atherosclerosis

Atherosclerosis newsletter

Simona Negrini and Arnold von Eckardstein

We are pleased to announce that since January 2020 *Atherosclerosis* has become a bimonthly journal. Newsletters will hence appear more frequently, but with smaller content.

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Familial hypercholesterolemia is one of the most important causes for manifestation of atherosclerotic cardiovascular disease at a young age. Nevertheless and despite being one of the most frequent monogenic diseases, it is still underdiagnosed and undertreated. Although the discovery of the LDL receptor as its genetic basis happened more than 35 years ago, research on FH is still ongoing to generate exciting findings. This issue of *Atherosclerosis* contains several articles on strategies to optimize early diagnosis and treatment of FH as well as novel findings from basic research on potential causes and therapeutics of FH.

Comparison of the characteristics at diagnosis and treatment of children with heterozygous familial hypercholesterolaemia (FH) from eight European countries

For children with heterozygous familial hypercholesterolaemia (HeFH), European guidelines recommend consideration of statin therapy by age 8–10 years for those with a low density lipoprotein cholesterol (LDL-C) >3.5 mmol/l, and dietary and lifestyle advice. While the European guidelines and country specific guidelines are relatively similar in their recommendations for total and LDL-C thresholds for a clinical diagnosis of FH and treatment strategies, adoption of these recommendations is likely to be influenced by local factors such as clinician and parental preferences and the different health care and reimbursement systems for lipid-lowering therapy. In this study, Ramaswami et al. compare the characteristics and lipid levels in HeFH children from Norway, UK, Netherlands, Belgium, Czech Republic, Austria, Portugal and Greece and they analyze the between-country similarities and differences in diagnostic and treatment strategies currently being used.

Fully-anonymized data were assessed at the London centre. Differences in registration and on treatment characteristics were compared by standard statistical tests.

Data was obtained from 3064 children. The median age at diagnosis differed significantly between countries (range 3–11 years), reflecting differences in diagnostic strategies. Mean LDL-C at diagnosis was 5.70 mmol/l, with 88% having LDL-C>4.0 mmol/l. The proportion of children older than 10 years at follow-up who were receiving statins varied significantly (99% in Greece, 56% in UK), as did the proportion taking ezetimibe (0% in UK, 78% in Greece). Overall, treatment reduced LDL-C by between 28 and 57%, however, in those >10 years of age, 23% of on-treatment children still had LDL-C>3.5 mmol/l and 66% of those not on a statin had LDL-C>3.5 mmol/l.

The results show that the age of HeFH diagnosis in children varied significantly across 8 countries, as did the proportion of those >10 years being treated with statin and/or ezetimibe. Approximately a quarter of the treated children and almost three quarters of the untreated children older than 10 years still had LDL-C over 3.5 mmol/l. These data suggest that many children with FH are not receiving the full potential benefit of early identification and appropriate lipid-lowering treatment according to recommendations.

Derivation and validation of SIDIAP-FHP score: A new risk model predicting cardiovascular disease in familial hypercholesterolemia phenotype

Familial hypercholesterolemia (FH) phenotype entails a high risk of atherosclerotic events. A patient-tailored assessment of risk, distinguishing primary and secondary prevention, would improve the clinical management of the FH population. Ramos et al. used data from the Catalan primary care system database (SIDIAP) of patients ≥18 years old with FHP in 2006–2013 to develop and validate two risk functions to predict incident and recurrent atherosclerotic cardiovascular disease (ASCVD), and to compare their predictive capacity with that of the SpAnish Familial hypErcHolEsterolemiA cohoRT (SAFEHEART) risk equation (SAFEHEART-RE), the first prospective tool to predict ASCVD risk in patients with FH.

The new model (SIDIAP-FHP) included age, diabetes, smoking, sex (male), hypertension, and baseline low-density lipoprotein cholesterol in the primary prevention cohort and age, diabetes, smoking, and disease characteristics (progressive, recent, polyvascular, or included myocardial infarction) in the secondary prevention cohort. The models demonstrated a fair fit: C-Statistic: 0.71 in primary prevention and 0.65 in secondary prevention (higher than that of SAFEHEART-RE: 0.64 and 0.55, respectively). The Brier scores obtained with the SIDIAP-FHP score were significantly lower than those obtained with SAFEHEART-RE in both the primary and secondary prevention cohorts.

The SIDIAP-FHP score provides accurate ASCVD risk estimates for primary and secondary prevention in the FHP population, with better predictive capacity than that of SAFEHEART-RE in this general population, especially in subjects with previous ASCVD.

Clinical significance of zero coronary artery calcium in individuals with LDL cholesterol ≥190 mg/dL: The Multi-Ethnic Study of Atherosclerosis

The 2018 multisociety cholesterol management guidelines identify individuals with low-density lipoprotein cholesterol (LDL-C) ≥190 mg/dL as a high-risk group for future atherosclerotic cardiovascular disease (ASCVD) events. Accordingly, the initiation of high-intensity statin therapy is strongly recommended in this population to achieve a ≥50% reduction in LDL-C. However, it is currently unknown if the risk for future cardiovascular disease (CVD) events is heterogeneous in this high-risk population. Coronary artery calcium (CAC) is a marker of coronary atherosclerosis burden and its presence is strongly associated with future CVD events. Additionally, important prognostic information is obtained from the absence of CAC, as a finding of zero CAC is associated with <1% risk of all-cause mortality over 10 years. Due to the strong prognostic information obtained when CAC is not present, it is possible that this non-invasive modality is able to further risk stratify subjects with LDL-C ≥190 mg/dL. Sandesara et al. aimed to assess the risk factors associated with CAC = 0 in middle-aged adults with LDL-C ≥190 mg/dL, and the predictive ability of CAC = 0 in these subjects.

Multi-Ethnic Study of Atherosclerosis (MESA) participants without clinical cardiovascular disease and baseline LDL-C ≥190 mg/dL were identified. Cardiovascular risk factors were compared between those with CAC = 0 and CAC >0. Multivariable Poisson regression was used to identify predictors of CAC = 0. Association of CAC = 0 with incident cardiovascular events over a median follow-up of 13.2 years was examined using multivariable-adjusted Cox regression.

Two hundred forty-six individuals with LDL-C ≥190 mg/dL were identified. Age <65 years, female sex, and no diabetes were associated with CAC = 0. Individuals with CAC = 0 had a lower risk for future cardiovascular events than those with CAC >0, suggesting the utility of CAC assessment to stratify risk in this high-risk group.

Predicted pathogenic mutations in *STAP1* are not associated with clinically defined familial hypercholesterolemia

Familial hypercholesterolemia (FH) is a genetic disorder characterized by very high plasma total cholesterol concentrations, due to increased low-density lipoprotein cholesterol (LDL-C), with a high risk of premature coronary heart disease (CHD). Autosomal dominant familial hypercholesterolemia (FH) is caused by mutations in *LDLR***,** *APOB* and *PCSK9.* Two new putative *loci* causing FH have been identified recently, the p.(Leu167del) mutation in *APOE* and new mutations in the signal transducing adaptor family member *STAP1*. The function of STAP1 is still unknown. It contains a domain with several tyrosine phosphorylation sites, which allows it to interact with the

membrane or with membrane proteins. Therefore, the involvement of STAP1 in cholesterol metabolism could be through interaction with membrane proteins. Lamiquiz-Moneo et al. aimed at investigating the role of *STAP1* mutations in the etiology of FH.

The authors sequenced *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *APOE*, *LIPA* and *STAP1* with the LipidInCode platform in 400 unrelated subjects from Spain with a clinical diagnosis of FH. All subjects carrying rare predicted pathogenic variants in *STAP1* gene, described as pathogenic by at least three bioinformatic analyses and having an allelic frequency lower than 1% in the general population, were selected for family study. Available relatives were recruited, including hypercholesterolemic and non-hypercholesterolemic family members.

Sequencing analysis of *STAP1* gene revealed seventeen rare variants, four of them being described as pathogenic by bioinformatic analysis. Cosegregation with hypercholesterolemia of four rare predicted pathogenic variants, c.-60A > G, p.(Arg12His), p.(Glu97Asp), p.(Pro176Ser) was studied in seven families. No cosegregation between genotype and phenotype was observed. Carriers of rare variants in *STAP1* had even lower LDL cholesterol levels than non-carriers.

These results would suggest that *STAP1* is not involved in hypercholesterolemia in these families. *STAP1* does not seem to play a major role in the etiology of FH.

PCSK9-D374Y mediated LDL-R degradation can be functionally inhibited by EGF-A and truncated EGF-A peptides: An *in vitro* study

PCSK9 (proprotein convertase subtilisin/kexin type 9) regulates LDL cholesterol (LDL-C) levels by interacting with the LDL receptor (LDL-R), through the LDLR epidermal growth factor-like repeat A (EGF-A) domain, and inducing its internalization and degradation. PCSK9 has emerged as a novel therapeutic target for hypercholesterolemia. Gain-of-function mutations of *PCSK9* cause hypercholesterolemia and early-onset coronary heart disease while loss-of-function mutations result in low plasma cholesterol levels and protection against coronary heart disease, without apparent negative consequences. Therefore, inhibition of PCSK9 is being pursued as an approach to reduce plasma LDL-C levels. PCSK9 inhibition strategies aim to disrupt the binding of PCSK9 to LDR to increase LDLR expression at the cell surface of hepatocytes and to promote LDL catabolism. Clinical studies with PCSK9 inhibiting antibodies have demonstrated strong LDL-c lowering effects, but other therapeutic approaches using small molecule inhibitors to target PCSK9 functions may offer supplementary therapeutic options. Valenti et al. aimed at disrupting the binding interface between LDL-R and the p.Asp374Tyr (D374Y) mutated PCSK9 variant, associated with severe hypercholesterolemia in humans, using two EGF-A analogs *in vitro*.

Huh7 human hepatoma cells were transiently transfected to overexpress the gain-of-function D374Y PCSK9 mutation. Transient transfection of cells with PCSK9-D374Y expression vector very effectively enhanced degradation of mature LDLR in Huh7. Treatment with both EGF-A and EGF-A truncated peptides inhibited this effect and showed increased LDLR protein in these cells in a concentration dependent manner. Huh7 transfected cells treated with increasing concentration of EGF-A analogs also showed an increased internalization of labeled DiI (1,1'-dioctadecyl- 3,3,3',3'-tetramethylindocarbocyanine perchlorate)-LDL.

The study shows that EGF-A analogs are able to effectively hamper the enhanced degradation of LDLR in liver cells expressing PCSK9-D374Y.

These results and alternatives to anti-PCSK9 antibodies are discussed in the editorial by Cariou and Dijk.