| *ABCA5* | | GGCTGCTATTCTGACCACTCACTATA | CAGAGGCTGTCTGTGATCGAGTAGC | TTAACTGCCCAGACACCATGAT | NM_018672 | 5462–5544 |
| *ABCA6* | | CCATGAGAAATGTCCAGTTTCCT | TCCTCAGAATCTGGGAAGGGTAGATAAA | TGCTGGGTTAAATTAGATATTGGTGTA | NM_080284 | 324–438 |
| *ABCA7* | | TTTCTCTGGGACATGTGTAACTACTTG | CCTTCCAGCAGAGGGCATATGTG | TGTGATCGACCAGCCATACAG | XM_055173 | 4337–4480 |
| *ABCA8* | | TGCTTAGTCCCTTTGCCTTCA | AATGGCCCAGCTTTTACACTTGGACTATGATT | GAGCCGTCCGATGGATGA | NM_007168 | 1226–1322 |
| *ABCA9* | | CATTCAGACTGACAGAAGCACATTT | TGGATTATGAGTATGGGTACCGAAGTAA | TCACCAATGCTGCTCATTGC | NM_080283 | 3152–3283 |
| *ABCA10* | | ATCACAAACTGCGTTTCTCCTTT | ATCGGCATGAGCAGCATCAGCGA | CAGAGGCCTGAAATCCATAACTG | NM_080282 | 3703–3794 |
| *ABCA12* | | CCTCCATTCAGCACCAAAGTCT | CCTACCTGTCACTCCTACGGGCACTCG | TGAGGTCACCCATGCCATT | NM_015657 | 4066–4141 |
| *ABCB1* | *MDR1* | GTCCCAGGAGCCCATCCT | TTGACTGCAGCATTGCTGAGAACATTGC | CCCGGCTGTTGTCTCCATA | NM_000927 | 3769–3836 |
| *ABCB2* | *TAP1* | GGGTGACGGGATCTATAACAACA | CATGGGCCACGTGCACAGCC | CCAAACAC CTCTCCCTGCAA | XM_042533 | 942–1007 |
| *ABCB3* | *TAP2* | ACAGCCAGGTGGTTTCAGTTG | CCTGTGCTGTTCTCCGGTTCT | GCCCATAAGCAATGTTGTTCCT | XM_165824 | 1724–1798 |
| *ABCB4* | *MDR3* | TTTTTACTTTCTTCCTTCAGGGTTTC | CGTTTGGGAAAGCTGGCGAGATCC | TAAAAGCCATTGACCGCAGTCT | XM_167466 | 2869–2949 |
| *ABCB5* | | GCTCTGGCCCCTCAAACC | CACCCTGACCTCCTGCTGCCTATGA | TGGCCTTGAGGTATGTGCAGTA | U66692 | 218–285 |
| *ABCB6* | *MTABC3* | TGAAAGAGGAGACAGAAGTGAAGGA | CTGGAGCAGGGCCCCTTCGC | AACTCAATACGGCCCTTCTGAA | XM_050891 | 1980–2052 |
| *ABCB7* | *ABC7* | GATCCGGCCTTTAGTCTCTGTTAG | AGGTCCGCAGTGGAGGCCACA | CCAAGGCGCCGAGTTG | XM_032877 | 88–155 |
| *ABCB8* | *MABC1* | GAAGCGAATGCTCACGAGTTC | TCACCAGCTTCCCCGAGGGCT | TCACCGACGACCGTGTTG | XM_032165 | 1775–1836 |
| *ABCB9* | | CGCCCACCTCCAACCA | AGGATGCGGCTGTGGAAGGCG | CGCAGATGTCCACACTCATGA | XM_038794 | 266–344 |
| *ABCB10* | *MTABC2* | GCTTCCGTAGGCATCAGTATGAT | TTTTTTGTCTCACCTAATCTGGCCACCTTTG | GGAGGCACCACGCTCAAA | XM_001871 | 941–1014 |
| *ABCB11* | *BSEP* | AGGGAGCTACCAGGATAGTTTAAGG | CTTCCATCCGGCAACGCTCCA | TCGTGCACCAGGTAAGAAAGC | XM_002644 | 2178–2253 |
| *ABCC1*[a] | *MRP1* | GAAGGCCATCGGACTCTTCA | CTCCTTCCTCAGCATCTTCCTTTTCATGTG | CAGCGCGGACACATGGT | L05628 | 3097–3166 |
| *ABCC2*[a] | *MRP2* | TGCAGCCTCCATAACCATGAG | AGAGAGAACAGCTTTCGTCGAACACTTAGCC | GATGCCTGCCATTGGACCTA | U63970 | 2728–2807 |
| *ABCC3*[a] | *MRP3* | CACACGGATCTGACAGACAATGA | CCAGTCACCTATGTGGTCCAGAAGCAGTTT | ACAGGGCACTCAGCTGTCTCA | AB_010887 | 2670–2745 |
| *ABCC4*[a] | *MRP4* | AAGTGAACAACCTCCAGTTCCAG | CAAACCGAAGACTCTGAGAAGGTACGATTCCT | GGCTCTCCAGAGCACCATCT | AF_071202 | 2026–2144 |
| *ABCC5*[a] | *MRP5* | TGAAAGCCATTCGAGGAGTTG | CTCGCAGCGTGCCCTTGACAAAG | CGGAAAAGCTCGTCATGCA | AF_146074 | 2979–3054 |
| *ABCC6*[a] | *MRP6* | AGACACGGTTGACGTGGACAT | CCAGACAAACTCCGGTCCCTGCTGAT | GCTGACCTCCAGGAGTCCAA | AF_168791 | 3156–3231 |
| *ABCC7* | *CFTR* | GGCACGAAGGAGGCAGTCT | TGATGACACACTCAGTTAACCAAGGTCA | TCGTGTGGATGCTGTTGTCTTT | XM_004980 | 2418–2508 |
| *ABCC8* | *SUR1* | CACCAATCAGCTCATGTGGTTT | CCAGTACAGATCATTGTGGGTGTGAT | GGCACTGACTCCGAGTATGTAGTAGA | XM_036347 | 1398–1506 |
| *ABCC9* | *SUR2* | CTACTCCTGTGTTCCTGGTTGCT | TCCTGCCCCTTGGTGTTGC | CAACCCGAAAGTATTTCTGGATAAA | XM_012243 | 3393–3467 |
| *ABCC10* | *MRP7* | GCGGGTTAAGCTTGTGACAGA | CTGCTGAGTGGCATTCGGGTC | CCCACCCGCAGAACTTGA | XM_052745 | 1585–1646 |
| *ABCC11* | *MRP8* | AGGGTCTACCACCACTACATCCA | CAGCTGGAGGTTACATGGTCTCTTGCATAATT | CGATCAGCACCACGAAGAAG | NM_033151 | 2721–2799 |
| *ABCC12* | *MRP9* | TTCATCCAAAGGCCTGTCATT | CATACATCATCCAGCTGAGCGGACTGCT | CCGTTCGCACACACACTTG | NM_033226 | 3505–3575 |
| *ABCD1* | *ALD* | CCTCTTTCTACAGCCTAATTTATTGGA | TCCCTATTCGTAGCCATCTCCG | TGGCACGGTAGTCACATTGG | NM_000033 | 3244–3316 |
| *ABCD2* | *ALDR* | GCTACCTTCGTCAACAGTGCAA | AGGTACCTGGAATGCAAATTGGCT | CGTGGTCTACTAGGCGAGTTCTG | NM_005164 | 1026–1104 |
| *ABCD3* | *PMP70* | ACCCCTCTCAGTCTGCAGTATTG | TGTTTAAAGTATATGTGCAGTCTTGCT | TGATACATGGTAACCCCTCCTTGT | XM_040463 | 2497–2574 |
| *ABCD4* | *PMP69* | GGCCCAGGTTAGATCTGCAA | CCAGCGGTTCCTGCAGATACTGA | TGATGACCAAGAAGGAAACAAAAAC | NM_005050 | 89–162 |
| *ABCE1* | *OABP* | GATCGCGTCATCGTTTTTGA | TGTTCCATCTAAGAACACAGTTGCAAAC | ATGCCAGCCAAAAGGGTTT | XM_017177 | 578–654 |
| *ABCF1* | *ABC50* | GAAGTTCAGCATCTCCGCTCAT | AGGAGCTGTTCGTCAATGCAGACCTGTAC | GGCGGCCGGCTACAA | NM_001090 | 901–971 |
| *ABCF2* | | TGGAGCAGGGAAGTCAACTCTT | TGAAGCTGCTAACTGGAGAGCTACTACC | TTTTCGGATCATGCCATCTG | NM_005692 | 1342–1414 |
| *ABCF3* | | CCGGGAGTTGTGGGTATGC | AAGGAGGCGGCGTCACCCG | GTACTGGTCAAATCCTCCTTCCA | U66685 | 769–832 |
| *ABCG1* | *White* | CCGACCGACGACACAGAGA | TCTGATCCAACCCCTAGAACCGCGT | GCACGAGACACCCACAAACC | XM_032950 | 2703–2772 |
| *ABCG2*[a] | *MXR* | CAGGTCTGTTGGTCAATCTCACA | CCATTGCATCTTGGCTGTCATGGCTT | TCCATATCGTGGAATGCTGAAG | AF_098951 | 1893–1969 |
| *ABCG4* | *White2* | GAGCCAGGGTCAGTGCATCT | CAAAGGCGTGGTCACCAACCTGATC | GCAAGCCGAGTCCCTTTAGA | XM_012099 | 823–893 |
| *ABCG5* | *White3* | TCTCTTGGCCCCCCACTTA | TTGGTGAATTTCTAACTCTTGTGCT | CTATATTTGGATTTTGGACGATACCA | NM_022436 | 1655–1728 |
| *ABCG8* | *White4* | TC GTACCCTCTCTACGCCATCT | CGTCATTGGCCTCAGCGGT | GGACACGTAGTACAGGACCATGAA | XM_055525 | 1999–2073 |

[a] Primers and probes were adapted from Taipalensuu et al. *(37)*.

300, 900 nM) of both forward and reverse primers with a constant probe concentration. The combination showing the highest fluorescence was tested thereafter at five different probe concentrations (50, 100, 150, 200, 250 nM).

Because almost all of the tested ABC transporter assays provided the best results at both the highest primer (900 nM) and probe (250 nM) concentrations, we chose these concentrations for further experiments.

### generation of calibration curves

To quantify the results obtained by real-time PCR, we used a calibration curve. For this purpose, we prepared a stock solution of cDNA from HepG2 total RNA from which we prepared serial dilutions in four steps. A calibration curve with 50, 25, 12.5, 6.25 ng of HepG2 total RNA was created for all ABC transporters except ABCA6, ABCA8, ABCA9, ABCA10, ABCB11, ABCC1, and ABCC7. These molecules are not expressed in HepG2 cells; therefore, the calibration curves were created from testis cDNA in a similar manner. When necessary, e.g., in the case of very low expression, we prepared additional dilutions to construct calibration curves covering the complete range of expression.

### TaqMan PCR

TaqMan PCR assays were performed on an ABI Prism 7900 HT Sequence Detection System (Perkin-Elmer Applied Biosystems). For quantification of each ABC transporter gene, we prepared a master mixture containing 10 μL of 2× TaqMan Universal PCR Master Mix, 1 μL of gene-specific forward and 1 μL of reverse primer (each at 18 μM), 1 μL of the gene-specific probe (5 μM), and 2 μL of sterile water and aliquoted it into the wells of a 384-well optical plate. A master mixture for the endogenous control *GAPDH*, containing 10 μL of 2× TaqMan Universal PCR Master Mix, 1 μL of predeveloped TaqMan assay reagents (PDAR) from the endogenous control reagent set, and 4 μL sterile water, was treated similarly. Finally, triplicates of cDNA templates equivalent to 50 ng of RNA were added to a final volume of 20 μL.

The thermal cycling conditions were 2 min at 50 °C and 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C.

### data analysis

The Sequence Detector Software SDS 2.0 (Applied Biosystems) was used for data analysis. The first step was to generate an amplification plot for every sample, which showed ΔRn on the *y* axis (where Rn is the fluorescence emission intensity of the reporter dye normalized to a passive reference, and ΔRn is the Rn of an unreacted sample minus the Rn value of the reaction) against the cycle number, displayed on the *x* axis. From each amplification plot, a threshold cycle (Ct) value was calculated, which is defined as the cycle at which a statistically significant increase in ΔRn is first detected and is displayed in the graph as the intercept point of the amplification curve with the threshold. The threshold is automatically calculated by SDS as the 10-fold SD of Rn in the first 15 cycles. The obtained Ct values were then exported to a Microsoft Excel spreadsheet for further analysis.

The next step was to construct calibration curve plots, using Microsoft Excel as recommended in User Bulletin 2 for the ABI Prism 7700 Sequence Detection System (Applied Biosystems), showing Ct values on the *y* axis and the logarithm of the input amount of cDNA (equivalent to the amount of total RNA) on the *x* axis.

All human ABC transporters were subsequently measured in the different human tissues, and the obtained Ct values were used to calculate the initial input amount. Thereafter the results were normalized to the endogenous control, *GAPDH*.

In the last step, we compared the expression of each individual ABC transporter in the complete tissue panel. Therefore, for each ABC transporter, the normalized amount of expression in the tissue that showed the lowest expression was used as a calibrator (set to 1), and the remaining tissue samples were displayed as fold changes.

## Results

### setup and optimization of TaqMan RT-PCR conditions for selected ABC transporters

The first step in developing a real-time quantitative RT-PCR based on the TaqMan chemistry for analysis of human ABC transporters involved the design of specific PCR primers and fluorogenic probes. After collection of the correct consensus sequences for all human ABC transporter mRNAs by GenBank screening, we used the Primer Express Software to design optimum and specific primer and probe combinations (Table 1). Except for ABCA13, primers and the corresponding TaqMan probes could be created for all 47 currently known human ABC transporters. Thereafter, experimental primer and probe concentrations were tested for 12 selected genes of the ABC transporter superfamily (Fig. 1). A primer matrix containing various concentrations of forward and reverse primers (50, 300, and 900 nM) was used for real-time PCR of ABC transporter transcripts in 50 ng of HepG2 cDNA (Fig. 1A). Similarly, several probe concentrations (50, 100, 150, 200, and 250 nM) with fixed amounts of cDNA (50 ng) and primers (900 nM each of the forward and reverse primers) were tested (Fig. 1B). As a result, the TaqMan PCR conditions that provided the highest possible ΔRn and lowest possible Ct values were achieved with highest primer (900 nM) and probe concentrations (250 nM). Therefore, these PCR conditions were used for all further assays with the remaining ABC transporters.

To construct calibration curves for relative quantification, we performed real-time amplification of several cDNA dilutions with ABC transporter genes and a reference gene. Shown in Fig. 2A are representative examples for *ABCB1* and *GAPDH*. The Ct values plotted vs the log concentrations of these calibration samples showed an inverse linear correlation; the correlation coefficient for each calibrator thus should be close to 1. The correlation coefficients for the

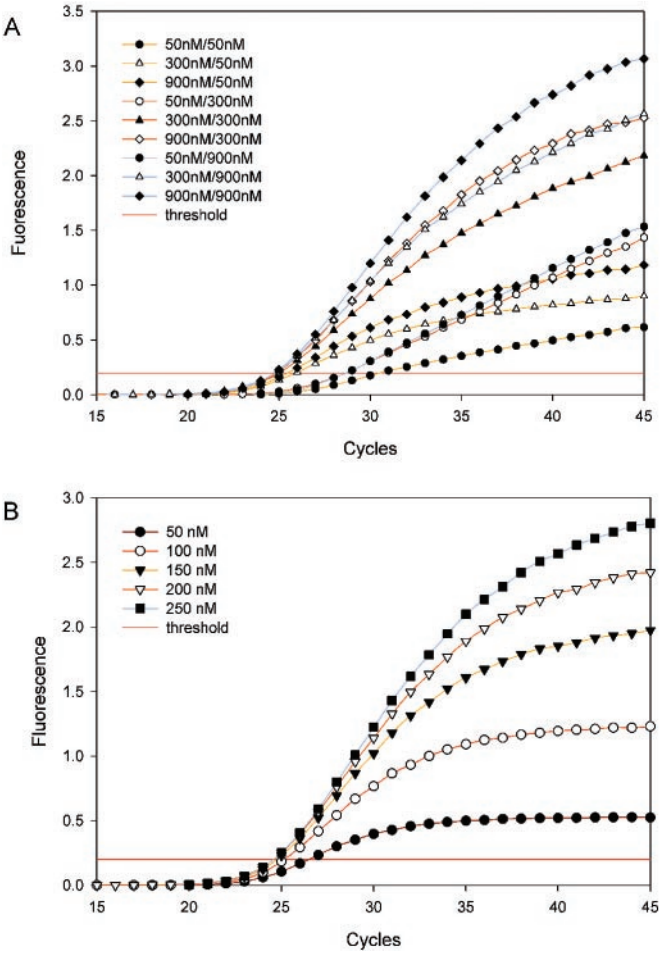



Fig. 1. Results of preliminary TaqMan assays to determine optimum primer and TaqMan probe concentrations.

Normalized fluorescence values (ΔRn) on the *y axis* were plotted against PCR cycles on the *x axis* in a linear scale. (*A*), various combinations of both forward and reverse primers at three different concentrations (50, 300, 900 nM) and a fixed probe concentration (250 nM) were tested with 50 ng of HepG2 cDNA for 12 ABC transporters. *Panel A* shows a representative amplification plot for *ABCB1* (*MDR1*). (*B*), the primer pair achieving the highest ΔRn and the lowest Ct value (900 nM each of forward and reverse primer) was chosen to evaluate the optimum probe concentration. Probe concentrations in a range between 50 and 250 nM in steps of 50 nM were tested. For all tested transporters, 250 nM was the optimum concentration, i.e., that provided the lowest Ct values.

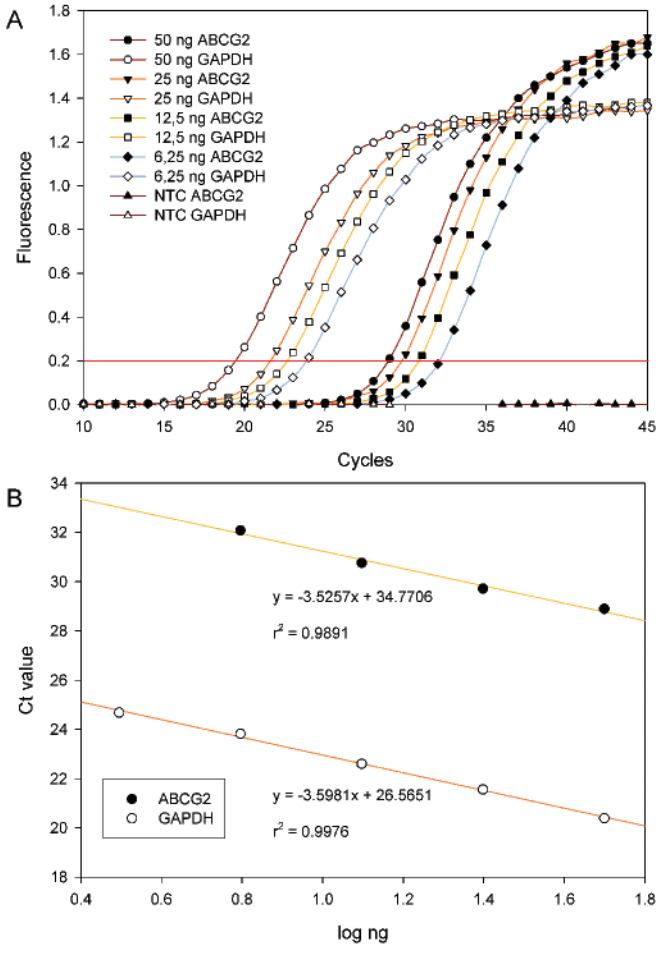



Fig. 2. Calibration curves constructed by measuring serial dilutions of HepG2 cDNA for the relative quantification of ABC transporter expression.

(*A*), amplification plots for *ABCG2* and *GAPDH* are shown, depicting normalized fluorescence values (ΔRn) plotted against the number of amplification cycles. The curves represent triplicate measurements of each sample dilution. (*B*), the Ct values obtained from the amplification plots were used to construct calibration curves for *ABCG2* and *GAPDH*. The Ct values (*y axis*) and log of sample concentrations (*x axis*) show a reverse linear correlation with nearly identical slopes and optimum correlation coefficients.

calibration curves of *ABCG2* and *GAPDH* (Fig. 2B) were 0.9891 and 0.9976, respectively. A similar relationship was observed in our experiments with all primers and probe sets used in mRNA expression analysis; the correlation coefficients were always >0.95. Similarly, the PCR efficiencies, assessed by the slope of the calibration curve, were optimal for all ABC transporter assays (data not shown). As shown in Fig. 1B, the slopes for *ABCG2* and *GAPDH* were very close to each other and near to the ideal value of −3.3, reflecting similar and optimum PCR efficiencies.

### Expression analysis of ABCG2 in 20 different human tissues

Real-time amplification profiles for ABCG2 in 20 human tissues are depicted as a representative example in Fig. 3A. Positive reactions, and thus expression, could be achieved for all tested tissues, with heart having the lowest and uterus the highest expression of ABCG2. To minimize interindividual variation in the expression of ABC transporters in the different tissues, pools from tissue samples from different donors were used. On the basis of earlier reports of mRNA expression analysis in human tissue samples and because of our own experiences with real-time RT-PCR, *GAPDH* was chosen as a reference gene *(15, 16)*. After we calculated the initial input amount from the determined Ct values, using the calibration curve method, we normalized the results to the endogenous control, *GAPDH*. The values shown in Fig. 3B represent the relative expression of ABCG2 compared with its expression in a tissue calibrator. The expression of the calibrator, which was the tissue with the

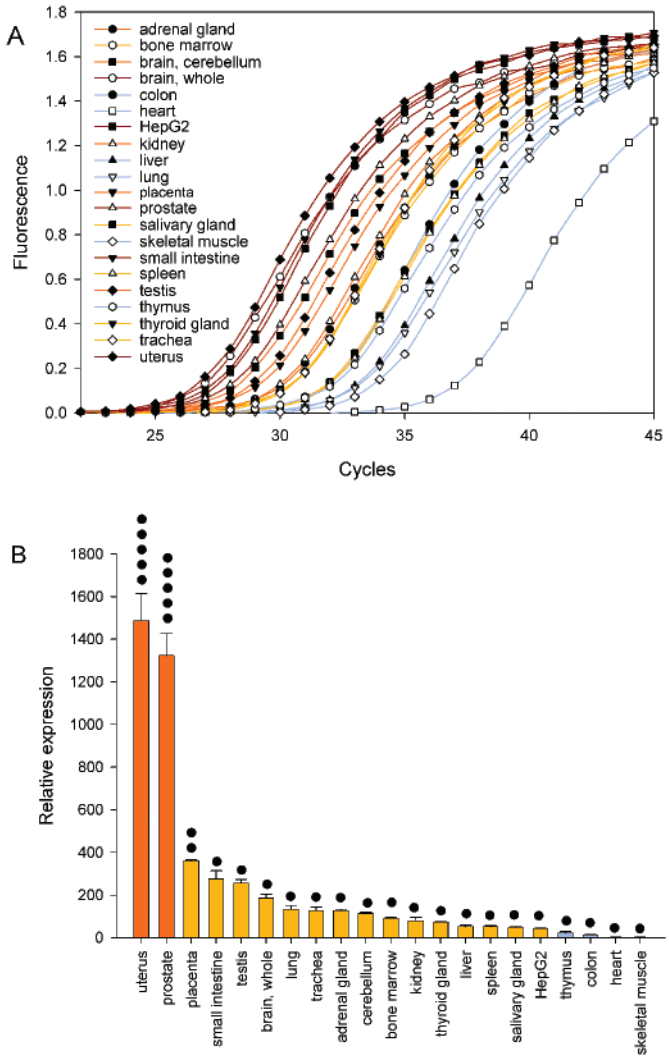



Fig. 3. Validation of the established RT-PCR assays for quantitative detection of *ABCG2* mRNA in 20 different human tissues.

(*A*), real-time amplification plot for *ABCG2* in various human tissues. cDNA equivalent to 50 ng of total RNA was used for each TaqMan PCR assay. (*B*), Ct values obtained with different tissues were used to calculate the input amount of template by the calibration curve method. After normalization to the endogenous control, *GAPDH* (data not shown), these values were expressed relative to the calibrator (tissue with lowest expression) and displayed as relative expression (fold change; indicated by the number of ● *above each column*).

lowest expression, was set as 1, and the remaining samples are diagrammed as fold changes. For a better presentation, these relative expression values were translated to a linear scale (0–5) and are shown as filled circles above the columns.

We selected ABCG2 as an example because its expression is relatively ubiquitous. Although the protein has been implicated in multidrug resistance and early hematopoiesis *(8)*, its physiologic function has not been determined. The results of our ABCG2 mRNA expression analysis (Fig. 3B and Table 2) agreed with the results obtained in earlier studies of ABCG2 mRNA as well as protein analysis in human tissues *(17, 18)*. The difference between triplicate TaqMan assays was generally <10% of the mean value [Fig. 3B and Data Supplement (available with the online version of this article at http://www.clinchem.org/content/vol49/issue2/)]. ABCG2 mRNA is highly expressed in male and female reproductive organs (uterus, prostate, and placenta) and in the small intestine, whereas the colon, heart, and skeletal muscle only marginally express this transporter mRNA. The presence of ABCG2 in these different examined tissues reveals that this molecule is involved in the protection and/or tropic function of the reproductive system and may regulate the extrusion of substrates from intestinal cells, e.g., chemicals or, because of its homology with other members of the ABCG family, lipids *(12, 19)*.

### COMPLETE EXPRESSION PANEL OF ALL HUMAN ABC TRANSPORTERS IN 20 DIFFERENT TISSUES

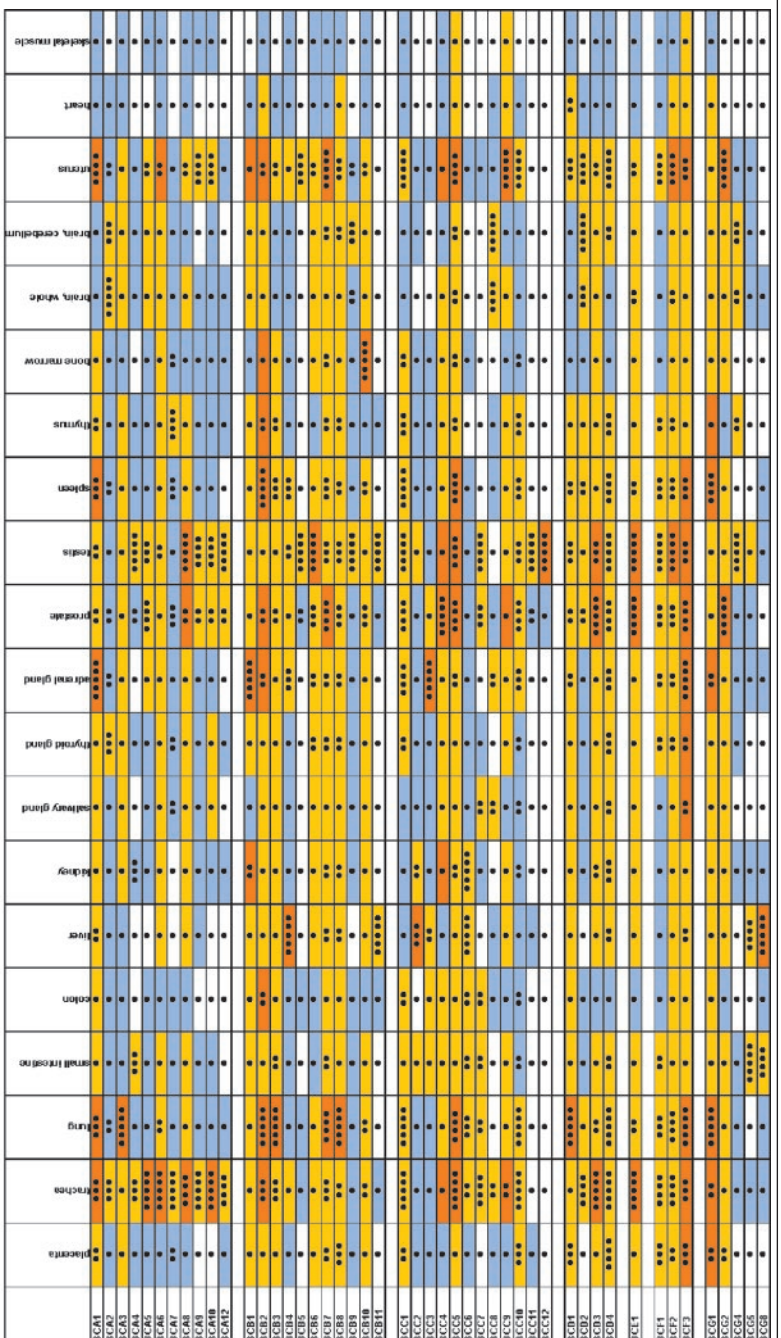
To provide a full overview of the expression profiles of all ABC transporter mRNAs, real-time RT-PCRs were performed, and data analysis was carried out as described above and in the *Material and Methods*. Because the expression analysis of 47 different target genes in 20 human tissues yields an immense amount of data, we sought a means to display these data in a preferably concise form. Therefore, single bar graphs with the relative expression values for all of the remaining transporters are given in the Data Supplement, and the final values were translated to a linear scale and are represented as filled circles in Table 2. This type of presentation allows assessment of the expression of individual ABC transporters in all examined tissues (horizontal analysis). To compare the mRNA expression of different ABC transporters in a given tissue (vertical analysis), we have developed a color code for high (red), medium (yellow), low (blue), and very low (white) expression of transporters, based on ΔCt values ($Ct_{transporter} - Ct_{GAPDH}$).

Analysis of all ABC transporters (Table 2) revealed that most of the genes display ubiquitous mRNA expression. However, some particular members are expressed in a very restrictive manner. This is especially true for ABCA2 in the brain, for ABCA3 in the lung, ABCB4 in the liver, ABCB11 in the liver and the testis, and ABCG5 and ABCG8 in the liver and the small intestine. The tissues with high expression of the seven ABC transporter subfamilies can thus be defined. It is intriguing that the tissues with highest ABC transporter expression are especially involved in secretory functions (adrenal gland) and the reproductive and tropic system (prostate, testis, uterus, and placenta) as well as barrier functions (lung, trachea, and small intestine) and metabolic activity (liver and kidney).

## Discussion

In the present study, we have shown the successful utilization of TaqMan real-time RT-PCR for mapping of ABC transporter mRNA expression profiles in 20 different human tissues. In addition to the optimization of primers and probe sets as well as the development of 47 RT-PCR

**Table 2. Expression profiles of all human ABC transporters in different tissues.**



assays, which could be particularly useful for the fields of ABC transporter research, clinical diagnosis, and drug monitoring and development, we provide comprehensive data on the expression of all human ABC transporter genes in various tissues. Because of its nature as a PCR-based method, TaqMan real-time RT-PCR is much more sensitive than conventional blot-based mRNA analysis tools, which means that the amount of starting material required (25–50 ng) is much lower than the amount needed for the most commonly used method, Northern blot analysis (5–20 μg). The assay thus allows monitoring of mRNA expression in samples with only very limited amount of RNA as, e.g., in clinical samples such as biopsies. In addition, the high specificity of the TaqMan primer and probe sets is especially useful when analyzing a complete superfamily, as in our case all human ABC transporter genes, and allows detection of splice variants of transcripts.

In our calibration curve method, we used several dilutions of HepG2 or testis cDNA rather than purified or cloned PCR products. The advantage of our method is that with only a few cDNA samples expressing the target genes, relative quantification of many genes is possible. Therefore, the time-consuming and laborious construction of cDNA plasmids for each individual ABC transporter can be avoided. However, if cDNA plasmids were used as the calibrators, an advantage would be that they can be easily prepared in large amounts and stored for long periods in the form of bacteria cultures.

The use of housekeeping genes as internal standards needs critical consideration *(20)*. Although the expression of *GAPDH* has been shown to vary under certain circumstances, e.g., under metabolic stimulation, in our hands, *GAPDH* was a suitable reference for expression analysis in human tissues. Certainly, alternative reference genes may be used for other cell systems.

The real-time analysis of PCR amplification also has several advantages over previous, mostly gel-based RT-PCR methods. Thus, TaqMan PCR is a closed homogeneous system without post-PCR processing and with a low contamination risk. Moreover, real-time PCR allows precise and reproducible quantification because it is based on Ct values rather than end-point detection, where the PCR components are rate limiting. Probably the most important advantage of the TaqMan chemistry and the 7900HT Sequence Detection System is the capability to rapidly monitor a large number of RNA samples in a 384-well format with minute amounts of starting material, enabling their use for high-throughput, routinely applicable assays in modern clinical laboratories. The use of additional automated equipment, such as pipetting robots and plate stackers, can further increase the number of assays to be performed.

The results obtained with our assay are consistent with those obtained in Northern blot and in situ hybridization studies with single ABC molecules *(2, 18, 19, 21–33)*, suggesting that real-time PCR is a reliable method to quantify ABC transporter gene expression. Moreover, the good concordance with earlier reports is particularly reflected by the expression pattern of ABC transporters with a very narrow expression profile. *ABCA2*, which has been identified as a highly expressed gene in oligodendrocytes *(34, 35)*, was also strongly expressed in our brain samples (Fig. 2). Similarly, ABCA3, a lamellar body component of human lung alveolar type II cells *(33, 36)*, was expressed exclusively in our lung samples, and the half-size transporters ABCG5 and ABCG8 were present only in liver and small intestine, as expected from previous reports *(24, 31)*.

Although our study provides a complete overview of ABC transporter expression profiles in 20 human tissues and thus could be helpful for structure/function analyses of currently insufficiently characterized ABC transporters, more human tissues not present in our panel and, in particular, different cell types of organs and various human cell lines await further analysis with our assay. Moreover, protein analysis tools could be used after quantitative mRNA analysis to strengthen the expression data because it has been shown that mRNA expression does not always reflect protein concentrations.

In conclusion, the real-time RT-PCR panel for all human ABC transporters presented in this study may be a highly useful tool for (*a*) detecting aberrant ABC transporter gene expression in various human diseases, especially in the field of lipid disorders and multidrug resistance; (*b*) monitoring the pharmacologic and metabolic modulation of ABC transporter expression; and (*c*) performing epidemiologic studies by exploring the effects of genetic variations in ABC transporter genes.

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