Transcriptional profiling of the ABC transporter family using TaqMan Low Density Array.

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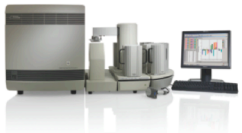
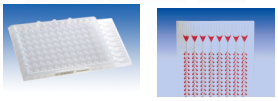
The ATP-binding cassette (ABC) transporters, a large family of transmembrane proteins, have essential physiological and protective functions in cells. ABC transporters are encoded by 50 identified genes in human and have been classified in seven subfamilies (A to G). Mutations in genes encoding these proteins are related to diverse genetic diseases in human. Most of these ABC proteins are involved in transport of a large variety of substrates (amino acids, lipids, ions, sugars, drugs…) across cellular membranes. Some ABC transporters have essential functions such as excreting liver or kidney toxins, or limiting penetration of toxic molecules in vital organs, such as brain or placenta. Multidrug resistance of cells is also correlated with the overexpression of several ABC transporters that induce drug-resistance phenotypes of tumor cells to anti-cancer chemotherapies. Studying expression of this gene family may help to explain their role in human diseases and drug resistance.

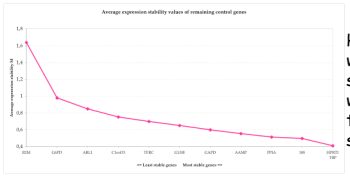
Methodology : High-throughput Quantitative PCR

To carry out ABC expression studies, we have developed high throughput quantitative PCR using TaqMan probes and ABI 7900HT QPCR instrument with automated robotic loading.

We have designed several sets of microfluidic cards (TLDA, TaqMan Low Density Array, applied Biosystems) that allow quantifying simultaneously the expression of the 50 human ABC genes or the murine ABC genes. These TLDA also contain 10 to 15 housekeeping genes for data normalization.

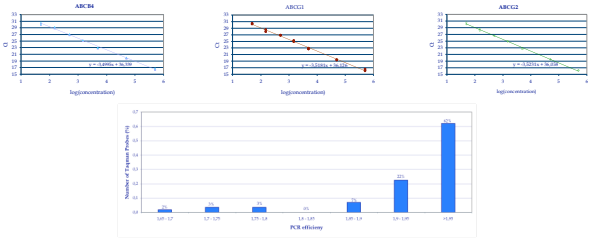






Housekeeping genes were selected with GeNorm and NormFinder software. Target genes expression was normalized using either one or the geometric mean of the 3-5 best selected genes.

QPCR efficiencies of the TaqMan probes were first controlled using the samples' standard curves, plasmid dilutions or linear regression calculations (LinRegPCR).



The relative quantification of target gene expression was calculated using the 2-ddCt method. Moreover, in the cell lines studies, the copy numbers of mRNA per cell were estimated. In order to assess these ABC transcripts numbers per cell, we used : equal threshold values, plasmid standard curves, number of cells used or number of 18S transcripts per cell.

Applications :

ABC Transporter expression in mice treated with colchicine.

Colchicine was proposed to induce ABC transporters in vivo. We have studied this hypothesis by analyzing the expression level of ABC Transporters in mice treated with colchicine.

Fig. 1 shows the expression of the ABC Transporters and other target genes. This cluster is the result of 6 000 PCR reactions (Color code : green for low expression, black for medium and red for high expression).

These experiments allowed us to measure the variations of gene expression, in lung and liver, induced by colchicine, as a function of time exposure and drug concentrations.

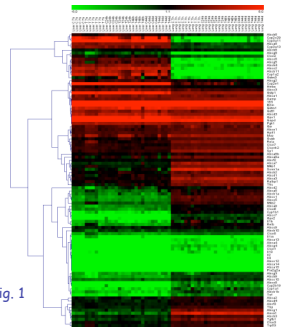


Fig. 1

ABC Transporter expression during liver development in human.

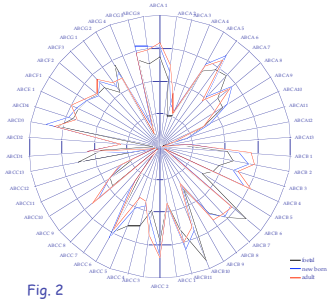


Fig. 2

We have mapped the expression of ABC transporters in human liver at different developmental stages.
Fig. 2 compares the expression level of ABC genes in fetal (dark) new-born (blue) and adult (red) livers. Circles indicate a 10 fold variation in expression.
Fig. 3/4/5 show three different examples of the observed modifications between 5 groups (from left to right : fetal, new-born 1-7 days, new-born 7-28 days, new-born 1-3 months, adult livers). Expression is constant (Fig. 2; ABCF1), increased (Fig. 3; ABCB1) or reduced (Fig. 4; ABCA7) during liver development in human. *Collaboration with T. Cresteil, ICSN.*

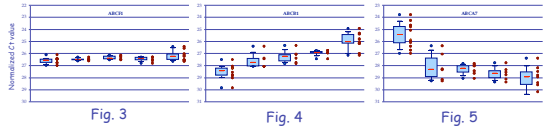
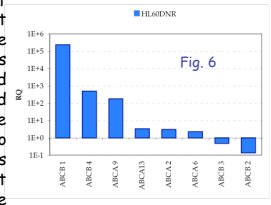


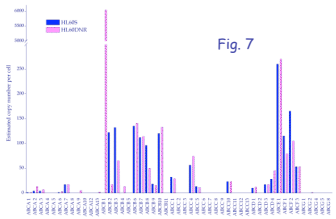
Fig. 3 Fig. 4 Fig. 5

ABC Transporter expression in human cell lines : RQ and mRNA copies per cell.

We compared the ABC expression pattern in human promyelocytic leukemia cells (HL60S) and its multidrug-resistant derivative HL60/DNR (daunorubicin). *Collaboration with J. Bignon, ICSN.*

Fig. 6 presents the relative expression of ABC transporters for the drug-resistant subline compared to the parental one. The fold change in ABC gene expression is indicated only if: 1) the observed variation is greater than a factor two, and 2) the ABC transcript level has a Ct value smaller than 30 in at least one of the two compared cell lines. These conditions exclude variations in gene expression that can be observed with very low abundance transcripts.





An estimation of the mRNA copy number per cell is represented in Fig. 7. This approximation provides a better overview of the ABC expression pattern in these cell lines. For example, ABCB2 has a low RQ value (RQ=-2) but this variation might be significant due to its high mRNA copy number in these cell lines.
Contrarily ABCB4 and ABCA9 show, respectively, RQ around 500 and 200 but less than 5 mRNA/cell. High RQ associated to only few mRNA copies must give us a warning about false-positive.