Submitter Name: Germán Gornalusse
Submitted email: ggornalu@fredhutch.org
PI Name: Florian Hladik
PI email: florian@uw.edu

Chronic heroin use generates distinct cleavage patterns of tRNA-Gly-GCC in semen

Germán Gornalusse[1*], Lucia Vojtech[1*], Ryan Spengler[2], Erin Sandford[2], Yeseul Kim[1], Claire Levy[1], Muneesh Tewari[2] and Florian Hladik[1,3,4]
* These authors contributed equally to the work

[1]Department of Obstetrics and Gynecology, University of Washington; [2]Medical School, University of Michigan; [3]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center; [4]Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington

In addition to their role in protein translation, transfer RNA (tRNAs) can be cleaved into shorter, biologically active fragments called tRFs. The generation of tRFs is highly regulated; alterations in tRF patterns are associated with stress and exposure to toxicants. Recent studies demonstrate a role for tRFs in spermatocytes to cause heritable metabolic disorder. This suggests tRFs are potentially a mechanism of epigenetic inheritance. As yet, the impact of opioid use on tRFs in germline cells has not been explored.
We ran an RNAseq experiment on RNA from highly purified spermatocytes from men who inject drugs and non-drug using controls. We validated results using droplet digital PCR, with specific reverse-transcription primers for long versus short tRF fragments.
We found that a tRF from Gly-GCC tRNA demonstrated different cleavage in spermatocytes from PWID compared to non-users. Over 90% of reads in non-drug using men mapped to shorter tRFs, while in PWID only 45% did. In contrast, only 4.1% of reads in controls mapped to a longer tRF species, compared to 45.6% in PWID. The long/short tRF ratio was significantly higher in PWID than non-drug users (0.65 vs. 0.14, p=0.03).
In summary, in semen samples from PWID, we observed an altered cleavage pattern of tRNA-Gly-GCC compared to that found in non-heroin users. This study lays the groundwork to investigate whether other tRFs or other epigenetic mechanisms like DNA methylation are changed following opioid use and whether these changes can be reversed by withdrawal or by opioid substitution therapies.