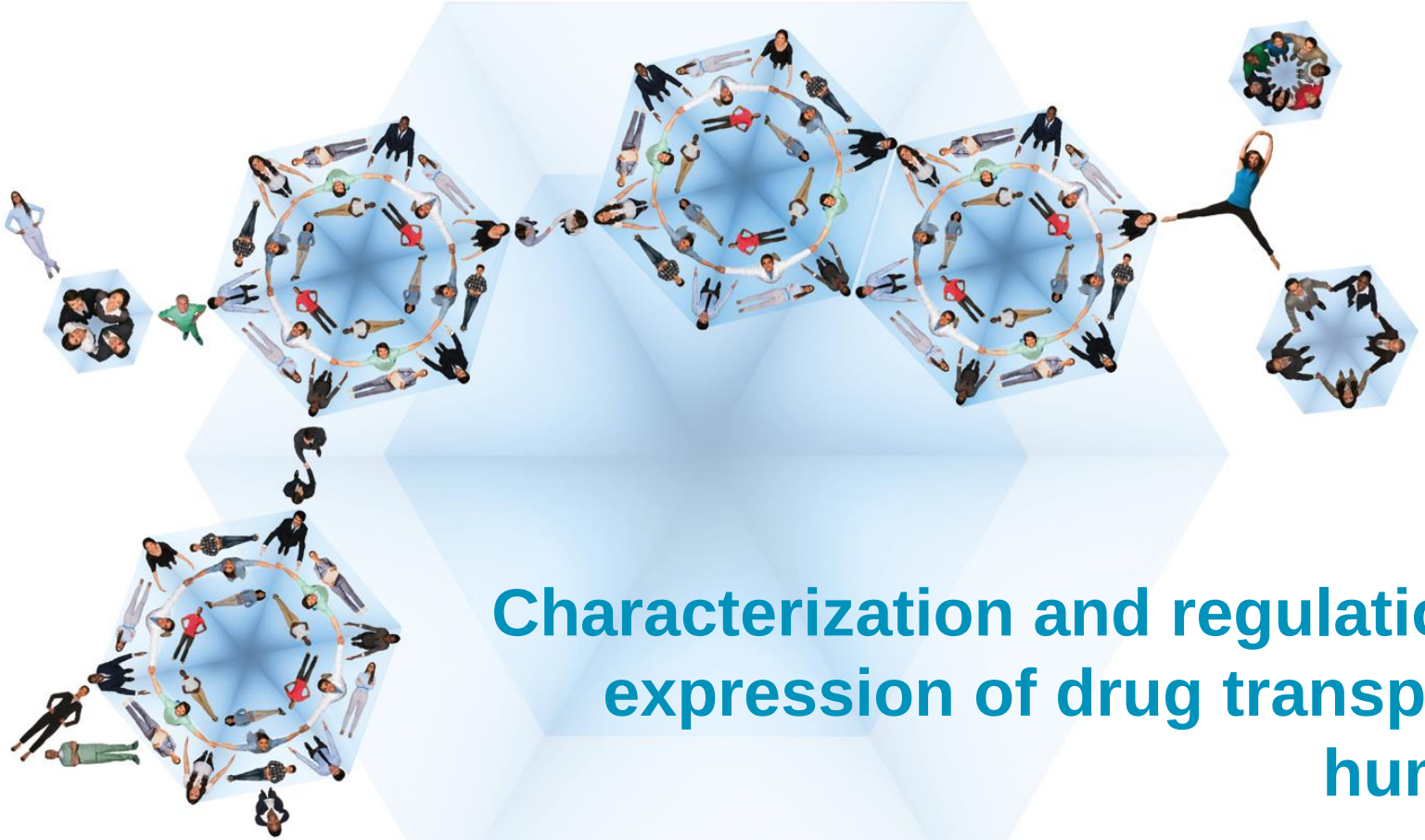

# Characterization and regulation of the expression of drug transporters in human skin

**Hanan Osman-Ponchet, PhD**

hanan.osman-ponchet@galderma.com

7th Euro-Global Summit on
Toxicology and Applied Pharmacology
Rome, Italy October 24-26, 2016

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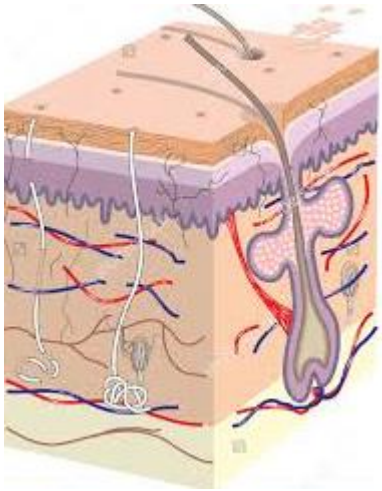
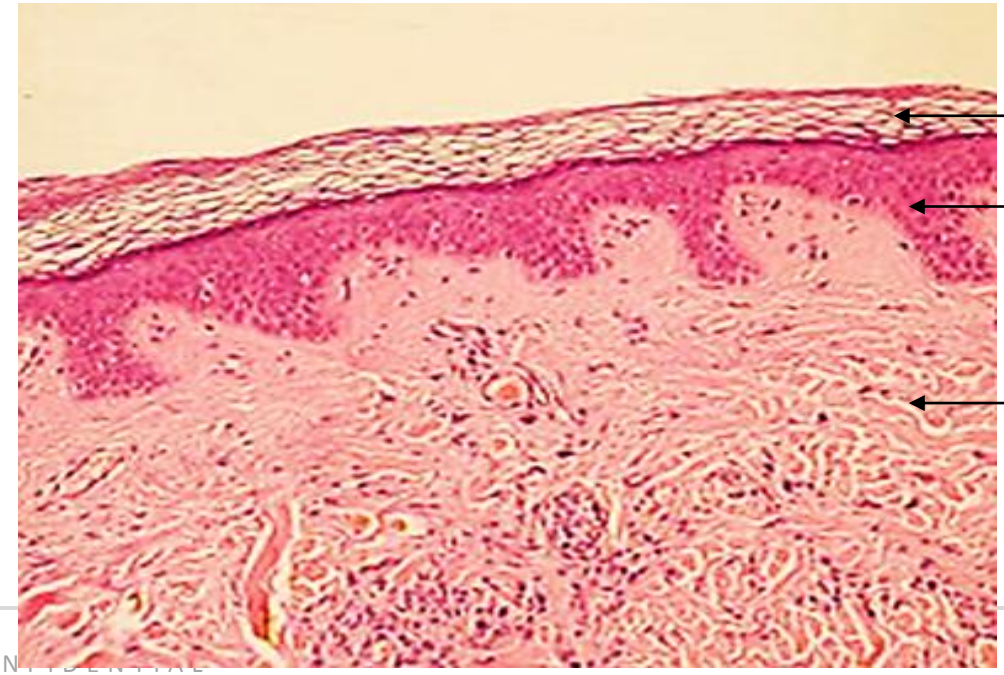
AZZALURE · Cetaphil · Clobex · Differin · Differin® · Emervel · Epiduo · Loceryl · Macrolane · metrogel 1% · metvix · Oracea · Restylane · Rozex® (metronidazole) · Silkis (calcitriol) · Tri-Luma Cream · Vectical

Committed to the future of dermatology
Science-based solutions for the skin, hair and nails

# Human skin

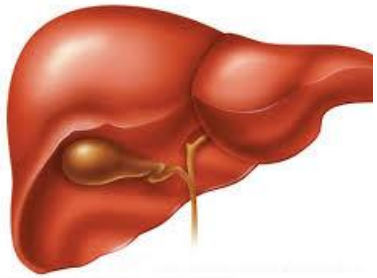
- Skin is the largest organ of the body
  - 2 $m^2$ surface area
  - 0.5 – 4 mm thickness
  - 16% body weight
- Skin plays a crucial role in body protection from:
  - damage, infection, and drying out

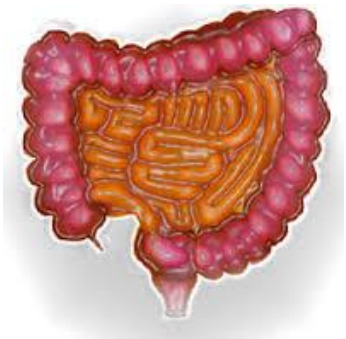
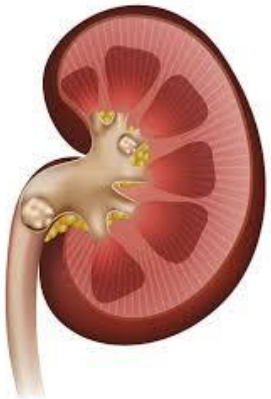


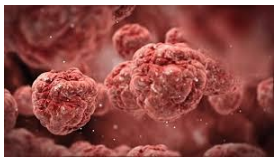


# Drug transporters

- Drug transporters are well characterized in many key tissues but very little is known about the skin



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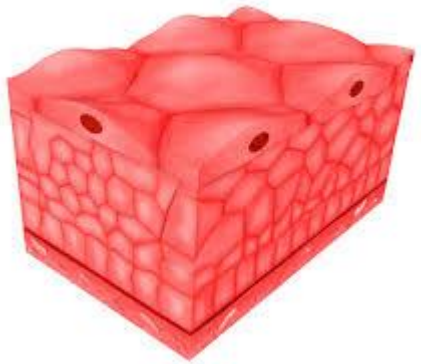


**SKIN**

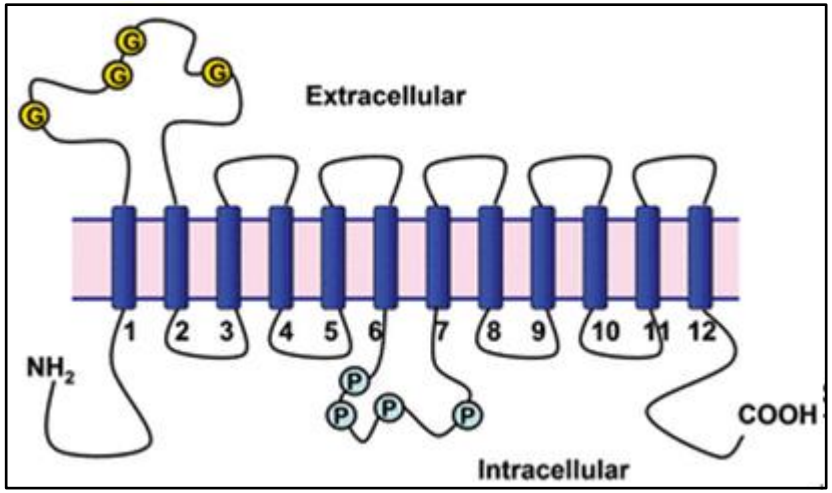
# Outline

- General overview of drug transporters
  - ABC and SLC transporters
  - Regulatory perspectives
- Characterization of drug transporters in human skin
  - Expression
  - Regulation
  - Localisation
  - Function
- Conclusion

# Drug transporters

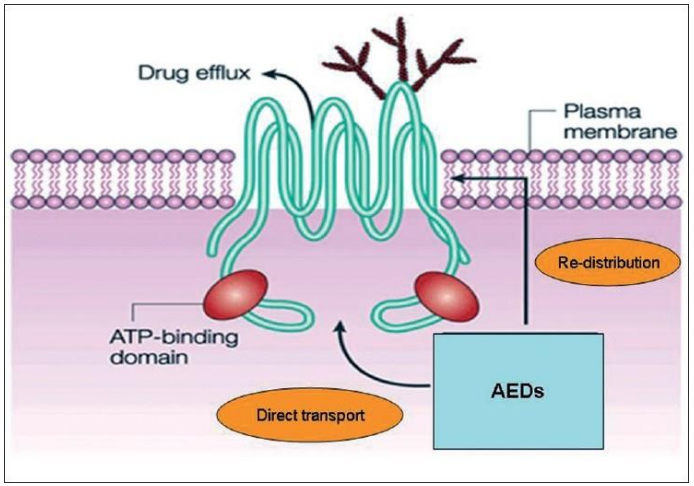
- Most identified drug transporters belong to 2 superfamilies:
  - ATP-Binding Cassette (ABC) → Drug Efflux (Out)
  - Solute Carrier (SLC) → Drug Uptake (In)

SLC




K. Sanjay et al; Physiological Reviews (2015)

ABC

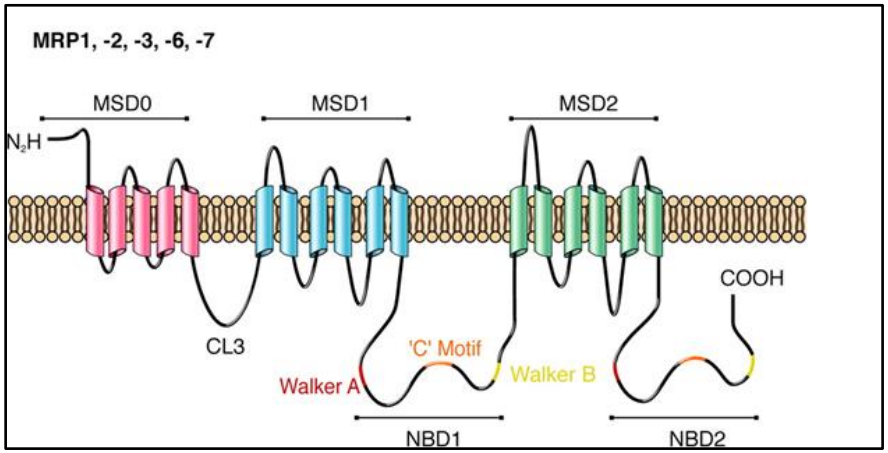



A. Das et al; Indian J Human Genetics (2011)

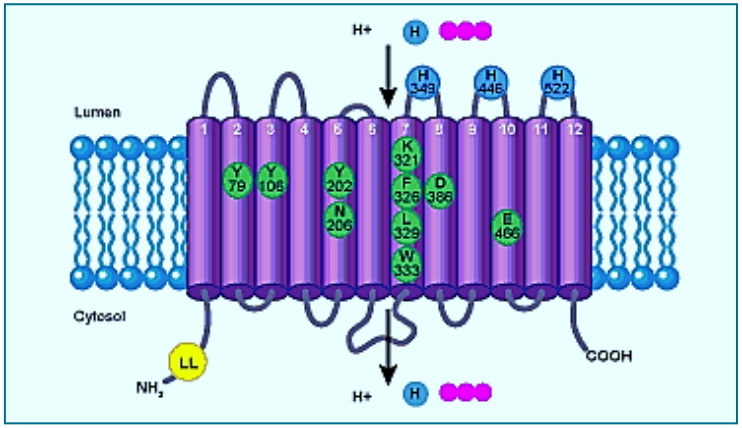
# Drug transporter Superfamily

- 49 human ABC genes grouped into seven subfamilies:
  - ABCB1 / MDR1 (Multi-drug resistance)
  - ABCC1 & 2 / MRP1 & 2 (Multidrug resistance-associated protein)
  - ABCG2/ BCRP (Breast cancer resistance protein)

- 386 SLC human genes grouped into 52 families
  - SLCO: Organic anion transporting polypeptide
  - SLC22: Organic anion/cation/zwitterion transporter
  - SLC47: Multidrug and toxin extrusion (MATE)



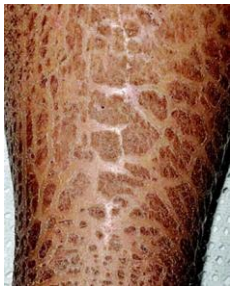

RG. Deeley et al., Physiological Reviews (2015)




Mutagenetix Labarchives

# ABC transporters and genetic diseases

- In humans, 15 severe genetic diseases are caused by the dysfunction of ABC transporters:
  - ABCA1 : Tangier disease
  - ABCA12 : Harlequin-type ichthyosis, Lamellar ichthyosis
  - ABCB4 : Progressive familial intrahepatic cholestasis
  - ABCC2 : Dubin–Johnson syndrome
  - ABCC6 : Pseudoxanthoma elasticum
  - ABCC7 : Cystic fibrosis



- Genetic polymorphisms (SNP, haplotypes) identified, with clinically relevance in drug pharmacokinetics

# Role of ABC and SLC transporters

- Tissue Distribution:
  - Highly abundant in the intestine, liver, kidney,
- Physiological role:
  - Transport of lipids, steroid conjugates, thyroid hormones, bile salts
- Drug disposition
  - Significantly modulate the absorption, distribution and elimination
  - Efficacy and toxicity of pharmacological agents
- Genetic variation contributes to inter-individual pharmacokinetic and pharmacodynamic variability
- Mediate drug-drug interactions

# Regulatory perspectives

- Due to contribution of drug transporters to drug-drug interactions, European and US regulatory agencies require evaluation of drug transporters for the substrate and inhibition potential of drug candidates.
- FDA (Guidance 2012):
  - 7 key transporters: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2
- EMA (Guidance 2013)
  - 9 key transporters: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2 and OCT1 (and BSEP)
- Very recently, evaluation of MATE transporters is required by the regulatory agencies.

# Regulatory perspectives

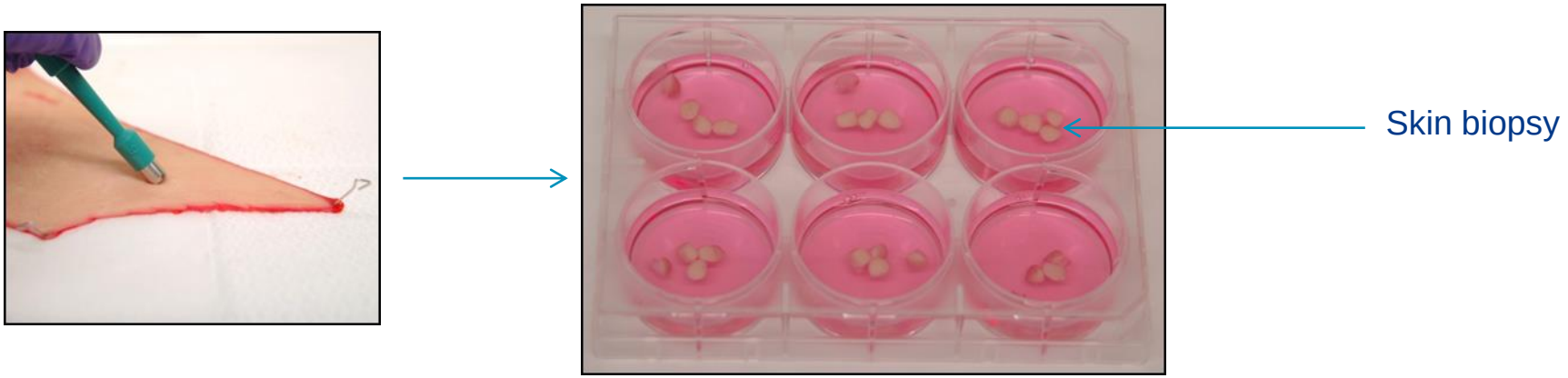
- ABC and SLC transporters are well characterized in liver, kidney and intestine but little is known about skin

- Objective of this work was the characterization of drug transporters in human skin :
  - Expression
  - Regulation
  - Localization
  - Role in drug disposition

- In order to meet regulatory agencies requirement for topically applied drugs, and to improve PBPK modeling

# Expression of ABC and SLC transporters in human skin

- Human skin biopsies in organ-culture for 3 days




- 4 skin biopsies (6 mm diameter) per well of 6-well plates.
- Culture medium: Long term skin culture medium (Biopredic, France).
- RNA extraction followed by quantitative real time RT-PCR (TaqMan technology).
- GAPDH gene used as housekeeping gene

# Expression of ABC and SLC transporters in human skin

- 11 SLC and 4 ABC transporters genes were evaluated
- Expression levels measured in human skin, and compared with human liver and kidney.

## SLC transporter genes

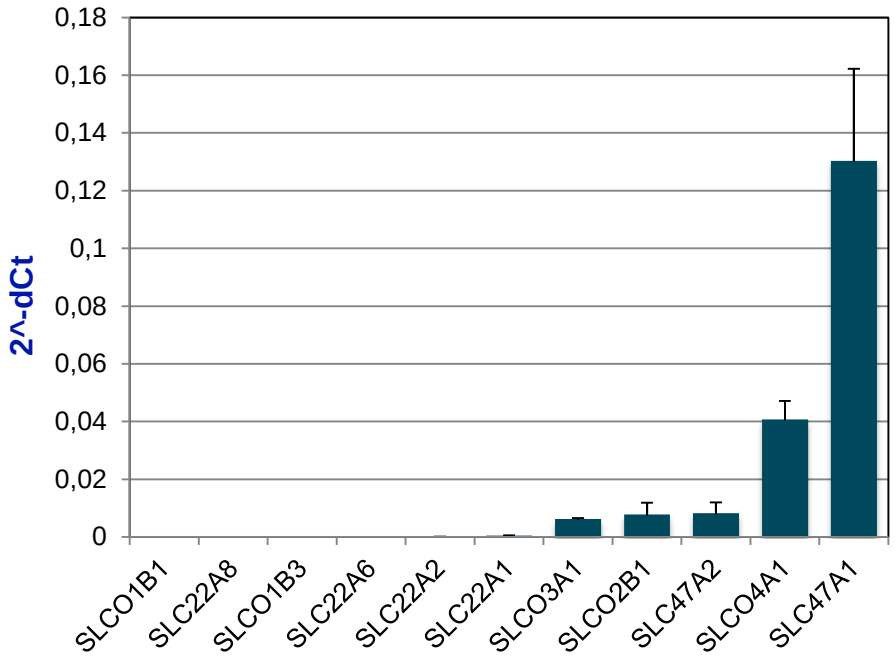
| Gene | Transporter |
|---|---|
| SLCO1B1 | OATP1B1 |
| SLCO1B3 | OATP1B3 |
| SLCO2B1 | OATPB |
| SLCO3A1 | OATPD |
| SLCO4A1 | OATPE |
| SLC22A1 | OCT1 |
| SLC22A2 | OCT2 |
| SLC22A6 | OAT1 |
| SLC22A8 | OAT3 |
| SLC47A1 | MATE1 |
| SLC47A2 | MATE2 |

## ABC transporter genes

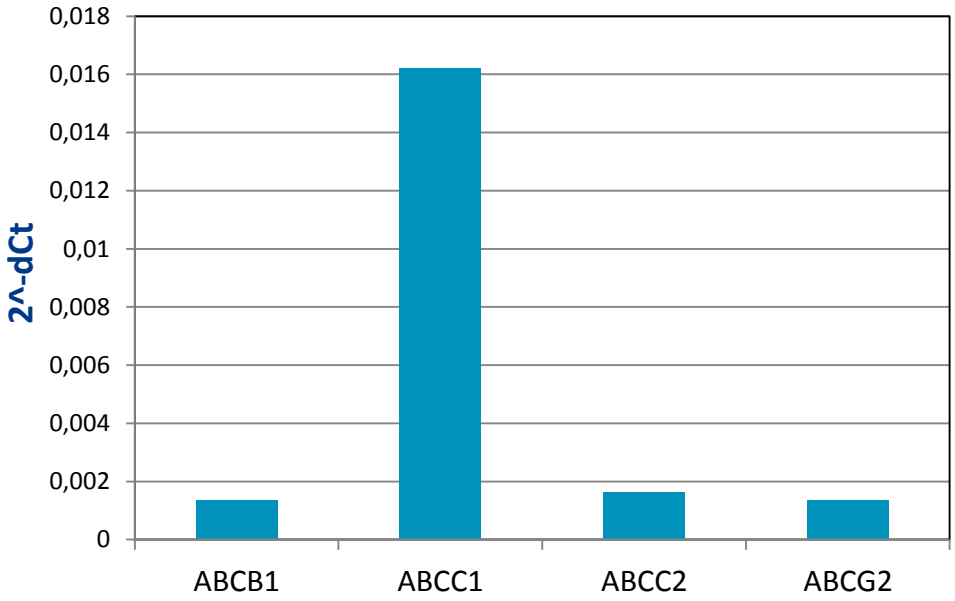
| Gene | Transporter |
|---|---|
| ABCB1 | MDR1 |
| ABCB1 | MRP1 |
| ABCC2 | MRP2 |
| ABCG2 | BCRP |

# Expression of ABC and SLC transporters in human skin

SLC transporters
in human skin
(N = 3 donors)

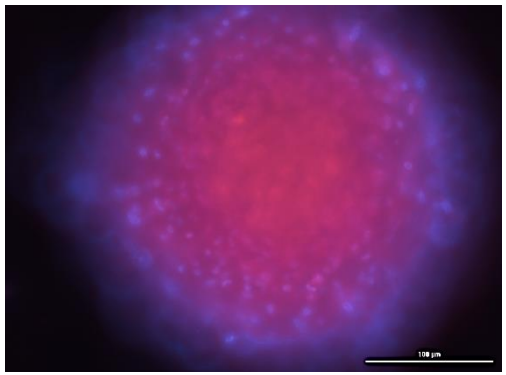


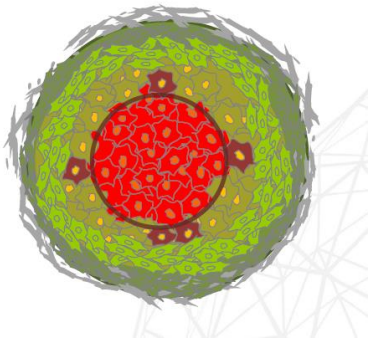

ABC transporters
in human skin
(N = 8 donors)

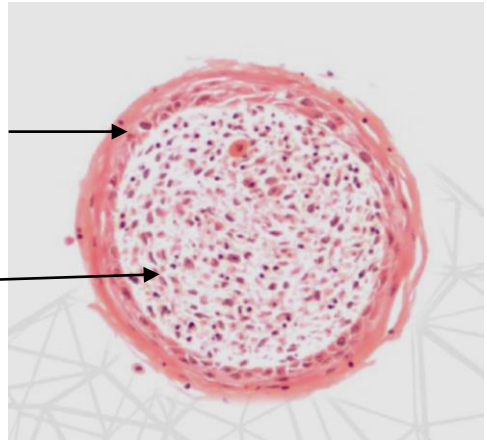


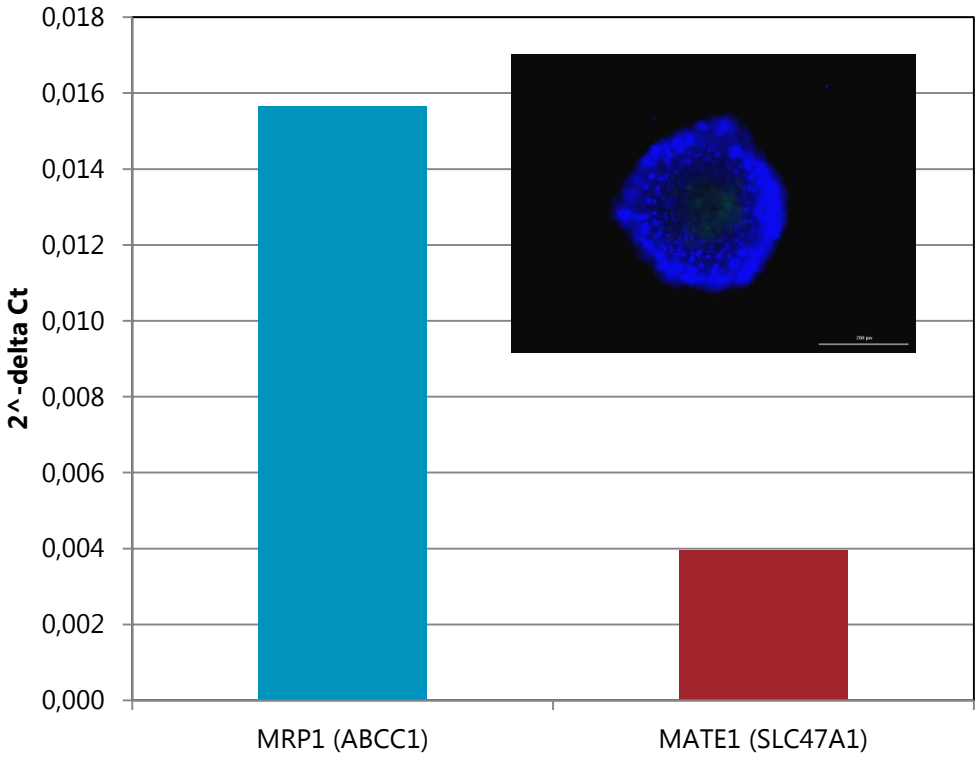

- 5 SLC transporters over 11 are expressed in human skin, MATE1 (SLC471) is the most abundant.
- All the 4 ABC transporters are expressed in human skin with MRP1 (ABCC1) is the most expressed.

# Expression of ABC and SLC transporters in 3D human skin microtissue



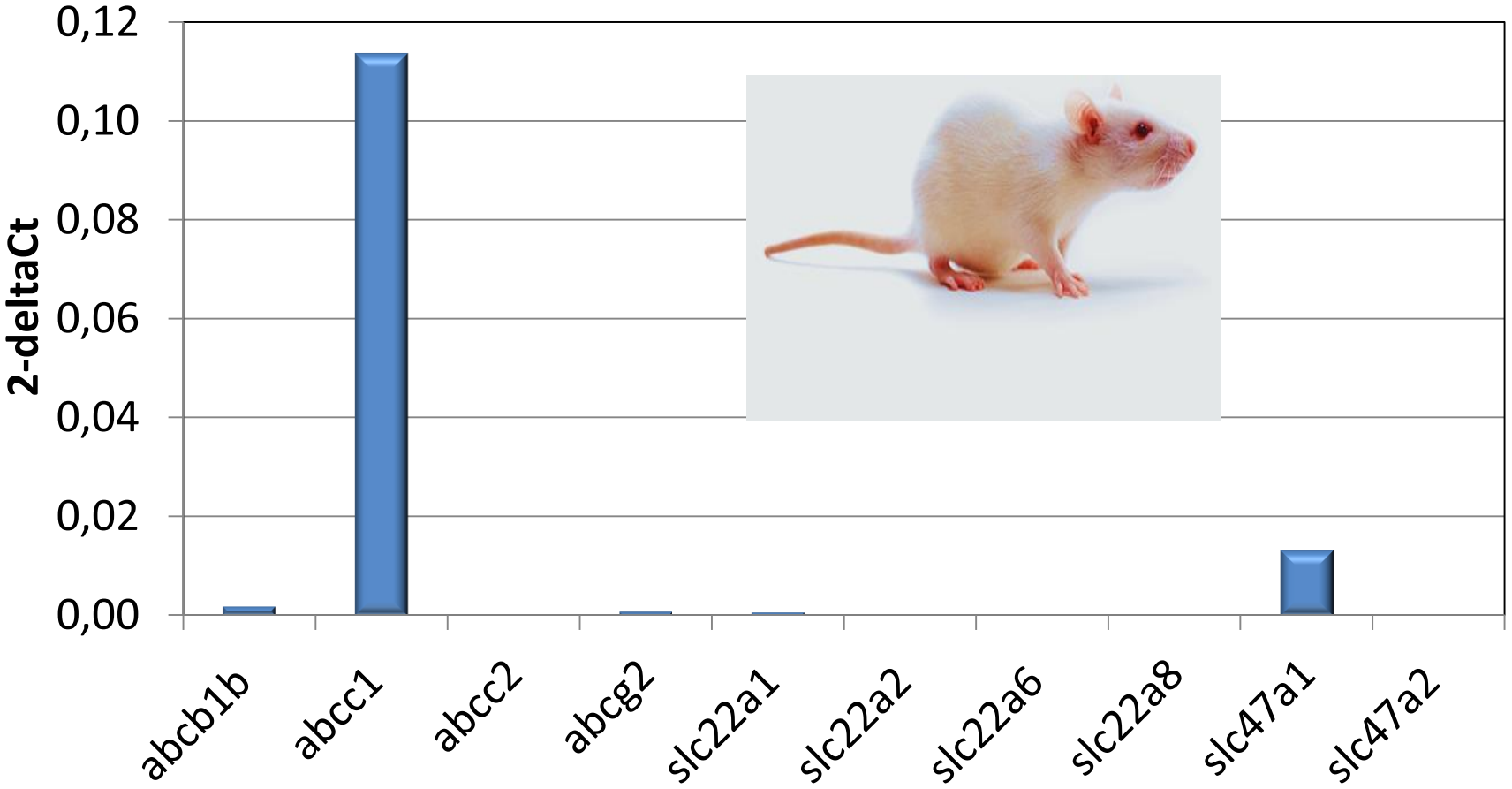




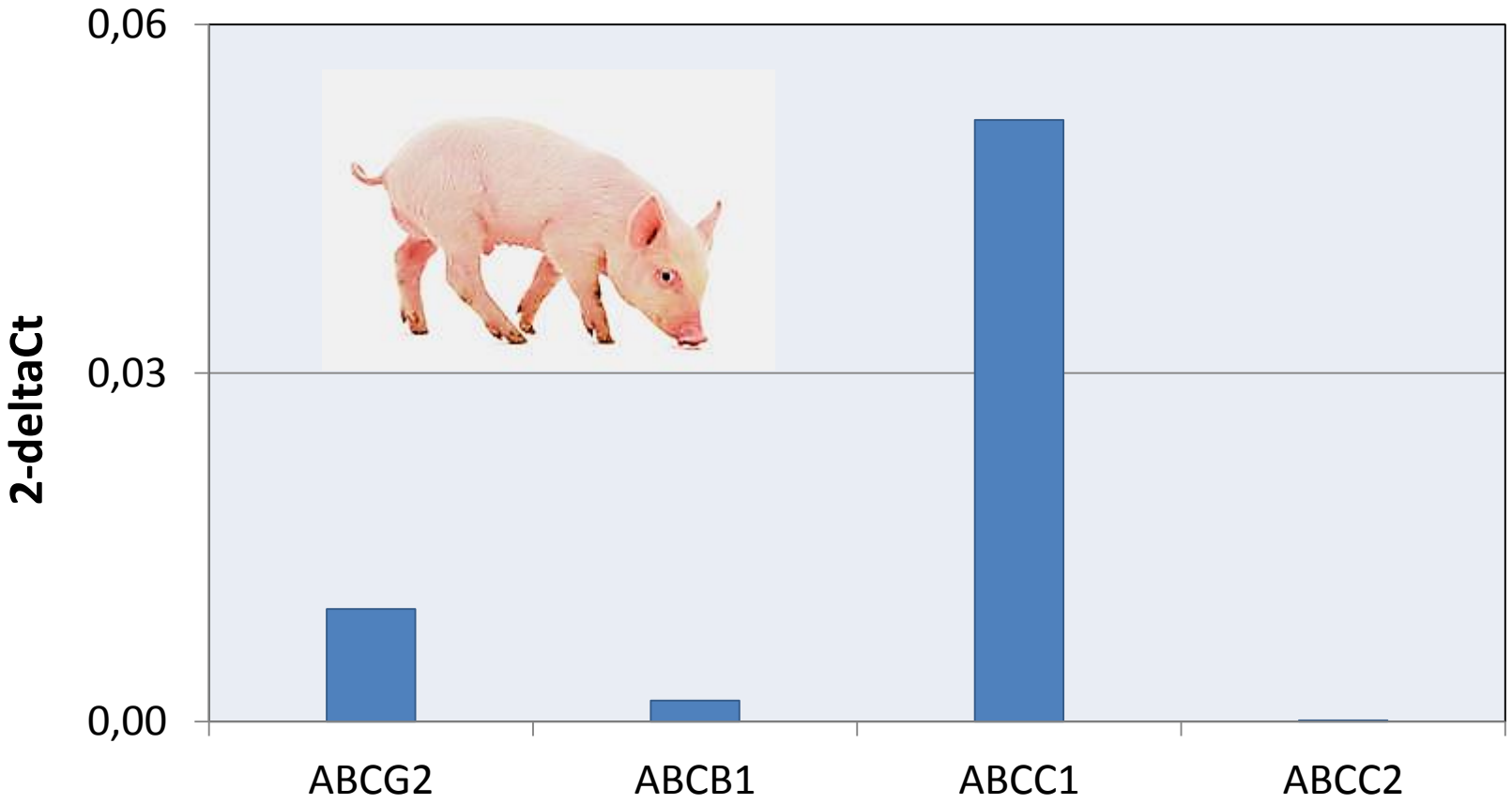

- MRP1 and MATE1 are expressed in 3D Human skin microtissue

# Expression of ABC and SLC transporters in Rat skin




- MRP1 and MATE1 are expressed in Rat skin

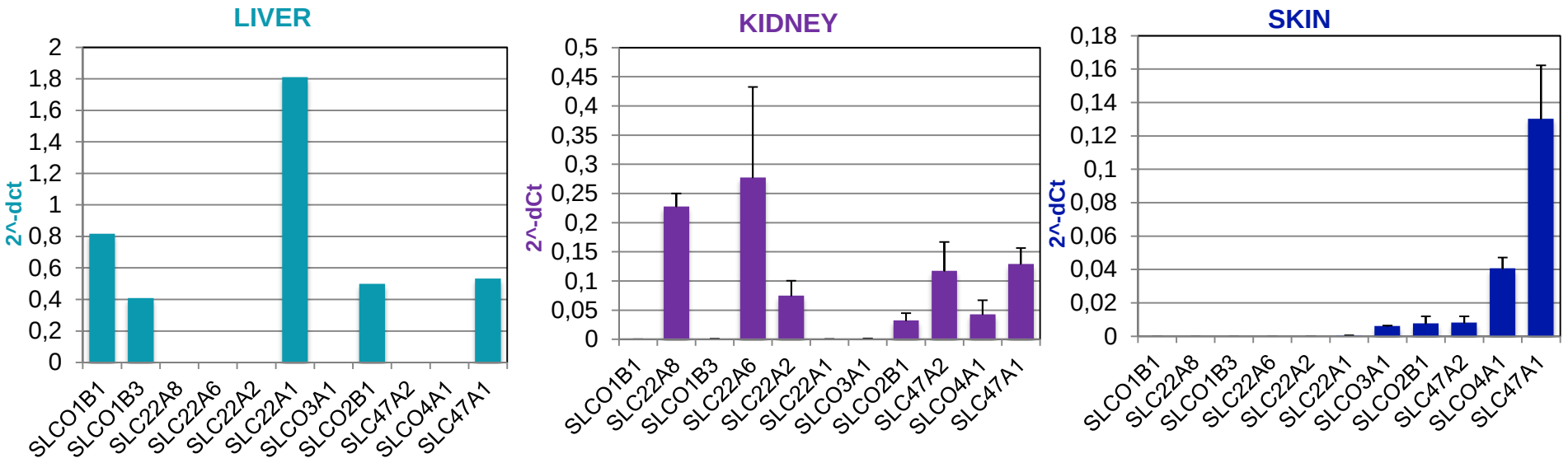
# Expression of ABC and SLC transporters in Minipig skin




- MRP1 is expressed in Minipig skin
- SLC transporters not detected in minipig skin.

# Expression of SLC transporters in different tissues in Human

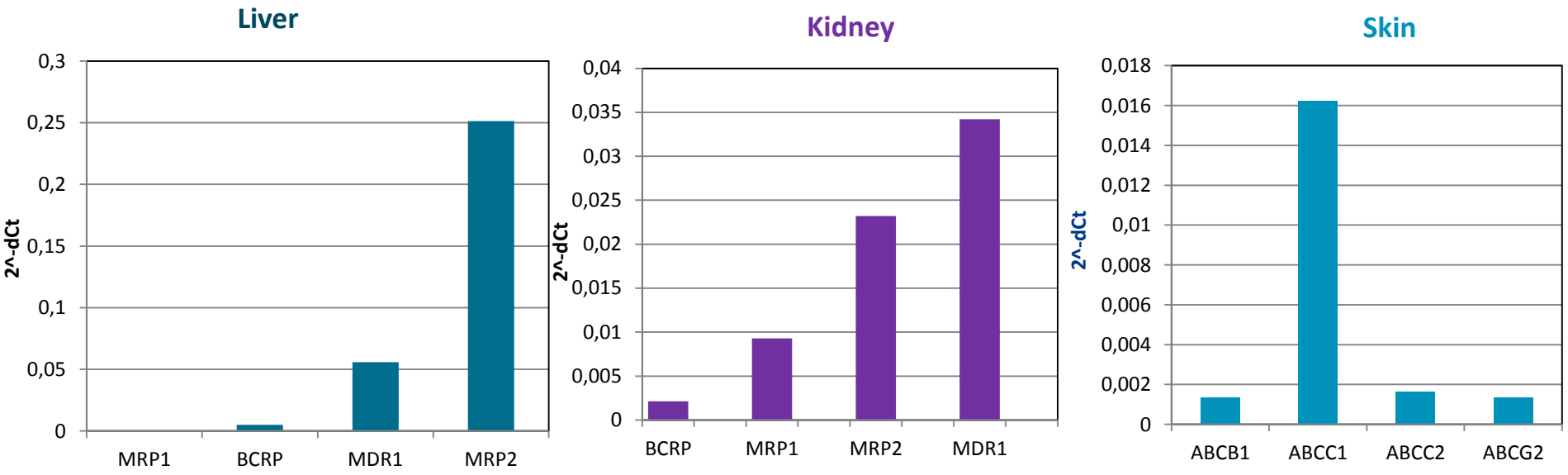
## Comparison of SLC transporters in Skin, Liver and kidney




- Expression profile very different according to tissue
  - SLC47A1 (MATE1) most expressed in skin
  - SLC22A1 (OCT1)  most expressed in liver
  - SLC22A6 (OAT1) most expressed in kidney

# Expression of ABC transporters in different tissues in Human

## Comparison of ABC transporters in Skin, Liver and kidney

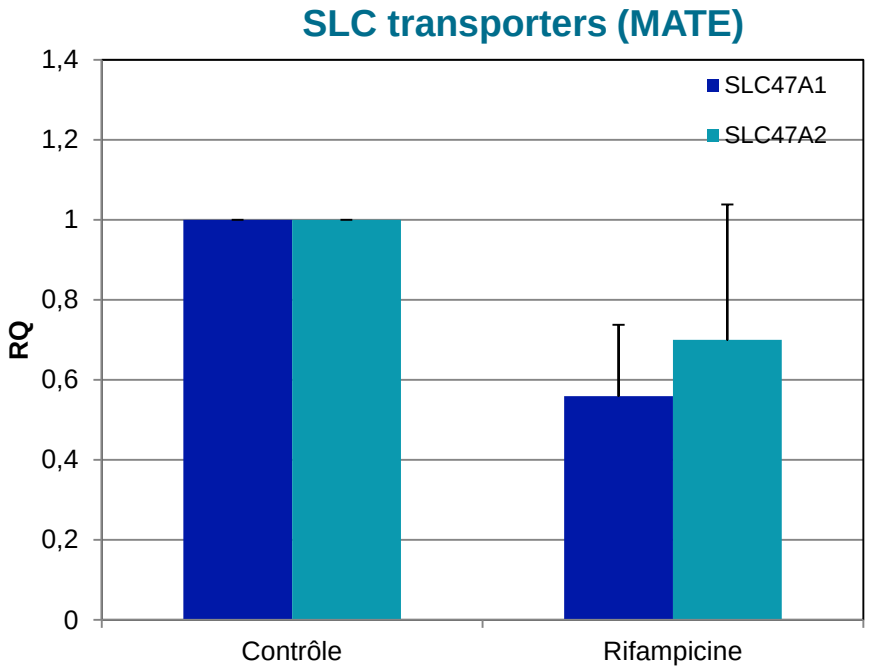


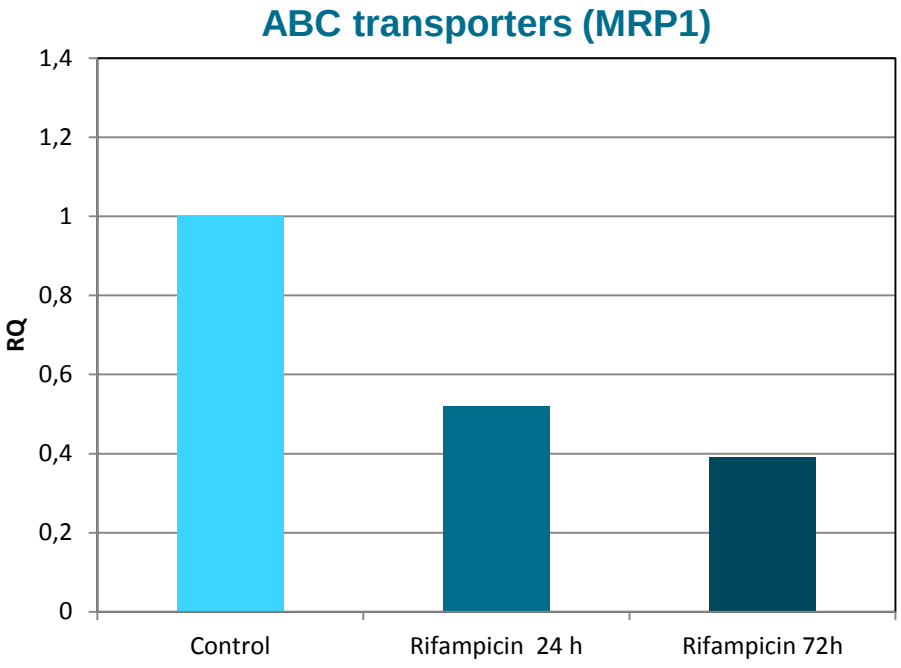

- Expression profile of ABC transporters very different according to tissue
  - ABCC1 (MRP1) most expressed in skin
  - ABCC2 (MRP2) most expressed in liver
  - ABCB1 (MDR1) most expressed in kidney

# Regulation of ABC and SLC transporters in human skin

## Effect of Rifampicin on ABC and SLC transporters in Skin

Rifampicin: 50 µM during 72 h
Human skin biopsies in organ-culture
N = 2 or 3 donors



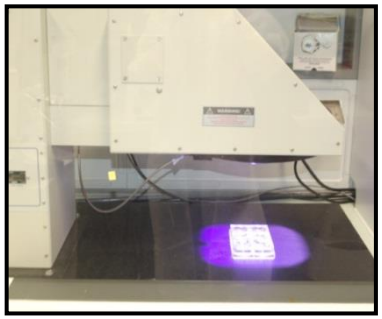




- Rifampicin markedly decreases expression of MATE and MRP1 transporters in human skin.

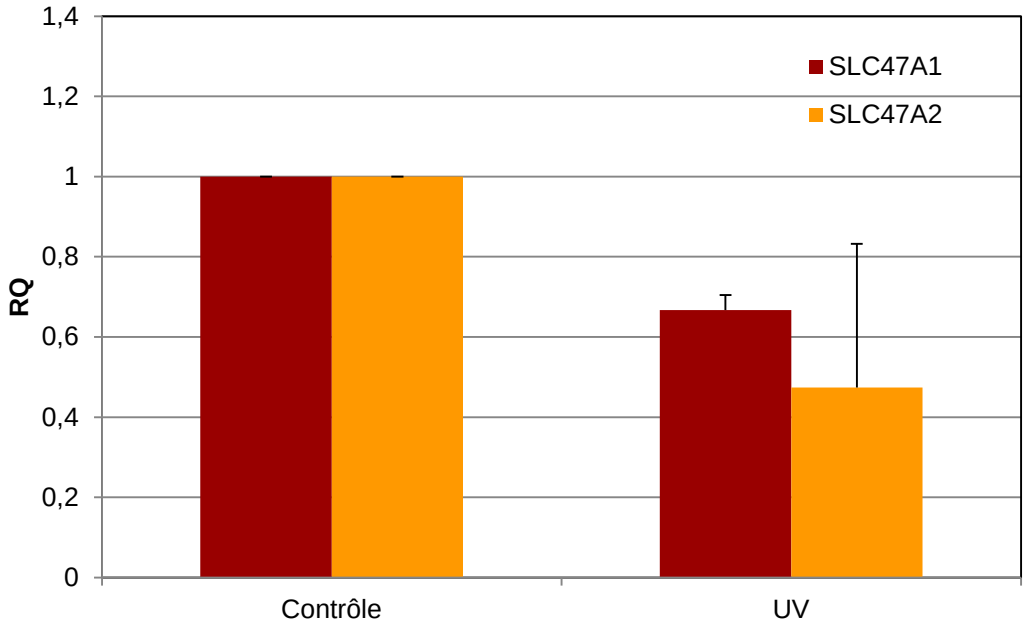
# Regulation of ABC and SLC transporters in human skin

## Effect of UV irradiation on MATE transporters

Human skin biopsies in organoculture
N = 2 donors
Solar simulator
Irradiation during 1 hour per day for 3 days.



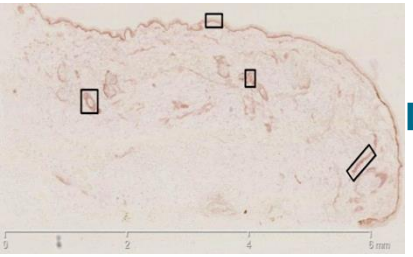
Solar simulator
(830W ; 47.8A;
UVA 110W/m²
UVB: 20W/m²)



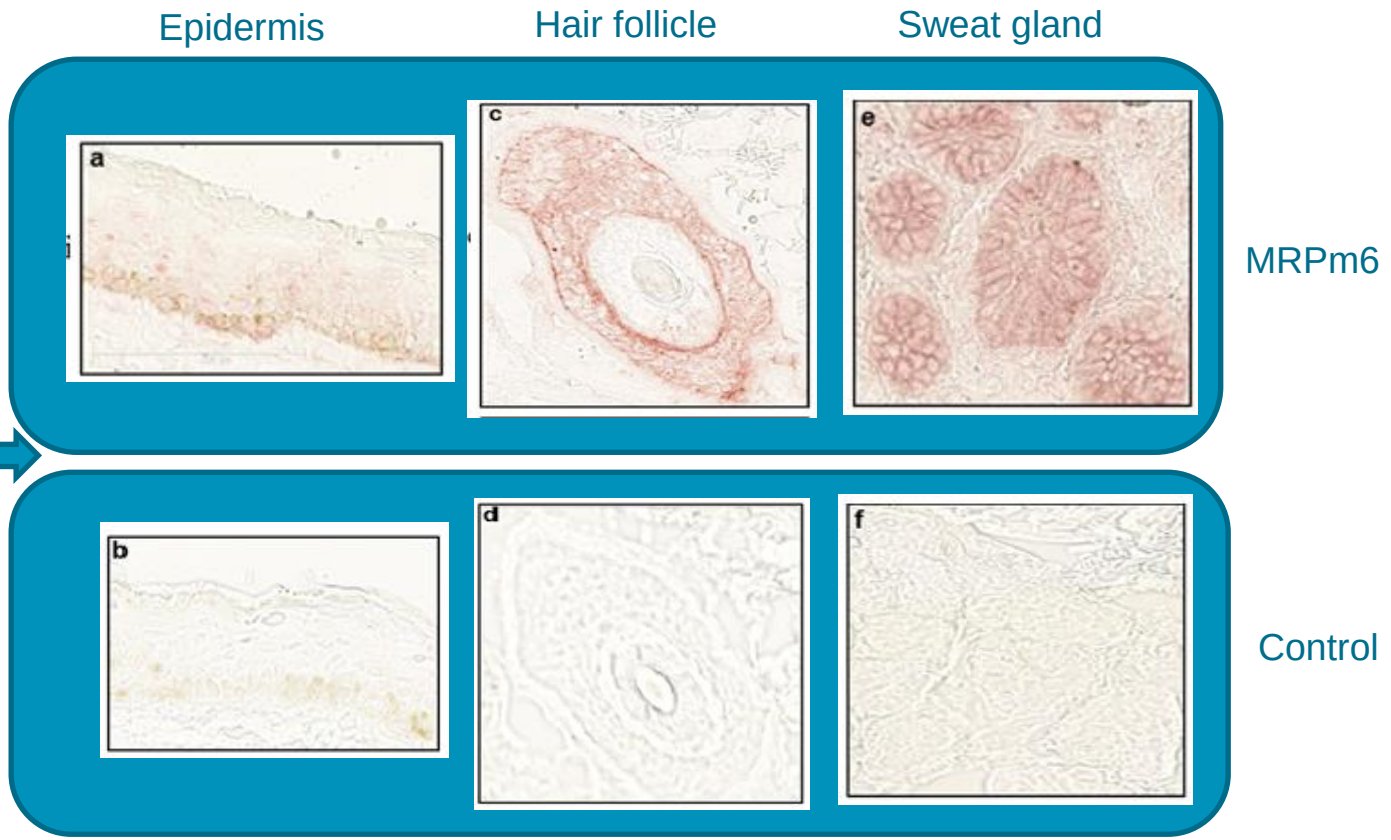

- UV irradiation markedly decreases expression of MATE1 and MATE2 transporters in human skin.

# Localization of MRP1 in human skin

- Immunohistochemical analysis of MRP1 in human skin
- Cryosection of skin, and MRPm6 monoclonal antibody.



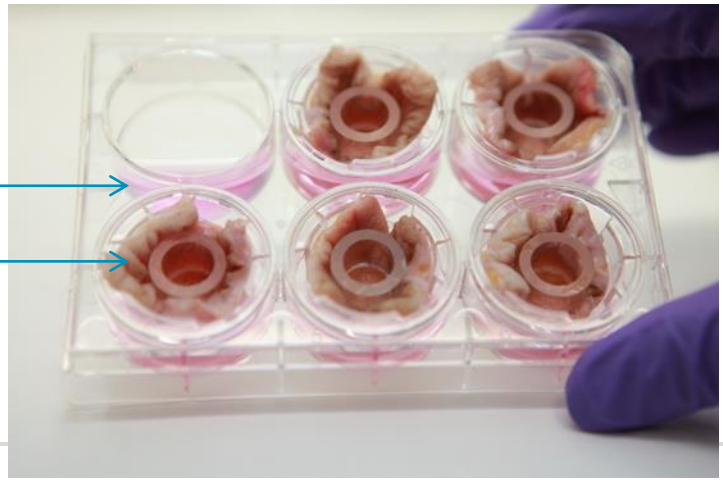
*Human skin cryosection*




- MRP1 is mainly localized in the hair follicle and sweat gland in the dermis

# Role of MRP1 transporter in drug absorption in the skin

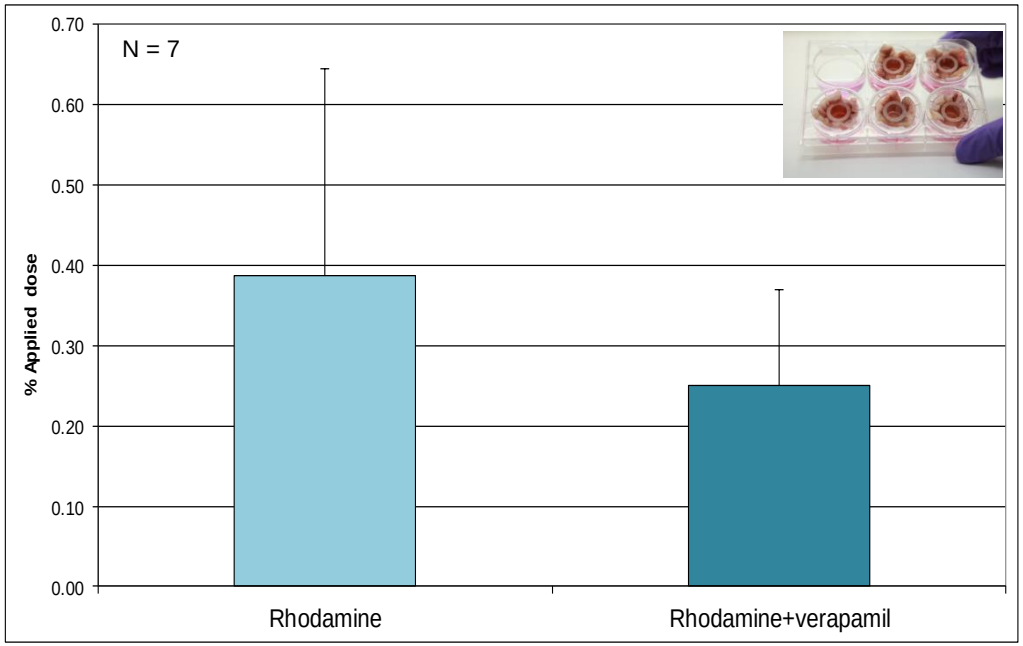
- Skin absorption and distribution of 3 couples of MRP1 substrates and inhibitors were evaluated:
  - Rhodamine 123 / Verapamil
  - [$^3$H]-Vinblastine / Verapamil
  - [$^3$H]-LTC4 / MK571
- *In vitro* model for skin absorption
  - Donor compartment (skin) on Transwell porous membrane
  - Receptor compartment (culture medium)



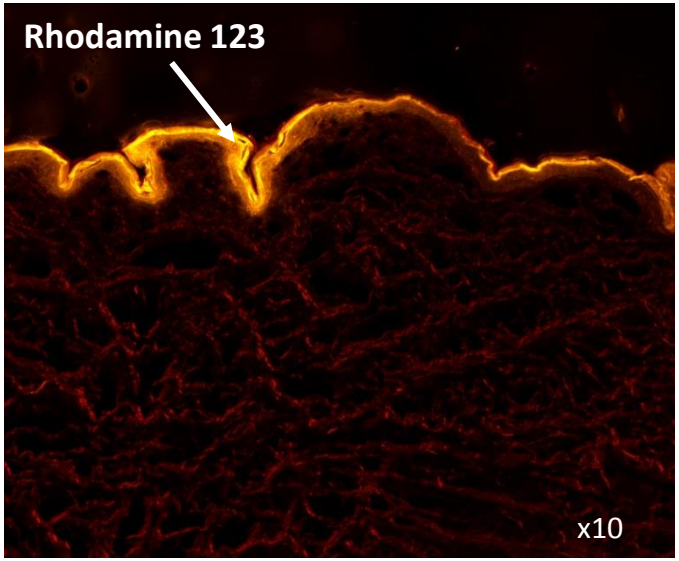

*In vitro* skin absorption model

# Skin absorption of Rhodamine 123

## Skin absorption



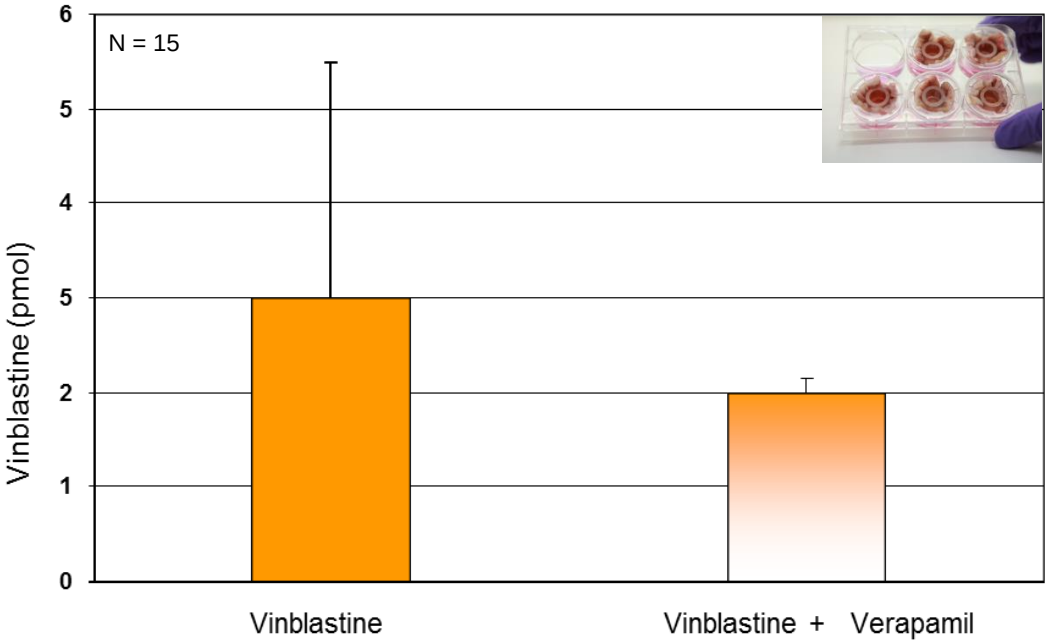

## Skin distribution
(Epifluorescent microscopy)



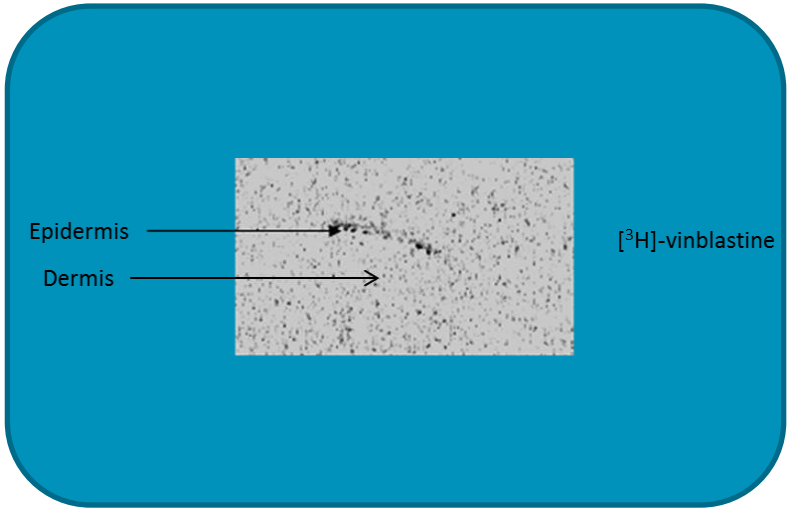

- Inhibition of MRP1 by verapamil significantly decreases skin absorption of Rhodamine 123.
- Rhodamine 123 mainly distributed in the epidermis.

# Skin absorption of Vinblastine

## Skin absorption



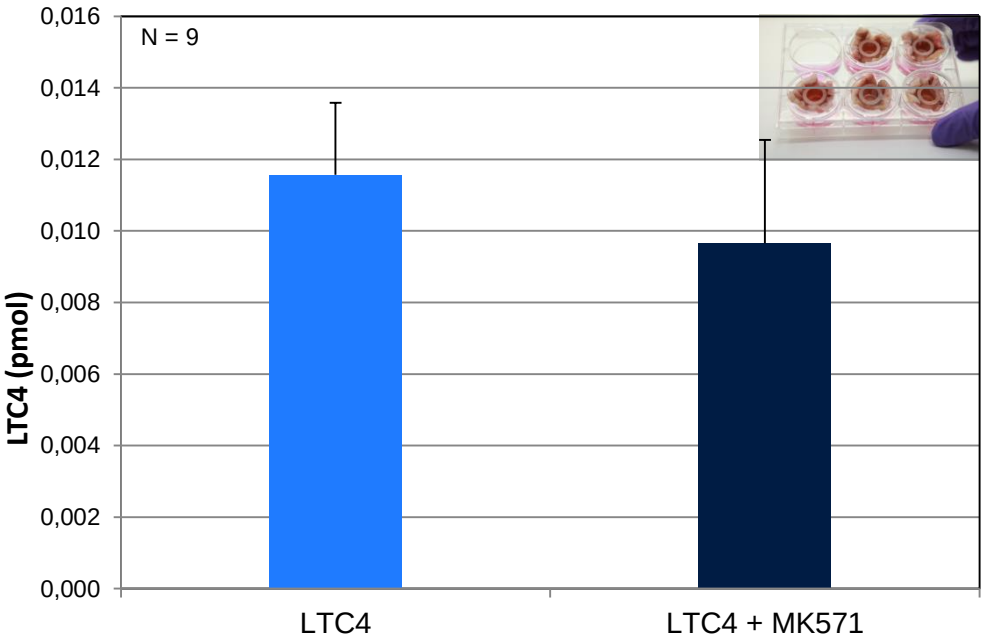

## Skin distribution (Autoradiography)



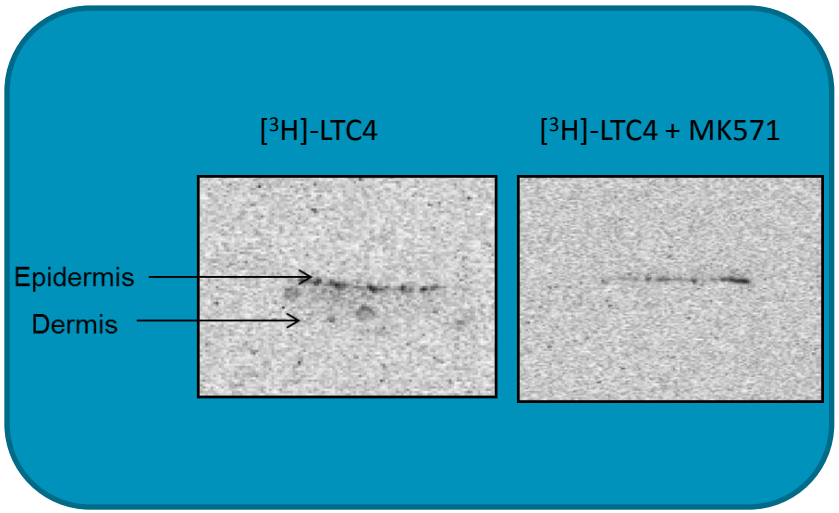

- Inhibition of MRP1 by verapamil significantly decreases skin absorption of vinblastine.
- Vinblastine mainly distributed in the epidermis

# Skin absorption of LTC4

## Skin absorption




## Skin distribution (Autoradiography)




- Inhibition of MRP1 by MK571 significantly decreases skin absorption of LTC4.
- Vinblastine distributed in the epidermis and the dermis.

# Conclusions

- Gene expression, localisation and functional studies clearly show that MRP1 plays a role in drug absorption in human skin
- Role and localization of of MATE1 to be clarified.
- Further studies needed to clarify the role of drug transporters in drug disposition and clinical drug-drug interactions with topically applied drugs.

# Thank you

# Thanks to my team

- Alexandre Gaborit
- Karine Sevin
- Anaïs Boulai
- Marion Alriquet
- Magali Kouidhi

  - ***2 publications,***
  - ***3 patents,***
  - ***3 posters***
  - ***3 oral presntations***