REPLY TO LIU ET AL.:

Tissue specificity of *SIM1* gene expression and erectile dysfunction

Eric Jorgenson[a,1], Navneet Matharu[b,c], Melody R. Palmer[d], Jie Yin[a], Jun Shan[a], Thomas J. Hoffmann[b,e], Khanh K. Thai[a], Xujia Zhou[b,c], James M. Hotaling[f], Gail P. Jarvik[d], Nadav Ahituv[b,c], Hunter Wessells[g], and Stephen K. Van Den Eeden[a,1]

In our study (1), we identify a locus in the human genome near the *SIM1* gene that is significantly associated with the risk of erectile dysfunction. Through differential enhancer assays, we demonstrate that the lead single-nucleotide polymorphism (SNP), rs17185536, alters enhancer activity. Furthermore, long-range chromatin interaction experiments show that the region containing the lead variant likely interacts with the promoter of the *SIM1* gene. Based on the evidence from these analyses, we hypothesize that a regulatory element harboring rs17185536 or other erectile dysfunction-associated SNPs in the locus affects *SIM1* expression.

Previous research found that male mice lacking the melanocortin 4 receptor (*Mc4r*) showed impaired copulatory behavior (2). Reexpressing MC4R in cells that also express SIM1, presumably neurons, can restore that function (3). Semple and Hill (3) observed colocalization and SIM1-dependent expression of *Mc4r* in the paraventricular nucleus of the hypothalamus and medial amygdala, suggesting two brain regions that could play a central role in erectile function.

In PNAS, Liu et al. (4) query the Genotype-Tissue Expression (GTEx) expression quantitative trait locus (eQTL) and NephQTL databases to determine whether rs17185536 is associated with gene expression differences in human tissues. They find a significant association between rs17185536 and *SIM1* gene expression in the hypothalamus in GTEx, but not in other tissues tested. This provides further support to our hypothesis that genetic variation in the erectile dysfunction risk locus alters the regulation of *SIM1* and is consistent with the findings of Semple and Hill (3) that the hypothalamus may harbor neurons that control erectile function.

There are a number of limitations to the eQTL findings. As Liu et al. (4) acknowledge, information on rs17185536 in GTEx is available in only 10 of 53 tissues. As a result, it is unclear whether rs17185536 is associated with *SIM1* expression in other, untested GTEx tissues. Among the 10 tissue types for which data were available, the statistical power to detect an association varies due to differences in the number of samples available for each tissue. Consequently, it is possible that eQTL associations in other tissue types may be missed. In addition, GTEx does not have expression data from amygdala tissue, so this region of the brain cannot be ruled out. Conversely, the hypothalamus contains a number of nuclei, and it is possible that rs17185536 may affect *SIM1* expression only in a subset of those nuclei, so the true effect of this locus may be larger but also more specific than the GTEx finding.

Future studies of regulatory elements in the erectile dysfunction locus can elucidate which specific region or regions of the brain control erectile function. Further functional characterization of these elements in cells and in mice has the potential to link the genetic variation in this region to altered *SIM1* expression and connect those changes to erectile function. An improved understanding of the function of this locus can open the door to new treatments for erectile dysfunction.

[a]Division of Research, Kaiser Permanente Northern California, Oakland, CA 94612; [b]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA 94158; [c]Institute for Human Genetics, University of California, San Francisco, CA 94158; [d]Division of Medical Genetics, University of Washington School of Medicine, Seattle, WA 98195; [e]Department of Epidemiology and Biostatistics, University of California, San Francisco, CA 94158; [f]Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT 84132; and [g]Department of Urology, University of Washington School of Medicine, Seattle, WA 98195

Author contributions: E.J., N.M., M.R.P., J.Y., J.S., T.J.H., K.K.T., X.Z., J.M.H., G.P.J., N.A., H.W., and S.K.V.D.E. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. Email: eric.jorgenson@kp.org or stephen.vandeneeden@kp.org.

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4 Liu G, Hu Y, Han Z, Jin S, Jiang Q (2019) Genetic variant rs17185536 regulates *SIM1* gene expression in human brain hypothalamus. *Proc Natl Acad Sci USA*, 10.1073/pnas.1821550116.