Human-specific abnormal alternative splicing of the wild-type *PKD1* gene induces premature termination of polycystin–1.

Wendy A. Lea, Stephen C. Parnell, Darren P. Wallace, James P. Calvet, Lesya V. Zelenchuk, Christopher J. Ward

The Jared Grantham Kidney Institute, University of Kansas Medical Center, Kansas City, KS 66160.

INTRODUCTION

Human *PKD1* is unusual in that it contains two long polypyrimidine tracts in introns 21 and 22 (2.5kb and 602bp, respectively, 97% C+T), whereas the mouse and other species lack these C+T rich regions. Western blot analysis of polycystin-1 (PC1), using a monoclonal antibody to the extreme N—terminus indicates that humans, but not mice, have a smaller EndoH sensitive product, termed Trunc_PC1. Here we show that Trunc_PC1 is the product of differential splicing across introns 21 and 22 and that 28.8-61.5% of human transcripts undergo splicing events that lead to premature translational termination. Thus, the presence of these polypyrimidine tracts leads to decreased levels of full length functional PC1 reducing the level of PC1 signaling from normal alleles and in the context of a mutant allele may force signaling below a critical `cystogenic' threshold.

METHODS

We used RT-PCR from exons 20-24 to quantify the number of splice forms terminating early using NanoPore sequencing. We also compared the number of copies PC1 mRNA per mg total RNA at the 5' and 3' end of the human transcript and compared this with the copy number from normal mouse kidneys. We also created a stable cell line that produces a C- terminally FLAG tagged PC1 cDNA that terminates after exon-20, the region where Trunc_PC1 is predicted to end.

RESULTS

Assaying seven adult kidneys showed that 62.2±12.6% of PC1 transcripts read through and had the accepted sequence while the remainder mis-spliced and truncated. We measured the PC1 mRNA copy number in nine adult human kidneys. For the 5' probe (exon 15) and 3' (exon 34) probes, there were $7.38\pm3.47\times10^5$ and $1.03\pm0.354\times10^6$ copies/mg, respectively — about 22–31 copies per cell. Mice had a similar number of total transcripts $9.59\pm2.30\times10^5$ copies/mg. The synthetic cDNA had an identical mass to Trunc_PC1 showing that they are the same species.

CONCLUSIONS

About 40% of human *PKD1* transcripts terminate early producing Trunc_PC1 implying that humans are dosage hypomorphs when compared to mice. Furthermore, the presence of an ER resident truncated form of the PC1 protein may interfere with the assembly of the polycystin complex.