TRAVEL GRANT REPORT

LS² – Physiology section

Monica Bulla, MD-PhD student

Group of Pr. Nicolas Demaurex

Department of Cell Physiology and Metabolism

Faculty of Medicine - University of Geneva

Monica.Bulla@unige.ch

Conferences attended :

Calcium Signalling (GRS)
Gordon Research Seminar

Intracellular Calcium Signalling in Normal Physiology and Disease

June 17-18, 2017
Il Ciocco, Lucca (Barga), Italy

Calcium Signalling
Gordon Research Conference

Intracellular Calcium Signals: Generation, Function and Therapeutic Intervention

June 18-23, 2017
Il Ciocco, Lucca (Barga), Italy

GRS and GRC – Calcium Signalling 2017

***ORAI1* Mutations with Distinct Channel Gating Defects Cause Tubular Aggregate Myopathy**

Calcium Signalling (GRS)
Gordon Research Seminar

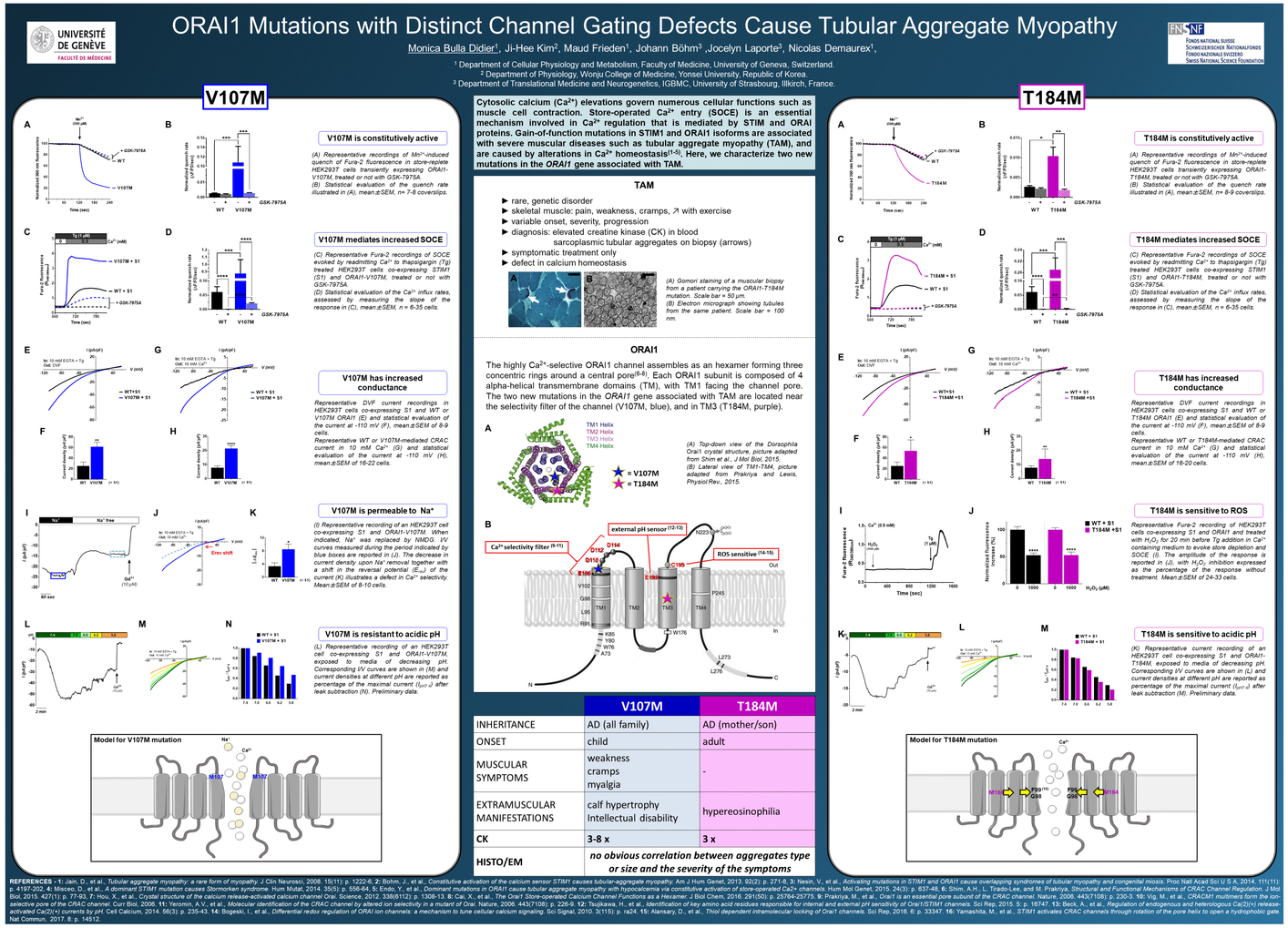
June 17-18, 2017
Lucca (Barga), Italy

Monica Bulla Didier
Group of Pr. Nicolas Demaurex

UNIVERSITÉ DE GENÈVE

FNS-NF Fonds national suisse Schweizerischer Nationalfonds Fondo nazionale svizzero Swiss National Science Foundation

My abstract entitled "*ORAI1* Mutations with Distinct Channel Gating Defects Cause Tubular Aggregate Myopathy" was selected for a talk at the GRS.

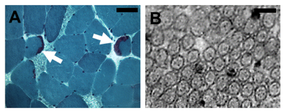


The poster I presented at the GRS and the GRC was awarded the prize for "the best GRS poster".

My poster, step by step (1)

TAM

- ► rare, genetic disorder
- ► skeletal muscle: pain, weakness, cramps, ↗ with exercise
- ► variable onset, severity, progression
- ► diagnosis: elevated creatine kinase (CK) in blood
  sarcoplasmic tubular aggregates on biopsy (arrows)
- ► symptomatic treatment only
- ► defect in calcium homeostasis

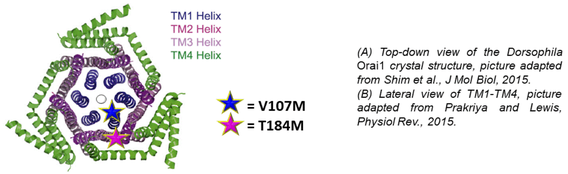


(A) Gomori staining of a muscular biopsy from a patient carrying the ORAI1-T184M mutation. Scale bar = 50 µm.
(B) Electron micrograph showing tubules from the same patient. Scale bar = 100 nm.

ORAI1

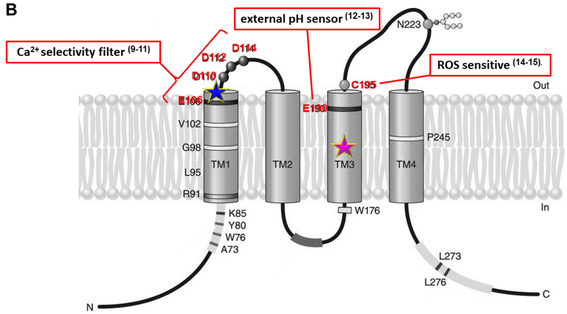
The highly Ca^{2+}-selective ORAI1 channel assembles as an hexamer forming three concentric rings around a central pore[6-8]. Each ORAI1 subunit is composed of 4 alpha-helical transmembrane domains (TM), with TM1 facing the channel pore. The two new mutations in the *ORAI1* gene associated with TAM are located near the selectivity filter of the channel (V107M, blue), and in TM3 (T184M, purple).

A



(A) Top-down view of the Dorsophila Orai1 crystal structure, picture adapted from Shim et al., J Mol Biol, 2015.
(B) Lateral view of TM1-TM4, picture adapted from Prakriya and Lewis, Physiol Rev., 2015.

B



	V107M	T184M
INHERITANCE	AD (all family)	AD (mother/son)
ONSET	child	adult
MUSCULAR SYMPTOMS	weakness cramps myalgia	-
EXTRAMUSCULAR MANIFESTATIONS	calf hypertrophy Intellectual disability	hypereosinophilia
CK	**3-8 x**	**3 x**
HISTO/EM	***no obvious correlation between aggregates type or size and the severity of the symptoms***	

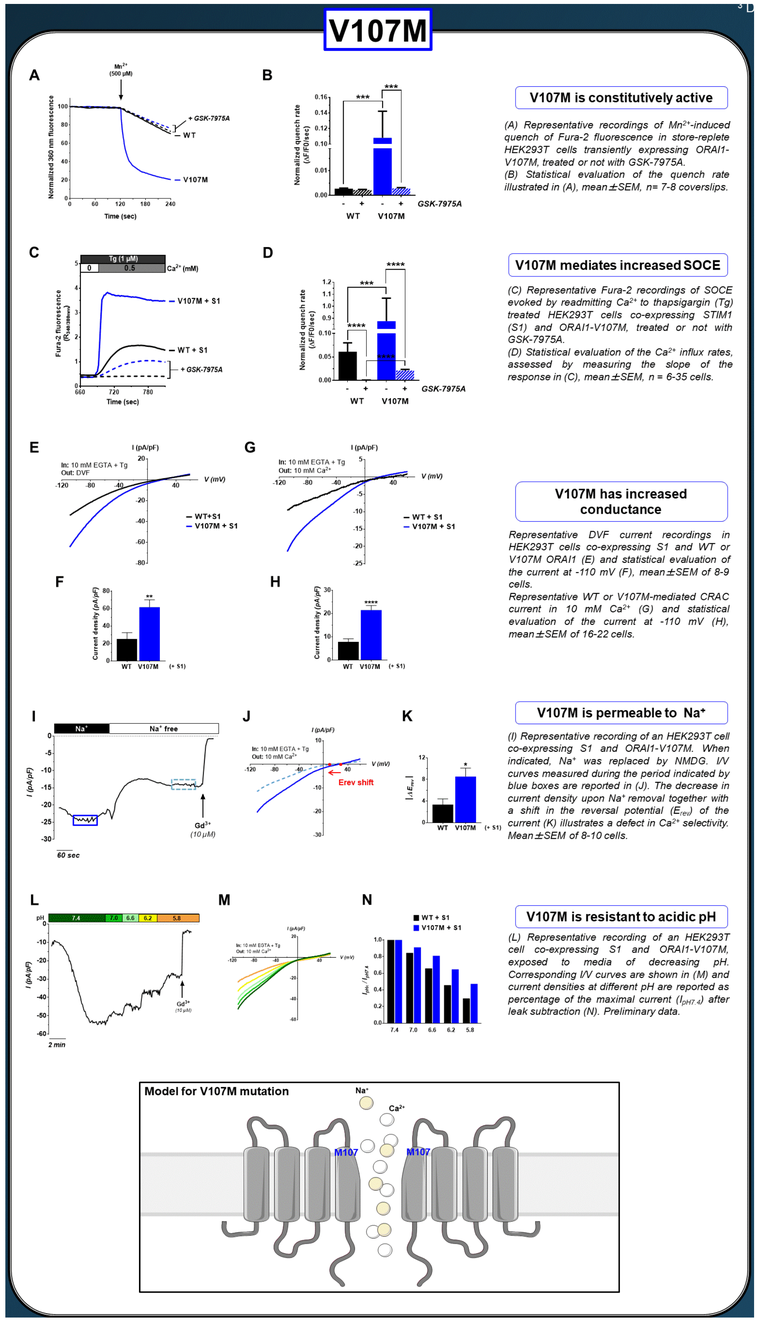
Tubular aggregate myopathy (TAM) is a rare, genetic disease affecting the skeletal muscle and caused by alterations in Ca^{2+} homeostasis. TAM leads to muscle pain, fatigue and cramps, increasing with effort. Symptoms, onset and prognosis are variable from a patient to another. Currently, no treatment is available aside from pain management. The diagnosis if offered by the observation of sarcoplasmic tubular aggregates (white arrows) in muscle biopsies, together with an increase in the level of circulating creatine kinase (CK), a marker of muscle stress.

Cytosolic calcium (Ca^{2+}) elevations govern numerous cellular functions such as muscle cell contraction. Store-operated Ca^{2+} entry (SOCE) is an essential mechanism involved in Ca^{2+} regulation that is mediated by STIM and ORAI proteins. Gain of function mutations in STIM1 and ORAI1 isoforms are associated with multiple muscular diseases such as TAM. In this study, we characterize two new mutations (V107M: blue, and T184M: purple) in the *ORAI1* gene associated with TAM. V107M faces the channel pore and relies in the Ca^{2+} selectivity region. T184M is located in the third transmembrane domain (TM3) of the protein, not far from two important residues conferring pH and ROS sensitivity to the channel.

These two TAM mutations lead to very different clinical features, with an early onset and very severe symptoms for patients carrying the mutation V107M, and almost no signs for the patient with mutation T184M. However, muscle biopsies and CK blood levels were comparable between the groups.

The two new disease-related ORAI1 mutations prompt us to test the channel properties, using Ca^{2+} imaging (Fura-2, AM) or electrophysiology. All experiments were performed in HEK293T cells overexpressing either the WT of the mutated forms of ORAI1, together with STIM1 when indicated.

My poster, step by step (2)



The manganese quench technic was used to determine the basal ion flow trough the pore of mutated channels, and showed that ORAI1-V107M was constitutively open at rest. However, the basal leak could be blocked by the SOCE inhibitor GSK-7975A.

The assessment of SOCE by Ca^{2+} imaging revealed that the ORAI1-V107M channel was overactive compared with the WT channel. This excessive activity was only partially inhibited by GSK-7975A.

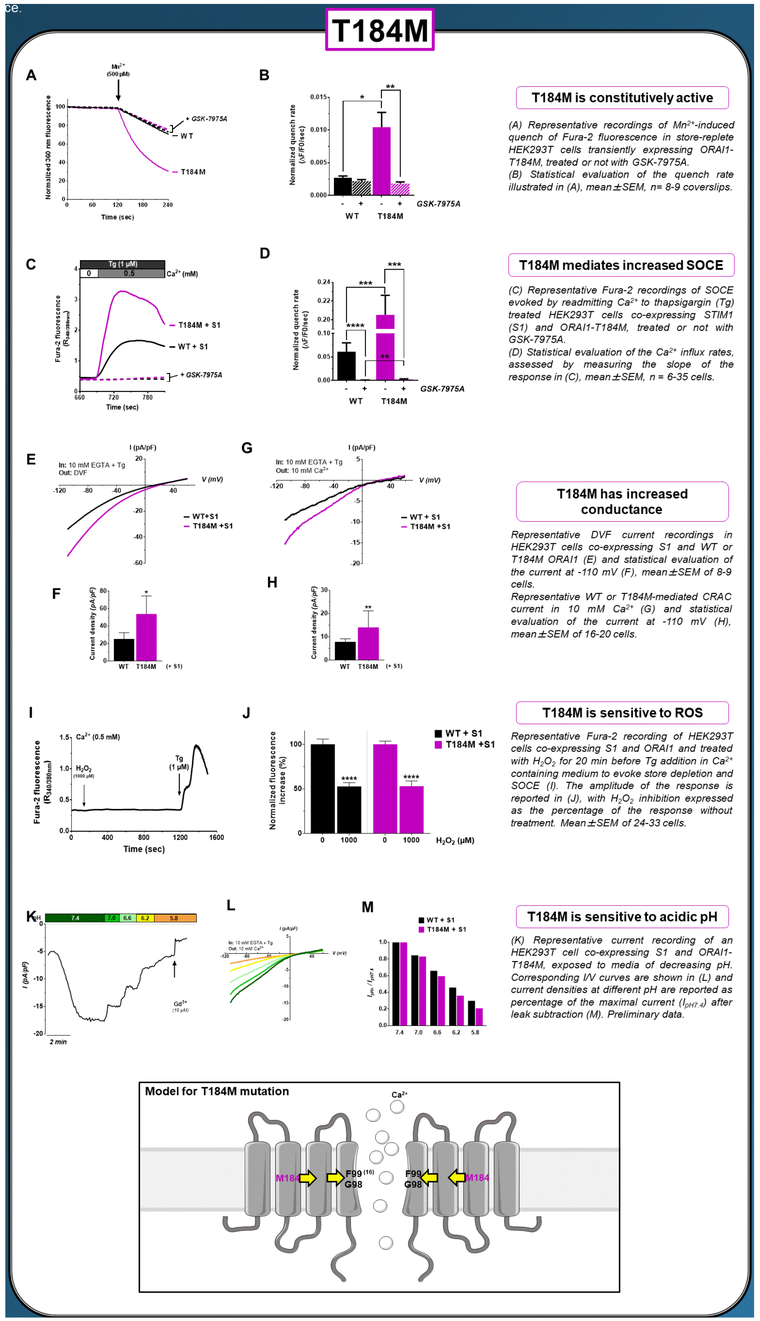
Patch clamp experiments confirmed the increased ion (Na^+ and Ca^{2+}) conductance of ORAI1-V107M.

The permeability of ORAI1-V107M to Na^+ ions was assess by switching external media from Na^+ containing to Na^+ free (replaced by NMDG). When Na^+ was removed, the current density decreased and the reversal potential (Erev) of the I/V relationship shifted to the left, indicating that ORAI1-V107M is less Ca^{2+} selective than the WT channel.

Bathing cells in media of decreasing pH showed that ORAI1-V107M was resistant to acidic pH inhibition, as compared with the WT channel (preliminary data).

Replacing the valine at the pore mouth by a big amino acid such as methionine might distort TM1 α helix, increasing the ion conductance of the channel at rest as well as when gated. The displacement of residues close to the pore mouth and providing the selectivity for Ca^{2+} ions to the channel would explain the increased permeability for Na^+ ions of ORAI1-V107M.

My poster, step by step (3)



The manganese quench technic revealed that ORAI1-T184M was also constitutively open when expressed in HEK-293T cells. The basal activity of the mutated channel could also be blocked by GSK-7975A.

Similar to ORAI1-V107M, SOCE was increased in the presence of ORAI1-T184M. However, contrary to V107M, this excessive activity was completely inhibited by GSK-7975A.

Electrophysiology experiments confirmed the increased ion (Na^+ and Ca^{2+}) conductance of ORAI1-T184M.

We first stipulated that a methionine substitution in the middle of TM3 would affect the overall TM domain structure and the exposure of the surface residue C195 responsible for ROS sensitivity. However, Ca^{2+} imaging experiments revealed that ORAI1-T184M was as sensitive as the WT channel to H_2O_2 inhibition. Moreover, C195 accessibility was assessed by molecular dynamic simulations and seemed to be conserved in the T184M context (not shown).

Bathing cells in media of decreasing pH showed that ORAI1-T184M was sensitive to acidic pH inhibition to the same extend than the WT channel (preliminary data).

Unlike what we originally hypothesize, the TAM mutation in the middle of TM3 did not alter the structure of TM3 itself but might alter neighboring TM α helixes, impacting on the channel pore and explaining the increased ion conductance of the resting or gated ORAI1-T184M channel.

Interestingly, the severity of the phenotypes observed in our experiments nicely correlated with the severity of the patients' clinical features, with a stronger effect for the V107M than for the T184M mutation.

Perspectives

The following perspectives were inspired by GRS/GRC talks or were suggested to me during the poster sessions:

1) Does STIM1 partially rescue V107M loss of selectivity for Ca^{2+} ions?

- Report the constitutive V107M current, without store depletion and intracellular Ca^{2+} buffering, in DVF and Ca^{2+} containing media (patch clamp).
- Repeat the Na^{+} to NMDG switch experiments in ORAI1-V107M expressing HEK-293T cells, without the co-expression of STIM1 (patch clamp): a bigger Erev shift than the one observed in ORAI1-V107M + STIM1 co-expressing cells would suggest that STIM1 partially rescues the loss of Ca^{2+} selectivity of mutant V107M.

2) Is the affinity for STIM1 increased in the context of the T184M mutation ?

- Use the gating-deficient STIM1-F394H mutant with decreased affinity for ORAI1 (Zhou et al., Nat Commun, 2015).
- Measure the colocalization of ORAI1-T184M and STIM1-F394H (confocal microscope): does the mutation T184M rescue the affinity of the mutated STIM1 to ORAI1?
- Measure the basal activity of ORAI1-T184M in STIM null cells with/without co-expression of STIM1-F394H (Mn^{2+} quenching): does the co-expression of STIM or STIM1-F394H increase the constitutive activity of ORAI1-T184M?
- Record the current of cells expressing STIM1-F394H in the presence of ORAI1-T184M (patch clamp): does the mutation T184M restore the CRAC current of the gating-deficient STIM1-F394H?

Supplemental Figure 1.
Attendees of the “GRS – Calcium signaling 2017”. The first line is composed of GRS speakers. The white arrow points towards Galina Schmunk, the GRS chair. The GRS invited speaker, Rosario Rizzuto (red arrow) gave a keynote lecture entitled “The Road to Discovery of MCU”. The green line circles Ananth Parekh (middle), Gary St. John Bird (right) and Mohamed Trebak (left), the chair and the two vice-chairs of the GRC, respectively. Monica Bulla is indicated with a black star. The picture was taken at the Renaissance Tuscany Marriott Hotel, in Il Ciocco, Lucca (Barga), Italy. Scale bar = 1 m.

Acknowledgements

I would like to thank all the members of the LS^2 - Physiology section board and Carolin Von Schoultz for approving my travel grant application and making my participation to this great meetings possible.

I would also like to thank my supervisor, Pr. Nicolas Demaurex, who supports me in my project, and who always encourages me to attend to conferences, to present my work and extent my network.