

DATE: April 14th, 2016
TO: UCH Medical Staff, Housestaff, Patient Care Centers, and Outpatient Clinics,
University Chicago Comprehensive Cancer Center
FROM: Larissa V. Furtado, MD, Jeremy P. Segal, MD, PhD, and Y. Lynn Wang, MD, PhD
RE: Launch of *APOLI* Genotyping Assay by Next-Generation Sequencing

Announcement

The Clinical Genomics Laboratory in the Division of Genomic and Molecular Pathology is pleased to announce the launch of the *APOLI* Genotyping Assay.

Two variants in exon 6 of the *APOLI* gene have recently been discovered to be significantly associated with increased risk of end-stage renal disease in African Americans: G1, which is a haplotype with two missense variants in near-complete linkage disequilibrium [c.(1027A>G; 1200T>G); p.(S342G; I384M); rs73885319 and rs60910145], and G2, which is an in-frame two amino acid deletion (c.1212_1217del; N388_Y389del; rs71785313). Specifically, patients who inherit two copies of these variants have a substantially increased risk of developing hypertension-attributed end-stage renal disease, focal and segmental glomerulosclerosis, and HIV-associated nephropathy compared to patients who inherit zero or one copy of the risk alleles. In addition, kidneys from donors with two *APOLI* risk alleles appear to have shorter renal allograft survival compared to kidneys from donors with zero or one *APOLI* risk alleles; recipient *APOLI* risk allele status does not appear to have an effect on allograft survival. Thus, genotyping of *APOLI* to determine whether patients have 0, 1, or 2 high-risk alleles may have important prognostic implications.

Test information

This is a next-generation sequencing assay for the qualitative detection of two *APOLI* nephropathy risk alleles (G1 and G2) in peripheral blood. This test is indicated for African-Americans with a clinical risk or family history of kidney disease and African-Americans being evaluated as living kidney donor. Results from this test are intended for use as an adjunct to existing clinical information.

The test procedure involves DNA extraction and quantity/quality assessment, PCR amplification of a portion of the *APOLI* exon 6 that contains the G1 and G2 nephropathy risk variants, library preparation, and pooling of patient libraries. Next-generation sequencing (NGS) is performed on the MiSeq system (Illumina) and downstream analysis for quality control and detection of mutations is performed via custom-design bioinformatics pipelines on a HIPAA-compliant high performance computing system within the Center for Research Informatics (CRI). *APOLI* variants are interpreted within the context of the reference transcript NM_003661.3.

Specimen Requirements

The test will be performed in peripheral blood specimens. Specimens should be collected in purple-top tubes (EDTA), and labeled with at least two patient identifiers. Samples may be transported to the laboratory at room temperature or refrigerated. The preferred age is less than 48 hours from the time of collection. Specimens collected in anticoagulants other than EDTA, severely hemolyzed, frozen or clotted specimens are unacceptable.

Test ordering

The test can be ordered through Epic using the **LABAPAPOL1B** code:

The screenshot shows the 'APOL1 Test by Next Generation Sequencing on PB' order entry form. The form includes a 'Process Inst.' field with a detailed description of the assay: 'This is a next-generation sequencing assay for the qualitative detection of two APOL1 nephropathy risk alleles: G1 which is a haplotype with two missense variants in near-complete linkage disequilibrium [c. (1027A>G; 1200T >G); p. (S342G; I384M); rs73885319 and rs60910145], and G2, which is an in-frame two amino acid deletion (c.1212_1217del; N388_Y389del; rs71785313) in peripheral blood specimens.' The 'Frequency' is set to 'ONCE'. The 'Starting' date is '2/25/2016' with 'Today' selected, and the 'At' time is '1103'. The 'First Occurrence' is 'Today 1103' and 'Scheduled Times' are 'Hide Schedule' with '2/25/16 1103' listed. The 'Type of Draw' is 'Peripheral'. The 'Ordering Physician pager number', 'Clinical Information/Reason for Test', and 'Institution where specimen was obtained?' (set to 'University of Chicago') fields are empty. The 'Comments (F6)' field has a rich text editor with 'Insert SmartText' and navigation icons. The 'Phase of Care' field is empty. The form has 'Accept' and 'Cancel' buttons at the top right and bottom right, and a 'Next Required' button at the bottom left.

Reporting and Test limitations

Only the *APOL1* nephropathy risk variants (G1 and G2) will be reported, if present. Other *APOL1* variants may be detected, but will not be reported. This test will not detect variants in areas outside the targeted genomic region (hg19 coordinates chr22: 36649117-36663577). Copy number alterations, translocations, and variants in other coding exons or non-coding regions that could affect gene expression will not be detected.

Testing Frequency and Turnaround Time

Testing will be performed at least once weekly, Monday through Friday during day shifts only. Expected turnaround time is 6-12 business days following receipt of adequate specimens.

Additional Questions

Additional questions may be directed to the Division of Genomic and Molecular Pathology at 773-702-4946 or Dr. Larissa Furtado at 773-702-2980 or Dr. Jeremy Segal at 773-702-3674.