Know FH: Mutation Spectrum and Utilization of Cascade Testing for Familial Hypercholesterolemia

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Background: Hypercholesterolemia is characterized by high levels of plasma low density lipoprotein (LDL) in the blood and an increased risk for coronary artery disease (CAD). Familial hypercholesterolemia (FH) caused by mutations in the *LDLR, APOB,* and *PCSK9* genes significantly increases risk for CAD and secondary comorbidities. It is estimated that up to 1/200 individuals have one *LDLR* mutation, and subclinical borderline cases may be missed leading to silent disease that goes undetected. Genetic testing can inform management and prognosis for both an affected proband and at-risk family members, and may be used to supplement biochemical screening during annual health checkups; however, it has been largely under-utilized in clinical practice. This report serves to describe the spectrum of FH mutations identified and utilization of cascade testing in at risk relatives at a clinical diagnostic laboratory.

Methods: Next-generation sequencing of *LDLR, APOB,* and *PCSK9,* plus deletion/duplication (del/dup) analysis of *LDLR* was performed on 217 probands referred for FH testing from April 2014-August 2016. Demographics and clinical information were obtained from test requisition forms and attached clinical records and pedigrees. Mutation characteristics and frequency were assessed, along with available data on familial mutation testing. Secondary finding reports from 2,614 probands who underwent whole exome sequencing (WES) for an unrelated indication were reviewed for FH mutation.

Results: Of 217 samples received, the majority were ordered by a cardiologist (61%), followed by a geneticist or genetic counselor (21%). Ninety patients in the FH cohort (41.5%) had a mutation identified, the majority of which were in *LDLR* (n=86). Five patients carried the known *APOB* p.R3527Q (c.10580G>A) missense mutation in exon 26. Among 2,614 probands with secondary finding reports, 7 heterogeneous *LDLR* mutations and 4 *APOB (*p.R3527Q) mutations were identified. As expected, significantly more *LDLR* (p=1.79e-94) and *APOB* (p=2.60e-05) mutations were identified in the FH cohort compared to incidental findings identified on exome. All probands with *APOB* p.R3527Q were of Caucasian descent, consistent with published frequency of this alteration in Amish and Western European groups. *LDLR* mutations were predominantly missense alterations (42.9%), with splicing alterations accounting for 17.6%, protein truncating alterations and in-frame del/dups accounting for 33.0% and gross del/dup accounting for 6.6% of mutations. The majority of mutations were unique to one proband, though several were seen in up to 3 probands. Mutations at the donor splice site in intron 3 of *LDLR* were observed in 5 probands in the FH cohort and in 1 from the exome cohort, identifying this as a potential hotspot for mutations. The mutations in *LDLR* were identified in the majority of exons, with the largest portion in EGF or EGF-like protein domains (n=40), followed by ligand binding domains (n=32), and cytoplasmic domains (n=3).

Of the 90 probands with mutations identified, 4 were related, leaving 88 apparently unrelated probands**.** Cascade testing for familial mutations occurred in 12 families (13.6%). Among 16 relatives of probands heterozygous for a *LDLR* or *APOB* mutation, 75% tested positive for the familial mutation, which is substantially higher than expected in cascade testing. Interestingly 83% of the 12 family members providing clinical data reported a diagnosis of high cholesterol prior to testing.

Conclusion: The frequency and type of mutations were consistent with previous reports and the overall high diagnostic yield of molecular diagnostics for FH. Preliminary data of cascade testing suggests a potential clinician or patient driven testing bias due to the greater likelihood of providers testing relatives known to have high cholesterol. This presents a possible paradigm between the utility of genetic testing in clinically unaffected at risk relatives and the uptake of testing by clinicians. Education regarding the benefit of testing family members for familial mutations may be needed, particularly as new therapeutic options for mutation carriers are emerging.