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Refinement of Variant Selection for the LDL Cholesterol Genetic Risk Score in the Diagnosis of the Polygenic Form of Clinical Familial Hypercholesterolemia and Replication in Samples from 6 Countries

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Guest:
Dr. Marta Futema is a Research Associate at the Department for Cardiovascular Genetics at the Institute of Cardiovascular Sciences, University College, London.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Familial hypercholesterolemia is a genetic disorder characterized by increased plasma concentrations of low density lipoprotein cholesterol and premature symptoms of coronary heart disease. Early identification of at-risk individuals allows changes in lifestyle including dietary intervention and drug treatment. In the January 2015 issue of *Clinical Chemistry,* a Special Issue devoted to Molecular Diagnostics, a group of researchers in a multi-center study describes a genetic risk score procedure that distinguishes familial hypercholesterolemia patients from healthy subjects.

In this podcast we're joined by one of the authors of that paper, Dr. Marta Futema. She is a Research Associate at the Department for Cardiovascular Genetics at the Institute of Cardiovascular Sciences, University College, London.

Dr. Futema, what are the characteristics of individuals affected by familial hypercholesterolemia, and how many people are thought to be affected?

Dr. Marta Futema: Familial Hypercholesterolemia, or FH, is a disease of the defective clearance of LDL cholesterol, which is also known as the bad cholesterol, from plasma. And this results in high LDL cholesterol levels from birth.

According to the UK FH diagnostic criteria, the affected individuals have LDL cholesterol above 4.9 mmol/L for adults, and above 4.0 mmol/L for children younger than 16 years. FH runs in families and in its classical form it is inherited as an autosomal dominant trait. Therefore FH patients have a family history of high cholesterol or premature myocardial infarction in their first or second degree relatives.

Individuals who present with this phenotype are clinically diagnosed with Possible FH. An additional sign of FH is a physical deposition of cholesterol-rich material in tendons called tendon xanthomas. Patients who have tendon xanthomas or a family history of such characteristics are diagnosed with Definite FH. In monogenic FH the faulty clearance of LDL cholesterol may result from mutations in genes that code for key proteins involved in the process of cholesterol metabolism, the cholesterol metabolism pathway. So the majority of FH mutations are located in the LDLR gene, which codes the LDL receptor protein. Mutations can also be found in the APOB gene, where a single common variant accounts for about 6 to 10% of all FH mutations. The least common is a gain-of-function mutation in the PCSK9 gene, which, however, leads to the most severe FH phenotype.

Now coming back to your question about the FH frequency, it is estimated that FH affects 1 in 500 individuals, however, several recent reports based also on next generation sequencing, suggest that the frequency is probably much higher, and the suggested numbers are around 1 in 200 or 1 in 250. Therefore it isn't really a rare condition; in fact FH frequency is very similar to Type 1 diabetes.

Bob Barrett: Doctor, in your study published this month in *Clinical Chemistry,* you have looked at the polygenic form of clinical familial hypercholesterolemia, what is the polygenic cause of the disease and how do you distinguish it from the monogenic FH?

Dr. Marta Futema: Sure! So with current standard molecular diagnostic methods an FH-causing mutation in the three genes which I have just mentioned, the LDLR, APOB or PCSK9 can be detected in about 20-30% of those that are diagnosed with Possible FH, and in about 70% to 90% of Definite FH cases.

Now, the majority of patients about two-thirds of them are diagnosed with Possible FH. So overall we can estimate that no mutations are detected in about 60% of individuals that are clinically diagnosed with FH.

Our original study that was led by Professor Philippa Talmud was published last year in *Lancet* showed that in a substantial proportion of patients diagnosed with FH, but with no mutation identified, the raised cholesterol levels can be explained by so called polygenes.

Now this means that a patient would inherit a high number of common polymorphisms distributed across the genome, each of them increasing the LDL cholesterol by a small fraction. Now to prove this, in the original study we have

calculated a SNP score for each individual from a cohort of a general population, and in FH patients without an identified mutation. The SNP score was based on 12 common polymorphisms that have been shown to have an effect on LDL cholesterol in very powerful genome-wide association studies.

And so the highest SNP score was observed in individuals with clinical FH, but with no mutation found; whereas the general population had a lowest score. This study provided us with the basis really for the development of a tool which is in form of the SNP score, thus then enabled us to define the polygenic cause of hypercholesterolemia.

Bob Barrett: You've refined the selection of SNPs in the score and overall the number of SNPs included in the score is reduced, is the 6 SNP score is efficient as the original 12 SNP score?

Dr. Marta Futema: We know that there are many more SNPs identified by GWAS as being associated with lipids. In our *Clinical Chemistry* study, we wanted to select the optimum set of SNPs for the score. Firstly, we thought we were going to add all the SNPs that were associated with LDL cholesterol, and additionally we added 21 LDL-associated SNPs to the original 12 SNP score. Our analysis showed that increasing the number of SNPs in the score did not improve the ability of the score to discriminate between healthy individuals and hypercholesterolemic patients with no identified monogenic cause/ or mutation. We then thought that reducing the number of SNPs in the score, by selecting only variants that are most frequent and have the strongest effect on LDL cholesterol, as indicated by GWAS meta-analysis, will provide us with the most time- and cost-efficient SNP score. We used area under the curve--the ROC curve--statistics to demonstrate that the 6 SNP score was performing as well as the original 12 SNP score.

We also thought that it was important for us to replicate our findings in other patients' cohorts. In collaboration with numerous clinicians, we genotyped and calculated the 6 SNP score in over 800 clinically diagnosed FH patients who did not have mutation detected, and in over 350 patients with monogenic FH. The patients' cohorts included individuals from Canada, Italy, Poland, Israel, adults and children from the Netherlands, and also children from Greece. The results were pretty consistent across all the cohorts confirming that the 6 SNP score is significantly higher in hypercholesterolemic patients that do not have a monogenic mutation or cause, and that the score analysis also worked in children's cohorts.

We also aimed to establish the score cut-off that could be used to diagnose individuals with polygenic

hypercholesterolemia. We assume, based on the results of several FH whole exome sequencing studies, that the frequency of the remaining undetected mutation would be very low, we estimated it to be 1 in 2000 (and this is assuming that if we have identified 75% of all FH mutations to be found). Based on that, we have calculated the probability of having a polygenic as opposed to a monogenic cause of hypercholesterolemia, which suggested that in individuals with SNP score above the first quartile of the score distribution, have more than 95% chance of having hypercholesterolemia due to a polygenic effect.

Bob Barrett: Well finally, doctor, just how important is it to distinguish the monogenic from the polygenic hypercholesterolemia?

Dr. Marta Futema: In families affected by the classical monogenic FH, there is a 50% chance that the first degree relative will inherit the disease. The polygenic hypercholesterolemia will not be passed on as an autosomal dominant trait, so the chance of inheriting the condition is much lower than 50%. This difference in this inheritance pattern will have a significant impact on the efficiency of cascade testing of FH in countries where DNA testing is not available.

The cascade testing on the basis of LDL cholesterol measurements will prove to be less effective than it should be. Our data supports the model used in the Netherlands and Wales, where cascade testing resources are used only in the families in which the proband has identified mutation for the monogenic FH, since in the majority of clinical FH patients with no detected single mutation the most likely explanation of high cholesterol is a polygenic cause.

In addition we thought that, we suggest that, any efforts to identify novel genes or mutations may be causing FH should be carried out really in the individuals who have SNP score in the bottom quartile, the lowest SNP score, since they are more likely to have a monogenic FH due to a single mutation.

Bob Barrett: Dr. Marta Futema is a Research Associate at the Department for Cardiovascular Genetics at the Institute of Cardiovascular Sciences, University College, London.

She has been our guest in this podcast from *Clinical Chemistry* on Molecular Technologies to Detect Familial Hypercholesterolemia. Her paper appeared in the January 2015 issue of *Clinical Chemistry,* a Special Issue devoted to Molecular Diagnostics.

I am Bob Barrett. Thanks for listening!