TRPM4 Promotes Psoriasiform Dermatitis (PsD) and is a Potential Therapeutic Target for Psoriasis

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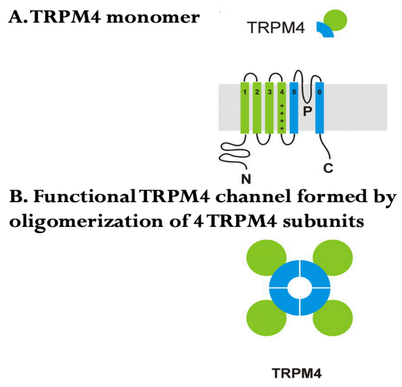
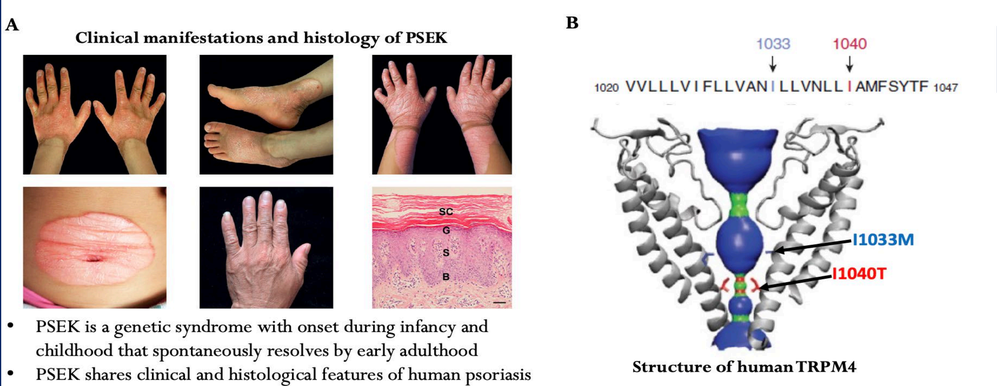
BACKGROUND

TRPM4 is a Calcium-Activated Non-Selective Cation Channel



- TRPM4 protein acts as a channel controlling the flow of positively charged atoms (cations) into and out of cells.
- TRPM4 channel plays key roles in cardiac cells, generating and transmitting electrical signals, maintaining a normal heart rhythm.
- Widely expressed with important function in the immune system.

Mutations in TRPM4 are the genetic causes of Progressive Symmetric Erythrokeratodermia (PSEK)



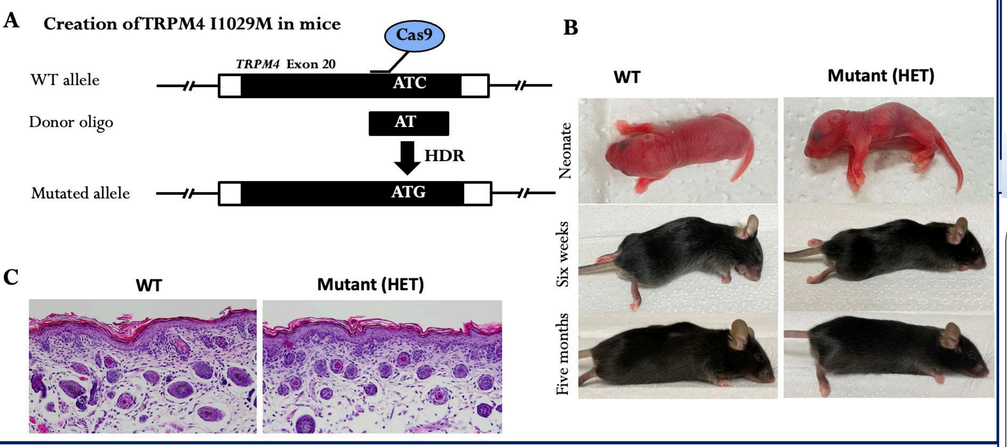
- PSEK is a genetic syndrome with onset during infancy and childhood that spontaneously resolves by early adulthood
- PSEK shares clinical and histological features of human psoriasis
- Genetic cause is I1033M or I1040T mutation of TRPM4

OBJECTIVES

- Examine the role of the gain-of-function TRPM4 channel mutation in the development of PsD using Imiquimod (IMQ) , Control Diet (CD), and Western Diet (WD) models.
- Explore the role of the gain-of-function (GoF) TRPM4 mutation in bone marrow dendritic cell (BMDC) differentiation and migration in the development of PsD.
- Target the TRPM4 channel for the treatment of psoriasiform skin conditions via TRPM4 inhibitors such as Glibenclamide (Gli) and "Compound 5", a halogenated anthranilic amide.

METHODS

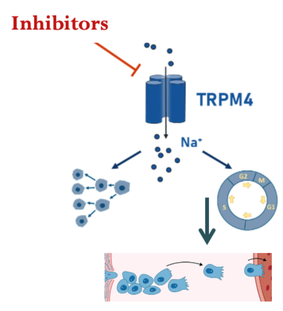
Mouse TRPM4 GoF (gain-of-function) mutant equivalent to human PSEK



Potential therapeutic strategy of applying TRPM4 inhibitors

➢ **Glibenclamide (Gli):**
A weak and non-selective TRPM4 inhibitor known as a drug for treating type-II diabetes.

➢ **Compound 5 (C5):**
Potent and selective small molecule inhibitor discovered by combining virtual screening and Na^+-influx validation



- To determine if TRPM4 plays a critical role in migration of skin DC's in response to chemotactic cytokines, and (b) to determine if BMDC proliferation is blocked by a potent TRPM4 inhibitor by in-vitro culturing primary cells from TRPM4 mutant and wild-type mice, we used a chemotaxis assay, which is used to conduct analysis of cell migration and differentiation.
- For BMDC studies, cells were cultured in standard culture conditions. Identical wells of BMDC were exposed to Gli and Compound 5 for 24 and 48 hours with dosing of drug agents at various concentrations. Cell proliferation was in turn measured by flow cytometry.
- Once effective dosing of TRPM4 inhibitors was identified, we tested nice models to obtain generalizable conclusions about the therapeutic effects of TRPM4 inhibitors on psoriasis models and then determined the optimal dose for ameliorating the symptoms with Gli and Compound 5 in mice induced with PsD.
- Lesional mRNA expression was quantified using RT-PCR methods. Histopathology was done using formaldehyde-fixed, paraffin-embedded skin samples stained with H&E.
- Student's t-test and ANOVA were used for comparisons between 2 treatment groups.

SUMMARY AND FUTURE WORK

- We created a TRPM4 GoF mouse that has a mutation equivalent to PSEK patients.
- The GoF mice showed exaggerated psoriasiform dermatitis upon Imiquimod (IMQ) application and Western Diet (WD) feeding.
- Keratinocyte proliferation and dendritic cell (DC) migration are associated with TRPM4 gain-of-function (GoF), and TRPM4 inhibitors Gli and Compound 5 decreased bone marrow dendritic cell (BMDC) migration.
- More studies are planned to test TRPM4 inhibitors in-vitro and in-vivo to develop effective therapeutics for the treatment of psoriasiform dermatitis.

BUILDING OFF PREVIOUS WORK

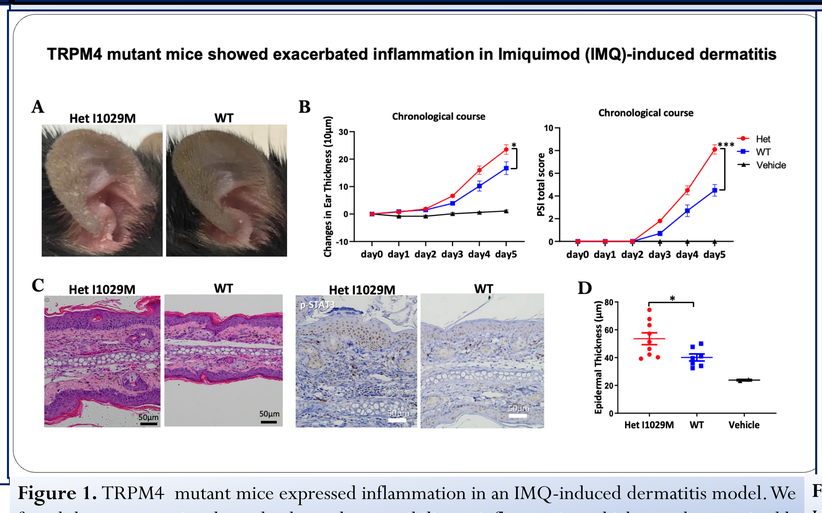


Figure 1. TRPM4 mutant mice expressed inflammation in an IMQ-induced dermatitis model. We found that mutant mice showed enhanced susceptibility to inflammation which was characterized by ear thickening, erythema, and scaling. All of these changes are greater in TRPM4 mutant mice.

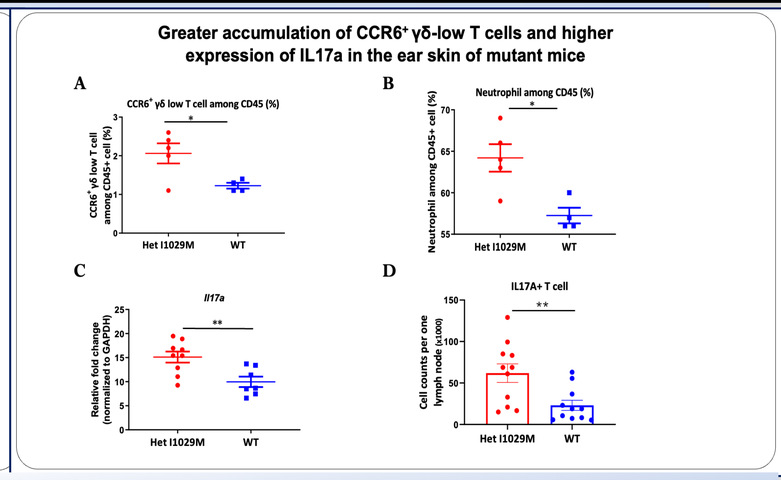


Figure 2. Mutant heterozygous mice expressed increased numbers of CCR6+ gamma delta-Low T cells and a greater number of neutrophils in draining lymph nodes, and had a higher expression of IL17a in the ear skin of mutant mice.

RESULTS

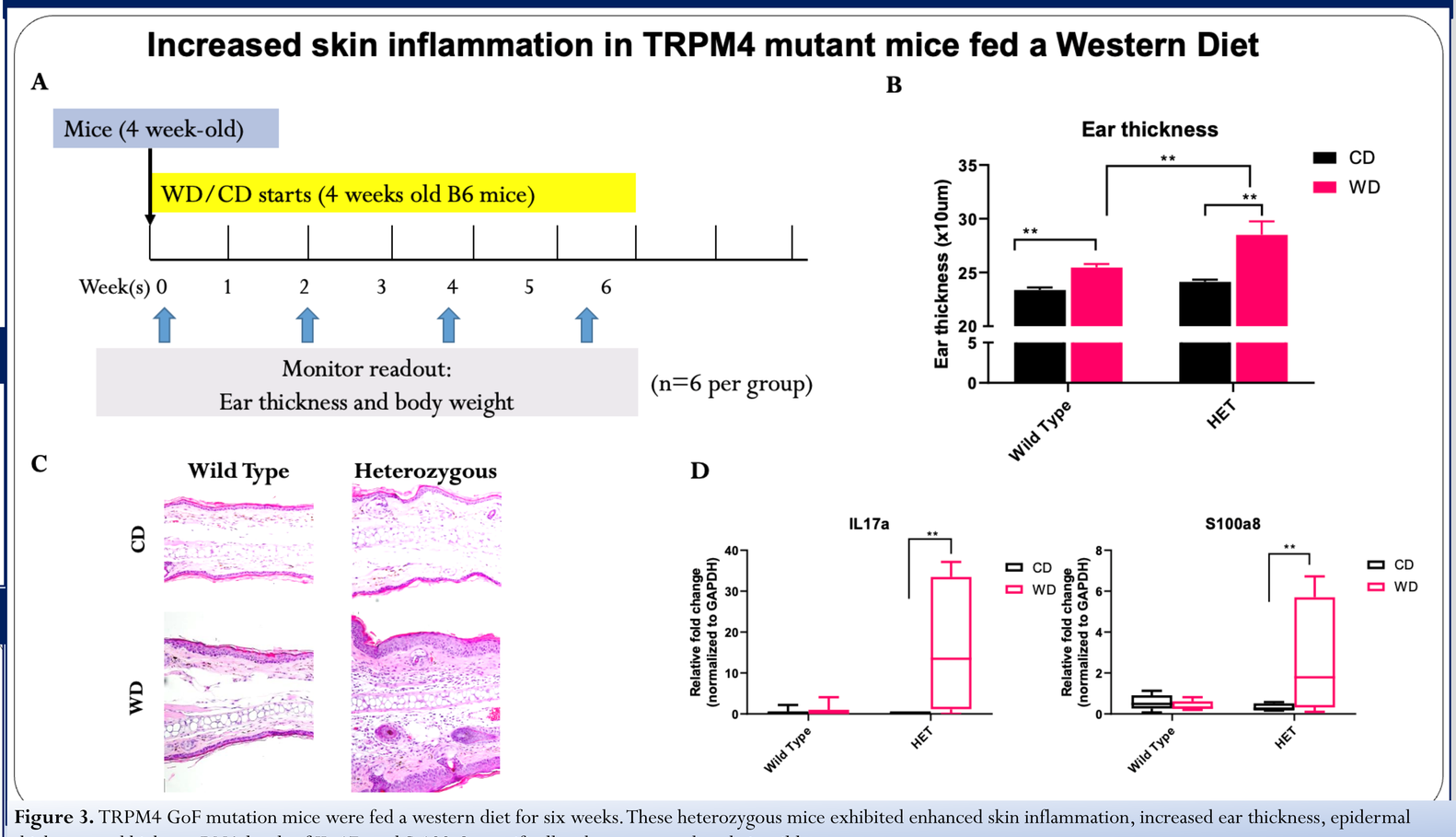


Figure 3. TRPM4 GoF mutation mice were fed a western diet for six weeks. These heterozygous mice exhibited enhanced skin inflammation, increased ear thickness, epidermal thickness, and higher mRNA levels of IL-17a and S-100a8, specifically when compared to their wild type counterparts.

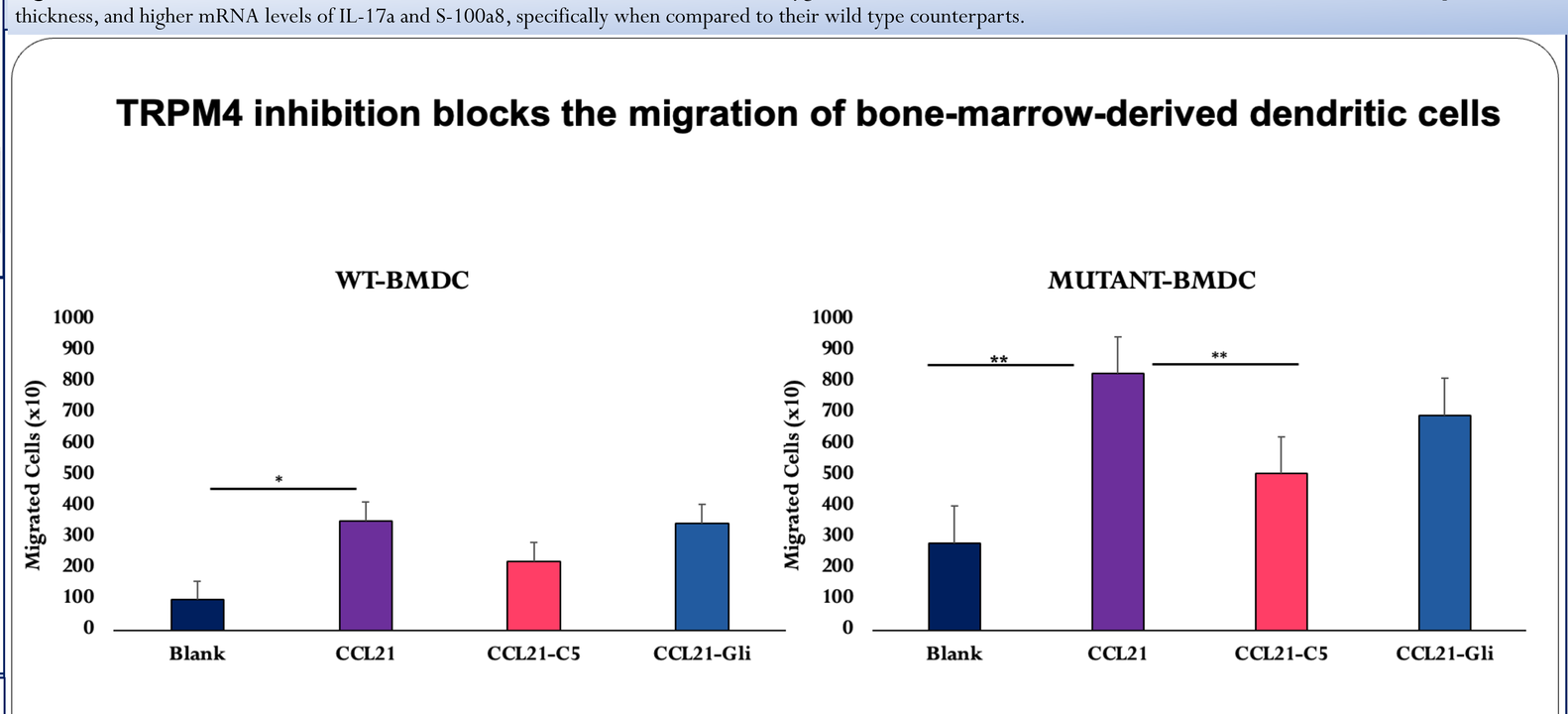


Figure 4. To see if TRPM4 inhibitors affected DC migration, we performed a chemotaxis assay with cultured BMDC from TRPM4 WT and mutant mice. Heterozygous DC had a greater migration in response to CCL21, with migration slightly inhibited by Gli and greater inhibition with Compound 5. This effect is more significant in migration of mutant cells.

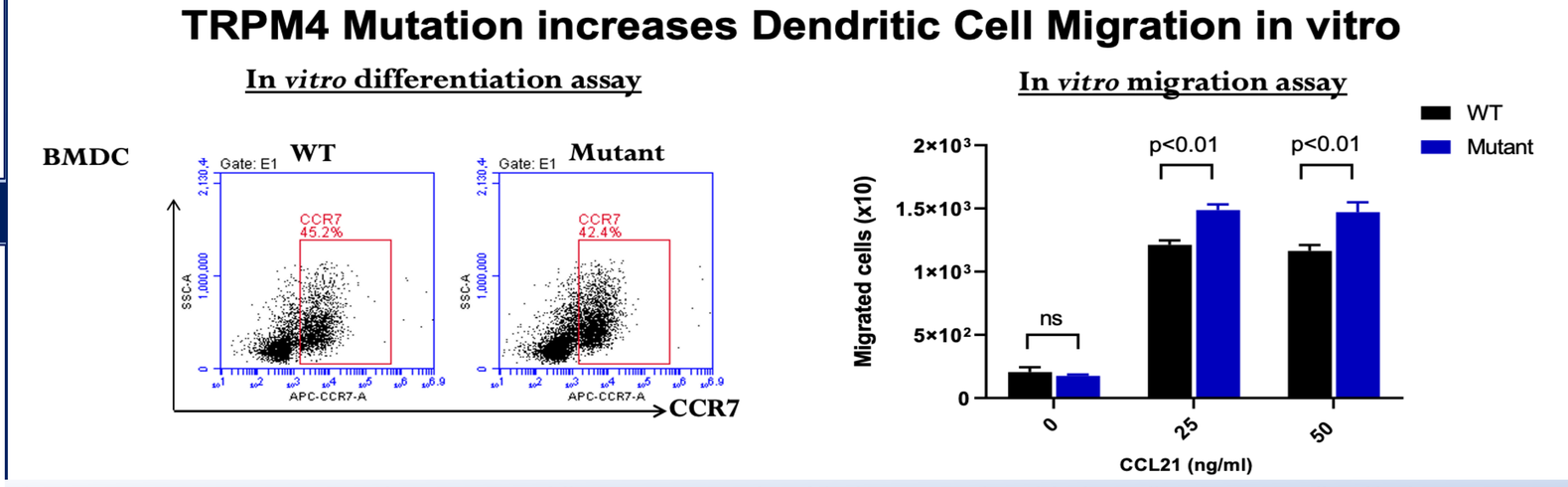


Figure 5. Using CCR7 as a maturation marker in our in vitro differentiation assay, we showed that bone marrow derived dendritic cell maturation is unaffected by the TRPM4 mutation. However, in in-vitro chemotaxis assays, we do find small but statistically significant increases in the chemotaxis migration assay of the TRPM4 mutant mice.