FUNCTIONAL ANALYSIS OF HUMAN MUTATIONS IN NAPI-IIC REVEALS IMPORTANT RESIDUES FOR SURFACE EXPRESSION AND SODIUM-PHOSPHATE CO-TRANSPORT

Clemens Bergwitz1 Graciana Jaureguiberry1, Thomas O Carpenter3, Stuart Forman2, Harald Jüppner1
1Endocrine Unit and 2Dept. of Anesthesiology, Massachusetts General Hospital, Boston, MA, United States; 3 Dept. of Pediatrics and Endocrine Unit, Yale School of Medicine, New Haven, CT, United States.

We recently reported homozygous and compound heterozygous loss-of-function mutations in SLC34A3/NaPi-IIc as the cause for hereditary hypophosphatemic rickets with hypercalciuria (HHRH). To better understand how some of the point mutations reported by us and others (T137M, S138F, S192L, G196R, R468W, del527L, R353L, D237N and A413E) lead to renal phosphate wasting, we generated expression plasmids encoding enhanced green-fluorescence protein (EGFP) concatenated to the N-terminus of wild-type or mutant human NaPi-IIc. After transient transfection in *Opossum* kidney cells G196R and R468W were not expressed in apical patches (the renal brush border membrane equivalent), nor were these mutants present in plasma membranes of *Xenopus* oocytes following cRNA injection (50 ng/oocyte). For S138F, S192L, R353L, D237N and A413E oocyte membrane fluorescence was reduced to <20% compared to EGFP-hNaPi-IIc, as quantified by confocal microscopy. The T137M and delL527L mutants were expressed in both kidney cells and oocytes, and sodium-dependent [33P]-uptake into oocytes corrected for surface expression was preserved. When further investigated, the Na:P-stoichiometry of simultaneous [^{33}P]- and [^{22}Na]-uptake was increased from 2.3±0.4 in wild-type to 7.1±3.6 and 23±13 for T137M and delL527L, respectively. Furthermore, two-electrode studies indicated that EGFP-hNaPi-IIc carrying these mutations remains non-electrogenic, like wild-type NaPi-IIc, but displayed a significant phosphate-independent inward-rectified sodium-leak current which appears to be insensitive to phosphonoformic acid (PFA), a known inhibitor of sodium-dependent phosphate co-transport. T137M and delL527L may thus reduce the rate of phosphate uptake by uncoupling sodium-phosphate co-transport, suggesting that these amino acid residues have an important functional role in human NaPi-IIc.