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The L530R variation associated with recurrent kidney stones impairs the structure and function of TRPV5

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ABSTRACT

TRPV5 is a Ca^{2+}-selective channel that plays a key role in the reabsorption of Ca^{2+} ions in the kidney. Recently, a rare L530R variation (rs757494578) of TRPV5 was found to be associated with recurrent kidney stones in a founder population. However, it was unclear to what extent this variation alters the structure and function of TRPV5. To evaluate the function and expression of the TRPV5 variant, Ca^{2+} uptake in *Xenopus* oocytes and western blot analysis were performed. The L530R variation abolished the Ca^{2+} uptake activity of TRPV5 in *Xenopus* oocytes. The variant protein was expressed with drastic reduction in complex glycosylation. To assess the structural effects of this L530R variation, TRPV5 was modeled based on the crystal structure of TRPV6 and molecular dynamics simulations were carried out. Simulation results showed that the L530R variation disrupts the hydrophobic interaction between L530 and L502, damaging the secondary structure of transmembrane domain 5. The variation also alters its interaction with membrane lipid molecules. Compared to the electroneutral L530, the positively charged R530 residue shifts the surface electrostatic potential towards positive. R530 is attracted to the negatively charged phosphate group rather than the hydrophobic carbon atoms of membrane lipids. This shifts the pore helix where R530 is located and the D542 residue in the Ca^{2+}-selective filter towards the surface of the membrane. These alterations may lead to misfolding of TRPV5, reduction in translocation of the channel to the plasma membrane and/or impaired Ca^{2+} transport function of the channel, and ultimately disrupt TRPV5-mediated Ca^{2+} reabsorption.

1. Introduction

TRPV5, one of the two epithelial Ca^{2+} channels with a high Ca^{2+} selectivity, is mainly expressed in the distal convoluted tubule (DCT) and connecting tubule (CNT) in the kidney [1,2]. It regulates the first step in transcellular Ca^{2+} transport and is considered as a gatekeeper for active Ca^{2+} reabsorption [3]. The knockout of TRPV5 in mice resulted in severe hypercalciuria and bone abnormalities [2]. Furthermore, altered expression of TRPV5 in mouse models of human diseases is associated with vitamin D-deficiency rickets, altered estrogen levels and postmepausal osteoporosis, and parathyroid hormone-related disorders [4].

Several nonsynonymous single nucleotide polymorphisms (SNPs) in TRPV5, including A8V (rs4252372), R154H (rs4236480), A563T (rs4252499) and L712F (rs4252509), have been found in African Americans with high allele frequency [5,6]. Our previous works indicate that the A563T variation increases TRPV5-mediated Ca^{2+} transport [6] and affects the structure and dynamics of the pore regions of TRPV5 [7]. This variation may contribute to an increased renal Ca^{2+} reabsorption in African Americans compared to Caucasians. The R154H variation, which is present in both African and non-African populations at a relatively high allele frequency, usually causes a lower Ca^{2+} uptake activity in TRPV5 when combined with other variations [6]. However, in most cases, the decrease in Ca^{2+} uptake does not reach statistical significance [6]. Interestingly, this SNP has been found to be associated with the occurrence of multiple stones in a cohort of 365 kidney stone

Abbreviations: TRPV5, transient receptor potential cation channel vanilloid subfamily member 5; TM, transmembrane; cRNA, capped complementary RNA; MD, molecular dynamics; RMSD, root mean square deviation; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; RDF, radial distribution function.

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patients in Taiwan [8]. Recently, a L530R variation (rs757494578) in TRPV5 was found to be associated with recurrent kidney stones in 2636 individuals from a founder population in Iceland [9]. This variation introduces a positive charged residue into a hydrophobic region of the pore helix and likely affects the function of TRPV5; however, the effects of this mutation have not been examined. To understand the role of TRPV5 in kidney stone disease, we assessed the Ca^{2+} transport function of the L530R variant by monitoring radiotracer Ca^{2+} uptake in *Xenopus* oocytes. To gain new insights into the structural changes produced in TRPV5 with the L530R variation, we performed molecular dynamic simulations of TRPV5 using the recently published crystal structure of TRPV6 [10], a close homologue of TRPV5 [11,12]. Our results are expected to provide further evidence for a relationship between genetic variations in TRPV5 and the risk of kidney stone disease.

2. Material and methods

2.1. Ca^{2+} uptake assay

Ca^{2+} uptake by *Xenopus* oocytes was performed as described previously [6,13]. The animal protocol used in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Alabama at Birmingham. The L530R variation of TRPV5 was generated using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) in the FLAG-tagged human TRPV5 and the mutation was confirmed by sequencing. The capped complementary RNAs (cRNAs) of FLAG tagged TRPV5 with L530 (reference) and R530 (variant) were prepared using mMESSAGE mMACHINE™ SP6 Transcription Kit (ThermoFisher). Oocytes were microinjected with either the reference or the variant cRNA at 12.5 ng/oocyte or water. Oocytes were cultured in 0.5 × L-15 solution and assayed 2 days after injection by radiotracer $^{45}Ca^{2+}$ uptake in standard Ca^{2+} uptake solution. After uptake, oocytes were washed six times and then lysed in 10% SDS solution. Radioactivity of each oocyte was determined using a scintillation counter. Statistical significance is defined as $P < 0.05$ by a Student's t-test.

2.2. Western blot analyses

Lysates were extracted from ten oocytes/group injected with water, L530 or R530 TRPV5 cRNA. Monoclonal anti-FLAG antibody (F7425, 1:1000 dilution) was purchased from Sigma–Aldrich (St. Louis, MO). Appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (1:10000 dilution) were purchased from Santa Cruz Biotechnology. Chemiluminescence signals were detected using SuperSignal West Pico Chemiluminescent Substrate kit (Pierce Biotechnology, Rockford, IL).

2.3. System modeling

The modeling of human TRPV5 was performed based on the structure of rat TRPV6 using MODELLER 9.13 software [14]. The template of TRPV6 with Ca^{2+} ions was obtained from the Protein Data Bank with an ID of 5IWP [10]. The sequence of TRPV5 used in modeling contains the linker helix (LH) 1–2, pre-transmembrane (TM) helix (pre-S1), TM helices 1–6 (S1-S6), and the TRP domain. The alignment of the human TRPV5 sequence used in the modeling and the corresponding rat TRPV6 sequence is presented in Fig. S1. Since we are only using human TRPV5 in this study, the species will not be specified hereafter. The identity between the sequences is 83.1%, which is much higher than the threshold of 30% that is considered to be reliable for modeling membrane proteins [15]. The modeled TRPV5 tetramer is shown in Fig. 1A. The L530R variation

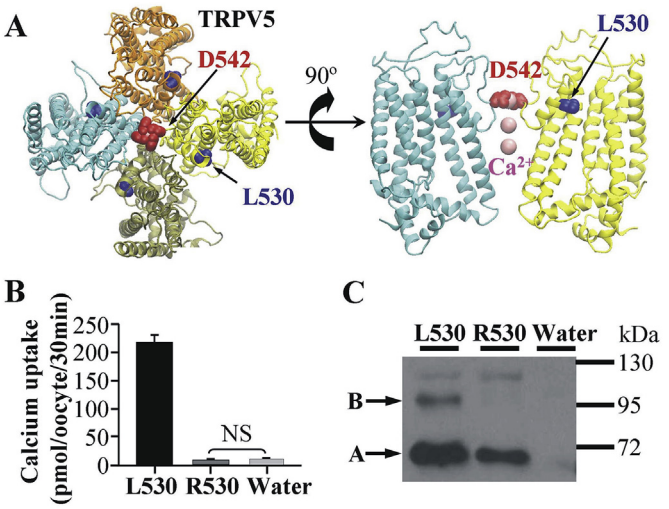

Fig. 1. The L530R variation abolishes the Ca^{2+} uptake activity of TRPV5. **(A)** The location of L530 in the modeled structure of the TRPV5 tetramer. The top view of the TRPV5 tetramer is shown on the left and only two monomers are shown in the side view (right panel) for clarity. The four monomers are shown in cyan, tan, yellow and orange, respectively. D542 in the Ca^{2+} selective filter, L530 where variation occurred, and Ca^{2+} ions are shown in red, blue and pink, respectively. **(B)** Ca^{2+} uptake in *Xenopus* oocytes expressing TRPV5 with L530, R530, or in control oocytes. Each group contained Ca^{2+} uptake values of 18 *Xenopus* oocytes from two frogs. NS means the difference is not significant. **(C)** Western blot analysis of TRPV5 variants (L530 and R530) expressed in *Xenopus* oocytes. Band B represents complex-glycosylated form of TRPV5, and band A represents core-glycosylated form. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was introduced into all four subunits of TRPV5 using the mutagenesis function of PyMOL [16]. The two TRPV5 systems containing leucine or arginine residues at amino-acid position 530 are denoted as **L530** and **R530**, respectively.

To mimic the membrane environment, the modeled TRPV5 structures were embedded in a lipid bilayer composed of 299 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) lipids using CHARMM-GUI membrane builder [17]. Each system (**L530** or **R530**) was then hydrated with a total of 32,854 TIP3P water molecules on both sides of the bilayer. The approximate dimensions of the resultant simulation box were 126 Å × 126 Å × 110 Å along the x, y, and z axes, respectively. Na^+ and Cl^- ions were added to the system to neutralize it and maintain a 150 mM NaCl concentration. The parameters of a FF14SB force field [18] were assigned to the protein, ions, and water molecules, and a FFlipid14 force field [19] was used for POPC lipids.

2.4. Molecular dynamic simulations

To investigate the effect of the L530R variation on the structural change in TRPV5, two 400 ns molecular dynamic (MD) simulations were performed using the AMBER14 simulation package [20]. The simulation protocol is similar to our previous studies [21–25], and it can be found in the *Supplementary Material*. Before data analysis, the root mean square deviation (RMSD) for the Cα atoms of TRPV5 was calculated to assess the equilibration of the simulation. Simulation reached an equilibration state after 200 ns in both **L530** and **R530** systems (Fig. S2). Thus, the last 200 ns simulations were used for analyses by using the CPPTRAJ program of AMBER14. The DSSP method was applied to determine whether an amino acid residue belonged to an α helix [26]. The electrostatic potential for TRPV5 was calculated by APBS [27]. The VMD software [28] was used for structure visualization.

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