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Original Article

Plasma inducible degrader of the LDLR, soluble low-density lipoprotein receptor, and proprotein convertase subtilisin/kexin type 9 levels as potential biomarkers of familial hypercholesterolemia in children

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BACKGROUND: Familial hypercholesterolemia (FH) in children is under-detected. Plasma biomarkers associated with low-density lipoprotein receptor (LDLR) function could help identifying FH children.

OBJECTIVES: We aim to assess the clinical value of inducible degrader of the LDLR (IDOL), soluble LDLR (sLDLR), and proprotein convertase subtilisin/kexin type 9 (PCSK9) plasma concentrations in children with FH compared with control children (CCh).

METHODS: This was a cross-sectional study performed in a Lipid Unit from a University hospital. The participants were 177 children distributed into FH (n = 77) and CCh (n = 100). Main outcomes were changes in IDOL, sLDLR, and PCSK9 plasma concentrations between children groups; secondary outcomes were the association between IDOL, sLDLR, and PCSK9 and lipid profile determined by 2-dimensional nuclear magnetic resonance.

RESULTS: The IDOL levels were higher in FH compared with CCh ($P = .007$). The PCSK9 levels were elevated in FH ($P < .001$). The sLDLR levels had no significant differences between groups. IDOL was significantly positively associated to total and LDL cholesterol and ApoB100 but not to LDL particle number. However, a robust correlation with Lp(a) ($P = .001$) was observed. PCSK9 had the strongest correlation with LDL-associated parameters including particle number. sLDLR

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was associated with triglyceride levels ($P < .001$) and triglyceride-rich particles and inversely to LDL size.

CONCLUSIONS: The IDOL and PCSK9 plasma levels are significantly higher in FH children. Interestingly, sLDLR was associated with atherogenic dyslipidemia components. IDOL concentrations show a robust association with Lp(a) levels. To study the role of plasma biomarkers associated with LDLR expression in FH is warranted.

Background

Familial hypercholesterolemia (FH) affects 1 of 250 children. FH is under-detected, particularly in pediatric age.[1,2] Gene defects in LDL receptor (*LDLR*), apolipoprotein B (*ApoB*), LDLR adaptor protein 1, and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) are clinically expressed as an FH phenotype.[3] The LDLR is the cornerstone molecule regulating the LDL cholesterol (LDL-C) plasma concentrations.[4] LDLR gene mutations, leading to a null or defective receptor, result in a substantial increase in the plasma LDL-C levels and higher cardiovascular risk.[5] On the other hand, drugs that induce LDLR upregulation decrease the LDL-C concentration, reducing cardiovascular event rates. The clinical significance of LDLR warrants interest in the mechanisms controlling its function and expression. Several proteins are involved in LDLR physiology. Its ligand, ApoB, mediates LDL capture. LDLR adaptor protein 1 anchors it to the cell membrane, contributing to LDLR activity. PCSK9 has emerged as a central LDLR functional regulator, and its expression parallels that of LDLR according to intracellular cholesterol content.[6] After being secreted into the bloodstream, PCSK9 binds to LDLR that is internalized with it. PCSK9 precludes LDLR molecule recycling, reducing the expression of LDLR at the hepatocyte membrane. Inhibition of PCSK9 by monoclonal antibodies leads to an increased number of receptors at the cell surface, resulting in a marked LDL-C reduction.[7] In the last few years, the inducible degrader of LDLR (IDOL), a new molecule involved in post-transcriptional LDLR expression regulation, has been identified.[8] IDOL is a sterol-regulated E3 ubiquitin ligase that labels LDLR with ubiquitin, driving it to degradation by proteasome cell mechanisms. Therefore, IDOL activity decreases the level of LDLR, similarly to PCSK9, albeit through a different mechanism. However, interdependent regulation mechanisms have been observed.[9] Furthermore, overexpression of both molecules is associated with nephrotic syndrome and hypercholesterolemia. Similar to PCSK9, IDOL also regulates the very low-density lipoprotein (VLDL) and apoE receptors.[10] Its expression shall be governed by the liver X receptor transcription factor.[11] Interestingly, genome-wide association studies have identified IDOL as a locus associated with circulating LDL-C concentrations.[12–14] Similar to PCSK9, IDOL gene loss-of-function mutations have been associated with low LDL-C levels in humans.[15] Because of all these factors, IDOL has attracted increasing interest as a putative target for hypercholesterolemia treatment.[16] Although IDOL function appears to be intracellular, a plasma-circulating fraction can be detected. Neither its function nor its association with the intracellular portion is known. The mechanisms involved in plasma secretion are equally unknown. However, because of IDOL tight relationship with LDLR expression, we hypothesize that it could be an LDLR functional biomarker. Also, it is known that soluble LDLR (sLDLR) is formed by cleavage of the extracellular domain and can also be detected in the serum of healthy subjects as a biomarker of diseases associated with triglycerides metabolism.[17,18]

To explore a possible role of these 3 circulating proteins as hypercholesterolemia biomarkers, we determined the IDOL, PCSK9, and sLDLR plasma levels in a cohort of FH children.

Subjects and methods

Study design and patients

This study was a cross-sectional study. One hundred seventy-seven children and adolescents, aged 4 to 18 year, who were participating in the "Early Familial Hypercholesterolemia Detection Program in Children" (DECOPIN) to investigate high cholesterol levels or because they were FH family members, were recruited. Children were distributed into 2 groups: (1) familial hypercholesterolemia (FH; n = 77), if they had a positive genetic study or LDL-C > 150 mg/dL if 1 of the parents had FH and (2) control children (CCh; n = 100) if they did not meet the previous criteria. At inclusion, no patient was receiving lipid-lowering therapy. The exclusion criteria were renal, hepatic, or thyroid chronic disease; type I diabetes mellitus; hypercalciuria; eating disorders; autoimmune disease; homozygous FH; and other chronic diseases. An exhaustive medical history, including the familial cardiovascular and dyslipidaemia history, complete physical examination, and anthropometry data, was collected. To calculate the body mass index (BMI) in children, we used the "BMI score" $(BMI_{children} - BMI_{50th\ percentile\ of\ Orbegozo's\ growth\ curves})/SD_{50th\ percentile\ of\ Orbegozo's\ growth\ curves}$. Also, a lifestyle evaluation, including nutritional and physical activity data, was recorded. The Hospital Ethical Committee approved this study, and patients or tutors, depending on