GDD1 has been found to be dominantly mutated in rare skeletal disorder Gnathodiaphyseal dysplasia (GDD) and recessively in Limb Girdle Muscular Dystrophy (LGMD2L) and Miyoshi myopathy (MMD3); however, protein function is still unknown. Due to similarity of predicted eight transmembrane in topology, GDD1 is classified as TMEM16E in TMEM16 family proteins. The first molecular identity for TMEM16 family proteins was the calcium-activated chloride channel (CaCC) in TMEM16A. Recessive mutation in TMEM16F was reported to cause Scott syndrome which exhibits blood coagulation disorder due to the defect of calcium-dependent phospholipid scramblase activity beside possible channel activity.

In this study, I characterized the stability, localization and possible Cl⁻ channel activity of TMEM16E using TMEM16A and TMEM16F as controls.

When the genes were expressed in HEK293T cells, TMEM16E showed lower protein expression than TMEM16A and TMEM16F. TMEM16E expression specifically accumulated with the inhibition of proteasome pathway comparing to the inhibition of lysosome pathway while TMEM16A and TMEM16F were up-regulated in both treatments. TMEM16E protein was rapidly decreased by treatment of protein synthesis inhibitor (Cyclohexaminde), implying TMEM16E is unstable and degraded by proteasome pathway. Besides proteasome inhibitor, PI3K kinase inhibitors (LY294002 and Wortmannin) also up-regulated TMEM16E and Sodium Butyrate worked as chemical chaperon to stabilize TMEM16E.

In contrast to plasma membrane (PM) localization of TMEM16A and TMEM16F, TMEM16E showed intracellular distribution. To determine the possible intracellular retention signal of TMEM16E, varied domains of TMEM16E were substituted to those of TMEM16A. The amino-terminus to first transmembrane (TM) domain or TM6 to TM8 domain from TMEM16E able to deprive PM distribution of TMEM16A, suggesting the structural intracellular propensity of TMEM16E. A₃E₅A and A₈E
(number indicated TM domain) trafficked to PM, implying these domain of TMEM16E have no role in cytoplasmic localization.

Next, I examined possible Cl⁻ channel activity of TMEM16E. Since the whole cell patch-clamping monitors only the channels on the plasma membrane, TMEM16E expressed in HEK293T cells exhibited no Cl⁻ current in accordance with its cytoplasmic localization. Because the domain from TM5 to TM6 was predicted to form channel pore in TMEM16 family proteins, we could test the channel activity of this domain of TMEM16E by PM trafficking A₅E₆A chimera. However, A₅E₆A expressed cells did not display any Cl⁻ current. By additional Thr612Cys mutation which mimics pore-forming domain of TMEM16A, A₅E₆CysA gained CaCC activity, implying polypeptides surrounding Thr612 of TMEM16E specifically defines the putative channel pore-forming domain character, that is, inability as Cl⁻ channel pore.

Since GDD disease was caused by Cys356 mutation in TMEM16E and TMEM16 family proteins conserve this cysteine, I tried to test effect of cysteine mutation on the function of well-characterized TMEM16A. The mutant designated TMEM16Aₕdd gained Cl⁻ channel activity in the absence of calcium. In the presence of calcium, the current profile was distinctively modified compared to that of TMEM16A. This suggests GDD associated mutation at cysteine commonly modifies the function of TMEM16E, resulted in the gain function in TMEM16Eₕdd ascribed to cause GDD.

Currently, the only cell that could detectably express and stabilize TMEM16E in vitro was myotube. Although TMEM16Eₕdd causes bone related disease, TMEM16E was unable to express in osteoblasts, chrodroblasts or osteosarcoma cells. In LGMD2L patient, defect of TMEM16E causes no symptom in any tissues except topical muscular dystrophy, which represents the protein is too unstable to function in tissues, except for muscles. In other word, the tissue specific stabilization is necessary for TMEM16E function which is not as a CaCC molecule. Since TMEM16E protein has no chance to function in GDD susceptible tissues such as bones, TMEM16Eₕdd might require ectopic stabilization to cause GDD disease through gain of function.