Additional protective effect of P2Y_{13} purinergic receptor in cardiometabolic diseases: role in Non-Alcoholic Fatty Liver Disease

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**Background and aim:** Non-Alcoholic Fatty Liver Disease (NAFLD) is now the most common cause of liver disease. NAFLD can progress to more serious forms of the disease, including Non-Alcoholic SteatoHepatitis (NASH), fibrosis, cirrhosis and cancer. Despite this major public health issue, there is no clear consensus on target strategy. We previously show that P2Y_{13} purinergic receptor is involved in HDL-mediated biliary lipid secretion and exerts antiatheroprotective functions. In this study, we investigate the potential protective role of P2Y_{13} receptor against NAFLD/NASH development.

**Method:** Wild-type (WT) and P2Y_{13} KO mice were fed a high-fat diet (HFD) for 16 weeks. During this period, body weight and plasma lipids levels were measured and glucose and lipid metabolisms were assessed. At sacrifice, hepatic gene expression measurement and histological liver analysis were performed.

**Results:** HFD-fed P2Y_{13} KO mice reveal increased susceptibility to body-weight gain and higher systemic inflammation, with impaired insulin-sensitivity. P2Y_{13} KO mice also display increased liver lipogenesis and stimulated lipolysis activity, associated to increased hepatic steatosis. Furthermore, we found increased expression of genes involved in liver inflammation and fibrosis, but without histological and biochemical translations at the end of the diet.

**Conclusion:** We hypothesize that P2Y_{13} deletion leads to lipolytic activity, furnishing substrates for liver with increased lipogenesis activity, leading to increased steatosis. All of these results support the protective role of P2Y_{13} against metabolism alterations and NAFLD/NASH development and suggest the relevance and potential of P2Y_{13} receptor as a drug target for NAFLD/NASH treatment.

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**Figure 1:** P2Y_{13} KO mice display increased body-weight gain susceptibility, increased systemic inflammation and impaired cholesterol secretion. (A) WT and P2Y_{13} KO mice body weight evolution throughout experiment. (B) Total caloric intake (C) WT and P2Y_{13} KO mice plasma lipids levels evolution throughout experiment. (D) Biliary cholesterol secretion. (E) Final plasma pro-inflammatory cytokines levels and relative plasma inflammatory index. Values are expressed as mean (+/- SEM). Data were analysed by Student’s t-test (*p < 0.05, ns not significant).

**Figure 2:** Increased steatosis and liver pro-inflammatory and pro-fibrotic gene expression in P2Y_{13} KO mice. (A) Liver histological staining in WT and P2Y_{13} KO mice. (B) Liver triglycerides and cholesterol content. (C) Liver weight. (D) Liver ROS fluorescence and pro-inflammatory gene expression levels. (E) Liver hydroxyproline content and pro-fibrotic gene expression levels. Values are expressed as mean (+/- SEM). Data were analysed by Student’s t-test (*p < 0.05, ns not significant).

**Figure 3:** Increased insulin resistance in P2Y_{13} KO mice. (A) Oral glucose tolerance test (OGTT). (B) OGTT-associated insulinemia. (C) Insulin resistance index. (D) Insulin tolerance test (ITT). Values are expressed as mean (+/- SEM). Data were analysed by Student’s t-test (*p < 0.05, ns not significant).

**Figure 4:** Increased liver lipogenesis, adipose tissue lipolysis and adipocyte intracellular cAMP levels in P2Y_{13} KO mice. (A) Liver lipogenesis genes expression. (B) In-vivo VLDL secretion rate. (C) Final adipose tissues weight in WT and P2Y_{13} KO mice. (D) Schematic lipolysis pathway activation by cAMP. (E) Global in-vivo lipolysis and ex-vivo perigonadic adipose tissue lipolysis. (F) Intracellular cAMP levels in perigonadic isolated adipocytes in basal and forskolin (FSK) (5µM, 30 min) stimulated conditions. Values are expressed as mean (+/- SEM). Data were analysed by Student’s t-test (*p < 0.05, ns not significant).

**Figure 5:** Working hypothesis. (A) In WT mice, P2Y_{13} negatively regulates adipose tissue lipolysis due to it small protein Gi coupling, and in liver, negatively regulates lipogenesis activity by a still unknown mechanism. (B) In KO mice, we hypothesize that P2Y_{13} deletion leads to increased perigonadic adipose tissue expansion and lipolytic activity, furnishing substrates for liver with increased lipogenesis activity, leading to increased steatosis.