

Can we extrapolate from a *Cmah*^{-/-}*Ldlr*^{-/-} mouse model a susceptibility for atherosclerosis in humans?

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We read with great interest the paper of Kawanishi et al. in PNAS (1). Humans lack a functional cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase (CMAH) controlling the synthesis of *N*-glycolylneuraminic acid (Neu5Gc). The possible role of anti-Neu5Gc antibodies—present in all human sera and resulting from diet-containing Neu5Gc—in the activation of endothelial cells displaying traces of diet-derived Neu5Gc and in triggering atherosclerosis in humans through “xenosialitis” is controversial (2, 3). In their article, Kawanishi et al. (1) state, “The underlying question that drove this study was the common observation that humans appear to be particularly prone to cardiovascular complications of atherosclerosis.” To reach their goal, they used *Cmah*^{-/-}*Ldlr*^{-/-} mice as a “humanized” model that would favor the investigation on a controversial deleterious vascular phenotype attributed to the lack of a functional CMAH. In their model, they identify “intrinsic” factors inherent to the engineered mice and “extrinsic” ones related to anti-Neu5Gc antibodies. We have several uncertainties regarding the proposed model both at the intrinsic- and extrinsic-level.

Indeed, the “strong diabetic phenotype in *Cmah*^{-/-}*Ldlr*^{-/-} mice,” an intrinsic factor of vascular diseases attributed by the authors to the loss of CMAH (1, 4, 5), was not observed in a detailed and dedicated study on pancreas histology and function in *Cmah*^{-/-} pigs (6), a species more related to humans. This makes it unlikely that *Cmah*^{-/-} mice display a universal phenotype that could be extended to humans. The lack of pancreatic abnormalities in CMAH-deficient pigs (6) may rather suggest a role for the C57BL/6 mouse

background that harbors sugar and lipid metabolism deregulations (reviewed in ref. 7), especially when fed with a high-fat diet as in the current study (1). Furthermore, the lack of functional LDL receptor predisposes mice to obesity and obesity-associated accelerated atherosclerosis (8). This ultimately adds to the “humanization” due to the lack of functional CMAH, an additional rare disease trait (one homozygous individual out of one million).

In addition, the experimental harnessing of the extrinsic arm side does not provide a surrogate model to study effects of diet-derived anti-Neu5Gc antibodies (present in all humans) since the *Cmah*^{-/-}*Ldlr*^{-/-} mice were actively immunized to produce elicited anti-Neu5Gc antibodies with different amounts (9), affinity, and repertoire (10). As such, the extrinsic arm of the model would rather fit with a model to explore the possible effect of anti-Neu5Gc antibodies elicited by xenotransplants, bioprostheses, or rabbit polyclonal IgG (9), where no vascular disease was noted in patients with extremely high anti-Neu5Gc titers (9). Furthermore, the immunization against Neu5Gc was performed with nine intraperitoneal injections with Freund adjuvant, a powerful innate immunity stimulant, superimposed on the disease-prone mouse model background.

In summary, the proposed model, tailored to overamplify the “xenosialitis” hypothesis of atheroma related to diet-derived anti-Neu5Gc antibodies, may lead to an overinterpretation of the possible deleterious effects associated with the loss-mutation of functional CMAH in humans, as suggested by the article title.

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