Genetic Instability and Polycystic Kidney Disease

Kenneth Liu,1,2 Lu Lu,1 Lisa Guay-Woodford,3 and John J. Bissler1,2
1Division of Nephrology and Hypertension, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA;
2College of Medicine, University of Cincinnati, Cincinnati, OH, USA;
3Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA.

Given the world’s estimated population, there are more than 6 million people with polycystic renal disease. We are interested in the mutagenic mechanism leading to these genetic disorders. Using the murine congenital polycystic kidney disease as a model, we identified a complex deletion at the cpk locus. We hypothesized that this mutation was the result of alternative DNA secondary structures such as cruciform or triplex conformation. To test this hypothesis, we cloned the murine sequence at the cpk locus into the chloramphenicol resistance gene in the vector pBR325. This construct could then be used to quantitate deletion by reversion back into chloramphenicol resistance. However, using host bacterial strains DH5α and Top10, we were unable to clone the full-length sequence because each isolate always contained deletion forms. Re-transformation failed to isolate the full-length tract as well. Furthermore, transformation into SURE bacterial strains, which lack the endonuclease SbcC, demonstrated tract instability, indicating that cruciform structures were not likely involved. Our current studies involve exploring melting curves of the sequence to characterize possible triplex structures, since triplex structure also appears to mediate mutations in the human PKD1 gene that lead to autosomal dominant polycystic kidney disease. Elucidating the mutagenic mechanism leading to polycystic kidney disease may not only lead to future treatments of the disease, but may also help researchers identify the mutagenic mechanisms of other diseases that involve alternative DNA structures.