



RAPID COMMUNICATION

Ferroptosis mediators vary in metabolic syndrome, type-2 diabetes, and hypercholesterolemia: A meta-analysis report

Metabolic syndrome (MetS) is a complex disorder characterized by the coexistence of phenotypes such as obesity, hypertension, hyperglycemia, high triglyceride level, and low level of high-density lipoprotein cholesterol. Inflammation majorly driven by oxidative stress has an overarching role in obesity and IR-mediated mechanisms leading to MetS. Besides these factors, the molecular linkages between the MetS components and prognostic biomarkers for the prediction of the progression of one component to the others are still elusive. MetS is linked to several morbidities such as type-2 diabetes (T2D), non-alcoholic fatty liver disease, and cardiovascular diseases. Recently, there has been a surge in evidence linking ferroptosis, through iron overload and lipid peroxidation-driven cell death, and diseases such as cardiovascular diseases and T2D. The role of ferroptosis in liver diseases is well characterized as hepatocytes play a major role in iron transport and metabolism. Collectively, the above findings indicate the likelihood of ferroptosis contributing to MetS pathology. Here we have systematically collated and analyzed the high throughput data on MetS generated using various platforms such as GWAS, RNA-seq, and microarrays using a non-quantitative meta-analysis approach to evaluate and compare the expression of molecular mediators of ferroptosis in MetS and its co-morbidities such as T2D and hypercholesterolemia (Fig. 1).

Plausible role of excess iron-mediated ferroptosis and mitophagy in MetS

Pathway enrichment analysis of genes obtained from microarray, RNA-seq, and post-GWAS analyses (See File S1

for detailed methods and results) commonly identified ferroptosis and mitophagy-related pathways, both of which are linked to increased intracellular iron accumulation. We postulate that excess iron contributes to MetS through ferroptosis and mitophagy and the proposed mechanism is illustrated in Figure 1B. The hypothesis is well supported by the regulation status of the pathway genes, inferred from RNA-seq analysis, as detailed below.

Iron metabolism

Cellular iron homeostasis is regulated by nuclear receptor coactivator 4 (*NCOA4*). In an iron-deficient state, *NCOA4* degrades iron-bound ferritin through ferritinophagy resulting in the release and accumulation of cellular iron. Under iron-replete conditions, E3 ubiquitin ligase (*HERC2*) degrades *NCOA4*.¹ *NCOA4* was found to be up-regulated; *HERC2* and *SLC40A1* (sole iron exporter in mammalian cells) were found to be down-regulated in MetS samples as per RNA-seq analysis (Fig. 1B; File S2). These observations are indicative of elevated cellular iron in MetS.

Glutathione metabolism

Excess iron favors ferroptosis through ROS-mediated lipid peroxidation. Glutathione peroxidase 4 (*GPX4*) represses ferroptosis by conversion of lipid peroxides to non-toxic lipid alcohol. *GPX4* activity is highly dependent on cellular cysteine uptake and glutathione metabolism. In our analysis, although we found higher *GPX4* expression; members of the cysteine uptake pathways (*SLC3A2*, *SLC7A11*, *GCLC*, and *GCLM*) were down-regulated in MetS samples. Additionally, molecules such as beclin 1 (*BECN1*) which degrades *SLC7A11*, and lysosomal associated membrane protein (*LAMP2A*) that enhances *GPX4* degradation were up-regulated (Fig. 1B; File

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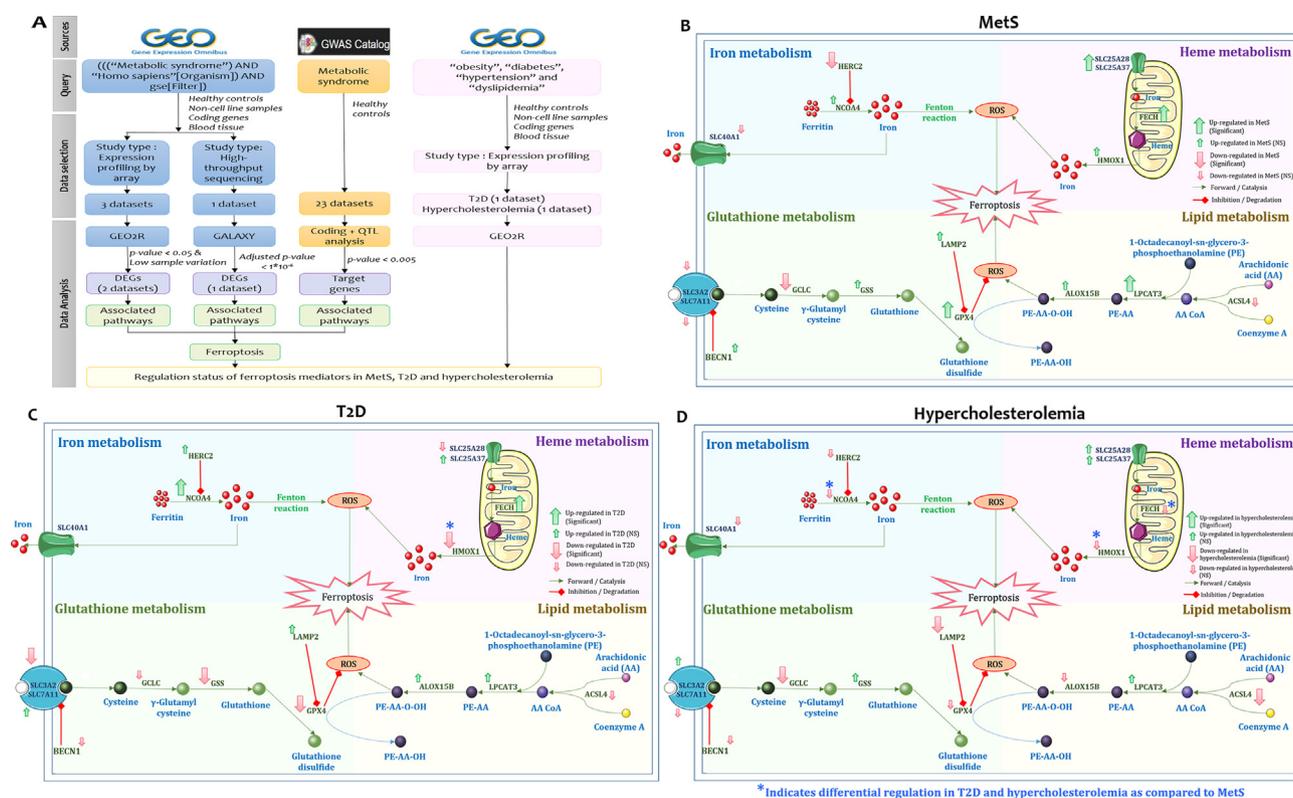


Figure 1 Overall workflow and results of the meta-analysis study. **(A)** Schema for data collection and meta-analysis. **(B)** Putative association of excess iron, ferroptosis, and mitophagy in metabolic syndrome (MetS). **(C)** Putative association of excess iron, ferroptosis, and mitophagy in type-2 diabetes (T2D). **(D)** Putative association of excess iron, ferroptosis, and mitophagy in hypercholesterolemia. The up and down arrows indicate the expression status of the genes in MetS, T2D, and hypercholesterolemia samples in contrast with healthy controls. The significance of the arrow indicates the significance of regulation. The LogFC and P-value for these genes can be referred to from File S2.

S2). Collectively, these events lead to lower cellular cysteine levels thereby inhibiting GPX4 activation and enhancing lipid peroxidation-mediated ferroptosis.

Lipid metabolism

Genes involved in the synthesis and oxidation of polyunsaturated fatty acids such as *LPCAT3* and *ALOX* genes and *POR* that enhance lipid peroxidation were found to be up-regulated in MetS samples based on RNA-seq analysis (Fig. 1B; File S2) suggesting the role of lipid-mediated ferroptosis in MetS.

Heme metabolism

Solute carrier family 25 member 37 (*SLC25A37*) and solute carrier family 25 member 28 (*SLC25A28*) are involved in mitochondrial iron transport and thus for heme synthesis. Enzyme ferrochelatase (*FECH*) catalyzes heme synthesis whereas heme oxygenase (*HMOX1*) degrades heme to ferrous iron. Elevated cellular iron leads to enhanced oxidative stress and mitochondrial iron influx thereby leading to mitophagy, ferroptosis, and autophagy.² The RNA-seq results revealed higher expression of *SLC25A37*,

SLC25A28, *FECH*, and *HMOX1* in MetS samples than in healthy controls (Fig. 1B; File S2).

The above findings from RNA-seq analysis indicate the positive contribution of iron-, glutathione-, lipid-, and heme-mediated ferroptosis, mitophagy, and autophagy in MetS pathology.

Comparison of ferroptosis molecular mediators in MetS, T2D, and hypercholesterolemia

Microarray datasets available in the GEO database for MetS-related morbidities such as obesity, T2D, hypertension, and dyslipidemia were queried, screened, and analyzed to compare the findings of MetS with these diseases. Two microarray datasets for hypertension and one each for T2D and hypercholesterolemia qualified the selection criteria for downstream data processing (Fig. 1A). Datasets of hypertension were not analyzed further due to disagreement in the regulation status of six signature hypertension genes³ (File S3). In T2D and hypercholesterolemia, similar to MetS, genes of glutathione metabolism were down-regulated, and lipid metabolism genes were up-regulated (Fig. 1C, D). *HMOX1* was down-regulated in both T2D and hypercholesterolemia; whereas, it was up-regulated in MetS. *NCOA4*

was down-regulated only in hypercholesterolemia. It was up-regulated in MetS and T2D.

In MetS, ferroptosis was contributed by i) inactivation of cysteine uptake in the cell; ii) increased ROS; iii) increased lipid peroxidation; and iv) increased cellular and mitochondrial iron. The causes of ferroptosis are grouped into four molecular mechanisms related to glutathione, lipid, iron, and heme metabolism. In MetS, we observed equal contributions of the four categories (Fig. 1B).

In the case of T2D, ferroptosis was due to the down-regulation of glutathione metabolism, increased lipid peroxidation, and increased cellular iron (Fig. 1C). These findings are in agreement with the inferences drawn from a recent review by He et al.⁴ The review recommended targeting ferritinophagy and ferroptosis for the treatment of T2D complications. The up-regulation of *FECH* and down-regulation of *HMOX1* observed from microarray analysis of T2D (Fig. 1C) implies reduced heme degradation to iron, thereby decreasing mitophagy-mediated cell death.

In the case of hypercholesterolemia, ferroptosis was mediated only by aberrations in glutathione and lipid metabolism. The microarray analysis did not indicate the presence of excess cellular iron in hypercholesterolemia (Fig. 1D). This observation is in agreement with the inferences drawn from a cross-sectional study on 290 individuals wherein He et al.⁵ found a negative correlation between dyslipidemia (high triglyceride and low high-density lipoprotein cholesterol levels) and serum iron. The absence of excess iron in hypercholesterolemia indicates the prognostic application of excess iron as a marker for the progression of hypercholesterolemia to MetS.

A meta-analysis was performed on publicly available microarray, RNA-seq, and GWAS data on MetS. The aim was to understand and compare the contribution of ferroptosis mediators in MetS, T2D, and hypercholesterolemia. Our analysis confirmed the contribution of many of the previously well-studied pathways in MetS such as insulin signaling, cholesterol metabolism, lipid metabolism, and inflammation. Interestingly, the ferroptosis pathway was found to be enriched in all three independent platforms; however, the mediators of ferroptosis in MetS were found to be distinct from T2D and hypercholesterolemia. It is to be noted that the observations and postulates raised in this study are based on mRNA-level evidence; it must be mapped to proteomics and metabolomics data of MetS samples for further confirmation. Likewise, the gene expression studies could not be classified based on the phenotype combinations of the five components of MetS. These limitations are due to the fact that the expected granularity of data, with respect to precise MetS phenotype combinations and the corresponding multi-omics data, is not available in the public domain. However, the limitations are offset by the strength of the study, which examines common findings across the heterogeneous, and large sample datasets from the three high throughput

approaches namely microarray, RNA-seq, and GWAS. These shared findings are highly unlikely to be random outcomes and are further supported by the orchestrated differential expression of several genes in the identified pathway networks. The observations and prognostic application of excess cellular iron in the progression of hypercholesterolemia to MetS merit further validation through animal models and clinical samples.

Ethics declaration

The ICMR-NIRRH Ethics Committee for Clinical Studies approved the exemption from ethics review (D/ICEC/Sci-121/125/2020).

Author contributions

S.I.-T. planned, designed, and supervised the study. I.K. and A.P. curated the data. I.K., A.P., and T.D. analyzed the data. I.K. and S.I.-T. wrote the manuscript. All authors read the manuscript and approved its publication on *Genes & Diseases*.

Conflict of interests

The authors declare no conflict of interests.

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Data availability

Publicly available online platforms and tools were used for data analysis and the results are collated and provided as supplementary information.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gendis.2023.06.024>.

References

1. Santana-Codina N, Mancias JD. The role of NCOA4-mediated ferritinophagy in health and disease. *Pharmaceuticals*. 2018; 11(4):E114.
2. Chang LC, Chiang SK, Chen SE, Yu YL, Chou RH, Chang WC. Heme oxygenase-1 mediates BAY 11-7085 induced ferroptosis. *Cancer Lett*. 2018;416:124–137.
3. Zhao XC, Yang SH, Yan YQ, et al. Identification of differential gene expression profile from peripheral blood cells of military pilots with hypertension by RNA sequencing analysis. *BMC Med Genom*. 2018;11(1):59.
4. He J, Li Z, Xia P, et al. Ferroptosis and ferritinophagy in diabetes complications. *Mol Metabol*. 2022;60:101470.
5. He L, Zhang Y, Ru D, Xue B, Wen S, Zhou H. Serum iron levels are negatively correlated with serum triglycerides levels in female university students. *Ann Palliat Med*. 2020;9(2): 414–419.

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