Targeted rescue of a polycystic kidney disease mutation by lysosomal inhibition

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic cause of end-stage renal disease. The molecular pathogenesis of ADPKD is not completely known, and there is no approved therapy. To date, there is limited knowledge concerning the molecular consequences of specific disease-causing mutations. Here we show that the ADPKD missense variant TRPP2^{D511V} greatly reduces TRPP2 protein stability, and that TRPP2^{D511V} function can be rescued *in vivo* by small molecules targeting the TRPP2 degradation pathway. Expression of the TRPP2^{D511V} protein was significantly reduced compared to wild-type TRPP2. Inhibition of lysosomal degradation of TRPP2^{D511V} by the US Food and Drug Administration (FDA)–approved drug chloroquine strongly increased TRPP2 protein levels *in vitro*. The validation of these results *in vivo* requires appropriate animal models. However, there are currently no mouse models harboring human *PKD2* missense mutations, and screening for chemical rescue of patient mutations in rodent models is time-consuming and expensive. Therefore, we developed a *Drosophila melanogaster* model expressing the ortholog of TRPP2^{D511V} to test chemical rescue of mutant TRPP2 *in vivo*. Notably, chloroquine was sufficient to improve the phenotype of flies expressing mutant TRPP2. Thus, this proof-of-concept study highlights the potential of directed therapeutic approaches for ADPKD, and provides a rapid-throughput experimental model to screen *PKD2* patient mutations and small molecules *in vivo*.

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ADPKD is the most common fatal monogenic disease in humans and a leading cause of end-stage renal disease. The 5-year probability of survival among ADPKD patients with end-stage renal disease is ≤40%.[1] There is no approved therapy to cure cystic disease, and current treatments include dialysis and transplantation, which are burdensome and costly. Mutations in *polycystic kidney disease 2* (*PKD2*) account for approximately 1 in 6 ADPKD cases, that is, about 2,000,000 cases worldwide. The *PKD2* gene product, transient receptor potential polycystin-2 (TRPP2), is a nonselective cation channel.[2] Several pathogenic missense mutations that impair TRPP2 ion channel function have been reported.[3,4] The confined impact of a single amino acid change makes missense mutations attractive candidates for corrective approaches using small molecules. A successful example of this strategy is the development of a therapeutic intervention for cystic fibrosis, which targets a specific causal mutation rather than disease symptoms.[5] Two classes of drugs have been developed for cystic fibrosis: (i) correctors, which target cellular misprocessing of cystic fibrosis transmembrane conductance regulator (CFTR), the protein mutated in cystic fibrosis; and (ii) potentiators, which restore CFTR ion channel activity at the cell surface.[6] The approval of ivacaftor by the FDA for the treatment of patients carrying the rare CFTR variant G551D highlights the feasibility of personalized therapies for inherited channelopathies such as cystic fibrosis and polycystic kidney disease.[5] Furthermore, the clinical use of ivacaftor is continuously expanding to larger pools of patients based on common pathogenic mechanisms between G551D and other CFTR missense mutations.[7] On the basis of this paradigm, we hypothesized that the analysis of allele-specific pathogenic mechanisms might reveal promising therapeutic targets for ADPKD. Here we show that the ADPKD patient missense mutation TRPP2^{D511V} greatly reduces cellular TRPP2 protein stability and that TRPP2^{D511V} function can be partially rescued *in vivo* by an FDA-approved drug targeting the default TRPP2 degradation pathway.

RESULTS

A *PKD2* missense mutation reduces TRPP2 protein abundance

Aspartate 511 of TRPP2 is highly conserved throughout evolution (Figure 1a). Non-synonymous substitution of this aspartate with valine has been reported to eliminate TRPP2 ion channel function *in vitro*.[4,8] We have recently shown that

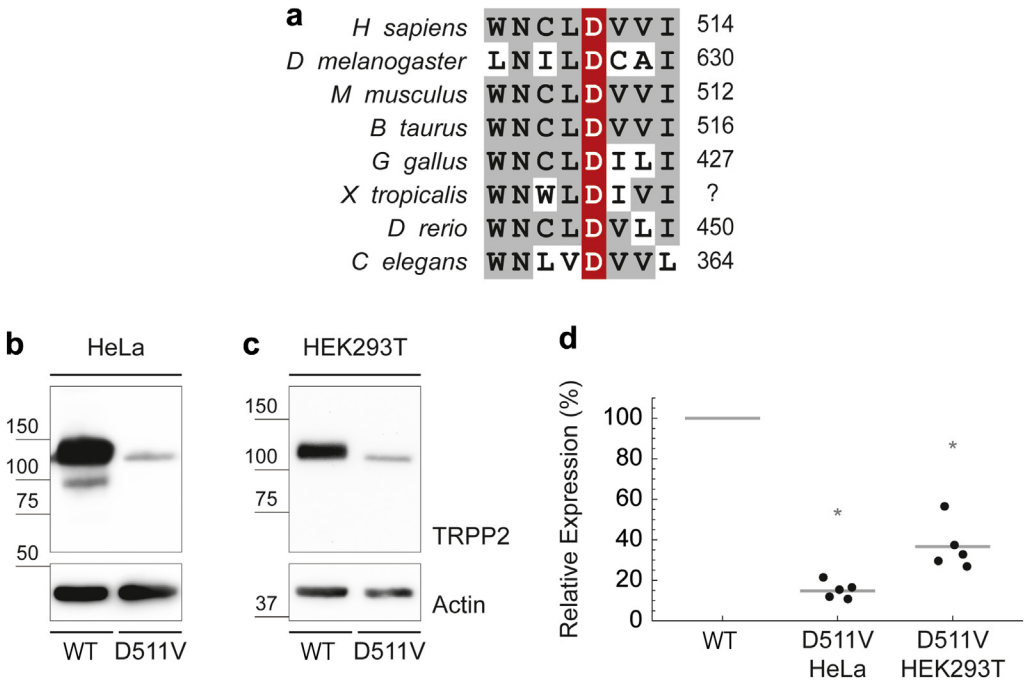

Figure 1 | The autosomal dominant polycystic kidney disease missense variant TRPP2^{D511V} reduces TRPP2 protein abundance. (**a**) The human TRPP2^{D511V} mutation is localized in the highly conserved third transmembrane segment (TRPP2$^{506–527}$) of TRPP2. (**b**) Western blot analysis of wild-type TRPP2 shows 2 distinct bands.[19] Compared to wild-type, TRPP2^{D511V} shows reduced protein levels in HeLa cells. (**c**) This effect is not cell type–specific, because a similar reduction is observed in HEK293T cells. (**d**) Group data from **b** and **c** show a significant reduction of TRPP2^{D511V} protein levels by 85% compared to wild-type TRPP2 in HeLa cells ($n = 5$; $P = 2 \times 10^{-6}$), and by 63% in HEK293T cells ($n = 5$; $P = 3 \times 10^{-4}$).

this substitution reduces protein abundance and ciliary localization of TRPP2 in *D melanogaster*, suggesting a pathogenic mechanism involving impaired protein processing in addition to defective channel gating *in vivo*.[8,9] To test whether protein expression of human TRPP2^{D511V} protein is also reduced and to characterize the molecular impact of the D511V substitution in more detail, we expressed wild-type and mutant protein in different cell lines. TRPP2^{D511V} showed a significant reduction in protein levels by 85% compared to wild-type TRPP2 in HeLa cells 48 hours after transfection (Figure 1b and d). This effect was not cell type–specific, because a similar reduction was observed in HEK293T cells (–63%) (Figure 1c and d). To ensure that this decrease was not caused by slower TRPP2 protein processing kinetics, we measured TRPP2 levels in HeLa cells 96 hours after transfection and found a similar reduction of mutant TRPP2 compared to wild-type (mean = –90.6%; $n = 4$; $P = 0.008$). Conceptually, either transcriptional downregulation or impaired protein processing might account for these significantly reduced TRPP2^{D511V} protein levels. However, *PKD2* mRNA transcription or stability was not impaired by the D511V mutation, suggesting that TRPP2^{D511V} reduces TRPP2 protein levels by impairing protein processing (Figure 2a).

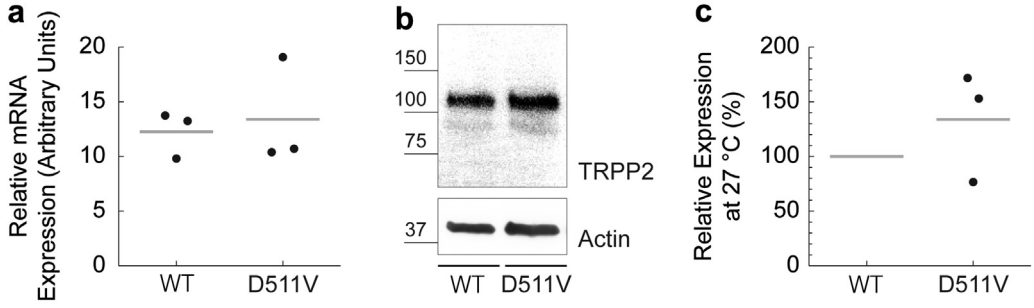

Figure 2 | TRPP2^{D511V} is temperature-sensitive. (**a**) mRNA of transiently transfected HeLa cells was isolated. TRPP2 wild-type and TRPP2^{D511V} mRNA abundance is similar as assessed by quantitative polymerase chain reaction. (**b**) Comparison of TRPP2 wild-type and TRPP2^{D511V} expression at 27 °C in HeLa cells by Western blot. (**c**) Group data from **b**.