# Investigation of the cardiac TRPM4 ion channel expression pattern in inbred mouse animal model strains

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## Background

### Biochemical properties of TRPM4

The non-selective Transient Receptor Potential Melastatin 4 (TRPM4) cation channel is expressed in a large variety of tissues, cardiomyocytes being among the major TRPM4 expression sites. The TRPM4 channel is involved in various physiological and pathological processes in cardiac cells (1). Specific domains function as binding sites for SUMO and caveolin (2). Phosphatidylinositolbisphosphate (PIP2) as well as caveolin interaction are involved in maintaining and enhancing the $Ca^{2+}$ sensitivity of the TRPM4 channels (3).

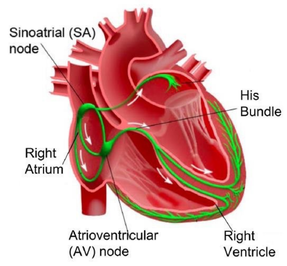


**Figure 1: Overview of human cardiac conduction system**

### TRPM4 under pathological conditions

A broad spectrum of conditions is known to correlate with TRPM4 channel activation. Conditions associated with TRPM4 include:

- **Hypertrophied cardiomyocytes**
- **Arrhythmias caused by hypoxia**
- **Inherited cardiac diseases:** Dozens of TRPM4 gene mutations have been linked to different kinds of cardiac arrhythmias, including conduction blocks, Brugada-Syndrome (BrS) and long QT syndrome.

The channel is involved in maintaining $Ca^{2+}$ homeostasis in cells, an overload of $Ca^{2+}$ is known to cause a TRPM4 overactivation. This activation causes an increasing $Na^{+}$ influx through the channel and can lead to electrical abnormalities in cardiomyocytes. As a result of AP prolongation and depolarization, a shift of the membrane potential can occur (1).

## Current research in the Sachse/Ritter group

### Current working hypothesis:

We propose that destabilization of the TRPM4 complex occurs through SUMOylation. The lower oxygen availability induces the SUMOylation enhancer RSUME expression, which strongly activates cellular SUMOylation through binding to the E2 ligase Ubc2. The release of caveolin results in TRPM4 activation. Finally, unbound TRPM4 ion channels can be maximally stimulated with phosphatidylinositol 4,5-bisphosphate (PIP2).
In combination, this leads to the current working hypothesis depicted in figure 2.

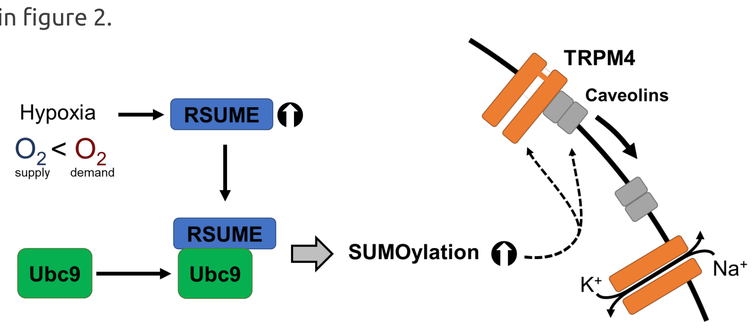


**Figure 2: Proposed mechanism of TRPM4 activation under hypoxic conditions**

## Objectives

This research project aims to quantify TRPM4 protein in cardiac mouse tissue and demonstrate how TRPM4 is characteristically distributed and expressed in different chambers of the heart. This mouse model includes four mouse strains: 129, FVB, DBA and C57BL/6. The main four hear chambers (right and left ventricles, the right and left atrium) were examined in an experimental study.

* RA = right atrium, LA = left atrium, RV = right ventricle, LV = left ventricle, M = male, F = female

## Method

**To detect TRPM4 expression in heart cells, the biochemical laboratory methods SDS-PAGE and western/immunoblot were used.**

Western blot is a technique to detect specific proteins from a complex mixture of proteins extracted from cells. This method has three main tasks: Separation by size, transferring to a solid support and marking the target protein, in this case TRPM4 and caveolin-3, by using a primary and secondary antibody.

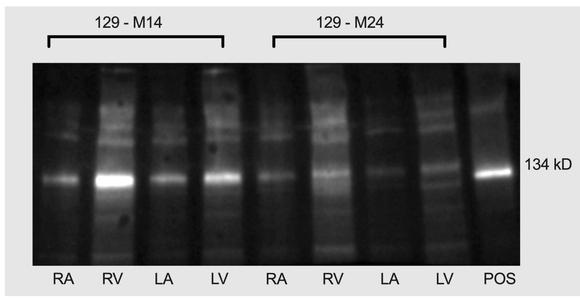


**Figure 3: Western blot depicting the distribution pattern of TRPM4 in the four heart chambers*. This blot contains two mice hearts from the strain 129.**

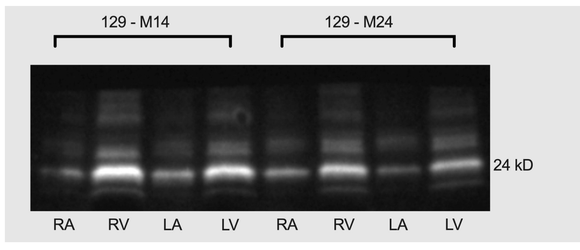


**Figure 4: Western Blot depicting the distribution pattern of caveolin-3 in the four hearth chambers*. This blot contains two mice hearts from the strain 129.**

## Results

Samples from 32 hearts were examined on 16 western blots in total. The collective results for all groups are displayed in table 1, given as mean corrected TRPM4 protein expression ± standard deviation.

**Table 1: Summery of all TRPM4 mean expressions**

| Strain | Sex | Heart Chambers | Sample Size N = x | TRPM4 Expression mean ± standard deviation |
|---|---|---|---|---|
| 129 | M | RA | 4 | 3,89 ± 0,58 |
| 129 | M | LA | 4 | 2,26 ± 0,39 |
| 129 | M | RV | 4 | 4,10 ± 0,94 |
| 129 | M | LV | 4 | 2,42 ± 0,43 |
| FVB | M | RA | 4 | 1,00 ± 0,21 |
| FVB | M | LA | 4 | 2,78 ± 0,84 |
| FVB | M | RV | 4 | 1,15 ± 0,31 |
| FVB | M | LV | 4 | 1,83 ± 0,19 |
| DBA | M | RA | 4 | 4,10 ± 1,15 |
| DBA | M | LA | 4 | 2,33 ± 0,51 |
| DBA | M | RV | 4 | 5,14 ± 1,83 |
| DBA | M | LV | 4 | 2,14 ± 0,38 |
| C57BL/6 | M | RA | 4 | 3,13 ± 0,90 |
| C57BL/6 | M | LA | 4 | 3,88 ± 1,52 |
| C57BL/6 | M | RV | 4 | 5,68 ± 2,34 |
| C57BL/6 | M | LV | 4 | 3,57 ± 1,23 |
| 129 | F | RA | 4 | 5,56 ± 1,47 |
| 129 | F | LA | 4 | 4,16 ± 1,36 |
| 129 | F | RV | 4 | 8,67 ± 2,67 |
| 129 | F | LV | 3 | 6,50 ± 1,75 |
| FVB | F | RA | 4 | 3,78 ± 1,11 |
| FVB | F | LA | 4 | 1,81 ± 0,38 |
| FVB | F | RV | 4 | 2,33 ± 0,69 |
| FVB | F | LV | 3 | 5,04 ± 1,31 |
| DBA | F | RA | 4 | 2,01 ± 0,66 |
| DBA | F | LA | 4 | 4,00 ± 1,00 |
| DBA | F | RV | 4 | 5,29 ± 1,32 |
| DBA | F | LV | 3 | 3,74 ± 1,19 |
| C57BL/6 | F | RA | 4 | 2,66 ± 0,49 |
| C57BL/6 | F | LA | 4 | 3,49 ± 1,01 |
| C57BL/6 | F | RV | 4 | 2,39 ± 0,32 |
| C57BL/6 | F | LV | 3 | 1,65 ± 0,42 |

Instead of showing arbitrary units, TRPM4 protein expression was normalized to the smallest value (FVB strain, male, RA).

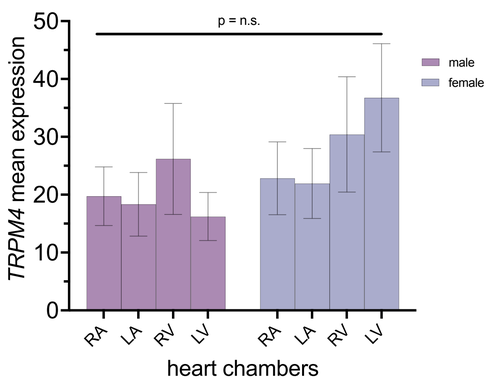


**Figure 5: TRPM4 protein expression for male and female heart chambers.**

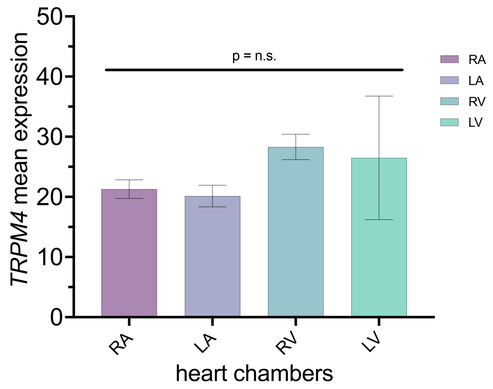


**Figure 6: TRPM4 protein expression for all four heart chambers.**

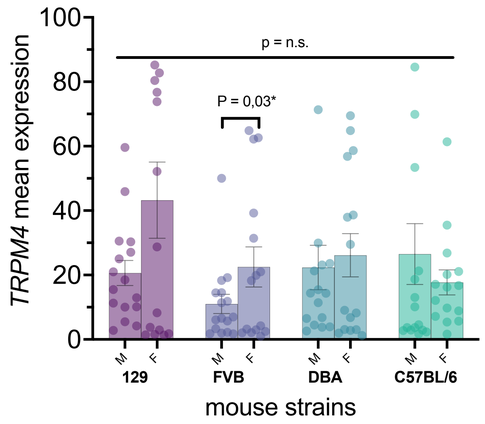


**Figure 7: TRPM4 protein expression in male and female mouse strains.**

### Multiple linear regression:

- The p-value of 0,1461 was calculated for the difference between male and female mice and their TRPM4 expressions in different heart chambers.
- The p-value of 0,6301 was calculated for the difference in TRPM4 expressions in different heart chambers of all samples independent of mouse strain or sex.
- The p-value of 0,2151 was calculated for TRPM4 expressions in different mouse strains. (*p value not significant after post-hoc correction and must be re-evaluated).

The results show that there was substantial TRPM4 expression in all chambers of the mouse hearts examined. This was independent of sex or mouse strain.

## Conclusion

Suggestions of improvement:

- Higher sample size: To generate more significant comparable data.
- Include areas in the conduction system: Purkinje fibers and septum.
- Normalize the quantity that is loaded into each lane for western blots.

Previous studies have shown the conduction system and atria as important physiological sites of TRPM4 activity, but most research is based on experimental work on cardiomyocytes from ventricular samples (4). TRPM4 studies make use of many different mouse strains, and often only examine mice of one specific sex.

This project shows that in the mouse model system, at least TRPM4 expression strength is not a concern when comparing results from different heart areas, such as atria and ventricles, and between mice of different sexes or laboratory strains.

These results are useful in analyzing past research, where data of possibly only one sex or mouse strain were used to generate results.
Also, this study could be beneficial in helping to choose an optimal mouse model for future TRPM4-related research.

**References:**

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