

Webinar around the Special Issue of Pharmaceutics  
**“Drug Delivery Across Physiological Barriers”**

**Effect of ultraviolet radiation on the expression of drug transporters in *in vitro* skin models**

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PKDERM

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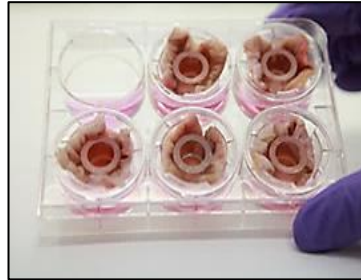


# PKDERM company

- PKDERM is a French company based in Grasse, known as the world's perfume capital
- PKDERM provides smart innovative *in vitro* solutions to evaluate the safety and efficacy of products likely to come into contact with the skin
- Area of our partners: pharmaceuticals, dermatology, cosmetics, chemistry, biocides, and medical devices

[www.pkderm.com](http://www.pkderm.com)

<https://www.linkedin.com/company/40768033/admin/>



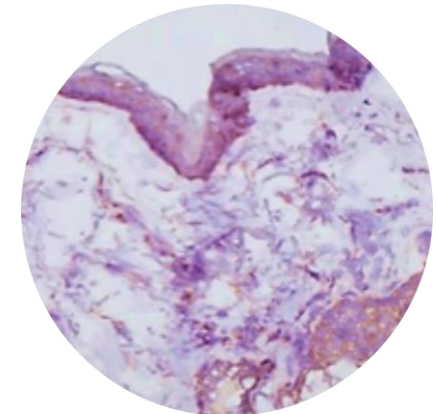
- Expertise & Services in dermal efficacy and safety testing
  - *In vitro* Efficacy
    - » Dermal absorption
    - » Anti-inflammation, Pigmentation
    - » Healing, Skin ageing
  - *In vitro* Safety
    - » Irritation, Cytotoxicity & Phototoxicity
    - » Sensitization (SenzaGen)
    - » Genetic toxicology (GenEvolutionN)
    - » Endocrine properties (WatchFrog)
- Available Biological Models
  - » Excised human skin
  - » 3D human skin equivalent
  - » 2D human skin cells (Keratinocytes, fibroblasts, melanocytes)
  - » 2D and 3D human hepatocytes
  - » 2D and 3D human lung models



# Outline

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- General overview of drug transporters
  - ABC and SLC transporters
  - Regulatory perspectives
- Characterization of drug transporters in human skin
  - Expression and function
    - 3D skin models and *ex vivo* human skin
- Effects of UVs on the expression of drug transporters
  - Normal human epidermal keratinocytes
  - Normal human dermal fibroblasts
- Conclusions and perspectives



# Drug transporters families

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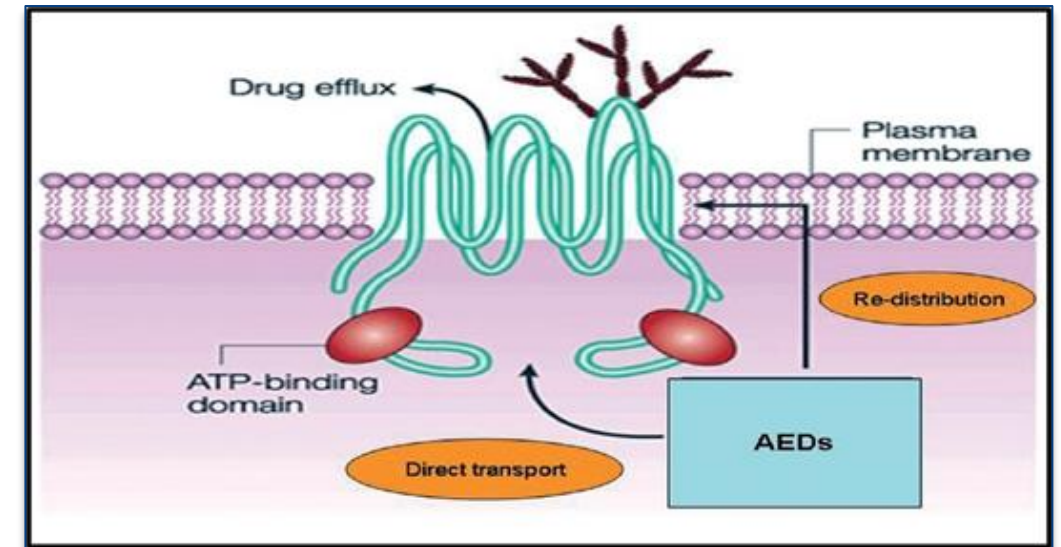
- ATP-binding cassette (ABC)
  - Efflux transporters, secretory transporters (Out)
- Solute carrier (SLC)
  - Influx transporters, absorptive transporters (IN)
- Drug transporters are involved in drug absorption, tissue distribution, excretion, and efficacy and toxicity

# ABC transporters

## ATP-binding cassette (ABC)

- **Drug efflux (Out)**

- ABCB1:
  - MDR1 (Multi-drug resistance) or P-gp
- ABCC1/2:
  - MRP1 (Multidrug resistance-associated protein)
  - MRP2 (Multidrug resistance-associated protein)
- ABCG2:
  - BCRP (Breast cancer resistance protein)



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

# SLC transporters

## Solute Carrier (SLC)

- **Drug uptake (IN)**

- SLCO:

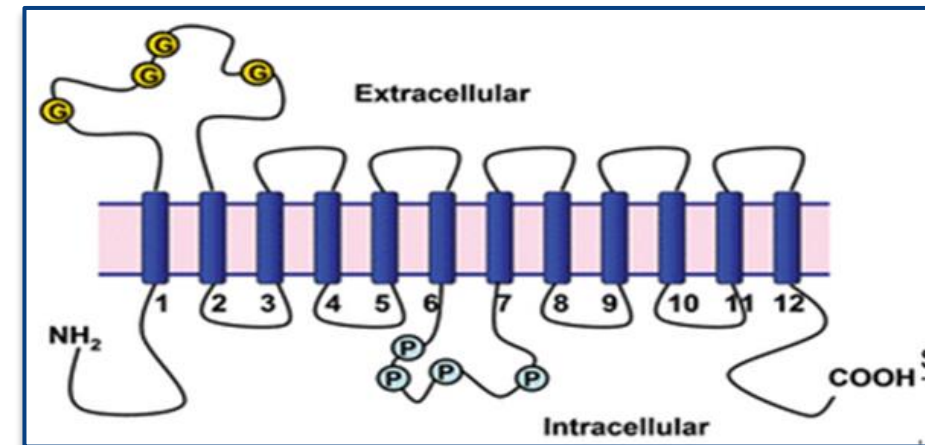
- OATP1B1/3 (organic anion transporting polypeptide) Liver

- SLC22:

- OCT1/2 (organic cation T) Kidney
- OAT1/3 (organic anion T) Kidney

- SLC47:

- MATE1/2-K (multidrug and toxin extrusion) Kidney, liver and skin



K. Sanjay et al; Physiological Reviews (2015)

# Role of drug transporters

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- **Physiological role**
  - Transport of glucose, lipids, creatinine, steroid conjugates, thyroid hormones, bile salts
- **Tissue distribution – drug disposition:**
  - Highly abundant in the gastrointestinal tract, liver, kidney, brain, skin
  - Significantly modulate the absorption, distribution and elimination
  - Efficacy and toxicity of pharmacological agents
- **Mediating drug interactions**
  - Result from altered tissue distribution of a drug that is a substrate of a transporter. May not be apparent by measuring systemic drug exposures

*Giacomini, Huang, et al. 2010; Brouwer, Keppler, et al. 2013; Giacomini and Huang 2013; Tweedie, Polli, et al. 2013; Zamek-Gliszczynski, Lee, et al. 2013.*

# Regulatory perspectives

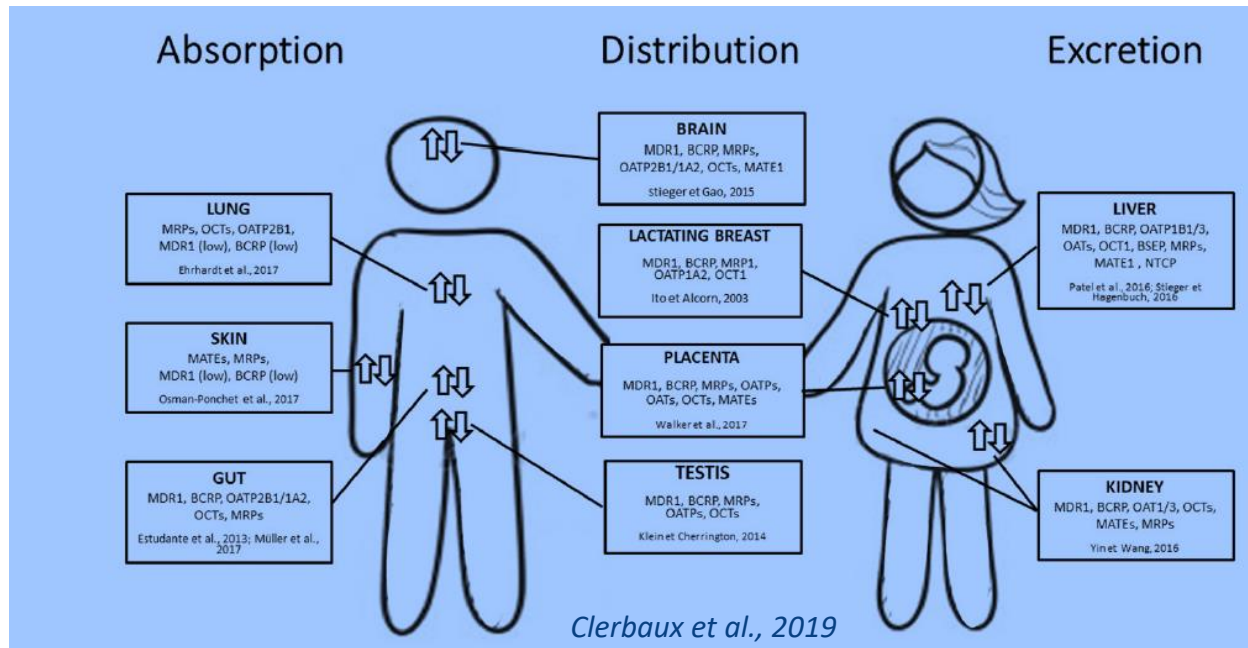
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- Due to contribution of drug transporters to drug-drug interactions, regulatory agencies require evaluation of key drug transporters during drug development
  - FDA (Draft Guidance October 2017):
  - EMA (Guidance 2013)
  - Japanese PMDA (Draft Guidance 2014)
- Key transporters:
  - MDR1 (P-gp), BCRP
  - OATP1B1/B3, OAT1/3, OCT1/2, MATE1/2-K, and BSEP

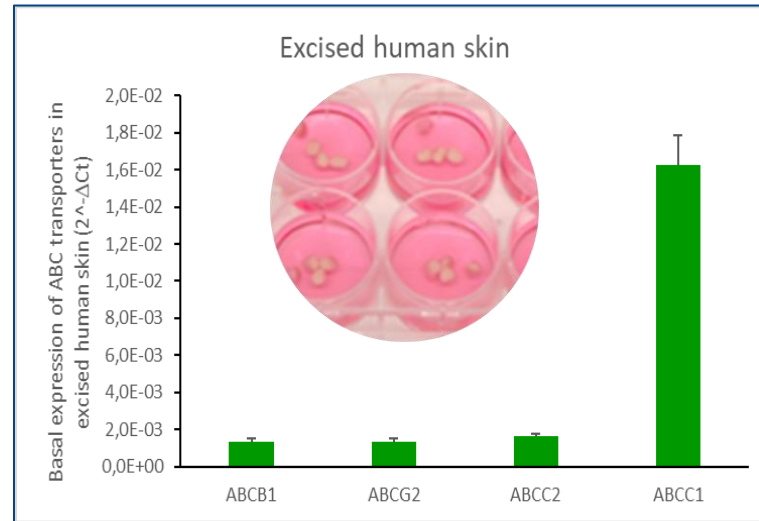
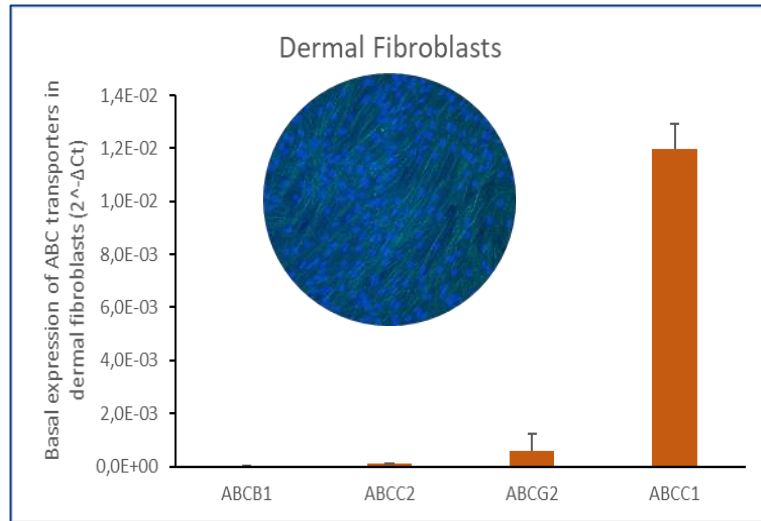
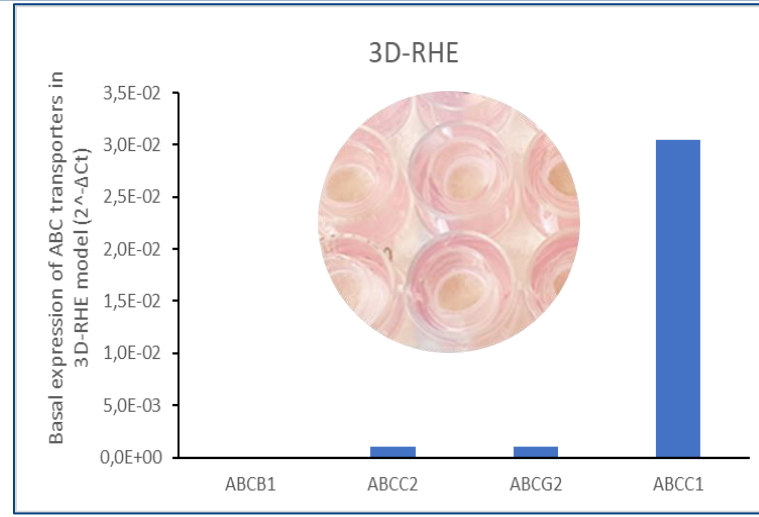
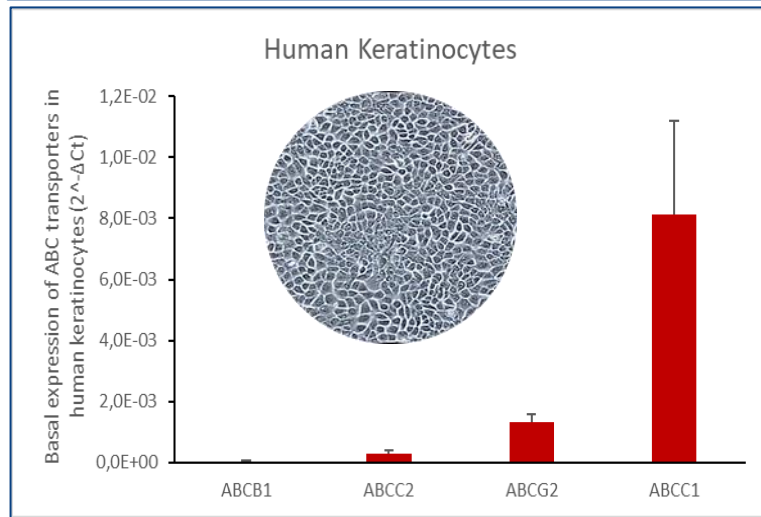


# Drug transporters characterization

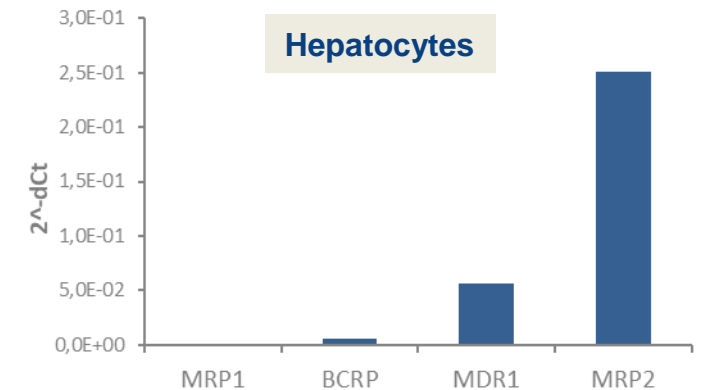
- Expression in tissues with barrier functions
  - liver, kidney, intestine, placenta, and brain
- Well characterized in barrier tissues
- Very little known about the role of drug transporters in the skin



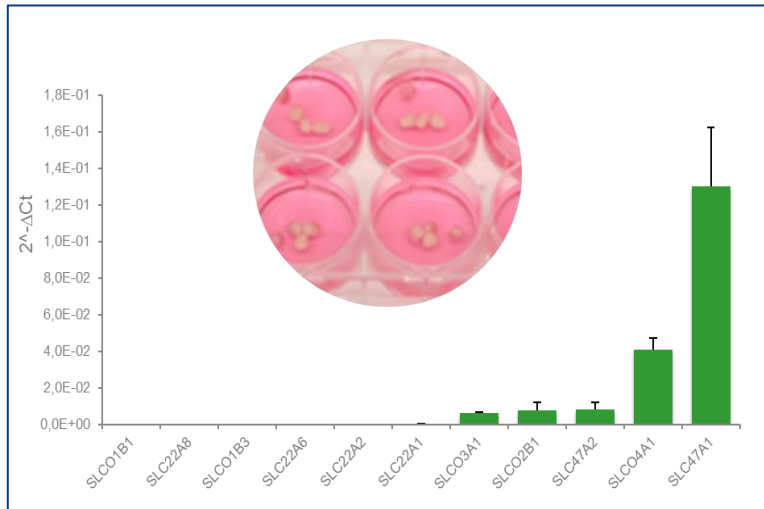
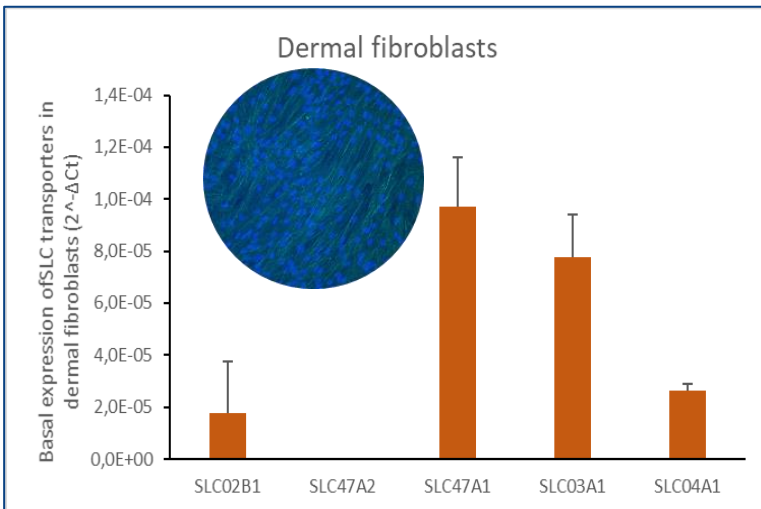
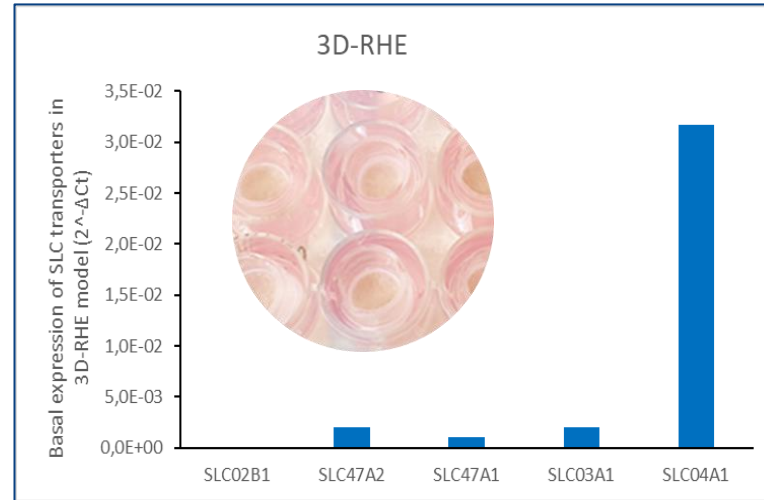
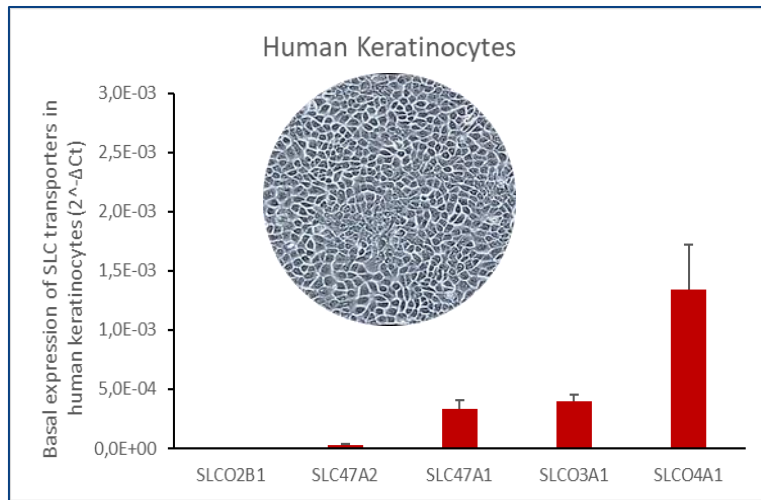
# Expression of ABC transporters in the skin



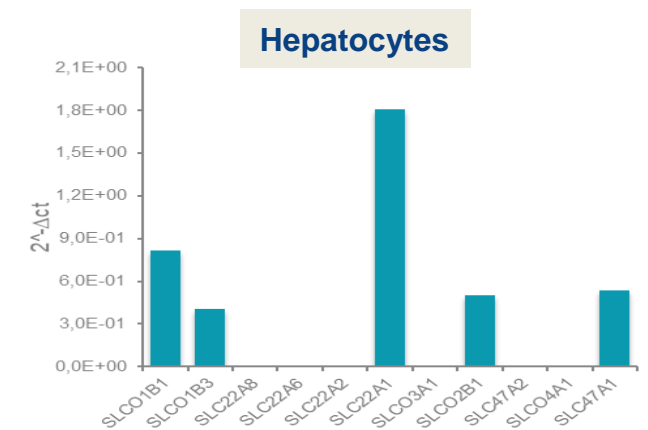
- ABC transporters are well expressed in human skin models
- Similar expression profile in
  - 2D skin cells
  - 3D skin model
  - Excised human skin
- Expression of ABCC1 (MRP1) is the highest in all skin models, different in hepatocytes



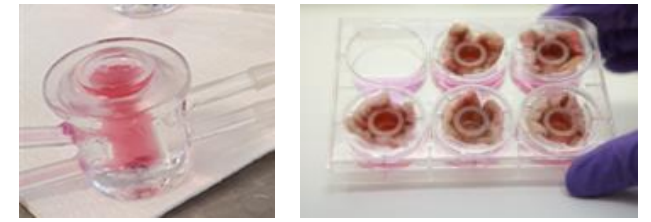
# Expression of SLC transporters in the skin



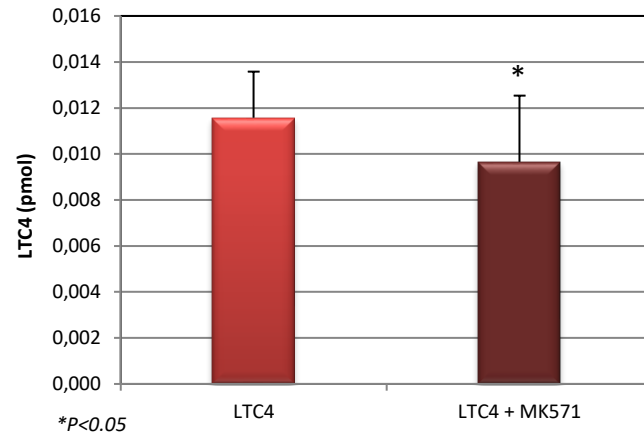
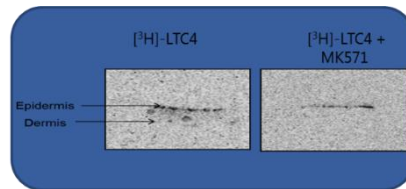
- SLC transporters are well expressed in human skin models
- Different expression profile in
  - 2D skin cells
  - 3D skin model
  - Excised human skin
- Different expression profile compared to hepatocytes



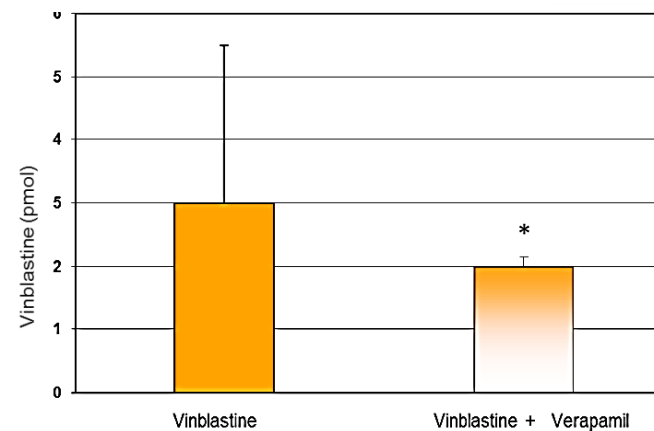
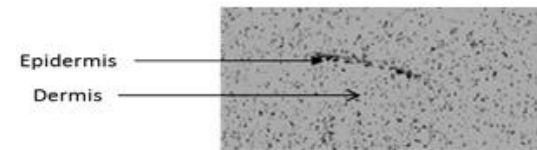
# Rôle of MRP1 in dermal absorption



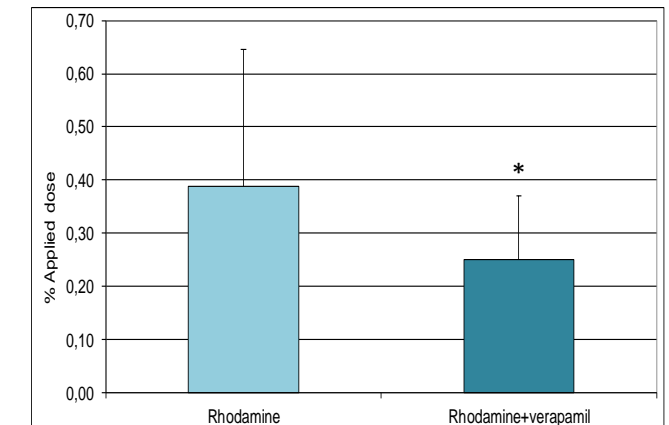
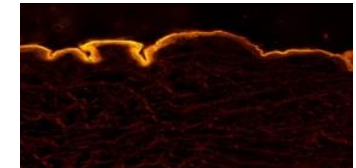
## LTC4/MK571



## Vinblastine/Verapamil

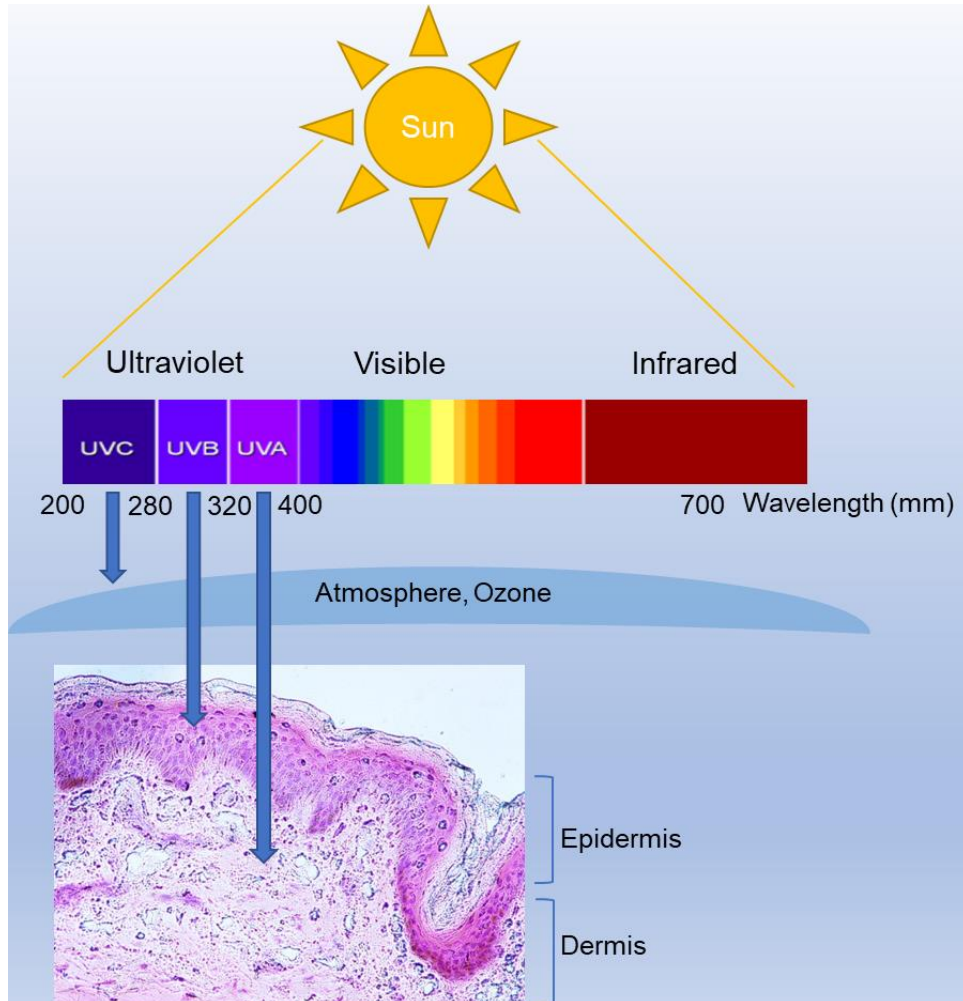


## Rhodamine 123/Verapamil



- MRP1 inhibitors significantly decrease dermal absorption of MRP1 substrates, showing the role of MRP1 in dermal absorption

# Effects of UVs on the expression of drug transporters



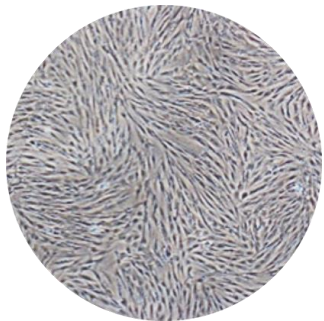
- Solar UV radiation is among the air pollutants that affect the skin
- Effects of UV radiation on human skin differ depending on the wavelength
- Over exposure to UV radiation leads to a variety of skin maladies including cancer and inflammation
- Little is known about the effects of inflammation induced by UV radiation on the expression of drug transporters in human skin
- **The purpose of this study was to investigate if UVA and UVB irradiation modulates the expression of ABC and SLC transporters in normal human keratinocytes and fibroblasts**

# Effects of UVs on the expression of drug transporters



UVB  
40mJ/cm<sup>2</sup>

Normal human epidermal keratinocytes



UVA  
30J/cm<sup>2</sup>

Normal human dermal fibroblasts



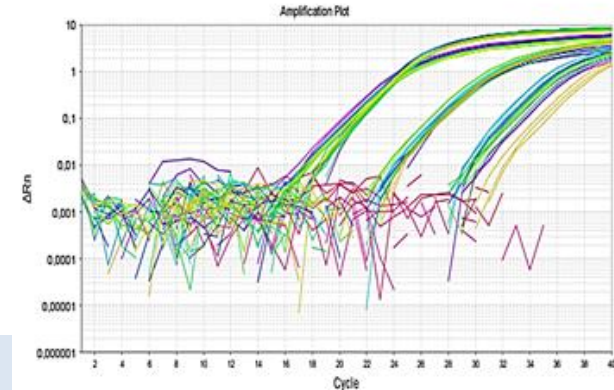
Incubation in cell incubator for 24 hours at 37°C and 5% CO<sub>2</sub>



IL-8, TNF- $\alpha$   
ABCB1, ABCC1, ABCC2, ABCG2  
SLCO2B1, SLCO3A1, SLCO4A1,  
SLC47A1, SLC47A2  
GAPDH



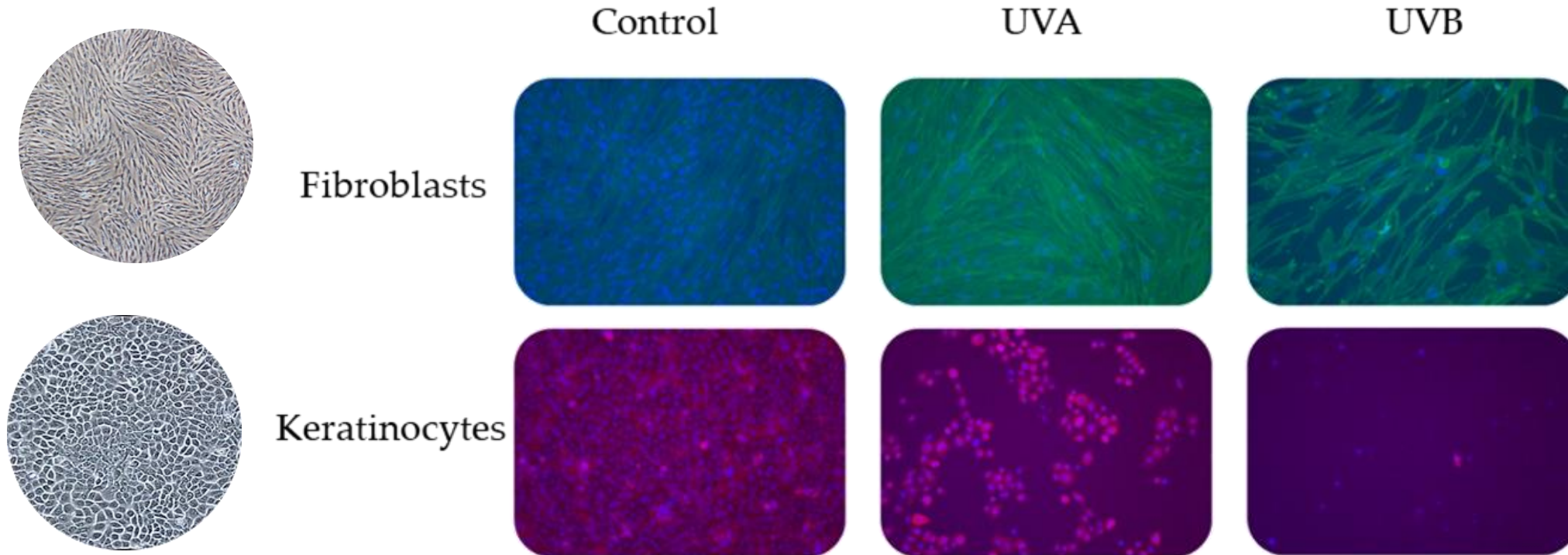
RNA isolation and quantification



RT-qPCR using TaqMan® technology

$$RQ = 2^{-\Delta\Delta CT}$$

# Effects of UVs on cell morphology and viability

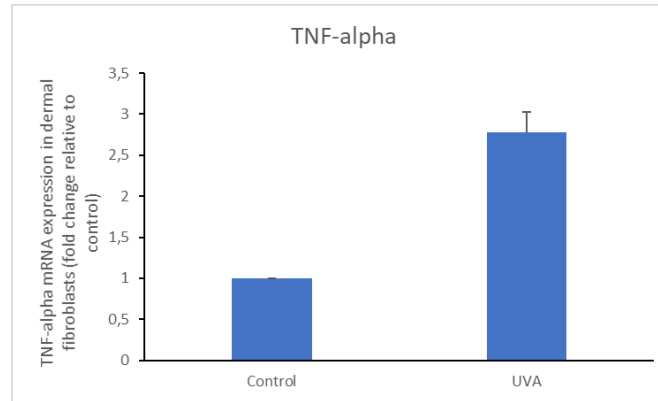
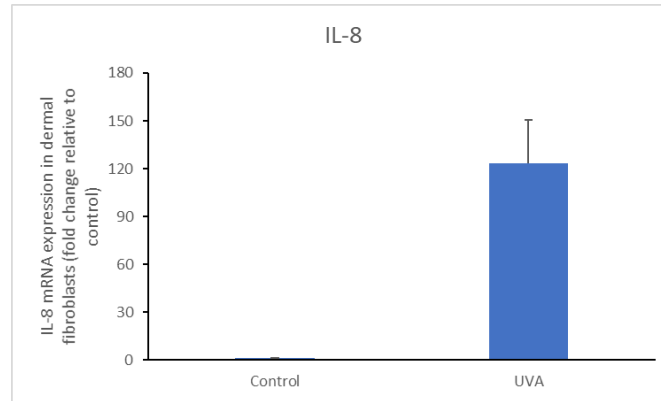


UV irradiation induced marked morphological changes in cultured keratinocytes and fibroblasts and marked reduction of cell number compared to control unexposed cells.

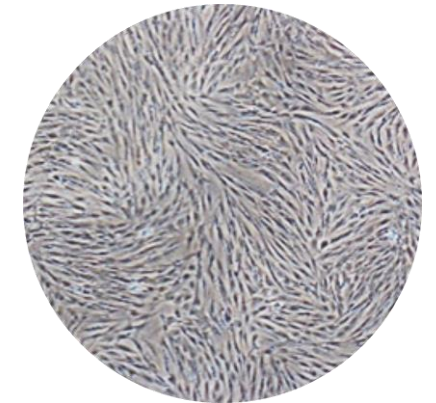
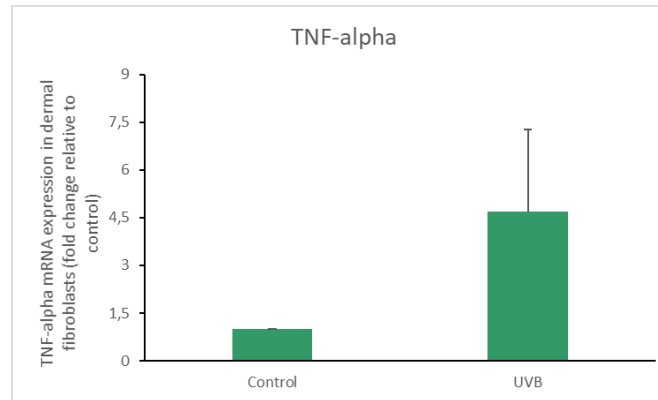
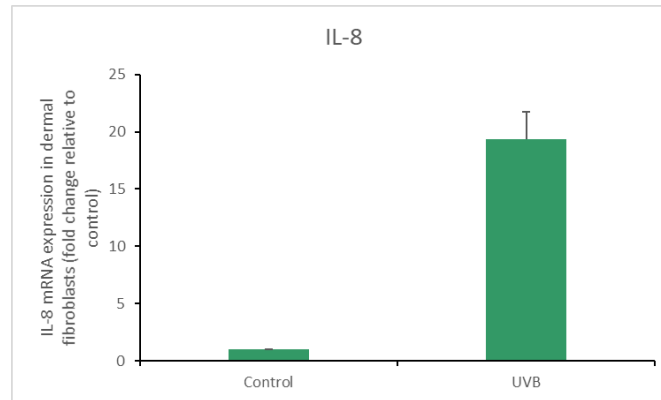
# Effects of UVs on inflammation markers



UVA



UVB



Normal human dermal fibroblasts

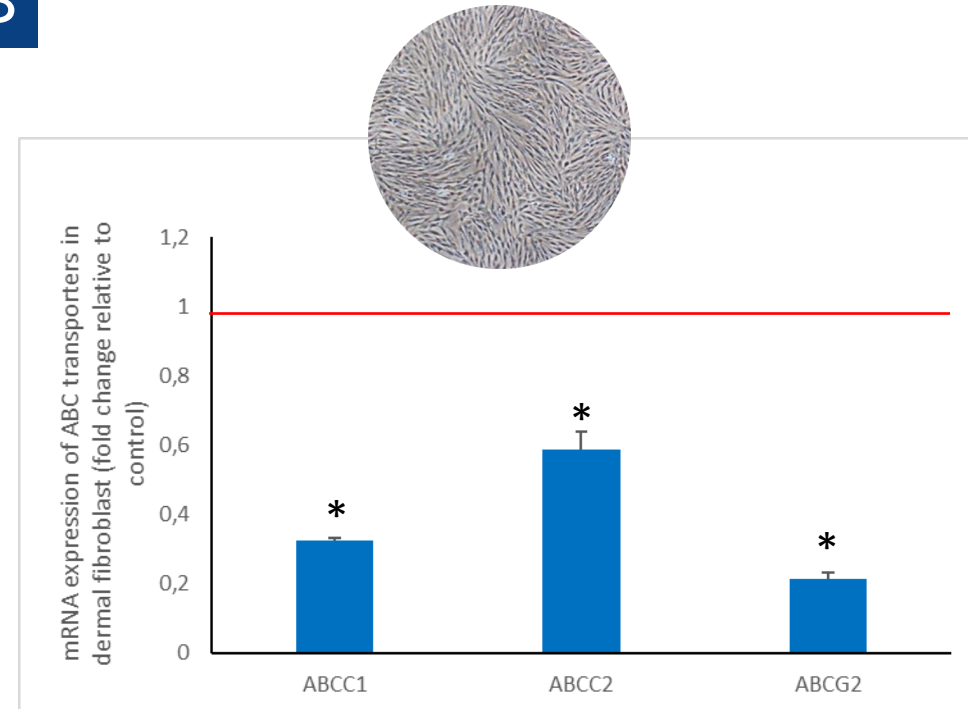
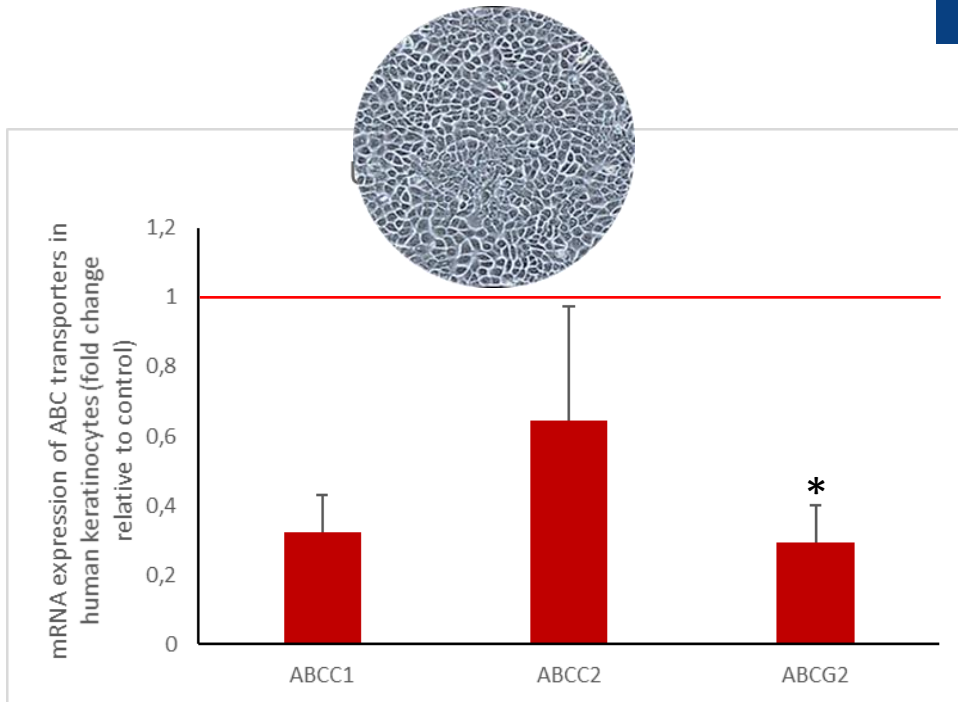
- UV irradiation induced increase of both IL-8 and TNF-alpha in normal human dermal fibroblasts.
- Fold increase ranged between 3 and more than 100-fold.
- Similar results seen in epidermal keratinocytes.



# Effects of UVs on ABC transporters



## UVB



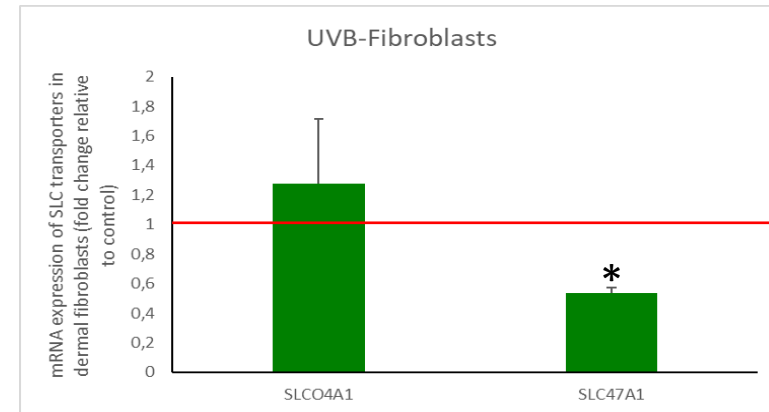
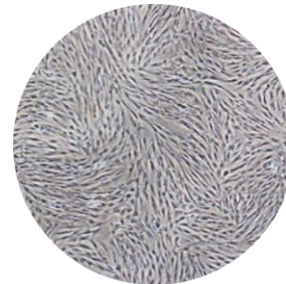
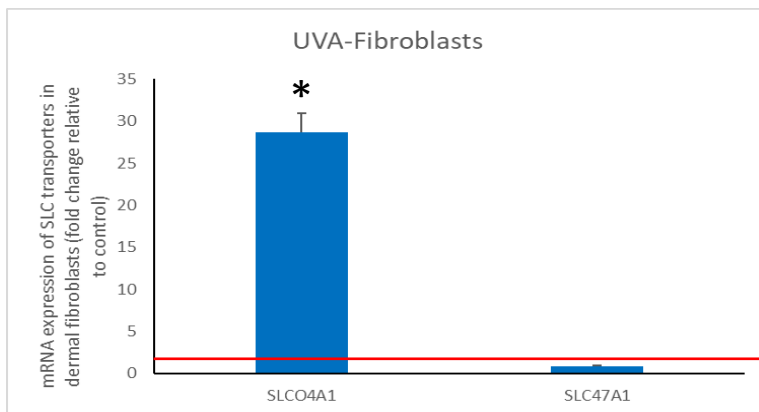
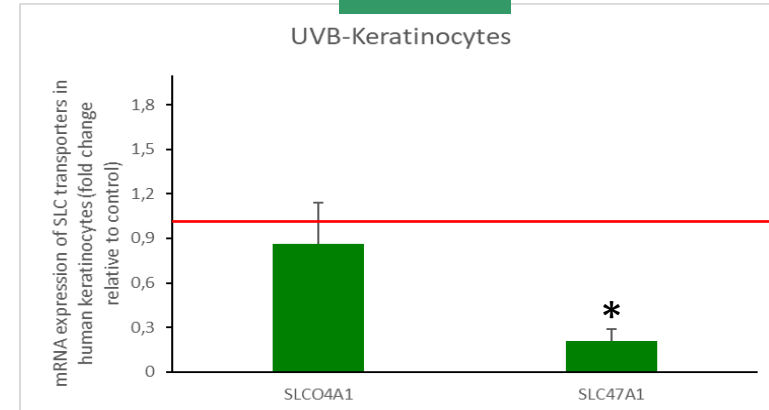
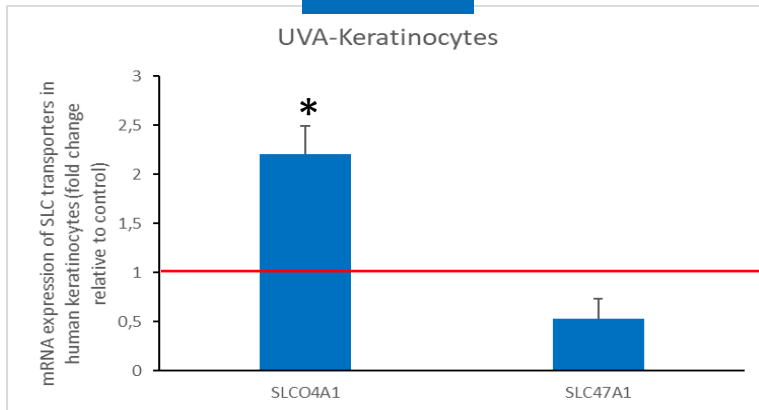
- UVB irradiation induced significant decrease of the expression of ABCC1, ABCC2 and ABCG2 in normal human dermal fibroblast and ABCG2 expression in normal human epidermal keratinocytes
- UVA irradiation had no significant effect on ABC transporters in both cell models

# Effects of UVs on SLC transporters



## UVA

## UVB



- UVA irradiation induced significant 2-fold and 29-fold increase of the expression of SLCO4A1 in keratinocytes and fibroblasts, respectively
- UVB irradiation induced significant decreased of the expression of SLC47A1 by 80% and 50%, in keratinocytes and fibroblasts, respectively

# Conclusion

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- Exposure to UV radiation increased production of cytokines and skin inflammation
- UV radiation significantly modulates expression of ABC and SLC transporters in human keratinocytes and fibroblasts
  - This finding may have an important impact on dermal absorption of topical products applied after acute exposure to sunlight
- We showed for the first time a significant regulation of the expression of SLCO4A1 in human dermal fibroblasts induced by UVA irradiation
  - Further investigations still needed to identify the potential role of this transporter in skin cancers induced by solar exposure and in pharmacokinetics of topical drugs
- Perspectives
  - Confirm our findings by functional investigations and proteomic approach

# THANK YOU

