Journal Pre-proof

Undifferentiated Induced Pluripotent Stem Cells as a Genetic Model for Nonalcoholic Fatty Liver Disease


PII: S2352-345X(22)00167-9
DOI: https://doi.org/10.1016/j.jcmgh.2022.07.009
Reference: JCMGH 1052

To appear in: Cellular and Molecular Gastroenterology and Hepatology
Accepted Date: 12 July 2022


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Title: Undifferentiated Induced Pluripotent Stem Cells as a Genetic Model for Nonalcoholic Fatty Liver Disease

Short title: iPSCs as a NAFLD genetic model

Authors: Antonio Muñoz¹, Elizabeth Theusch¹, Yu-Lin Kuang¹, Gilbert Nalula¹, Caitlin Peaslee², Gabriel Dorlhiac³, Markita P. Landry⁴,⁵ Aaron Streets³,⁵,⁶, Ronald M. Krauss¹,⁷, Carlos Iribarren⁸, Aras N. Mattis²,⁹, Marisa W. Medina¹*

Affiliations:
1. Department of Pediatrics, University of California San Francisco, San Francisco, CA 94143, USA.
2. Department of Pathology, University of California San Francisco, San Francisco, CA 94143, USA.
3. Biophysics Graduate Group, University of California Berkeley, Berkeley, CA 94720, USA.
4. Department of Chemical and Biomolecular Engineering, University of California Berkeley, Berkeley, CA 94720, USA.
5. Chan Zuckerberg Biohub, San Francisco, CA 94158, USA.
6. Department of Bioengineering, University of California Berkeley, Berkeley, CA 94720, USA.
7. Department of Medicine, University of California San Francisco, San Francisco, CA 94143, USA.
8. Kaiser Permanente Division of Research, Oakland, CA 94612, USA.
9. Liver Center, University of California San Francisco, San Francisco, CA 94143, USA.

* Corresponding Author. Email: marisa.medina@ucsf.edu

Funding: This work was supported by the National Institutes of Health P50 GM115318 (MWM, RMK), R01DK127718 (MWM, ANM), P30 DK026743 (ANM), the National Science Foundation 1845623 (AS), and the Program for Breakthrough Biomedical Research, which is partially funded by the Sandler Foundation (MWM, ANM). The funding agencies had no role in the study design, analysis or interpretation of data.

Disclosures: ANM is a consultant for Hepatx, Ambys Medicines, and BioMarin. All other authors have nothing to disclose.

Author contributions:
Conceptualization: MWM, ANM
Methodology: AM, MWM, ANM
Investigation: AM, ET, YK, CP, GN, ANM
Visualization: AM, GD, ANM, AS, ML
Funding acquisition: MWM, RMK, CI, ANM
Project administration: MWM, ANM
Supervision: MWM, ANM
Writing – original draft: MWM, AM
Writing – review & editing: MWM, AM, RMK, ET, CI, ANM

Data and materials availability:
All data are available in the main text or the supplementary materials. Cell lines are available upon request.
Patient-derived induced pluripotent stem cells (iPSCs) have been transformational in biomedical research for their ability to differentiate into any cell type while retaining the genetic information of the donor individual, for example iPSC-derived hepatocyte-like cells (iPSC-Heps) for studies of nonalcoholic fatty liver disease (NAFLD)\(^1\). However, differentiation protocols are time-intensive, use costly reagents, require highly specialized training, and can result in heterogeneous cultures that are limited in number\(^2\). Thus, iPSC-Heps are poorly suited for studies of genetic variation which require scalability and reproducibility. In contrast, iPSCs exhibit self-renewal, can be cryopreserved, have standardized and robust protocols available for their generation and culturing, and are substantially less expensive to produce. We tested whether iPSCs in their undifferentiated state may be an informative to model genetic factors underlying NAFLD. NAFLD is initiated by hepatic steatosis, often attributed to excess synthesis, retention, or uptake of fatty acids by the liver, where they are stored as triglycerides within lipid droplets. As nearly all cells can take up fatty acids, synthesize triglycerides and create lipid droplets\(^3\), we sought to determine whether iPSCs could model fatty acid induced lipid accumulation.

Authenticated iPSCs (table S1) were previously described\(^4\). We confirmed that a representative iPSC accumulates intracellular lipids in response to 24hr oleate challenge in a dose dependent manner, with lipids detected by two neutral lipid stains (Nile Red and LipidTox Red) and through Simulated Raman Spectroscopy, a highly specific detection method for unlabeled triglycerides\(^5\), Fig. 1A, B. To improve quantitation accuracy, we developed a flow cytometry-based assay (Fig. 1C), resulting in highly reproducible measures (Fig. 1D) which confirmed that oleate treatment increased intracellular lipids in cell lines from 30 donors (2.0±0.11 fold mean ± SE, p=4.0e-10, Fig. 1E, table S2).
We next compared the degree of oleate-induced lipid accumulation in iPSCs from eight donors both in their undifferentiated state and after differentiation into iPSC-Heps through a 23-day protocol as we previously described. iPSC-Heps were authenticated by expression of hepatocyte markers and secretion of albumin into the culture media (fig. S1A, S1B). There were no differences in the levels of intracellular lipids in the isogenic iPSCs and iPSC-Heps, either with values expressed as absolute levels or the magnitude of change between oleate vs. BSA treated cells (fig. S1C-E).

Variants in TM6SF2 (rs58542926), PNPLA3 (rs738409), GCKR (rs1260326) and MBOAT7 (rs641738) are all associated with NAFLD in multiple independent cohorts, and have published effect sizes for their association with hepatic fat. All four genes had detectable expression in undifferentiated iPSCs, unlike lymphoblastoid cell lines, another patient-derived cell line (fig. S2). Importantly, iPSCs carrying increasing numbers of rs58542926 and rs738409 NAFLD risk alleles had greater intracellular lipid accumulation with an additive relationship observed (p=1.4e-5, Fig. 2A). The magnitude of this effect was nearly identical between the two risk alleles, consistent with their reported effect sizes (Fig. 2B). Moreover, we found a significant positive correlation (r²=0.60 p=4.8e-7) between oleate-induced intracellular lipid accumulation and a weighted genetic risk score based on the reported associations of TM6SF2 rs58542926, PNPLA3 rs738409, GCKR rs1260326 and MBOAT7 rs641738 alleles with hepatic fat (Fig. 2C.)

Here we show that patient-derived iPSCs in their undifferentiated state can be used to model genetic factors that influence individual-level variation in fatty-acid induced lipid accumulation, critical in NAFLD pathobiology. Compared to iPSC-Heps or liver organoids, iPSCs are significantly more scalable, enabling their use for genetic discovery. This could support future use of iPSCs for identifying high risk individuals, testing variation in response to treatment, and informing the development of precision medicine guidelines for NAFLD prevention and management. Our results also raise the possibility of using iPSCs for investigating genetic influences on other diseases characterized by excess lipid storage. Notably
both the *TM6SF2* rs5854296 and *PNPLA3* rs738409 risk variants are thought to cause lipid accumulation in hepatocytes by impairing intracellular lipid transport and reducing triglyceride secretion in APOB-containing lipoprotein particles \(^8,^9\), processes that has not been identified in iPSCs. Additional study is needed to assess the mechanisms underlying these relationships and determine the extent to which NAFLD relevant pathways can be modeled in the iPSC. Lastly, these findings challenge the current paradigm of iPSC use which assumes that cells must be differentiated to be informative, highlighting the potential utility of undifferentiated patient-derived iPSCs as a cellular model of individual level disease risk.

**References**


**Acknowledgments**

We thank all of the POST participants without whom this study would not be possible, as well as Meng Lui and Gabriela Sanchez for their assistance with recruitment. Kristin Stevens assisted with RNAseq library preparation, and some iPSCs were generated by the University of Florida iPSC Core. AS and ML are Chan Zuckerberg Biohub Investors. AS is a Pew Biomedical Scholar.
Figure Legends

Fig. 1. iPSCs accumulate intracellular lipids when challenged with oleate. A) Images taken at 100x magnification of an iPSC line challenged for 24hr with (0-100µM) sodium oleate conjugated to BSA. Cells were stained with 10µg/mL of Nile Red (pink) to visualize lipid droplets and Hoescht (blue) to stain nuclei. 10µm size bars shown. B) Oleate vs. BSA treated iPSCs were stained with Nile Red or LipidTox Red and visualized via fluorescence microscopy. A separate aliquot of cells was left unstained and subjected to SRS microscopy, in which unstained triglycerides are visualized as white areas. 50µm size bars shown. C) Representative histogram of Nile Red fluorescence values of BSA and 100µM oleate treated iPSCs. Cells were stained with Nile Red prior to quantitation by flow cytometry. D) Biological replicate measures of intracellular lipid levels in iPSCs from three donors, n=4. E) Geometric means of the Nile Red fluorescence values indicative of intracellular lipids in 30 iPSC lines treated with BSA and 100µM oleate.

Fig. 2. The magnitude of oleate-induced intracellular lipid accumulation in undifferentiated iPSCs is correlated with NAFLD genetic risk. Oleate-induced intracellular lipid accumulation was quantified in iPSCs from 30 donors as described in Figure 1, and the fold change in lipid accumulation was plotted separated by the number of NAFLD risk alleles for TM6SF2 and/or PNPLA3 together (A) or separately (B). Linear regression (panel A) and ANOVA with posthoc multiple comparisons against the 0 allele carrier group was performed with adjusted p-values (panel B) are shown. C) Correlation of intracellular lipid accumulation with 4-SNP NAFLD genetic risk score.

Supplementary Materials

Materials and Methods
Figures S1, S2
Tables S1, S2
References