INSTITUT DES MALADIES GÉNÉTIQUES

Proposal for the 2020 International PhD programme

Laboratory: **Laboratory of genetic skin diseases: from disease mechanisms to therapies**
Head of laboratory: Prof. Alain Hovnanian
Project and student supervisor: Alain Hovnanian (HDR)
Number of HDR in the lab: 3
Field of research: Genetic skin diseases
Number and names of PhD students currently in the lab in 2020: 0
Number and names of PhD students under the Imagine program: 0
Project Title: Integrated gene and proteomic signatures in Darier and Hailey-Hailey diseases

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Darier disease (DD) and Hailey-Hailey disease (HHD) are rare and severe dominant genetic skin diseases characterized by acantholysis (cell-to-cell separation) of the epidermis, the uppermost layer of the skin. Both diseases can manifest as extensive skin lesions, are worsened by triggering factors and cause a considerable burden. We and others have shown that DD and HHD are caused by haploinsufficiency of Ca^{2+} pumps of the endoplasmic reticulum (*ATP2A2* encoding SERCA2) and the Golgi apparatus (*ATP2C1* encoding SPCA1), respectively (Sakuntabhai *et al.* 1999, Hu *et al.* 2000, Sudbrak *et al*. 2000). There is currently no specific treatment and both diseases are orphan diseases which cause considerable distress with major unmet medical need.

Despite the discovery of their defective genes, the pathogenesis of DD and HHD remains largely unknown. SERCA2 and SPCA1 refill ER and Golgi Ca^{2+} stores, respectively, by pumping Ca^{2+} back from the cytosol following Ca^{2+}release from these organels. In addition, SPCA1 also transports Mn^{2+} and maintains Mn^{2+} concentrations in the Golgi. Functional analyses have shown that *ATP2A2* and *ATP2C1* mutations lead to loss of calcium transport, supporting haploinsufficiency. Murine models defective for *Serca2* or *Atp2c1* do not faithfully reproduce the human disease phenotype and develop skin cancers (Shull *et al*. 2011).

Given the importance of Ca^{2+} homeostasis in the epidermis, and in particular of ER and Golgi Ca^{2+} and Mn^{2+} concentrations in post-translational modifications (proteolytic processing, glycosylation, trafficking and/or sorting) of transmembrane, membrane-associated or secreted proteins, it is likely that key biological events in epidermal cell-to-cell adhesion and/or differentiation are impaired. It is also expected that impaired intracellular signaling deregulates calcium dependent gene expression, including in particular epidermal differentiation genes. In Darier disease, various studies have pointed to ER stress secondary to calcium store reduction, abnormal trafficking of E-cadherin and desmoplakin underlying adherens junction and desmosomal destabilisation through the modulation of PKC alpha, or to sphingolipid pathway abnormalities (Dhitavat *et al.* 2003 ; Hobbs *et al*. 2011 ; Celli *et al.* 2012 ; Savignac *et al.* 2014). In Hailey-Hailey disease, a recent transcriptomic study reported increased oxidative stress and Notch1 activation in cultured keratinocytes upon *ATP2C1* inactivation and in cultured keratinocytes from lesional skin from 3 HHD patients (Cialfi *et al*. 2016). However it is likey that the disease mechanisms involved are multiple and complex.

We propose to use an unbiased and combinatory approach to capture the gene and proteomic signatures of DD and HHD *in vivo* and *in vitro* in comparison with healthy controls, using a unique cohort of patients with DD and HHD. To this aim, the candidate will perform:

1. ***In vivo* comparative studies using mRNA sequencing and proteomic studies from lesional and non lesional DD and HHD skin**, and healthy controls. Skin biopsies from 10 patients with HHD and DD will be compared with 10 age, sex and anatomic site matched controls. Six patients who show **segmental DD** corresponding to mosaic forms of the disease will also be studied. These samples will allow to overcome inter-individual genetic variations. These samples have already been collected after consent was obtained. They have been sequenced by mRNA sequencing at the Imagine plateform. Quality controls are fully satisfactory and the data are ready for analysis and interpretation. The samples for proteomic studies using high resolution mass spectrometry are ready to be processed. A global and N-glycosylation study will be performed, considering the importance of Ca^{2+} and Mn^{2+} concentrations in the ER and the Golgi apparatus in the glycosylation processes of proteins. Differentially expressed gene and protein analyses will be performed and the Ingenuity pathway will be used to identify biological cascades, gene and protein networks involved. mRNA and protein data will be confirmed by q-RT-PCR, immunostaining and/or western-blot analyses as

appropriate. Inter-individual variability will be taken into account during statistical analysis. Single-cell transcriptomic landscape will also be considered. A combinatory and integrated approach will aim at defining the global *in vivo* biological signature of lesional and non lesional skin of both diseases and has the potential to identify therapeutic targets.

2. ***In vitro* comparative studies using the same gene and proteomic approaches applied to DD and HHD patient keratinocytes in culture** compared to healthy controls. This approach will help deciphering the intrinsic biological signature of DD and HHD keratinocytes in the absence of other cell types and skin inflammation observed *in vivo*. To overcome inter-individual variability, the effect of partial inactivation of SERCA2 in healthy control keratinocytes by the pharmacological inhibitor thapsigargin, or Crispr/Cas9 invalidation of *ATP2A2* or *ATP2C1* will be compared with chronic haploinsufficency of *ATP2A2* and *ATP2C1* which is a hallmark of DD and HHD patient keratinocytes.
3. **Functional studies aiming at testing existing compounds, biotherapies and gene therapy** will be developed. These studies will use DD and HHD keratinocytes grown in culture and in an *in vitro* organotypic 3-D skin model allowing full terminal epidermal differentiation. Libraries of existing drugs and small components will be screened, using readouts defined during mRNA and proteomic analyses as well as intracellular Ca^{2+} measurements. *In vivo* and *in vitro* gene therapy approaches will also be developed aiming at restoring *ATP2A2* or *ATP2C1* expression at sufficient levels to restore the normal phenotype by counteracting the haploinsufficiency mechanism of DD and HHD. Murine models defective for *Serca2* or *Atp2c1* will also be considered to develop strategies to prevent the development of skin cancer which can develop in DD and HHD patients.

These analyses will be performed at the Imagine Institute and at the Federative Structure for Reseach (SFR) at Necker campus. Hovnanian laboratory has an extensive experience in keratinocyte culture, reconstructed skin, mRNA sequencing, qRT-PCR, immunostaining, western-blot analyses and gene therapy strategies using gene addition and/or CrisprCas9 technology. Transcriptomic analyses will be performed at the Genomic and Bioinformatic plateform at the Imagine Institute. Proteomic studies will be carried out in the context of existing collaborations with a highly performing Proteomic plateform run by Dr Chiara Guerrera at the SFR. Clinical Bioinformatics and Combinatorial Omics will be performed in collaboration with Dr Antonia Raussell laboratory at Imagine.

This proteogenomic approach should allow to identify key biological processes involved in the pathogenesis of DD and HHD. This study should help identifying a disease signature for DD and for HHD, and reveal possible overlaping signaling pathways between the two diseases. This project also holds the promise of identifying new therapeutic targets. This pre-clinical project could therefore be the foundation for a rational therapeutic design for these severe orphan diseases which cause a significant disability.

Lab members

Hovnanian A. (Director), Kayser-Guélin V. (PA), Gaucher S. (Assoc. Prof.), Bachelez H. (Prof.), Titeux M. (INSERM Researcher), Gouin O. (post-doc), Barbieux C. (post-doc), Petrova E. (post-doc), Izmiryan A. (RE INSERM), Pironon N. (RE Univ.), Bonnet des Claustes M. (RE), Berthault C. (RE), Leturcq F. (Tech), Miskinyte S. (Tech Univ).

Major Publications

1. Sakuntabhai A, Ruiz-Perez V, Carter S, Jacobsen N, Burge S, Monk S, Smith M, Munro CS, O'Donovan M, Craddock N, Kucherlapati R, Rees JL, Owen M, Lathrop GM, Monaco AP, Strachan T and **Hovnanian A**. Mutations in *ATP2A2*, encoding a Ca2+ pump, cause Darier disease. *Nat Genet*. 1999. 21 : 271-7.
2. Sakuntabhai A, Burge S, Monk S and **Hovnanian A**. Spectrum of novel *ATP2A2* mutations in patients with Darier's disease. *Hum Mol Genet* . 1999. 8 : 1611-9.
3. Sudbrak R, Brown J, Dobson-Stone C, Carter S, Ramser J, White J, Healy E, Dissanayake M, Larregue M, Perrussel M, Lehrach H, Munro CS, Strachan T, Burge S, **Hovnanian A** and Monaco AP. Hailey-Hailey disease is caused by mutations in *ATP2C1* encoding a novel Ca(2+) pump. *Hum Mol Genet*. 2000. 9 : 1131-40.
4. Sakuntabhai A, Dhitavat J, Burge S and **Hovnanian A**. Mosaicism for *ATP2A2* mutations causes segmental Darier's disease. *J Invest Dermatol.* 2000. 115 : 1144-7.

5. Dobson-Stone C, Fairclough R, Dunne E, Brown J, Dissanayake M, Munro CS, Strachan T, Burge S, Sudbrak R, Monaco AP, **Hovnanian A**. Hailey-Hailey disease: molecular and clinical characterization of novel mutations in the *ATP2C1* gene. *J Invest Dermatol*. 2002. 118 : 338-43.
6. Sheridan AT, Hollowood K, Sakuntabhai A, Dean D, **Hovnanian A**, Burge S. Expression of sarco/endo-plasmic reticulum Ca2+-ATPase type 2 isoforms (SERCA2) in normal human skin and mucosa, and Darier's disease skin. *Br J Dermatol.* 2002. 147 : 670-4.
7. Dhitavat J, Macfarlane S, Dode L, Leslie N, Sakuntabhai A,∗ Saihan EM and **Hovnanian A**. Acrokeratosis verruciformis of Hopf is caused by mutation in *ATP2A2*: evidence that it is allelic to Darier's disease. *J Invest Dermatol*. 2002. 120 : 229-32.
8. Fairclough RJ, Dode L, Vanoevelen J, Andersen JP, Missiaen L, Raeymaekers L, Wuytack F, **Hovnanian A**. Effect of Hailey-Hailey Disease mutations on the function of a new variant of human secretory pathway Ca2+/Mn2+-ATPase (hSPCA1). *J Biol Chem.* 2003. 278 : 24721-30.
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10. Dode L, Andersen JP, Leslie N, Dhitavat J, Vilsen B, **Hovnanian A**. Dissection of the functional differences between sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) 1 and 2 isoforms and Characterization of Darier disease (SERCA2) mutants by steady-state and transient kinetic analyses. *J Biol Chem.* 2003. 278 : 47877-89.
11. Dhitavat J, Cobbold C, Leslie N, Burge S, **Hovnanian A**. Impaired trafficking of the desmoplakins in cultured Darier's disease keratinocytes. *J Invest Dermatol.* 2003. 121 : 1349-55.
12. Dhitavat J, Fairclough RJ, **Hovnanian A**, Burge SM. Calcium pumps and keratinocytes: lessons from Darier's disease and Hailey-Hailey disease. *Br J Dermatol.* 2004. 150 : 821-8.
13. Fairclough RJ, Lonie L, Van Baelen K, Haftek M, Munro CS, Burge SM, **Hovnanian A**. Hailey-Hailey disease: identification of novel mutations in *ATP2C1* and effect of missense mutation A528P on protein expression levels. *J Invest Dermatol.* 2004. 123 : 67-71.
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23. Zhang A, Duchatelet S, Lakdawala N, Tower RL, Diamond C, Marathe K, Hill I, Richard G, Diab Y, Kirkorian AY, Watanabe F, Siegel DH, **Hovnanian A**. Targeted Inhibition of the Epidermal Growth Factor Receptor and Mammalian Target of Rapamycin Signaling Pathways in Olmsted Syndrome. JAMA Dermatol. 2020 Jan 2. doi: 10.1001/*JamaDermatol*.2019.4141
24. Lévy R, Béziat V, Barbieux C, Puel A, Bourrat E, Casanova JL, **Hovnanian A**. Efficacy of Dupilumab for Controlling Severe Atopic Dermatitis in a Patient with Hyper-IgE Syndrome. *J Clin Immunol.* 2020 Jan 28. doi: 10.1007/s10875-020-00751-4. [Epub ahead of print]