



Hyundai Hope on Wheels Hyundai Scholar Research

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Dr. Druley is a board-certified pediatrician and faculty member in Pediatric Hematology and Oncology at St. Louis Children's Hospital. He is a general oncologist who also participates in the care of bone marrow transplant recipients. His research is based in the hypothesis that much of pediatric cancer predilection and treatment is heavily influenced by the unique combination of rare inherited DNA polymorphisms within each person.

Dr. Druley's research is conducted in Washington University's Center for Genome Sciences, which is composed of a multidisciplinary group of investigators focused on utilizing the latest genomic technologies to improve the understanding of a wide array of fundamental genetic processes and their roles in human diseases. Dr. Druley has helped design and validate new high-throughput DNA sequencing technology and plans to use this technology to characterize the degree of germline DNA variation inherent in a variety of diseases, particularly pediatric cancer. The long term goals are to better understand the pathophysiology of pediatric cancer as well as to identify all of the pertinent germline variants within a pediatric cancer patient prior to starting therapy in order to provide a genetically customized treatment plan designed to maximize anti-cancer activity while minimizing toxicity and morbidity.

Background

Despite decades of careful research and clinical trials, there remains no clear understanding as to why the overwhelming majority of children with cancer developed their disease. We believe that children who develop cancer or experience significant morbidities from therapy are likely to harbor a unique collection of rare, inherited DNA polymorphisms that predispose to disease and/or difficulties with medication metabolism. Rare DNA polymorphisms have historically been exceedingly difficult to identify using traditional DNA sequencing methods due to the relatively low data yield compared to cost. This obstacle has been largely overcome with the advent of so-called "next-generation" DNA sequencing, which has delivered exceptionally high throughput DNA sequencing at a fraction of prior costs.

It is also important to note that while any single polymorphism is individually rare, rare variants are collectively quite common, especially when considering that a whole gene or entire metabolic pathway might harbor many different variants in different individuals, all of which result in the same effect on health. In addition, because they are rare, rare variants are more likely to have an adverse effect on an individual and act in a dominant fashion in the context of disease.

To speed the identification of rare variants and lower the attendant costs, we designed a pooled-sample DNA sequencing approach. In this method, we create a normalized pool of genomic DNA from a cohort of individuals, which can range in size from less than 100 to over 1000. We then target specific regions of the human genome by performing a single PCR reaction on that region from the pooled DNA. We then create a second normalized pool of PCR products and prepare the DNA for next-generation sequencing. We then created and validated novel bioinformatic computer software to accurately sift through the billions of nucleotides generated by the DNA sequencing. In our pilot experiment with 1,111 individuals in a single pool, we targeted four genes – one of which had been sequenced individually in every person using traditional sequencing methods. We identified 64 polymorphisms, 61% of which occurred at a frequency of $\leq 1\%$ in the population, and were able to accurately detect a single variant gene copy in a background of 2221 wild-type alleles. Our algorithm demonstrated a sensitivity of 100%, specificity 99.8% and the correlation of allele frequency estimates compared to individual genotyping was $r^2=0.96$. All of this was achieved at one-fiftieth of the cost of individual sequencing and completed in one-sixth of the time.

Intended Research

Now that the tools are in place, we are ready to apply our method to the study of pediatric leukemia. The Hyundai Scholars award will support our effort to conduct the largest deep resequencing project in pediatric precursor B acute lymphoblastic leukemia (ALL) that has been performed to date. In conjunction with the Children's Oncology Group, we have obtained 100 matched leukemia and non-leukemia DNA samples from children with high-risk leukemia, all of whom have been treated according to a common treatment protocol.

The first step is to pool the non-tumor DNA samples as well as a similar pool of unaffected, random individuals; target 55 genes that have previously been demonstrated to be associated with ALL; perform pooled-sample, next-generation DNA sequencing on these two cohorts. These results will hopefully identify genes that exhibit an increased amount of germline variation in the ALL patients, suggesting that disruption of these genes is associated with ALL.

The second step is to validate these findings in a second population of high-risk ALL patients followed by a retrospective chart review for each patient with the intent of identifying clinical patterns that may correlate with validated loci demonstrating significantly different patterns of genetic variability.

Future directions would then be to demonstrate functional consequences of the validated loci via the appropriate in vitro cell-based assays. Such assays would likely combine a variety of techniques such as RT-PCR, immunofluorescence, CHIP-seq and flow cytometry. Additionally, we will begin a similar evaluation of the matched leukemia DNA samples in an effort to identify and characterize whether somatic mutation at any of these loci contributes to disease onset or outcome. Finally, our method can be readily adapted for bisulfite treated DNA in order to study methylation patterns in these same specimens.