Supplementary Online Content

Duchatelet S, Pruvost S, de Veer S, et al. A new TRPV3 missense mutation in a patient with Olmsted syndrome and erythromelalgia. *JAMA Dermatol*. Published online January 22, 2014. doi:10.1001/jamadermatol.2013.8709

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

DNA Extraction

Genomic DNA was extracted from EDTA anticoagulated peripheral venous blood samples following a standard phenol chloroform procedure. DNA integrity was evaluated by 0.8% agarose gel electrophoresis. DNA purification was performed prior to whole-exome sequencing using Amicon Ultra 30K centrifugal filter units (Millipore). DNA samples were stored at −20°C until used.
Written informed consent was obtained from the parents of the patient for publication of clinical details and accompanying images.

Exome Sequencing

Agilent SureSelect libraries were prepared from 3 µg of genomic DNA sheared with a Covaris S2 Ultrasonicator as recommended by the manufacturer. Exome capture was performed with the 50 Mb SureSelect Human All Exon kit (Agilent technologies) using a multiplex approach with molecular barcodes for traceable ID of samples. Sequencing was carried out with the SOLiD5500 (Life Technologies) on a pool of barcoded exome librairies. 75 bases reads were generated and mapped on human genome reference (NCBI build37/hg19 version) using LifeScope (Life Technologies). We achieved an average coverage of 106 fold of the mappable, targeted exome sequences and 80% of the exome was covered at least fifteenfold. SNPs and indels calling were made using GATK tools. An in-house software (PolyWeb) was used to annotate and filter the variants.

Exome Analysis

To distinguish potentially pathogenic mutations from others variants, we focused on variants with coding consequences (non-synonymous, nonsense, frameshift, start or stop-lost) or affecting splice-acceptor or -donor sites. We also presumed that the causative variant would be rare and likely absent in databases. Variations were filtered by the dbSNP135, the 1000 Genomes Project, and the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/).

Sanger Sequencing

Sanger sequencing was performed to confirm the results from whole-exome sequencing. The previously reported PCR primers were used to validate the candidate mutation in TRPV3 exon 15 and to test the two parents.[1] Sequencing reactions were performed using big-dye terminator chemistry using standard protocols and run on an Applied Biosystems 3130xl genetic analyzer.

RNA Extraction

Total RNA was isolated from the patient's confluent cultured keratinocytes using a RNeasy Mini kit (Qiagen) according to the manufacturer's instruction. After reverse transcription, the PCR primers 5'-GTGTTTTTGCTTGGATTTGGA-3' and 5'-GCTCCTCAGCCATTCTGGTA-3' were used to validate the presence of the identified mutation in mRNA.

TRPV3 Homology Modeling Analysis

The structure of TRPV1 has been studied by single-particle electron cryomicroscopy and resembles a classical tetrameric, six transmembrane domain ion channel.[2] However, a high-resolution TRPV channel structure is currently unavailable. Thus, a homology model for TRPV3 was generated. The model template was selected by searching SWISS-MODEL (http://swissmodel.expasy.org/workspace/) with the sequence of the human TRPV3 channel-forming segment (residues 566-690). The match returned was the recent crystal structure of the Na_vRh voltage-gated sodium channel (PDB ID 4DXW) which shows higher sequence similarity to TRPV1-6 across the channel forming region than $K_{v1.2}$ (used to produce existing TRPV models).[3] A TRPV3 model was subsequently built encompassing residues 591-680 (based on Na_vRh residues 137-222) using SWISS-MODEL. Alignment between TRPV3 and Na_vRh was refined in DeepView4.0.4 (http://www.expasy.ch/spdbv/rnainpage.html) and subsequently used to produce a second-generation model (Phe_{590} to Ser_{685}) which was fitted to each

subunit of the Na_vRh complex to generate a tetrameric TRPV3 model. Side chain clashes were relieved by step-wise relaxation in YASARA Dynamics (http://www.yasara.com/servers.htm). All structural figures were produced using CCP4MG software (http://www.ccp4.ac.uk/MG/).

References

1. Lin Z, Chen Q, Lee M, et al. Exome sequencing reveals mutations in TRPV3 as a cause of Olmsted syndrome. Am J Hum Genet. 2012;90(3):558-564.
2. Moiseenkova-Bell VY, Stanciu LA, Serysheva, II, Tobe BJ, Wensel TG. Structure of TRPV1 channel revealed by electron cryomicroscopy. Proc Natl Acad Sci U S A. 2008;105(21):7451-7455.
3. Zhang X, Ren W, DeCaen P, et al. Crystal structure of an orthologue of the NaChBac voltage-gated sodium channel. Nature. 2012;486(7401):130-134.

eFigure. Modeling of TRPV3

TRPV3 590-685 model (blue) superimposed on the template structure (NavRh, silver). Non-S6 segments are transparent to highlight S6 helices.