G4 DNA structures and "second hit" mutagenesis

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Most tumor suppressor genes follow a second hit inactivation pathway, where a pathogenic allele is inherited but a phenotype, or tumor risk, is not realized until a mutational event in a somatic cell disrupts the remaining normal copy, causing biallelic inactivation of the gene in that cell. The second hit hypothesis was first described by Alfred Knudson in 1971 for the retinoblastoma gene, but the reason why some genes are prone to second hit inactivation has remained a mystery. Using the *PKD1* tumor suppressor as a model, we have identified one mechanism for second hit mutagenesis that implicates G4 DNA structures in the pathway. Results were published January 2nd, 2025, in *Nature Communications.*

Polycystic kidney, human. Credit, Darren Wallace, KU PKD research consortium

Loss of the *PKD1* gene causes Autosomal Dominant Polycystic Kidney Disease (ADPKD), a common and lethal renal disease. It runs in families, where one pathogenic allele is inherited and the remaining copy is lost through second hit mutagenesis, causing a cyst to form. This happens thousands of times in ADPKD, for unknown reasons, resulting in thousands of renal cysts. A major clue to the mechanism comes from mice where, unlike humans, *Pkd1* heterozygotes are normal and do not form cystic kidneys. This implies a difference between the genes. Our team compared *PKD1* orthologs and found an abundance of guanine repeats for the human gene. Tandem repeats of guanine support guanine quadruplex (G4) DNA structures, four stranded conformations that cause mutations by interfering with replication and transcription. Human *PKD1* has about 10 times more G4 sequences compared to mouse *Pkd1*, and G4 structures form within the gene and inside cells as measured by chromatin immunoprecipitation and fluorescent microscopy. Most importantly, structure formation enforced via the G4-stabilizing ligands Phen-DC3 or CX-5461 resulted in DNA breaks within human *PKD1*, but not at control loci. Mouse *Pkd1* also did not show DNA breaks, consistent with a lack of G4 sequences in that gene. Therefore, a model emerges whereby the formation of G4 DNA within human *PKD1* leads to an increase in breaks and loss of heterozygosity, explaining cystogenesis in ADPKD patients.

The discovery is profound. Up to 1/400 people are affected by ADPKD but there are no treatments available to prevent cystogenesis. Our results identify G4 DNA as a molecular target. Since retaining one functional copy of *PKD1* can compensate for an inherited mutant allele, a strategy that reduces structure formation would in turn prevent *PKD1* mutagenesis and preserve a cellular barrier to cystogenesis. In the same vein, this strategy may apply broadly to other guanine rich genes connected with inherited diseases, like ALS, repeat expansions, and certain cancer predispositions. This research was funded by WMed and the NIH, NIH/NIDDK R15DK119864.