Gain-of-Function Claims for Type-2-Diabetes-Associated Coding Variants in SLC16A11 Are Not Supported by the Experimental Data

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Cell Reports

Matters Arising

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Highlights

- The mutant model system used by Zhao et al. may not pertain to human SLC16A11 physiology
- The mouse mutations tested do not correspond to the human SLC16A11 T2D-risk haplotype
- Gain-of-function claims for Slc16a11 variants are not supported by data in the paper
- Conclusions about physiological impact of human T2D variants are not justified

Authors

Eitan Hoch, Jose C. Florez, Eric S. Lander, Suzanne B.R. Jacobs

Correspondence

sjacobs@broadinstitute.org

In Brief

Hoch et al. discuss the analysis of coding variants of SLC16A11 in mice and humans in light of a recent *Cell Reports* publication. This Matters Arising paper is in response to Zhao et al. (2019a), published in *Cell Reports*. See also the response by Zhao et al. (2019b), published in this issue.

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Gain-of-Function Claims for Type-2-Diabetes-Associated Coding Variants in SLC16A11 Are Not Supported by the Experimental Data

Eitan Hoch,[1,7] Jose C. Florez,[1,2,3] Eric S. Lander,[4,5,6] and Suzanne B.R. Jacobs[1,2,8,*]
[1]Programs in Metabolism and Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
[2]Diabetes Unit and Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA 02114, USA
[3]Department of Medicine, Harvard Medical School, Boston, MA, USA
[4]Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
[5]Department of Biology, MIT, Cambridge, MA 02139, USA
[6]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA
[7]Present address: Jnana Therapeutics, Boston, MA 02210, USA
[8]Lead Contact
*Correspondence: sjacobs@broadinstitute.org
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SUMMARY

Human genetic variants in *SLC16A11* are associated with increased risk of type 2 diabetes (T2D). We previously identified two distinct mechanisms through which co-inherited T2D-risk coding and non-coding variants disrupt *SLC16A11* expression and activity, thus implicating reduced SLC16A11 function as the disease-relevant direction of effect. In a recent publication, Zhao et al. (2019a) argue that human SLC16A11 coding variants confer gain of function, basing their conclusions on phenotypic changes observed following overexpression of mutant murine Slc16a11. However, data necessary to demonstrate gain-of-function activity are not reported. Furthermore, several fundamental flaws in their experimental system—including inaccurate modeling of the human variant haplotype and expression conditions that are not physiologically relevant—prevent conclusions about T2D-risk variant effects on human physiology. This Matters Arising paper is in response to Zhao et al. (2019a), published in *Cell Reports*. See also the response by Zhao et al. (2019b) in this issue of *Cell Reports*.

The recent paper "Gain-of-Function Mutations of SLC16A11 Contribute to the Pathogenesis of Type 2 Diabetes" by Zhao et al. (2019a) follows up on our report of two mechanisms by which type 2 diabetes (T2D)-associated genetic variants disrupt the function of SLC16A11 (Rusu et al., 2017).

In our paper, we demonstrated that human variants that decrease SLC16A11 levels (i.e., hypomorphic alleles) induce metabolic changes associated with insulin resistance and T2D, suggesting that therapies aimed at increasing SLC16A11 activity may be a beneficial treatment for T2D.

In their recent paper, Zhao et al. (2019a) created and studied various mutations of *Slc16a11* in C57BL/6 mice. Specifically, they generated a global *Slc16a11* knockout mouse model to examine the physiological consequences of *Slc16a11* loss on glucose metabolism. In *Slc16a11* knockout mice fed either a normal chow diet (NCD) or high-fat died (HFD) for 3 months, they report only minimal metabolic effects. They then used adeno-associated virus (AAV; introduced through tail vein injection into the knockout model) to re-express either wild-type *Slc16a11* or a mutated form of the gene intended to model the human T2D-risk coding variants (Figure 1). In knockout mice expressing mutant *Slc16a11*, they report slightly elevated triglycerides, along with marginal glucose intolerance and insulin resistance. Follow-up experiments in HepG2 cells overexpressing mutant Slc16a11 again showed a slight increase in triglycerides. Based on these findings, they conclude that T2D-risk-associated coding variants in Slc16a11 "produce a gain-of-function mutant protein," thereby "challenging the concept of enhancing SLC16A11 function to treat T2DM."

For various reasons, explained below, it is impossible to draw any conclusions about the impact of human *SLC16A11* T2D-risk variants on molecular function or human physiology from this paper.

The Control Animals Do Not Show the Expected Metabolic Response to a High-Fat Diet

The authors fed their animals an HFD, which is a well-documented approach to induce glucose intolerance and insulin resistance in some mouse strains (Surwit et al., 1988). Yet, their HFD-fed, wild-type mice, despite gaining weight and exhibiting elevated triglycerides, display neither insulin resistance nor glucose intolerance. (If anything, the opposite phenotype is observed; the HFD-fed mice appear more glucose tolerant than younger mice fed an NCD, though interpretation is further complicated because the authors do not directly report glucose levels but only proportional changes from an unspecified baseline.)

The likely explanation for the failure would depend on the C57BL/6 substrain used, which the authors unfortunately do not report. If they used C57BL/6J (which are particularly

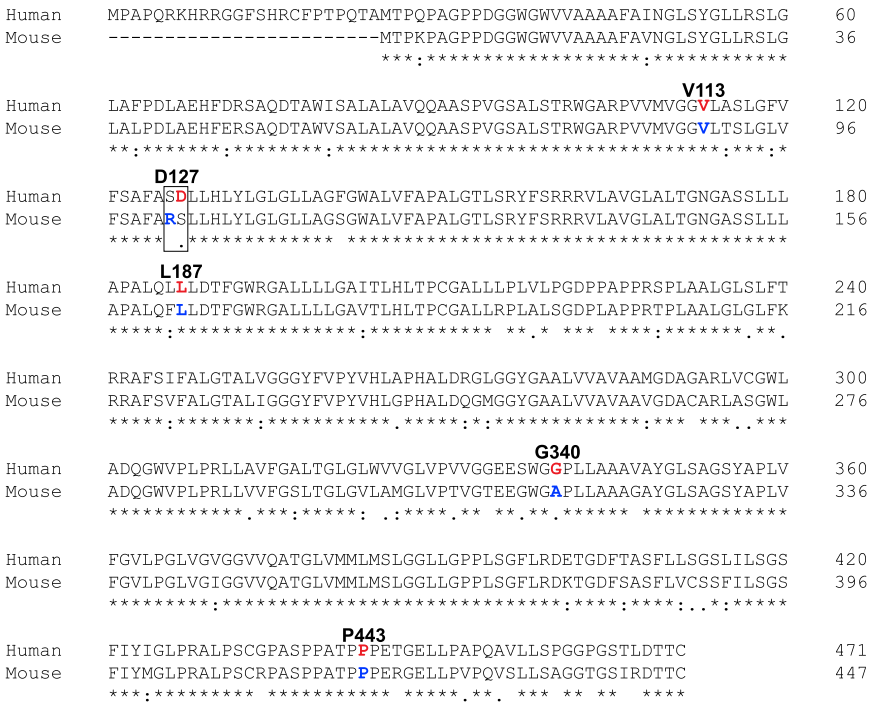

Figure 1. Alignment of Human and Mouse SLC16A11 Protein Sequences

Comparison of human and mouse SLC16A11 protein sequences indicates a high degree of conservation, with no deletions or insertions. Amino acids harboring T2D-associated coding variants in human SLC16A11 are labeled and shown in red. Residues mutated by Zhao et al. (2019a) in mouse Slc16a11 (intended to correspond to the human T2D-risk variants) are shown in blue. Box indicates human residue D127 corresponds to mouse S103, showing weak similarity (indicated by .), but Zhao et al. incorrectly mutated mouse R102. Sequence alignment was produced using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) using UniProt sequences Q8NCK7 (human) and Q5NC32 (mouse).

susceptible to HFD-induced glucose intolerance), then their metabolic assay failed. If they used a strain such as C57BL/6N (which typically has a lesser glycemic response to HFD), then the choice of substrain was problematic. Whatever the reason, the lack of insulin resistance and glucose intolerance following HFD feeding confounds interpretation of data from genetic perturbations.

Another SLC16 Family Member Is Significantly Upregulated in Their *Slc16a11* Knockout Mouse Model

The authors report that levels of *Slc16a6* are elevated ~2.5-fold in the livers of *Slc16a11* knockout mice; however, they do not explore this further or discuss the potential impact of this finding on their interpretation of data obtained from their knockout mice. In particular, they do not address the possibility that their inability to detect metabolic phenotypes could be due to *Slc16a6* compensating for loss of *Slc16a11*.

The Mutated Version of *Slc16a11* Does Not Correspond to the Human T2D-Risk Haplotype

The human and mouse SLC16A11 protein sequences are highly conserved and show perfect alignment with no deletions (Figure 1). The position of the human D127G variant clearly corresponds in mouse to S103, yet the authors mutated the previous amino acid, creating an R102G allele. The substitution thus occurs at a non-homologous position (102 versus 103), which encodes a negatively charged, basic amino acid rather than a positively charged, acidic amino acid, as in human. This major discrepancy makes it impossible to draw conclusions about the impact of the human T2D-risk coding variants on physiology.

The Overexpression System Used to Investigate the Metabolic Impact of Slc16a11 Expression Is Neither Properly Controlled Nor Calibrated to Draw Physiological Conclusions

In both their *in vivo* and *in vitro* models, the *Slc16a11* expression levels reported are not physiologically relevant; it is 5-fold higher than endogenous *Slc16a11* levels in liver and >150-fold above background in cells. In addition to the five coding mutations, the human T2D-risk-associated haplotype also includes non-coding variants that decrease *SLC16A11* expression. Experimental models to ascertain disease-relevant physiological effects must account for this difference in expression between the reference and the T2D-risk SLC16A11 protein; in the absence of this, conclusions about the impact of *SLC16A11* T2D-risk coding variants on disease physiology are not warranted. The authors may claim effects of overexpression of the mutant protein in mice and cells but not conclude this is relevant to the human context.

Though a liver-specific TBG promoter was used for the AAV-based re-expression studies, the authors do not provide data to exclude the possibility of ectopic *Slc16a11* expression in tissues where it is not normally expressed or to demonstrate that expression levels of other Slc16 family members are unaffected, as they observed for *Slc16a6* in the knockout. Given the restricted expression profile of endogenous *Slc16a11*, these data are crucial to support any conclusion that the observed phenotypes indeed arise from Slc16a11 activity in the liver.

The Paper by Zhao et al. Presents No Experimental Data to Support Their Conclusion—Stated in the Paper's Title—that Variants in the SLC16A11 Coding Region Confer a Gain of Function

The authors' conclusion that their mutant Slc16a11 protein exhibits gain-of-function activity appears to be based on the observation of marginal increases in triglycerides and *Lipin1* expression. However, increases in a downstream phenotype do not indicate the nature of a mutation (that is, whether it abolishes, decreases, increases, or qualitatively changes

function)—especially when the mutant protein is expressed in non-physiological conditions. In fact, the authors do not directly assess the molecular or biochemical activity of their mutant Slc16a11 transporter. Without such information, it is impossible to infer the nature and directionality of effect of variants on protein function—that is, whether they are loss-of-function (null), hypomorphic (reduced levels), hypermorphic (increased level), or gain-of-function (completely new function) alleles.

For all the reasons above, no conclusions regarding the physiological consequences of human T2D-risk variants can be drawn from the recent report.

AUTHOR CONTRIBUTIONS

E.H. and S.B.R.J. performed the sequence alignment analysis. E.H., J.C.F., E.S.L., and S.B.R.J. wrote and edited the manuscript.

DECLARATION OF INTERESTS

E.S.L. serves on the board of directors for Codiak BioSciences and Neon Therapeutics and serves on the scientific advisory board of F-Prime Capital Partners and Third Rock Ventures; he is also affiliated with several non-profit organizations, including serving on the board of directors of the Innocence Project, Count Me In, and the Biden Cancer Initiative and the board of trustees for the Parker Institute for Cancer Immunotherapy. He has served and continues to serve on various federal advisory committees. The Broad Institute has filed a patent application related to work discussed in this article; E.H. and S.B.R.J. are inventors on that application.

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REFERENCES

Rusu, V., Hoch, E., Mercader, J.M., Tenen, D.E., Gymrek, M., Hartigan, C.R., DeRan, M., von Grotthuss, M., Fontanillas, P., Spooner, A., et al. (2017). Type 2 diabetes variants disrupt function of SLC16A11 through two distinct mechanisms. Cell *170*, 199–212.e120.

Surwit, R.S., Kuhn, C.M., Cochrane, C., McCubbin, J.A., and Feinglos, M.N. (1988). Diet-induced type II diabetes in C57BL/6J mice. Diabetes *37*, 1163–1167.

Zhao, Y., Feng, Z., Zhang, Y., Sun, Y., Chen, Y., Liu, X., Li, S., Zhou, T., Chen, L., Wei, Y., et al. (2019a). Gain-of-function mutations of SLC16A11 contribute to the pathogenesis of Type 2 diabetes. Cell Rep. *26*, 884–892.e884.

Zhao, Y., Feng, Z., and Ding, Q. (2019b). Type 2 diabetes variants in the *SLC16A11* coding region are not loss-of-function mutations. Cell Rep. *29*, this issue, 781–784.