The Role of FOG2 S657G on Hepatic Lipid Metabolism, Insulin Resistance, and Fatty Liver Disease

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INTRODUCTION

- Metabolic dysfunction-associated Fatty Liver Disease (MAFLD) is a leading cause of liver disease characterized by excess lipid accumulation in the liver[1].
- Friend of GATA 2 (FOG2) is a transcriptional co-regulator which regulates hepatic lipid metabolism and accumulation[2] and is also associated with Metabolic dysfunction-associated Steatohepatitis (MASH) in humans[3].
- We have identified a coding variant of FOG2 (rs28374544, A1969G, S657G) which is present in individuals of African ancestry (minor allele frequency ~0.2) and is associated with angiogenesis and decreased fatty acid oxidation (FAO) (Guerraty MedRxiv).

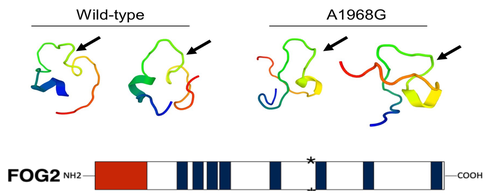


Figure 1: Schematic of FOG2 & FOG2 S657G. (A) Pepfold3 simulation shows polar amino acids forming loop domain in WT. Introduction of Ser657Gly disrupts local structure. (B) Schematic of protein domains. * = region of S657G. Red = repressor domain. Blue = Zinc finger domains.

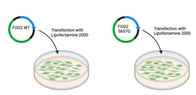
- FOG2 S657G is associated with diagnosis of liver failure/cirrhosis (p=0.0053, Genebass – 395k individuals in UK Biobank) and with decreased plasma Triglyceride levels (p=0.039,Global Lipids Genetics Consortium).

Hypothesis:

FOG2 S657G regulates hepatic lipid metabolism to promote Metabolic dysfunction-associated Fatty Liver Disease (MAFLD).

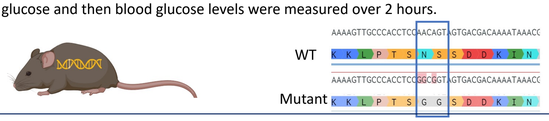
METHODS

1) **Transient lipid-based transfection of human hepatoma cell line Huh7 with FOG2 WT and FOG2 S657G**
   - i) Huh7 cells were transfected with Empty Vector (EV), FOG2 Wild Type (WT), and FOG2 S657G (MUT), lysed, separated by SDS-PAGE and blotted with antibodies against FOG2 and Actin or RNA was extracted and qRT-PCR was performed.
   - i) Total cell TG was extracted from cells and measured using Infinity TG Reagent



2) **Interrogate previously published[4] RNAseq & Genotype dataset of Human induced pluripotent stem cell (iPSC) differentiated into hepatocytes (iHeps).**
   - i. Differential Gene Expression (DESeq2, Partek Flow Software) was performed on 24 iHeps lines (16 lines = FOG2 wild type genotype (AA) & 8 lines = FOG2 heterozygous for FOG2 S657G (AG)) from African-American female descent
   - ii. Gene Set Enrichment Analysis using Hallmark Pathway
   - iii. Hierarchical Clustering of Genes in the MTORC1 pathway

3) **Developed novel mouse model with mutation analogous to S657G (termed FOG2 MUT)**
   - i) RT-PCR and Western Blot Analysis of mouse livers of fed mice
   - ii) Glucose Tolerance Test (GTT) was performed on 9 month old mice on chow-diet. Mice were fasted for 6 hours and blood glucose measurements were taken at baseline. Male and female Fog2 MUT and WT mice were injected IP with 2g/kg glucose and then blood glucose levels were measured over 2 hours.



RESULTS

I) Overexpression of FOG2 S657G in Huh7 Cells shows increased expression of de novo lipogenesis genes and increased TG mass.

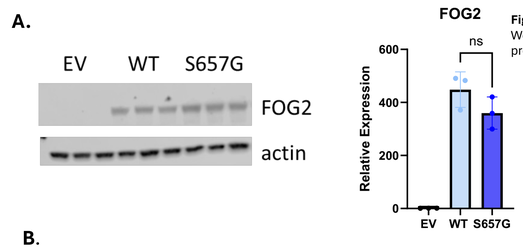


Figure 2A: Transient transfection of FOG2 WT vs S657G in Huh7 Cells. Western blot and qRT-PCR show increased expression of FOG2 WT vs S657G protein and gene, respectively, relative to empty vector control (EV).

Figure 2B: qRT-PCR data of de novo Lipogenesis Genes. RNA expression of DNL genes in WT and S657G transfected Huh7 cells show significantly increased expression of key regulators of DNL. N=6/group, *-P<0.05 by student's t-test

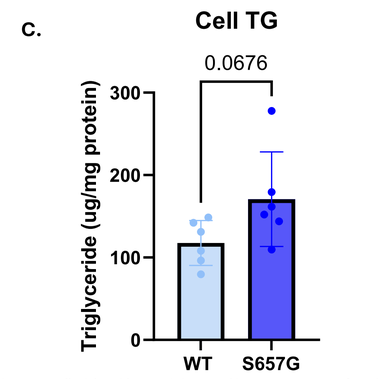


Figure 2C: Cell Triglyceride Mass is increased in FOG2 S657G transfected cells as compared to WT. TG was normalized to total protein as measured by BCA and expressed as Triglyceride per mg protein. N=6/group

II) Genomic analyses of iPSC-hepatocytes from individuals with and without FOG2 S657G variant.

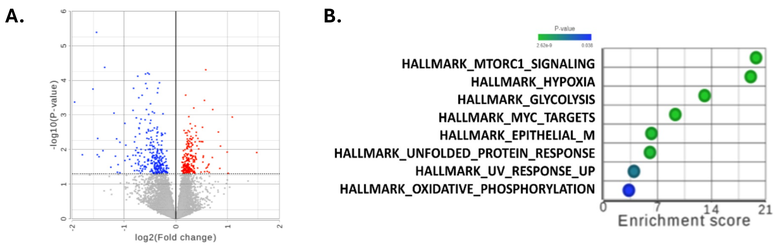


Figure 3: MTORC1 pathway was differentially regulated between between iPSC-hepatocytes from cell lines from individuals with and without the variant. A) Volcano Plot showing 228 genes upregulated & 288 genes downregulated in differential gene expression analysis (with nom p <0.05 and Fold change > 1.2). B) Gene Set Enrichment Analysis (GSEA) using Hallmark Pathway gene sets identified MTORC1 signaling as the top pathway. C) Heat map summarizing genes differentially regulated in the MTROC1 pathway showing changes consistent with MTROC1 activation in iHeps with AG genotype.

IIIa) A Novel Fog2 MUT mouse model does not affect Fog2 expression in the liver and recapitulates changes seen in human FOG2 S657G.

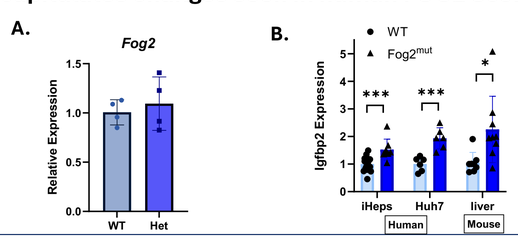


Figure 4: Expression of FOG2 and IGFPBP2 in FOG2mut mice. A) Gene expression of FOG2 was similar in WT vs mice heterozygous for the mutation. B) IGFBP2, a gene strongly associated with MAFLD, was differentially regulated in Huh7 and was one of the top differentially regulated genes in iHeps. Livers from FOG2 Mut mice have similar pattern of IGFBP2 expression.

RESULTS

IIIb) Increased de novo lipogenesis and increased mTORC1 signaling in livers of Fog2 MUT mice.

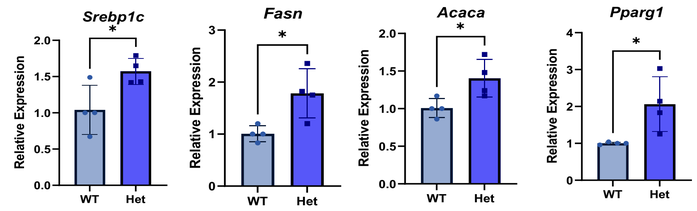


Figure 5: qRT-PCR of DNL genes using RNA from WT and HET mouse livers. Relative expression of canonical DNL genes (i.e.: Srebp1c, Fascn, Acaca, & Pparg1) is significantly increased in fed mice, further supporting that S657G increases DNL even in our novel mouse model. N=4 mice/group, *p<0.05 by student's t-test

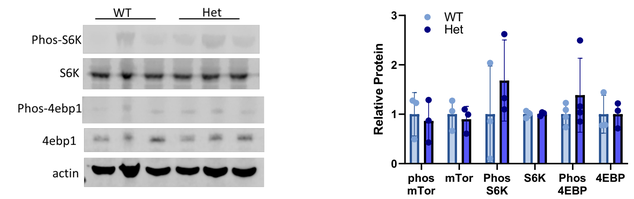


Figure 6: MTORC1 Signaling in livers from mice with FOG2 vs FOG2mut mice. Western Blot with quantification shows a trend of increased phosphorylation of mTORC1 targets including phosphorylated-S6K and phosphorylated-4-EBP.

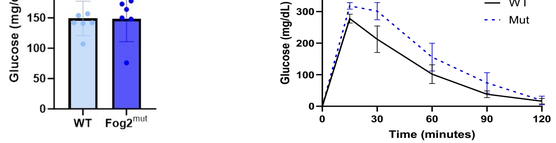


Figure 7: Fasting glucose and Glucose Tolerance Test in FOG2 vs FOG2mut mice. A) There was no difference in fasting glucose level. B) FOG2mut mice had increased glucose during IP GTT which was most pronounced post-prandially and resolved by 120 minutes. N=6-7/group, mean and SEM are displayed, *p<0.05 and +p=0.1 by student's t test relative baseline.

CONCLUSIONS

- Overexpression of FOG2 S657G in Huh7 cells increases expression of de novo lipogenesis genes and increased TG mass.
- iHeps from individuals with FOG2 S657G variant have differential gene expression consistent with increased mTORC1 signaling.
- Novel FOG2 MUT mouse model shows increased expression of DNL and trends toward increased liver TG, activation of MTORC1 signaling, and impaired insulin sensitivity.

These data are consistent with the model whereby FOG2S657G may promote MAFLD through increased de novo Lipogenesis (DNL) and drive increased Insulin Resistance.

Future Directions: Additional experiments are necessary to determine whether these changes are mediated through transcriptional changes or through changes in mTORC1 signaling. Future experiments will interrogate the role of FOG2mut in relation to FAO, FA uptake, TG secretion, and whole-body insulin sensitivity.

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