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**Abstract**

**Key** **words**

**Introduction**

These findings improve our understanding of how changes in temperature affect mammals by putting the responses of individual tissues into the context of the whole body

The hypothalamus is the central regulating unit in the brain for maintenance of energy homeostasis, including body temperature.

We ([Chevalier et al., 2020](https://elifesciences.org/articles/78556#bib9); [Chevalier et al., 2015](https://elifesciences.org/articles/78556#bib8); [Spiljar et al., 2021](https://elifesciences.org/articles/78556" \l "bib30)) and others ([Simcox et al., 2017](https://elifesciences.org/articles/78556#bib26)) observed that cold (4–10°C), and warm exposures close to thermoneutrality (33–34°C) also affect organs apart from the adipose tissues, including the intestine, bone, and immunologic tissues.

The ambient temperatures where metabolic rate is at a minimum is the usual definition of a thermoneutral zone, and it is in the interval of 29 °C in the light phase and up to 33 °C during dark ([Škop et al., 2020](https://elifesciences.org/articles/78556" \l "bib27)). The typical housing of mice is at room temperature (RT) of 22 °C, which is below their thermoneutrality, and a substantial amount of their energy expenditure is devoted to maintaining core body temperature. The above data suggest that thermal variations exert a whole-body functional reprogramming; however, no systematic transcriptomics analysis has addressed to what extent organs undergo changes induced by temperatures below the thermoneutral zone.

RNA sequencing (RNASeq) is the most widely used quantitative approach to assess the global gene expression and its alterations under different conditions, since it determines subtle molecular changes that may contribute to acquisition of certain phenotypes ([Carninci et al., 2005](https://elifesciences.org/articles/78556" \l "bib5); [Grada and Weinbrecht, 2013](https://elifesciences.org/articles/78556" \l "bib11)). It has been shown that RNAseq can reflect tissue specificity; as such, highly regulated genes might represent characteristic functions of the tissues ([Breschi et al., 2016](https://elifesciences.org/articles/78556" \l "bib3); [Sonawane et al., 2017](https://elifesciences.org/articles/78556" \l "bib28)). However, due to a deluge of data, particularly when several transcriptomic data sets are obtained, the combined analysis of several datasets (meta-analysis) remains challenging ([Sudmant et al., 2015](https://elifesciences.org/articles/78556" \l "bib32)). This analysis becomes even more challenging if one aims to interconnect the variations in the meta-data with the complex molecular basis of phenotypic changes.

In this study, we conducted a systematic transcriptomics analysis across 11 mouse tissues at RT and cold (10 °C), and across additional 7 tissues at mild warm close to the mouse thermoneutral zone (33–34°C). We establish a common expression signature of differentially expressed genes in BAT during various cold exposure experimental setups across seven studies (six previously published RNAseq datasets and this work). Further, we provide a comprehensive resource dataset of comparative transcriptomics across 11 mouse tissues describing the transcriptional landscape at room temperature (RT) and at 10 °C, and supplement these analyses with a multi-tissue transcriptomics profiling at 34 °C. We systematically investigate how differential expression impacts specific cellular functions across the tissues and identify shared, specific, and inversely regulated gene signatures and gene set enrichments under cold exposure and at 34 °C compared to RT housing. Our work shows that adipose tissues undergo most severe transcriptome changes, followed by the immune tissues and the CNS cluster. With this resource and the applied bioinformatics methods, we characterize tissue-specific expression patterns and detect temperature-dependent gene expression profiles; provide insights into the tissue-specific adaptation mechanisms associated with temperature variations; and place the adaptive role of each tissue in a whole-organism perspective to comprehend the tissue-specific organization of the biological processes after 12 °C increase or decrease of environmental temperature compared to RT housing.

**Methods**

*Peromyscus eremicus, RNA Extraction, and Sequencing*

Captive born, sexually mature, non-reproductive healthy male and female P. eremicus were reared in an environmental chamber designed to simulated the Sonoron desert (Blumstein, Blumstein, Colella, [Kordonowy](https://www.biorxiv.org/content/10.1101/2023.07.03.547568v1.full#ref-37)). Mice were provided a standard diet and fed ad libitum (LabDiet® 5015\*, 26.101% fat, 19.752% protein, 54.148% carbohydrates, energy 15.02 kJ/g, food quotient [FQ] 0.89). Animals were randomly selected and assigned to the two water treatment groups (n=9 of each treatment, female mice with water, female mice without water, male mice with water, and male mice without water, total n=36). At the start of the experiment (day 0, time 0hr, 10:00), mice were weighed (rounded to the nearest tenth of a gram) on a digital scale, a temperature-sensing passive integrated transponder (PIT) tag (BioThermo13, accuracy ±0.02°C, BioMark®, Boise, ID, USA) was implanted subdermally, water was removed from chambers corresponding to those animals in the dehydration group. Mice were metabolically phenotyped for the duration of the experiment (Bumstein and MacManes 2023) using a pull flow-through respirometry system from Sable Systems International (SSI). Rates of CO2 production, O2 consumption, and water loss were calculated using equations 10.6, 10.5, and 10.9, respectively, from [Lighton (2018)](https://www.biorxiv.org/content/10.1101/2023.07.03.547568v1.full" \l "ref-40). Respiratory quotient (RQ, the ratio of VCO2 to VO2) and Energy expenditure (EE) kJ hr-1was calculated as in [Lighton (2018](https://www.biorxiv.org/content/10.1101/2023.07.03.547568v1.full" \l "ref-40), eq. 9.15). For downstream analysis, we calculated the mean of the last hour of water loss, EE, and RQ for each mouse.

At the conclusion of the experiment (day 3, time 72hr, 12:00) described in Blumstein and MacManes (2023), body temperature was recorded via a Biomark® HPR Plus reader, mice were weighed, animals were euthanized with an overdose on isoflurane and 120 µl of trunk blood for serum electrolyte measurement using an Abaxis i-STAT® Alinity machine using i-STAT CHEM8+ cartridges (Abbott Park, IL, USA, Abbott Point of Care Inc). We measured the concentration of sodium (Na, mmol/L), potassium (K, mmol/L), blood urea nitrogen (BUN, mmol/L), hematocrit (Hct, % PCV), ionized calcium (iCa, mmol/L), glucose (Glu, mmol/L), osmolality (mmol/L), hemoglobin (Hb, g/dl), chlorine (Cl, mEq/L), total CO2 (TCO2, mmol/L), and Anion gap (AnGap, mEq/L). Using Na, Glu, and BUN, we calculated serum osmolality. To test for statistical significant (p < 0.05) differences we used a student’s two-tailed t-test (stats::t.test) between the sexes for each experimental group in R v 4.0.3 (R [**Core Team 2020**](https://www.biorxiv.org/content/10.1101/2023.07.03.547568v1.full#ref-69)).

The lung, liver, kidney, a section of the gastrointestinal tract, and hypothalamus were collected fixed in an RNA later (Ambion) solution and stored at 4°C for 12hr before being frozen at −80°C for long-term storage. Prior to RNA extraction, the tissues were removed from RNA later and a small section was dissected off. Tissues were mechanically lysed using a Bead Beater, and RNA was then extracted using a standardized Trizol protocol. RNA libraries were prepared using standard poly-A tail purification, prepared using Illumina primers, and individually dual-barcoded using a New England Biolabs Ultra Directional kit. Individually barcoded samples were pooled and sequenced paired end and 150 bp in length on \_\_\_\_\_ lanes of a Novaseq at the University of New Hampshire Hubbard Genome Center.

*Genome Alignment and Differential Gene Expression*

The P. eremicus genome was indexed and reads were aligned to the genome using STAR version () allowing a 10 base mismatches (--outFilterMismatchNmax 10), a maximum of 20 multiple alignments per read (--outFilterMultimapNmax 20), and discarding reads that mapped at <30% of the read length (--outFilterScoreMinOverLread 0.3). Aligned reads were counted using htseq-count version 0.11.3 (Anders et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1111/mec.16024#mec16024-bib-0002)).

Counts from htseq-count were exportaed as csv files and all downstream data manipulation and statistical analyses were conducted in R v 4.0.3 (R [Core Team 2020](https://www.biorxiv.org/content/10.1101/2023.07.03.547568v1.full#ref-69)). Counts were merged into a gene-level count. Low expression genes were filtered and removed if the gene had 10 or less counts in 8 or more individuals experiment wide. Differential gene expression analysis was conducted in R using DESeq2 (). For the dataset as a whole, we compared three models that tested for the effects of sex, water access, and tissue type. For each tissue we compared two models, testing the effect of and identify genes specific to sex and water access with a Wald test. Results were visualized using ggplot2 (Wickham, [2011](https://onlinelibrary.wiley.com/doi/full/10.1111/mec.16024#mec16024-bib-0094)).

*Weighted Gene Correlation Network Analysis*

To identify the regulation of gene expression associated with responses to water access we performed a network-based statistical approach that identifies clusters of genes with highly correlated expression profiles (modules) called a weighted gene correlation network analysis (WGCNA) () for each tissue independently. This approach allows us to relate gene expression with performance-level phenotypes and physiology (mean EE, water loss, RQ, total weight loss, proportional weight loss, sex, body temperature, water access, and the panel of electrolytes). Prior to WGCNA read counts were normalized using DESeq2. Module detection was done using WGCNA::blockwiseModules with netowrkType set to “signed” but otherwise default parameters. We estimated a soft threshold power (β) for each tissue dataset by plotting this value against mean connectivity to determine the minimum value at which mean connectivity asymptotes, which represents scale-free topology (liver = 15, kidney = 21, GI = 14, lung = 20, hypothalamus = 14).

*Canonical Correlation Analysis*

We used CCA () implemented in the R package *vegan* () to investigate multivariate correlation of gene expression, by tissue, water access, and sex, with metabolic variables (mean EE, mean RQ, mean water loss, body temperature, and proportional weight loss) and display the three levels of information in an ordination diagram, also called a triplot. We used and ANOVA to identify what response variables were significant. Significant response variables were graphed as vectors and allowed up to identify their correlative nature, vectors pointing in the same direction are positively correlated while vectors pointing in opposite direction are negatively correlated. To identify genes of interest we selected genes that graphed two standard deviations away from the mean on CCA1 and CCA2.

*Gene Ontology*

To examine gene ontology of DE genes, WGCNA modules, and genes identified as outliers in the CCA we cross-referenced our gene IDs with *Homo sapiens* gene IDs via Ensembl before running Gene Ontology (GO) analyses. Each analysis above resulted in a list of genes that was used as input for the GO analysis and *gprofiler*::*gost* () using *Homo sapiens* set as the organism ().

**Results**

*Phenotypes*

When comparing males and females separately, the following electrolytes showed significant differences with and without access to water; NA (male and female Na p = 0.0016 and p = 0.0026 respectively), BUN (p = 0.001/0.003), Hct (p = 0.002/0.001), osmolality (p = 8.2-05/0.0001), Cl (p = 0.02/0.007), Hb (p = 0.017/0.009), and TCO2 (female p = 0.017). When comparing males to females for either water treatment (with or without access to water), no significant differences were found in the electrolyte levels. Both males and females experienced significant weight loss (p = 0.001, 0.005) and proportional weight loss (p = 2.2e-16 ,2.2e-16) at the end of the experiment. Body temperature followed a similar pattern as described above and were significantly lower for mice without access to water for females but not males (p = 0.0003). Finally, within sex, RQ and WLR were significantly different between water groups (male and female RQ p = 2.2e-16 and 0.001, WLR p = 8.473e-15 and 1.721e-13) but EE was only significantly different between males not females (p = 0.008919).

*Differential Gene Expression*

We obtained an average of 91.44 million reads (+- 11.6 SD) per sample. On average, 78.33% of reads were uniquely mapped per sample (+- 2.12% SD). Data on the number of reads and mapping rate per sample are located in Supplemental file X and all gene expression count data and code used to analyze the data are located at the GitHub repository (). After cross-referenced our gene IDs with *Homo sapiens* gene IDs via Ensembl and filtering out genes present in less than 8 samples with counts of 10 or more, 14998 genes remained. Patterns of gene expression data are largely driven by tissue type (PC1: 42% variance and PC2: 26% variance).

A graph of red and green dots

Description automatically generatedWe then conducted all downstream analysis (except for CCA, see below) on each tissue independently. Count and sample data were filtered to include only the tissue of interest and then genes were then further filtered using the filtering technique described resulting in 11609 genes in the kidney (PC1- 38% and PC2-14%), 12105 genes in the GI (PC1- 28% and PC2-21%), 12904 genes in the hypothalamus (PC1- 64% and PC2-21%), 10654 genes in the liver (PC1- 52% and PC2-11%), and 12404 genes in the lung: PC1- 46% and PC2-13%, Supplemental Figure X). Within each tissue we found many differentially expressed genes (p < 0.05) between water treatments (Table x) and few differentially expressed genes between sex (Table X).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tissue | DE between water treatments | | | DE between sexes | | |
|  | Up regulated | Down regulated | Total | Up regulated | Down regulated | Total |
| Kidney | 732 | 1026 | 1758 | 5 | 7 | 12 |
| Liver | 868 | 1090 | 1958 | 1 | 3 | 4 |
| Lung | 2064 | 2137 | 4201 | 5 | 5 | 10 |
| Hypothalamus | 3 | 9 | 12 | 1 | 2 | 3 |
| Gastrointestinal tract | 855 | 662 | 1517 | 1 | 1 | 2 |

This could be suplamental and then I can pick 5-10 terms that are lit

A close-up of a chart

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*Weighted Gene Correlation Network Analysis*

A total of 17012 genes identified in the kidney were successfully assigned into 33 modules with the number of genes per module ranging from 20 – 11012. A full list of gene assignments is available in supplemental (). Of the 33 modules identified, 12 modules were significant for three or more phenotypes (supplemental ()). We identified 24 individual modules using 12622 genes for the GI. Modules contained 33-3530 genes per module (supplemental ()). Of these modules, 14 modules were significantly correlated with three or more phenotypes (supplemental ()). In the lung 12942 genes assigned to 13 modules. Each module contained 23-7116 genes (supplemental ()). Nine modules were significant for three or more phenotypes (supplemental ()). A total of 13389 genes were assigned to 18 different modules in the hypothalamus. Modules contained 30-2319 genes (supplemental ()). Of the 18 modules, 3 modules were significant for three or more phenotypes (supplemental ()). Finally, 11125 genes assigned to 18 modules in the liver with the number of genes per module ranging from 28-3116 (supplemental ()). Of the 18 modules, 13 modules were significant for three or more phenotypes (supplemental ()).

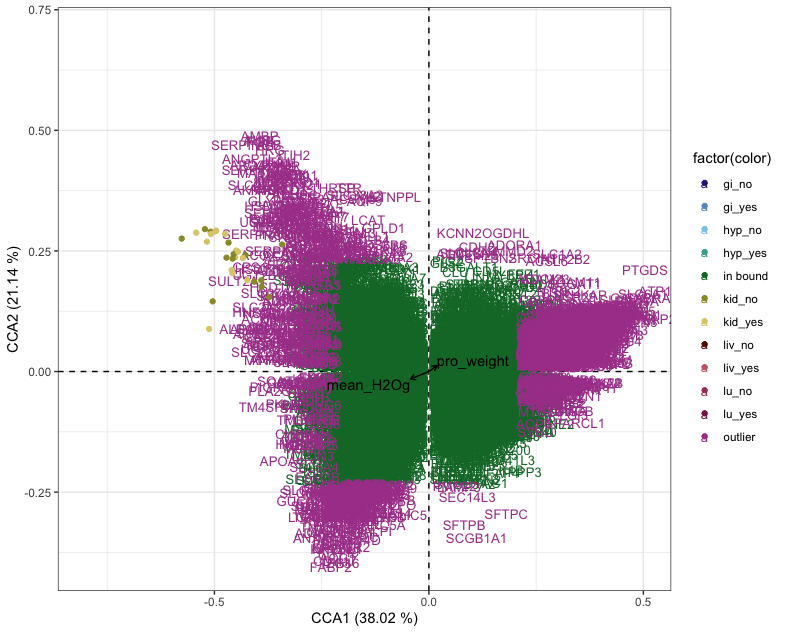
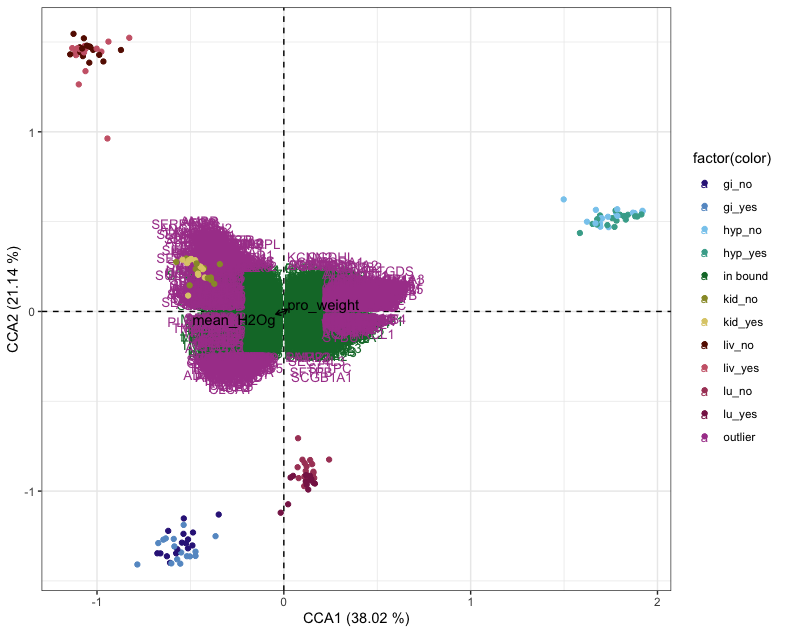
Choose the GO terms wisely (decision rule: GO for modules that overlap three diff phenotypes?)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trait | Kidney | Gastrointestinal tract | Liver | Lung | Hypothalamus |
| Sex | 0 | 0 | 0 | 1 | 0 |
| Delta weight | 5 | 13 | 9 | 7 | 2 |
| Proportional weight loss | 8 | 13 | 9 | 8 | 2 |
| Na | 8 | 14 | 12 | 9 | 2 |
| BUN | 9 | 14 | 8 | 8 | 3 |
| AnGap | 2 | 0 | 0 | 0 | 0 |
| K | 4 | 0 | 0 | 0 | 0 |
| Cr | 2 | 3 | 2 | 5 | 4 |
| Htc | 9 | 12 | 10 | 8 | 3 |
| Cl | 8 | 12 | 12 | 7 | 2 |
| Glu | 3 | 0 | 0 | 0 | 0 |
| Hb | 9 | 12 | 10 | 8 | 3 |
| TCo2 | 8 | 9 | 10 | 6 | 2 |
| iCa | 2 | 0 | 4 | 2 | 0 |
| RQ | 5 | 1 | 1 | 1 | 2 |
| EE | 1 | 0 | 2 | 1 | 1 |
| WLR | 6 | 3 | 6 | 6 | 1 |
| Body temperature | 4 | 3 | 9 | 5 | 2 |
| Water access | 7 | 12 | 9 | 7 | 1 |
| Total modules | 33 | 24 | 18 | 13 | 18 |

*Canonical correspondence analysis*

We examined the relationship between phenotypes (EE, RQ, WLR, water access, proportional weight, body temperature, and tissue type) and gene expression using canonical correspondence analysis (CCA) ([Oksanen et al. 2007](javascript:;)). CCA suggests a significant overall association between the phenotypes and gene expression across tissues (F = 105.45, p = 0.001; CCA1 – 38.02% and CCA2 – 21.14%, Figure x, table X). We found that genes located two standard deviations from the origin (# of genes) mirrors our finding from the individual WGCNA analyses (# genes also in each WGCAN analysis). Here, the proportional weight loss and WLR domains are significantly corelated (both p=0.001, table x) with shared genes in the hypothalamus and GI (figure x) but there is no overlap between the genes and the two domains. This suggests that proportional weight loss and WLR exert different effects on gene expression for the hypothalamus and the GI.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| model: cca(formula = gene expression ~ water access + proportional weight loss + rq + ee + wlr + body temperature + sex + tissue) | | | | | |
|  | Df | ChiSquare | F | Pr(>F) |  | |
| water access | 1 | 0.0001207 | 6.4369 | 0.001 | \*\*\* | |
| proportional weight loss | 1 | 0.00009 | 4.8006 | 0.001 | \*\*\* | |
| rq | 1 | 0.0000186 | 0.9896 | 0.407 |  | |
| ee | 1 | 0.0000276 | 1.4738 | 0.179 |  | |
| wlr | 1 | 0.0000372 | 1.9856 | 0.096 | . | |
| body temperature | 1 | 0.0000227 | 1.2123 | 0.261 |  | |
| sex | 1 | 0.0000324 | 1.7296 | 0.12 |  | |
| tissue | 4 | 0.0214075 | 285.3174 | 0.001 | \*\*\* | |
| model | 11 | 0.0217569 | 105.45 | 0.001 | \*\*\* | |
| residual | 147 | 0.0027574 |  |  |  | |



Genes identified in all analysis (i.e. DE genes, assigned to module in WGCNA, and two SD from the origin in CCA)

A screenshot of a chart

Description automatically generated

Graph the data from the globslly normalized data

**Discussion**

**Genes in hyp don’t appear to be DE but there are other analysis that allow us to uncover some potential functional things, such as WGCNA and CCA**

**Right now the story is vascularization and blood things**

Overall, adipose tissues experienced the biggest changes in gene levels between different temperatures, followed by tissues involved in immune responses, and the brain and spinal cord tissues. Each organ changed gene expression levels in its own way. , and this was not due to the different intimate gene expression profile between the various organs.

Good go terms from my go plot:

* regulation of vascular development (up, kid)
* histone modification (up, liv)
* glucose metabolic process (up, liv)
* angiogenesis (up, lu)
* hemopoiesis (down, liv)

AGT:

* The protein encoded by this gene, pre-angiotensinogen or angiotensinogen precursor, is expressed in the liver and is cleaved by the enzyme renin in response to lowered blood pressure. The resulting product, angiotensin I, is then cleaved by angiotensin converting enzyme (ACE) to generate the physiologically active enzyme angiotensin II. The protein is involved in maintaining blood pressure, body fluid and electrolyte homeostasis, and in the pathogenesis of essential hypertension and preeclampsia
* Essential component of the renin-angiotensin system (RAS), a potent regulator of blood pressure, body fluid and electrolyte homeostasis. ( [ANGT\_HUMAN,P01019](https://www.uniprot.org/uniprot/P01019#function) ). Angiotensin-2]: Acts directly on vascular smooth muscle as a potent vasoconstrictor, affects cardiac contractility and heart rate through its action on the sympathetic nervous system, and alters renal sodium and water absorption through its ability to stimulate the zona glomerulosa cells of the adrenal cortex to synthesize and secrete aldosterone (PubMed:[10619573](https://pubmed.ncbi.nlm.nih.gov/10619573/), [17138938](https://pubmed.ncbi.nlm.nih.gov/17138938/)).

CYP2E1:

* This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and is induced by ethanol, the diabetic state, and starvation. The enzyme metabolizes both endogenous substrates, such as ethanol, acetone, and acetal, as well as exogenous substrates including benzene, carbon tetrachloride, ethylene glycol, and nitrosamines which are premutagens found in cigarette smoke. Due to its many substrates, this enzyme may be involved in such varied processes as gluconeogenesis, hepatic cirrhosis, diabetes, and cancer. [provided by RefSeq, Jul 2008]
* A cytochrome P450 monooxygenase involved in the metabolism of fatty acids (PubMed:[10553002](https://pubmed.ncbi.nlm.nih.gov/10553002/), [18577768](https://pubmed.ncbi.nlm.nih.gov/18577768/)). Mechanistically, uses molecular oxygen inserting one oxygen atom into a substrate, and reducing the second into a water molecule, with two electrons provided by NADPH via cytochrome P450 reductase (NADPH--hemoprotein reductase) (PubMed:[10553002](https://pubmed.ncbi.nlm.nih.gov/10553002/), [18577768](https://pubmed.ncbi.nlm.nih.gov/18577768/)). Catalyzes the hydroxylation of carbon-hydrogen bonds

CYP4B1:

* This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum. In rodents, the homologous protein has been shown to metabolize certain carcinogens; however, the specific function of the human protein has not been determined. Multiple transcript variants have been found for this gene. [provided by RefSeq, Jan 2016]

INMT:

* N-methylation of endogenous and xenobiotic compounds is a major method by which they are degraded. This gene encodes an enzyme that N-methylates indoles such as tryptamine. Alternative splicing results in multiple transcript variants. Read-through transcription also exists between this gene and the downstream MINDY4 (aka FAM188B) gene. In rodents and other mammals such as cetartiodactyla this gene is in the opposite orientation compared to its orientation in human and other primates and this gene appears to have been lost in carnivora and chiroptera. [provided by RefSeq, Jul 2019]
* Functions as thioether S-methyltransferase and is active with a variety of thioethers and the corresponding selenium and tellurium compounds, including 3-methylthiopropionaldehyde, dimethyl selenide, dimethyl telluride, 2-methylthioethylamine, 2-methylthioethanol, methyl-n-propyl sulfide and diethyl sulfide. Plays an important role in the detoxification of selenium compounds (By similarity). Catalyzes the N-methylation of tryptamine and structurally related compounds. ( [INMT\_HUMAN,O95050](https://www.uniprot.org/uniprot/O95050#function) )

MTARC1:

* Enables molybdenum ion binding activity; molybdopterin cofactor binding activity; and oxidoreductase activity, acting on other nitrogenous compounds as donors. Contributes to nitrite reductase (NO-forming) activity. Involved in cellular detoxification of nitrogen compound; nitrate metabolic process; and nitric oxide biosynthetic process. Located in mitochondrion. Part of nitric-oxide synthase complex. [provided by Alliance of Genome Resources, Apr 2022]
* Catalyzes the reduction of N-oxygenated molecules, acting as a counterpart of cytochrome P450 and flavin-containing monooxygenases in metabolic cycles (PubMed:[19053771](https://pubmed.ncbi.nlm.nih.gov/19053771/), [21029045](https://pubmed.ncbi.nlm.nih.gov/21029045/), [30397129](https://pubmed.ncbi.nlm.nih.gov/30397129/)). As a component of prodrug-converting system, reduces a multitude of N-hydroxylated prodrugs particularly amidoximes, leading to increased drug bioavailability (PubMed:[19053771](https://pubmed.ncbi.nlm.nih.gov/19053771/)). May be involved in mitochondrial N(omega)-hydroxy-L-arginine (NOHA) reduction, regulating endogenous nitric oxide levels and biosynthesis (PubMed:[21029045](https://pubmed.ncbi.nlm.nih.gov/21029045/)). Postulated to cleave the N-OH bond of N-hydroxylated substrates in concert with electron transfer from NADH to cytochrome b5 reductase then to cytochrome b5, the ultimate electron donor that primes the active site for substrate reduction (PubMed:[21029045](https://pubmed.ncbi.nlm.nih.gov/21029045/), [19053771](https://pubmed.ncbi.nlm.nih.gov/19053771/)). ( [MARC1\_HUMAN,Q5VT66](https://www.uniprot.org/uniprot/Q5VT66#function) )

PCK1:

* This gene is a main control point for the regulation of gluconeogenesis. The cytosolic enzyme encoded by this gene, along with GTP, catalyzes the formation of phosphoenolpyruvate from oxaloacetate, with the release of carbon dioxide and GDP. The expression of this gene can be regulated by insulin, glucocorticoids, glucagon, cAMP, and diet. Defects in this gene are a cause of cytosolic phosphoenolpyruvate carboxykinase deficiency. A mitochondrial isozyme of the encoded protein also has been characterized. [provided by RefSeq, Jul 2008]
* Cytosolic phosphoenolpyruvate carboxykinase that catalyzes the reversible decarboxylation and phosphorylation of oxaloacetate (OAA) and acts as the rate-limiting enzyme in gluconeogenesis (PubMed:[30193097](https://pubmed.ncbi.nlm.nih.gov/30193097/), [24863970](https://pubmed.ncbi.nlm.nih.gov/24863970/), [26971250](https://pubmed.ncbi.nlm.nih.gov/26971250/), [28216384](https://pubmed.ncbi.nlm.nih.gov/28216384/)). Regulates cataplerosis and anaplerosis, the processes that control the levels of metabolic intermediates in the citric acid cycle (PubMed:[30193097](https://pubmed.ncbi.nlm.nih.gov/30193097/), [24863970](https://pubmed.ncbi.nlm.nih.gov/24863970/), [26971250](https://pubmed.ncbi.nlm.nih.gov/26971250/), [28216384](https://pubmed.ncbi.nlm.nih.gov/28216384/)). At low glucose levels, it catalyzes the cataplerotic conversion of oxaloacetate to phosphoenolpyruvate (PEP), the rate-limiting step in the metabolic pathway that produces glucose from lactate and other precursors derived from the citric acid cycle (PubMed:[30193097](https://pubmed.ncbi.nlm.nih.gov/30193097/)). At high glucose levels, it catalyzes the anaplerotic conversion of phosphoenolpyruvate to oxaloacetate (PubMed:[30193097](https://pubmed.ncbi.nlm.nih.gov/30193097/)). Acts as a regulator of formation and maintenance of memory CD8(+) T-cells: up-regulated in these cells, where it generates phosphoenolpyruvate, via gluconeogenesis (By similarity). The resultant phosphoenolpyruvate flows to glycogen and pentose phosphate pathway, which is essential for memory CD8(+) T-cells homeostasis (By similarity). In addition to the phosphoenolpyruvate carboxykinase activity, also acts as a protein kinase when phosphorylated at Ser-90: phosphorylation at Ser-90 by AKT1 reduces the binding affinity to oxaloacetate and promotes an atypical serine protein kinase activity using GTP as donor (PubMed:[32322062](https://pubmed.ncbi.nlm.nih.gov/32322062/)). The protein kinase activity regulates lipogenesis: upon phosphorylation at Ser-90, translocates to the endoplasmic reticulum and catalyzes phosphorylation of INSIG proteins (INSIG1 and INSIG2), thereby disrupting the interaction between INSIG proteins and SCAP and promoting nuclear translocation of SREBP proteins (SREBF1/SREBP1 or SREBF2/SREBP2) and subsequent transcription of downstream lipogenesis-related genes (PubMed:[32322062](https://pubmed.ncbi.nlm.nih.gov/32322062/)). ( [PCKGC\_HUMAN,P35558](https://www.uniprot.org/uniprot/P35558#function) )

SLC27A2:

* The protein encoded by this gene is an isozyme of long-chain fatty-acid-coenzyme A ligase family. Although differing in substrate specificity, subcellular localization, and tissue distribution, all isozymes of this family convert free long-chain fatty acids into fatty acyl-CoA esters, and thereby play a key role in lipid biosynthesis and fatty acid degradation. This isozyme activates long-chain, branched-chain and very-long-chain fatty acids containing 22 or more carbons to their CoA derivatives. It is expressed primarily in liver and kidney, and is present in both endoplasmic reticulum and peroxisomes, but not in mitochondria. Its decreased peroxisomal enzyme activity is in part responsible for the biochemical pathology in X-linked adrenoleukodystrophy. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Apr 2009]
* Mediates the import of long-chain fatty acids (LCFA) into the cell by facilitating their transport across cell membranes, playing an important role in hepatic fatty acid uptake (PubMed:[20530735](https://pubmed.ncbi.nlm.nih.gov/20530735/), [22022213](https://pubmed.ncbi.nlm.nih.gov/22022213/), [24269233](https://pubmed.ncbi.nlm.nih.gov/24269233/), [10198260](https://pubmed.ncbi.nlm.nih.gov/10198260/), [10749848](https://pubmed.ncbi.nlm.nih.gov/10749848/), [11980911](https://pubmed.ncbi.nlm.nih.gov/11980911/)). Also functions as an acyl-CoA ligase catalyzing the ATP-dependent formation of fatty acyl-CoA using LCFA and very-long-chain fatty acids (VLCFA) as substrates, which prevents fatty acid efflux from cells and might drive more fatty acid uptake (PubMed:[20530735](https://pubmed.ncbi.nlm.nih.gov/20530735/), [22022213](https://pubmed.ncbi.nlm.nih.gov/22022213/), [24269233](https://pubmed.ncbi.nlm.nih.gov/24269233/), [10198260](https://pubmed.ncbi.nlm.nih.gov/10198260/), [10749848](https://pubmed.ncbi.nlm.nih.gov/10749848/), [11980911](https://pubmed.ncbi.nlm.nih.gov/11980911/)). Plays a pivotal role in regulating available LCFA substrates from exogenous sources in tissues undergoing high levels of beta-oxidation or triglyceride synthesis (PubMed:[20530735](https://pubmed.ncbi.nlm.nih.gov/20530735/)). Can also activate branched-chain fatty acids such as phytanic acid and pristanic acid (PubMed:[10198260](https://pubmed.ncbi.nlm.nih.gov/10198260/)). May contribute to the synthesis of sphingosine-1-phosphate (PubMed:[24269233](https://pubmed.ncbi.nlm.nih.gov/24269233/)). Does not activate C24 bile acids, cholate and chenodeoxycholate (PubMed:[11980911](https://pubmed.ncbi.nlm.nih.gov/11980911/)). In vitro, activates 3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholestanate (THCA), the C27 precursor of cholic acid deriving from the de novo synthesis from cholesterol (PubMed:[11980911](https://pubmed.ncbi.nlm.nih.gov/11980911/)). However, it is not critical for THCA activation and bile synthesis in vivo (PubMed:[20530735](https://pubmed.ncbi.nlm.nih.gov/20530735/)). ( [S27A2\_HUMAN,O14975](https://www.uniprot.org/uniprot/O14975#function) )
* [Isoform 1]: Exhibits both long-chain fatty acids (LCFA) transport activity and acyl CoA synthetase towards very long-chain fatty acids (PubMed:[21768100](https://pubmed.ncbi.nlm.nih.gov/21768100/), [10198260](https://pubmed.ncbi.nlm.nih.gov/10198260/)). Shows a preference for generating CoA derivatives of n-3 fatty acids, which are preferentially trafficked into phosphatidylinositol (PubMed:[21768100](https://pubmed.ncbi.nlm.nih.gov/21768100/)). ( [S27A2\_HUMAN,O14975](https://www.uniprot.org/uniprot/O14975#function) )
* [Isoform 2]: Exhibits long-chain fatty acids (LCFA) transport activity but lacks acyl CoA synthetase towards very long-chain fatty acids. ( [S27A2\_HUMAN,O14975](https://www.uniprot.org/uniprot/O14975#function) )

SLC38A4:

* SLC38A4 is found predominantly in liver and transports both cationic and neutral amino acids. The transport of cationic amino acids by SLC38A4 is Na(+) and pH independent, while the transport of neutral amino acids is Na(+) and pH dependent (Hatanaka et al., 2001 [PubMed 11342143]).[supplied by OMIM, Mar 2008]
* Symporter that cotransports neutral amino acids and sodium ions from the extraccellular to the intracellular side of the cell membrane (PubMed:[11342143](https://pubmed.ncbi.nlm.nih.gov/11342143/), [19015196](https://pubmed.ncbi.nlm.nih.gov/19015196/), [33928121](https://pubmed.ncbi.nlm.nih.gov/33928121/)). The transport is electrogenic, pH dependent and partially tolerates substitution of Na(+) by Li(+) (PubMed:[11414754](https://pubmed.ncbi.nlm.nih.gov/11414754/)). Preferentially transports smaller amino acids, such as glycine, L-alanine, L-serine, L-asparagine and L-threonine, followed by L-cysteine, L-histidine, L-proline and L-glutamine and L-methionine (PubMed:[11414754](https://pubmed.ncbi.nlm.nih.gov/11414754/), [33928121](https://pubmed.ncbi.nlm.nih.gov/33928121/)). ( [S38A4\_HUMAN,Q969I6](https://www.uniprot.org/uniprot/Q969I6#function) )

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**Author Contributions**

Conceptualization: M.D.M.; Methodology: D.M.B., M.D.M.; Formal analysis: D.M.B., Investigation: D.M.B., Resources: M.D.M.; Writing - original draft: D.M.B.; Writing - review & editing: D.M.B., M.D.M.; Visualization: D.M.B; Supervision: M.D.M.; Project administration: M.D.M.; Funding acquisition: M.D.M.

**Competing Interests**

No competing interests declared.

**Data Availability**

**References**

**Supplemental**