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

Jean-Paul Soulillou[a,b,1], Emanuele Cozzi[c], Cesare Galli[d], and Jean-Marie Bach[e]

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-factor 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.

[a]Centre de Recherche en Transplantation et Immunologie, INSERM, Université de Nantes, 44093 Nantes, France; [b]Institut de Transplantation Urologie Néphrologie, Centre Hospitalier Universitaire Nantes, 44093 Nantes, France; [c]Department of Cardiac, Thoracic and Vascular Sciences, Transplant Immunology Unit, Padua University Hospital, 35128 Padova, Italy; [d]Avantea, 26100 Cremona, Italy; and [e]Cellular and Molecular Immuno-Endocrinology Research Unit, Oniris, Institut National de la Recherche Agronomique, Université Bretagne Loire, 44307 Nantes, France

Author contributions: J.-P.S., E.C., C.G., and J.-M.B. wrote the paper.

The authors declare no competing interest.

[1]To whom correspondence may be addressed. Email: jean-paul.soulillou@univ-nantes.fr.

First published January 21, 2020.

1 K. Kawanishi *et al.*, Human species-specific loss of CMP-*N*-acetylneuraminic acid hydroxylase enhances atherosclerosis via intrinsic and extrinsic mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 16036–16045 (2019).
2 T. Pham *et al.*, Evidence for a novel human-specific xeno-auto-antibody response against vascular endothelium. *Blood* **114**, 5225–5235 (2009).
3 L. Le Berre *et al.*, Elicited and pre-existing anti-Neu5Gc antibodies differentially affect human endothelial cells transcriptome. *Xenotransplantation* **26**, e12535 (2019).
4 S. Kavaler *et al.*, Pancreatic beta-cell failure in obese mice with human-like CMP-Neu5Ac hydroxylase deficiency. *FASEB J.* **25**, 1887–1893 (2011).
5 D. N. Kwon *et al.*, CMP-Neu5Ac hydroxylase null mice as a model for studying metabolic disorders caused by the evolutionary loss of Neu5Gc in humans. *BioMed Res. Int.* **2015**, 830315 (2015).
6 A. Salama *et al.*, Neu5Gc and α1-3 GAL xenoantigen knockout does not affect glycemia homeostasis and insulin secretion in pigs. *Diabetes* **66**, 987–993 (2017).
7 D. A. Fontaine, D. B. Davis, Attention to background strain is essential for metabolic research: C57BL/6 and the International Knockout Mouse Consortium. *Diabetes* **65**, 25–33 (2016).
8 S. A. Schreyer, T. C. Lystig, C. M. Vick, R. C. LeBoeuf, Mice deficient in apolipoprotein E but not LDL receptors are resistant to accelerated atherosclerosis associated with obesity. *Atherosclerosis* **171**, 49–55 (2003).
9 A. Salama *et al.*, Anti-Gal and anti-Neu5Gc responses in nonimmunosuppressed patients after treatment with rabbit antithymocyte polyclonal IgGs. *Transplantation* **101**, 2501–2507 (2017).
10 R. Amon *et al.*, Glycan microarray reveal induced IgGs repertoire shift against a dietary carbohydrate in response to rabbit anti-human thymocyte therapy. *Oncotarget* **8**, 112236–112244 (2017).