SUPPLEMENTARY RESULTS

for

"Dominant negative mutation in oxalate transporter *SLC26A6* associated with enteric hyperoxaluria and nephrolithiasis" by Nicolas Cornière, R. Brent Thomson, Stéphanie Thauvin, Bruno O. Villoutreix, Sophie Karp, Diane W. Dynia, Sarah Burlein, Lennart Brinkmann, Alaa Badreddine, Aurélie Dechaume, Mehdi Derhourhi, Emmanuelle Durand, Emmanuel Vaillant, Philippe Froguel, Régine Chambrey, Peter S. Aronson, Amélie Bonnefond, & Dominique Eladari

Detailed In Silico analyses of the effects of the p.R507W mutation on SLC26A6

Human SLC26A6 residue p.R507 was found fully conserved in an interspecies comparison. The conservation of this residue was also very high in this family of proteins (*i.e.,* the score at position 507 was equal to 8, the maximum is 9, via the Consurf server). We built a homology model using as experimental template the mouse Slc26a9 homodimer anion transporter 3D structure (Fig. 1). The modeling was possible as the overall sequence identity between the two proteins is around 40% (Suppl. Fig. S6). One large insertion loop in SLC26A6 could not be predicted in the STAS domain but this area is far away from position 507 (Suppl. Fig. S6, Fig. 1).

The human SLC26A6 model structure positioned into a membrane is shown in Fig. 5. p.R507 is located on the C-terminal side of the last transmembrane helix (Suppl. Fig. S6), prior to the STAS domain. This residue is essentially buried, at the interface with residues from the second subunit (Fig. 1). It makes a salt bridge with p.D682 of the other subunit, and has polar interactions with p.Q715 of the other subunit and p.N470 of the same subunit. It has also hydrophobic/aromatic contacts with p.F680 of the other subunit, which is distant from the expected Cl^- channel (Fig. 1). The region of p.R507 should be essentially rigid (PredyFlexy computation), and thus not very tolerant to the p.R507W substitution. Interactive structural analysis via PyMol and Chimera highlights that the replacement of p.R507 with a tryptophan damages the above-mentioned hydrogen bonds and salt-bridge while creating steric clashes with the surrounding amino acids.

ΔΔG stability studies (Suppl. Fig. S7), computed with DUET and SAAFEC, also underline an important change in stability suggesting that the mutant protein will be

affected as compared to the wild-type. We used the RING sever to compute a residue interaction network that we visualized in Cytoscape. Residue p.R507 is not highly connected (Suppl. Fig. S8) but many residues around make numerous non-covalent interactions with the surrounding (*e.g.*, with p.T508) and as such, the R to W is expected to alter the structure of the last helix of this domain. The clashes resulting from the p.R507W substitution together with the loss of hydrogen bonds and salt-bridge should damage this region of the mutant protein and most likely perturb correct interaction with the membrane. These data are summarized in the flower-donut-traffic-light chart presented in Suppl. Fig. S7.