Transient Receptor Potential Channel TRPM4:

PredictingPathogenic/Likely Pathogenic Status for Variants of

Uncertain Clinical Significance

TRPM4 is a calcium-activated channel that selectively permeates

monovalent cations. Genetic variantsin the hTRPM4 channel in cardiomyocytes are associates with various heart disorders, such as progressive familial heart block and Brugada syndrome.As of May 2023, over 1,350 variants of the TRPM4 channel are listed in the ClinVar database. Among these, clinical significance is uncertain for over 750 variants (VUS) and only 12 variants are described as pathogenic. The very small proportion of pathogenic

variants is a serious problem in diagnostics and treatment of inherited heart diseases.

Methods and Results. Here we employed bioinformatics to reclassify some VUS variants as pathogenic/likely pathogenic (P/LP) variants. We used databases ClinVar, Humsavar and Ensembl to compose a dataset of missenseP/LP and VUS variants in the superfamily of TRP channels. Using this dataset, we tested 22 bioinformatic tools with various thresholds of pathogenicity on their capability to predict pathogenicity for known

P/LP variants. We found that ClinPred is the best-performing tool for TRP channels. In the set of 582 uncharacterized missense variants of TRPM4, ClinPred predicted pathogenicity of 299 variants. Among these, 12 variants are also categorized as P/LP variants in at least one paralogue of TRPM4. We further used the cryo-EM structure of hTRPM4 to locate scores of

contact pairs between parental (wild type) residues of VUSs for which ClinPred predicts a high probability of pathogenicity of variants for both contact partners and propose that 68 respective missense VUSs could be also reclassified as P/LP variants.

Conclusions. Bioinformatic tool ClinPred, the paralogue annotation method and analysis of intersegment contacts in the cryo-EM structure of the hTRPM4 channel suggest that a total of 80 missense VUSs may be reclassified as P/LP variants. This number is much larger than 13 P/LP variants currently listed in the ClinVar database, among which only two variants are missense ones. The results are expected to improve diagnostics and treatment of several heart diseases. Our bioinformatic pipeline may be used to reclassify VUSs and

variants with conflicting interpretation of pathogenicity as P/LP variants in other TRP channels.