Abstract

Most types of hypercholesterolemia are of polygenic origin. Some genes related to hypercholesterolemia are known, although all genes responsible for cholesterolemia regulation have not been characterised yet. To identify these new genes, animal models with spontaneous defects in cholesterol metabolism could be very useful. Moreover, a number of variations and polymorphisms have been found to influence blood cholesterol levels in humans. Some may also affect cholesterolemia responsiveness to dietary fat.

The Prague hereditary hypercholesterolemic (PHHC) rat is a unique model of hypercholesterolemia induced by dietary cholesterol alone (without administration of cholic acid or thyrotoxic drugs). It exhibits modestly increased cholesterolemia when fed chow and responds to a diet containing cholesterol with a several-fold increase of cholesterolemia to concentrations comparable to those observed in hypercholesterolemic patients. Hypercholesterolemia in this model is characterised by accumulation of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) enriched by cholesterol.

In an experiment with tyloxapol (an inhibitor of lipoprotein lipase) we found that PHHC rats on a cholesterol diet incorporated twice as much cholesterol into VLDL as Wistar rats, although liver cholesterol remained the same. When labelled with ^{125}I, these cholesterol-rich VLDL of PHHC rats were catabolised *in vivo* more slowly than ^{125}I-labelled VLDL of Wistar rats and accumulated in circulation. The increased incorporation of cholesteryl esters (CE) into VLDL in PHHC rats could not be explained by differences in acyl-CoA:cholesterolacyltransferase (ACAT) or microsomal triglyceride transfer protein (MTP) activities and gene expression. Furthermore, we found no differences between PHHC and Wistar rats in the response of the hepatic transcriptome (as determined using AffymetrixGeneChip® arrays) to dietary cholesterol. However, several genes were differently expressed between both strains, independent of diet. Of those, we studied *Apof*, *Aldh1a7* and corresponding proteins in detail. We could not ascribe any role to these genes in hypercholesterolemia pathogenesis. We were able to explain the aetiology of hypercholesterolemia in the PHHC rat, although the related genetic defects need to be clarified.

Cholesterol 7α-hydroxylase (CYP7A1), a key regulatory enzyme in bile acid biosynthesis, plays an important role in cholesterolemia regulation. The -203A>C

polymorphism (rs3808607) in the CYP7A1 gene (*CYP7A1*) promoter is involved in cholesterolemia determination and its responsiveness to diet. This polymorphism is in close linkage disequilibrium with the -469C>T polymorphism (rs3824260).

Firstly, using dual luciferase assay, we demonstrated that expression of the -203C (-203C, -469T) allele was markedly increased compared to the -203A (-203A, -469C) allele, caused by the nucleotide in position -203. The alleles neither responded to stimulation with insulin nor PPARα agonists (WY-14643 or fenofibrate). Secondly, we analysed diurnal variation of CYP7A1 after enzyme activity upregulation (cholestyramine) and suppression (chenodeoxycholic bile acid, CDCA) in healthy men homozygous for the -203A or -203C allele. As expected, CYP7A1 activity was upregulated after treatment with cholestyramine and suppressed after treatment with CDCA. There were no differences between -203A and -203C homozygous subjects in the response of enzyme activity to both drugs. Importantly, in the control experiment, CYP7A1 in -203A allele carriers displayed diurnal variation, but not in -203C carriers. The differences in diurnal variations of enzyme activity may partly explain the role of the CYP7A1 polymorphism in the regulation of cholesterolemia and its responsiveness to diet.