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Title page

MULTISPECIFIC DRUG TRANSPORTER *Slc22a8* (*Oat3*) REGULATES MULTIPLE METABOLIC PATHWAYS

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Running Title Page

Running title: *Oat3 handling of endogenous metabolites and flavonoids*

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Abstract

Multispecific drug transporters of the SLC and ABC families are highly conserved through evolution, but their true physiological role remains unclear. Analyses of the *organic anion transporter 3* (*Slc22a8/Oat3*, originally *Roct*) knockout mouse have confirmed its critical role in the renal handling of common drugs (e.g., antibiotics, antivirals, diuretics) and toxins. Previous targeted metabolomics of the knockout of the closely related *Oat1* have demonstrated a central metabolic role, but the same approach with *Oat3* failed to reveal a set of endogenous substrates. Nevertheless, the *Oat3* knockout is the only Oat described so far with a physiologically significant phenotype suggesting the disturbance of metabolic and/or signaling pathways. Here we analyzed global gene expression in *Oat3* knockout tissue, which implicated *Oat3* in Phase I and Phase II metabolism as well as signaling pathways. Metabolic reconstruction with the recently developed "mouse Recon1" supported the involvement of *Oat3* in the aforementioned pathways. Untargeted metabolomics were used to determine whether the predicted metabolic alterations could be confirmed. Many significant changes were observed; several metabolites were tested for direct interaction with mOAT3, whereas others were supported by published data. *Oat3* thus appears critical for the handling of Phase I (hydroxylation) and Phase II (glucuronidation) metabolites. *Oat3* also plays a role in bioenergetic pathways (e.g., TCA cycle) as well as those involving vitamins (e.g., folate), steroids, prostaglandins, cyclic nucleotides, amino acids, glycans and possibly hyaluronic acid. The data also suggests that *Oat3* is essential for the handling of dietary flavonoids and antioxidants.

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Introduction

Oat3 (*Slc22a8*), originally identified as *Roct* (Brady et al., 1999) is a multispecific organic anion drug transporter expressed in renal proximal tubule cells, endothelial cells of the brain and cells of the choroid plexus, where it is believed to comprise part of the "classic" organic anion handling mechanism responsible for the rate-limiting steps in the movement of solutes across the blood-urine, blood-cerebrospinal fluid and blood-brain barriers. While *Oat3* shares several characteristics with related transporters, such as *Oat1* (*Slc22a6*) (Lopez-Nieto et al., 1996; Lopez-Nieto et al., 1997), including the presence of twelve membrane-spanning helices, with several consensus extracellular glycolsylation and intracellular PKC sites (Eraly et al., 2004; You, 2004; Klaassen and Aleksunes, 2010; VanWert et al., 2010; Wu et al., 2011), it is phylogenetically and structurally unrelated to other transport proteins, such as the *Oatps* and drug transporting *ABCs* (Hagenbuch and Meier, 2003). Its nearest non-Oat *SLC22* relations are the organic cation- and carnitine-transporting *Octs* (Burckhardt and Wolff, 2000; Sweet et al., 2001) and *Octns*, as well as the *Flipts* (fly-like putative transporters) (Eraly and Nigam, 2002; Enomoto et al., 2003). As with these related transporters, OAT3 is a multispecific transporter (Kusuhara et al., 1999; Sweet et al., 2003), coupling organic anion influx to dicarboxylate efflux (Sweet et al., 1997; Sweet et al., 2003; Zhou and You, 2007; Anzai et al., 2012), manifesting the physiological properties expected for a transporter underlying "classic" organic anion uptake.

Oat3 is a predominant component of renal organic anion transport apparatus believed to account for the transport of one-third to one-half of the most commonly prescribed drugs (i.e., penicillins, NSAIDs, cephalosporins, ACE inhibitors, diuretics, smallpox and HIV antivirals, methotrexate, statins as well as many toxins (Eraly et al., 2003; Eraly et al., 2004)). In addition, we have analyzed the knockout of *Oat3*, which are born at expected male/female ratio and are fertile (Sweet et al., 2002) and the resulting mutant mice, while free of obvious morphological abnormalities, manifested a distinct physiological phenotype characterized by a loss of organic

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anion transport in the kidney and choroid plexus (Sweet et al., 2002). In addition, basal glomerular filtration rate (determined by ^{3}H-inulin clearance) as well as PAH clearance were not different between WT control and *Oat3* deficient mice, suggesting that *Oat3* is not absolutely required for renal PAH secretion (Sweet et al., 2002). *Oat3*-deficient mice also display systolic blood pressure 10 to 15% lower than wild-type mice, raising the possibility that OAT3 mediates the specific transport of an endogenous compound(s) involved in the regulation of blood pressure (Vallon et al., 2008a). Nevertheless, despite its potential considerable pharmaceutical and pharmacological worth, representing a high priority target of interest (Giacomini et al., 2010), the endogenous physiological function(s) of this highly conserved transporter remains to be fully elucidated. In contrast to the *Oat1* knockout (Eraly et al., 2006) targeted metabolomics analysis of the *Oat3* knockout have revealed surprisingly few changes (Eraly et al., 2008; Vallon et al., 2008a; Vallon et al., 2008b; Vallon et al., 2012).

A role for *Oat3* and other SLC and ABC multispecific drug transporters in remote sensing and signaling during inter-organ communication has been proposed (Ahn and Nigam, 2009; Wu et al., 2011); implicit in this hypothesis is a central role for “drug” transporters like OAT3 in regulating key metabolites and signaling molecules. By using a systems-biology approach combining computational and wet-lab “omics” data obtained from the *Oat3* deficient mouse, we observed altered molecular gene networks and concentrations of a battery of cellular metabolites involved in energy production and cell growth, as well as plasma accumulation of a large number of secondary metabolites (Phase II) of plant ingredients of dietary origin, particularly those of flavonoid antioxidants. Metabolic reconstruction using transcriptomic data from the knockout and wild type was then performed, followed by testing of predictions by two different untargeted metabolomics methods (Figure 1). In general, the data are consistent with a central role for *Oat3* in regulating the cellular metabolism and remote communication.

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Materials and Methods

Materials- Chemicals used in in vitro tests were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were analytical grade pure.

Animal- Oat3/Slc22a8 deficient mice were born with expected sex ratio. Male mice between 12 and 20 weeks of age were used in these experiments. For microarray analysis, *Oat3/Slc22a8* deficient mice were backcrossed to C57BL/6J for five generations. For metabolomic analysis, *Oat3* deficient mice were backcrossed to C57BL/6J for a total of ten generations and both control and *Oat3* deficient mice were fed the same standard diet. Blood and urine samples were collected and plasma was isolated and stored at −80 °C until analysis was carried out.

Microarray analysis- Total RNA was prepared and purified from wild-type (n=3) and knockout kidneys (n=3) and microarray analyses were performed as previously described (Wikoff et al., 2011). The amplified RNA was labeled by incorporation of biotinylated nucleotides during in vitro transcription and then hybridized to Affymetrix microarrays, washed, and scanned per the standard Affymetrix protocol. Hybridization and scanning were carried out at the UCSD/Department of Veterans Affairs Medical Center (VAMC) GeneChip core laboratory (http://www.vmrf.org/research-websites/gcf), and microarray data analysis was performed as described (Tsigelny et al., 2008).

Recon 1 analysis. A variation of Recon 1 (Duarte et al., 2007), a global human metabolic network reconstruction, was used to analyze the differential transcriptomic data from the wild-type and *Oat3* deficient animals. As described previously (Ahn et al., 2011), the NCBI Homologene Database (www.ncbi.nlm.nih.gov/homologene) was used to map human Entrez Gene IDs to their mouse homologs. Specific WT and *Oat3* deficient models were created using the transcription profiles (binary classification using Affymetrix MAS5.0 presence/absence calls) as data with the GIMME algorithm (Becker et al., 2007) and the COBRA toolbox

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(Schellenberger et al., 2011). The mouse biomass pseudo-reaction was used as the objective function. Identical generalized uptake conditions were used for both models and flux spans were calculated following Flux Variability Analysis (FVA) (Mahadevan and Schilling, 2003; Ahn et al., 2011).

Predicted changes in the metabolic capabilities of the two models were classified into two categories; the active reactions that were shared between the models and the reactions that were not shared between KO and WT. The most substantive flux changes for the set of shared reactions were identified by rank ordering the reactions and identifying reactions with flux spans that either increased by 2-fold or decreased by 50%. Of note, changes in the flux spans will not necessarily be reflected as increases or decreases in flux, but rather increases or decreases in the range of possible flux for a particular reaction.

Untargeted Metabolomics- 950 MHz NMR: Blood and spontaneous urine samples (at the time of blood collection) from adult male wild-type control and *Oat3* deficient mice (n=3) were obtained and individual, unpooled samples were subjected to 950 MHz NMR analysis at University of North Carolina metabolomics laboratory (Research Triangle Park, NC 20779).

An untargeted, mass-spectrometry-based LC/MS approach for metabolomics was carried out by the core facility at Scripps Center for Metabolomics and Mass-spectrometry. Plasma samples were obtained from adult male *Oat3* deficient and control wild-type mice (n=3) and prepared in a manner similar to that previously described for mOAT1 metabolomics (Wikoff et al., 2011). Mass-spectrometry data were evaluated using XCMS (https://xcmsonline.scripps.edu/), with nonlinear data alignment together with intensity integration. METLIN database (http://metlin.scripps.edu/) was then used for compound identification. KEGG was used for pathway analysis. Plasma samples from *Oat3* deficient mice were compared with plasma samples from wild-types. The samples for metabolomics were then run together and analyzed as a single group. The concentration ratios of revealed metabolites

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indicate those molecules with altered plasma distribution in the knockout versus wild type samples.

Cellular uptake assay- As previously described, confluent monolayers of Chinese hamster ovary cells permanently expressing mouse OAT3 (mOAT3-CHO) growing in 96-well tissue culture plates were employed in uptake assays using 5-carboxyl fluorescein as a tracer molecule (Ahn et al., 2011).

Statistical Analysis- The statistical analysis was performed using unpaired t-test. The values are expressed as the means ± standard deviation.

Ethics Statement- All animals were handled in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines (http://iacuc.ucsd.edu/index.aspx).

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Results

Detailed transcriptomic analyses revealed altered expression of genes involved in key metabolic pathways in the Oat3 deficient kidney. *Oat3* is predominantly expressed in the kidney where it mediates the uptake of organic anionic solutes in proximal tubular cells (VanWert et al., 2010). Despite the importance of *Oat3* in the uptake and elimination of a wide variety of drugs and toxins (Table 1), *Oat3* deficient animals are healthy and viable. In order to determine if a molecular compensatory mechanism exists in the kidney of *Oat3* deficient mice, a microarray analysis was carried out comparing renal gene expression in the knockout to that of wild-type controls. Among the profiled genes, significant expression changes (≥2-fold change in expression either up or down) were observed for more than 100 genes, including *Oat3/Slc22a8* (absent in in the *Oat3* deficient mice). Detailed annotation of 67 of those gene transcripts that were most significantly elevated (>2.5X) in the *Oat3* deficient kidney revealed that at least 23 of them encode gene products related to metabolism suggesting elevated enzymatic activities (Table 2). For example, expression of the gene related to the human cytochrome P450 2b10 isoform 1, a Phase I drug metabolizing enzyme, was significantly elevated (4.2 fold) in the *Oat3* deficient kidney (Table 2). In addition, the expression of Ugt2b5 homolog, a Phase II drug metabolizing enzyme which catalyzes glucuronide conjugation, was also elevated (over 300 fold), while elevated expression was also observed for 3-oxo-5-alpha-steroid 4-dehydrogenase 2 (an enzyme involved in steroid metabolism) (3.7 fold). Taken together, these expression changes indicate that *Oat3*-deficiency results in alterations in the expression of Phase I and Phase II drug metabolizing enzymes, as well as Phase III drug transporters and appears to link Oat3 to the normal physiological functions of metabolic enzymes and transporters.

Utilizing available bioinformatic tools including GeneSpring, GOBY and Ingenuity IPA, the genes were further analyzed in order to investigate the possibility that networks of genes involved in drug metabolism and transport were altered in the knockout animals. These

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analyses revealed that TCA cycle and energy metabolism, as well as nucleotide and amino acid metabolism, were among some of the most significantly impacted pathways (Table 3). In addition to the core cellular functions implicated, altered functionality was also observed in vitamin and mineral metabolism. Thus, our analyses not only link *Oat3* to the normal physiological functions of metabolic enzymes and transporters, but they also suggest the involvement of *Oat3* in the handling of nutrients and vitamins.

Metabolomic Reconstruction of potential physiological roles of Oat3 by Recon 1. To further investigate this possibility as well as decipher the cellular response to *Oat3* deficiency, global transcriptomic clustering was followed by pathway analysis using mouse Recon 1, a global metabolic network reconstruction largely based on human Recon 1 which is comprised of known biochemical and physiologic data (Sigurdsson et al., 2010; Bordbar et al., 2012). These computational analyses revealed alterations in a number of metabolic pathways related to transcriptional regulation, solute handling, and endogenous enzymatic activities (also see Table 2). Among the 285 reactions linked to transcriptomic alteration in *Oat3* deficient kidneys by mouse Recon 1, 220 were related to the cell mass growth functionality that includes energy (ATP) production and metabolism of nucleic acids, amino acids and fatty acids, raising the possibility that compensatory molecular responses in cell growth and bio-mass occur in the kidneys of *Oat3* deficient mice. This genome-wide reconstruction of mouse metabolism based on transcriptomic data also identified 19 additional processes/reactions, including bile acid synthesis, glucuronidation, sulfation, prostaglandin synthesis, hyaluronan metabolism and steroid metabolism, as well as vitamin metabolism, including folate (vitamin B9 derived from dietary sources) (Table 4). As an example, the affected folate metabolism pathway is depicted here and three reactions involved in the metabolism of folate (DHFR, MTHFD and MTHFC) which, based on mouse Recon1 reconstruction, should be enhanced in *Oat3* deficient kidney are highlighted (Figure 2). An overview of the predicted changes in metabolic pathways reactions can be found in Supplemental Figure 1 and Supplemental Tables 1-4 respectively.

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Thus, comparison of the WT and *Oat3* deficient metabolic networks using transcriptomic data highlighted alterations in multiple areas of metabolism, including amino acid, fatty acid, cholesterol, and nucleotide pathways. Moreover, hydroxylation as well as glucuronidation and conjugation reactions (which are usually involved in Phase I and Phase II drug metabolism) were among the largest differences noted between the WT and *Oat3*-deficient mouse.

Metabolomic analysis revealed altered urinary excretion of cellular metabolites in Oat3 deficient mice. In contrast to the *Oat1* deficient animal, targeted metabolomics analysis of the *Oat3* deficient animal revealed minimal alterations in a set of about 30 of the most abundant organic anionic endogenous metabolites (Vallon et al., 2008a). Taken together with subsequent analyses which revealed modest alterations in the concentration of urate, thymidine and flavin mononucleotide in the *Oat3* deficient animals (Eraly et al., 2008; Vallon et al., 2008a; Vallon et al., 2008b), it seems likely that the metabolic pathways disturbed in the *Oat3* deficient mouse are different from those altered in the *Oat1* deficient mouse. In order to confirm and validate the systems biology predictions from the analyses described above (Table 3), global untargeted metabolomics analyses were performed on serum and urine samples derived from wild type and *Oat3* deficient mice. After normalization of the concentrations of the detected metabolite to creatinine, 950 MHz NMR-based untargeted metabolomics of urine revealed significant concentration changes in metabolites related to cellular energy metabolism, (e.g., α-ketogluterate, citrate, 4-hydroxyphenylacetate), and growth related metabolites such as amino acids (e.g. valine) (Figure 3), providing support for the Recon 1 predictions in Table 3. However, 950 MHz NMR-based untargeted metabolomics of serum samples did not yield broad concentration changes in the *Oat3* deficient animal (data not shown), although non-significant reductions in the concentrations of several amino acids, including valine, were detected in the *Oat3* deficient animal.

Global untargeted mass-spectrometric metabolomic profiling in Oat3 deficient mice revealed significant plasma accumulation of metabolites conjugated by Phase I and Phase II

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reactions, including derivatives of plant flavonoids. A global untargeted, mass-spectrometry-based analysis of blood samples derived from adult *Oat3* deficient mice verses wild-type controls was also performed. Plasma samples from adult C57/BL6 control mice and comparable *Oat3* deficient mice were obtained. These samples were subject to LC/MS untargeted metabolomics analysis at Scripps Centers for Metabolomics and Mass Spectrometry and the METLIN metabolomics database was used for compound identification. The plasma concentrations of 1538 features were found to be significantly different between the *Oat3* deficient and wild type control mice, 982 of which were altered 1.5 fold or higher (either up or down). Of these 982 features, 220 of them were associated with known molecular fragments, of which 30 were clearly identified as known metabolites (Figure 4 and supplemental table 5).

Consistent with metabolic reconstructions, behenic acid (a cholesterol-raising saturated fatty acid in humans), as well as a propionoate (a metabolite linked to carboxylic acid metabolism) and modified serotonin was significantly reduced (11 fold and 5 fold decrease relative to Wt, respectively) in the plasma of *Oat3* deficient mice (Figure 4a). In addition, a glucuronidated molecule (2-Amino-3-hydroxy-5-nitrobenzophenone glucuronide) was also among the highest accumulated in the *Oat3* deficient mice, consistent with the Recon 1 analysis (Figure 2, Table 4). Furthermore, methylguanosine, a uremic toxin, was also found to be significantly accumulated in the plasma of *Oat3* deficient mice. The two molecules which were found to be most highly accumulated in *Oat3* deficient plasma were pongamoside A and 9-amino-nonanoic acid, both of which are derived from plants (Figure 4a). Pongamoside A is a plant derivative flavonoid antioxidant, while 9-amino-nonanoic acid is a modified nonanoic acid derived from soybeans.

Known mass-spect features associated with a group of metabolites that are dietary phenolic derivatives. The potential molecules associated with the mass-spectrometry fragment features that were most highly accumulated in the Oat3 deficient mice were also examined. Of the top ten features with a minimum of a 5-fold increase in their concentrations in the *Oat3*

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deficient mice (Top 4 are listed in Table 5), at least 7 of them were found to be associated with metabolites of plant origin. For example, feature M457T_1, elevated 13.8 fold *Oat3* deficient plasma, is associated with a group of metabolites of dietary phenolic derivatives including Epicatechin 3-O-(3-O-methylgallate), also known as an internal metabolite of the ester of epigallocatechin and gallic acid (epigallocatechin gallate, EGCG). EGCG is a potent antioxidant found in a number of plants, but it is the most abundant catechin in tea leaves. Interaction of EGCG with OAT3 was confirmed in a cell based assay that employed mOAT3-expressing CHO cells (mOAT3-CHO). In this assay, EGCG, as well as typical OAT3 substrates (ie., estrone sulfate) were tested for their ability to inhibit uptake of a preferential OAT3 tracer 5CF. The uptake of 5CF in mOAT3-CHO cell was inhibitable by epicatechin (Sigma 49045-U), as well as epicatechin gallate (Sigma 49060-U) (Figure 5). Thus, the data support the notion that the broad accumulation of multiple phytophenolic metabolites of dietary origin is most likely the result of a deficiency in OAT3-mediated transport.

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Discussion

Utilizing a systems biology approach comparing wild-type and *Oat3* deficient mice, the correlation of transcriptomic, computational pathway and metabolomics analyses (950 MHz NMR plus LC/MS) revealed that the absence of this transporter leads to significant alterations in: 1) a number of cellular metabolic pathways (e.g., TCA cycle, nucleotide and amino acid metabolism) (Table 2-3 and Figure 2 and 3, Supplemental Figure S1); 2) the expression of genes encoding enzymes with critical roles in Phase I and Phase II xenobiotic metabolism, as well as Phase III drug transport (Table 2 and 4); 3) pathways involved in the regulation of secondary metabolites, including endogenous signalling molecules (e.g., prostaglandins and steroids), and dietary plant derivatives (e.g., vitamins) (Table 2-4, and Figures 2 and 4); and 4) pathways involved in the handling of dietary flavonoids (Table 5, Figure 4 and 5). Among the dietary flavonoids found to accumulate in the plasma of the *Oat3*-deficient animal, epicatechin is interesting as this molecule has been suggested to impact blood pressure (and have potential cardiovascular benefits), and the *Oat3* deficient mice have decreased blood pressure (Vallon et al., 2008a). Nevertheless, taken together, the data help to define the role of this transporter in normal physiology by linking its activity to a variety of pathways regulating levels of key metabolites and signalling molecules.

Oat3 is expressed in the proximal tubules of the kidneys, where it plays an important role in the elimination of numerous organic anions of physiological, pharmacological and toxicological relevance (Burckhardt and Burckhardt, 2003; Eraly et al., 2004; Nigam et al., 2007; Ahn and Bhatnagar, 2008; Di Giusto et al., 2008; Ahn and Nigam, 2009; Wu et al., 2009; Wu et al., 2011). There is significant overlap in the expression of *Oat3* and its close homolog, *Oat1*, with many proximal tubular cells expressing both of these transporters (Lopez-Nieto et al., 1997; Hwang et al., 2010). Nevertheless, immunocytochemical localization reveals that OAT1 appears to be expressed preferentially in the S1 and S2 segments while OAT3 is found more in

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the S2 and S3 segments (Hwang et al., 2010), a finding consistent with their functional localization in knockout animals (Truong et al., 2008). In addition to its renal expression, *Oat3* is also expressed in the brain (on the endothelial cell as part of blood brain barrier), as well as in choroid plexus where it is believed to be part of the transport apparatus mediating the elimination of solutes from the brain and central nervous system (Sweet et al., 2002; Vanwert et al., 2007; Truong et al., 2008; Vallon et al., 2008a; Vallon et al., 2008b; VanWert and Sweet, 2008; Ose et al., 2009; Nagle et al., 2011; Sweeney et al., 2011; Xue et al., 2011).

While it is believed that OAT3 is involved in mediating the disposition, distribution and elimination of a wide variety of common pharmaceuticals (VanWert et al., 2010; Burckhardt and Burckhardt, 2011), the role of *Oat3* in normal physiology has been less clearly defined. In a recent targeted metabolomics study, of the 30 most abundant plasma small organic anion molecules investigated, none were found to accumulate in the plasma of *Oat3* deficient mice, which is contrary to the finding from *Oat1 deficient* mice (Vallon et al., 2008a). The data from this study indicate that *Oat3*-deficiency leads to changes in the concentration of a variety of small organic metabolites in the blood. Furthermore, transcriptomic and pathway analyses revealed alterations in Phase I and Phase II xenobiotic metabolism such as sulfation and glucuronidation a notion supported by metabolomics analyses which revealed alterations in the levels of sulfated and glucuronidated small molecules, including components of bio-mass cell growth and flavonoid antioxidants of dietary origin (Table 6). Thus, *Oat3* is not only likely key to the handling of endogenous metabolites and signaling molecules with important physiological functions, but it is also important in the handling of metabolites derived from the gut microbiome. The results seem generally compatible with the proposed role of *Oat3* and remote sensing and signaling (Kaler et al., 2006; Ahn and Nigam, 2009; Wu et al., 2011).

Since this set of metabolites was not found to be altered in a untargeted metabolomics analysis of plasma from *Oat1*-deficient animals (Ahn et al., 2011; Wikoff et al., 2011), the data presented above support the notion that *Oat3* plays a critical and perhaps primary role in the

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uptake and elimination of these dietary antioxidants. A metabolic reconstruction of the *Oat1* deficient animal using similar transcriptomic and metabolomics data revealed alterations in largely different but somewhat overlapping set of pathways (Ahn et al., 2011). Considering the importance of OAT3 in the uptake and elimination of a wide variety of commonly prescribed drugs (e.g. antibiotics, antivirals used for HIV, diuretics, NSAIDs, methotrexate) (Giacomini et al., 2010; Klaassen and Aleksunes, 2010; VanWert et al., 2010; Wu et al., 2011), together with the fact that the use of dietary supplements, including herbal medicines, botanicals, and probiotics, is increasing worldwide (Bardia et al., 2007; Williamson et al., 2007; Wong et al., 2011a; Wong et al., 2011b), the data raise the possibility of competition between dietary metabolites and drugs for access to OAT3. Moreover, based on the expression of *Oat3* such metabolite-drug interactions are likely to affect multiple tissues (Figure 6).

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Authorship Contributions

Participated in research design: Wu, Jamshidi, Palsson, Eraly, and Nigam.

Conducted experiments: Wu, Jamshidi, Eraly, Bush and Liu.

Contributed new reagents or analytic tools: Palsson, and Nigam.

Performed data analysis: Wu, Jamshidi, Eraly, Bush and Liu.

Wrote or contributed to the writing of the manuscript: Wu, Jamshidi, Bush, Palsson, and Nigam.

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Footnotes

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The abbreviations used are: Oat3, organic anion transporter 3/Slc22a8; Oat1, organic anion transporter 1/Slc22a6; EGCG, Epigallocatechin gallate.

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Figure Legends

Figure 1. **Overall strategy of this study.**

Figure 2. **Graphic pathway diagram depicting altered components (enhanced) of folate metabolism in *Oat3* knockout kidney generated by Recon 1 analysis.** The enhanced pathways of DHFR, MTHFC and MTHFD were also listed in table 4. This diagram is a portion of the global overview of predicted global changes in pathways by Recon 1 analysis, which can be found in Supplemental Figure 1.

Figure 3. ***Oat3*-deficiency results in alterations in the urinary concentration of metabolites.** Relative urine concentrations of selected compounds in wild-type and *Oat3* deficient mice. Spontaneous urine samples were obtained from adult mice and subjected to untargeted metabolomic analysis using $^{(1)}$H nuclear magnetic resonance (NMR) spectroscopy and multivariate statistical analyses to generate metabolic fingerprints. Urine concentrations of metabolites were normalized to creatinine. n=3. *, $p< 0.05$.

Figure 4. ***Oat*3-deficiency results in the plasma accumulation of a variety of chemical compounds.** A. List of significantly altered (>2.5X) natural metabolites in the plasma of *Oat3* deficient mice by untargeted LC-MS analysis. Additional significantly altered mass-spect features are listed in Supplemental Table 5. B. Boxplots of relative plasma concentrations for four most highly accumulated compounds in *Oat3* deficient plasma. Pongamoside A (*p*-value $=3.3 \times 10^{-2}$) is a flavonoid metabolite of dietary origin.

Figure 5. **Inhibitory effects of metabolites on uptake of 5CF in Chinese hamster ovary cell permanently expressing mouse OAT3 (mOAT3-CHO).** mOAT3-CHO cells were plated in 96-

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well plate overnight and uptake of an OAT3-preferred tracer (5-carboxyl fluorescein, 20 microM) was carried out for 5 min at room temperature. (A) Bar graph illustrating inhibitory effects of epicatechin and epicatechin gallate on OAT3-mediated uptake of 5CF. As expected, probenecid inhibits the uptake in a range that is consistent with previous data. (B-C) Molecular structures and some chemical properties of epicatechin (B) and epicatechin gallate (C). n=3; *p<0.01.

Figure 6. **Diagram depicting potential interaction of commonly prescribed pharmaceuticals and endogenous substrates at the site of organic anion transporter 3 for excretion.**

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Tables

TABLE 1
Known Functional Alterations in the Oat3 Knockout Kidney

Molecule	Class	Reference
Methotrexate	Drug	VanWert and Sweet, 2007
Penicillin G	Drug	VanWert et al., 2007
Furosemide	Drug	Vallon et al., 2008
Bendroflumethiazide	Drug	Vallon et al., 2008
Ciprofloxacin	Drug	VanWert et al., 2008
Urate	Metabolite	Eraly et al., 2008
Ro 64-0802 (active form of Oseltamivir)	Drug	Ose et al., 2009
Aristolochic acid	Toxin	Xue et al., 2011
Zidovudine	Drug	Nagle et al., 2011
Acyclovir	Drug	Nagle et al., 2011
Tenofovir	Drug	Nagle et al., 2011
Lamivudine	Drug	Nagle et al., 2011
Creatinine	Metabolite	Vallon et al., 2012

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TABLE 2
Partial List of Enzyme Transcripts (including some Ests) Upregulated in *Oat3*-KO Kidney

Affy. ID	Gene Name	Description	Fold Change
1423397_at	Ugt2b5	UDP glucuronosyltransferase 2B5	371.9
1440339_at	Enpp1	ectonucleotide pyrophosphatase/phosphodiesterase 1	120.2
1459311_at	Est	cAMP-specific 3',5'-cyclic phosphodiesterase 4D-like	14.6
1449486_at	Ces1	carboxylesterase 1	4.5
1422257_s_at	Cyp2b10	cytochrome P450, family 2b10 isoform 1	4.2
1440463_at	Est	similar to peptide N-glycanase (Ngly1) gene	4.0
1422960_at	Srd5a2	3-oxo-5-alpha-steroid 4-dehydrogenase 2	3.7
1444032_at	Keg1	glycine N-acyltransferase-like protein Keg1	3.7

NOTE. Multiple probes for Phase I (i.e., cytochrome P450, family 2b10 isoform 1) and Phase II (i.e., UDP glucuronosyltransferase 2B5) drug metabolizing enzyme displayed similar levels of expression change. The elevated expression observed for 3-oxo-5-alpha-steroid 4-dehydrogenase 2 suggests enhanced enzymatic metabolism for steroid hormones, many of which are known OAT3 substrates.

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TABLE 3
Subsystem Prediction of Altered Metabolic Pathways in *Oat3* Knockout

Up	Down
Arginine and Proline Metabolism	Galactose metabolism
Blood Group Biosynthesis	Glutathione Metabolism
Chondroitin/heparan sulfate biosynthesis	Glycine, Serine & Threonine Metabolism
Fatty Acid Metabolism	Glycolysis/Gluconeogenesis
Galactose metabolism	IMP Biosynthesis
Glycerophospholipid Metabolism	Pentose Phosphate Pathway
Inositol Phosphate Metabolism	Pyrimidine Biosynthesis
Nucleotides	Pyruvate Metabolism
O-Glycan Biosynthesis	Transport, Extracellular
Sphingolipid Metabolism	Vitamin B12 Metabolism
Steroid Metabolism	

Note. Transcriptomic analysis was carried out using Affymetrix mouse gene expression array. The most significantly altered sybsystems were listed. It appears that Arginine and Proline Metabolism, Blood Group Biosynthesis, Fatty Acid Metabolism and others were predicted to be increased in *Oat3* deficient kidney; While Glutathione Metabolism, Glycine, Serine, and Threonine Metabolism and others were predicted to be reduced in the *Oat3* deficient kidney.

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TABLE 4
Enhanced RECON 1 Enzyme Reactions in the *Oat3* Knockout

Enzyme Reactions	Reaction Name	Subsystem
P4508B11r	Sterol 12-alpha-hydroxylase	Bile Acid Biosynthesis
P4508B13r	Sterol 12-alpha-hydroxylase (nadh)	Bile Acid Biosynthesis
B3GNT51g	UDP-GlcNAc:bGal b-1,3-N-acetylglucosaminyltransferase 5	Glycan Biosynthesis
ST3GAL31g	ST3 beta-galactoside alpha-2,3-sialyltransferase 3	Glycan Biosynthesis
HMGCOASim	Hydroxymethylglutaryl CoA synthase (ir)	Cholesterol Metabolism
GALT2g	UDP-D-galactose:galactosylxylose galactosyltransferase, Golgi	Chondroitin/heparan sulfate biosynthesis
S3T2g	heparin-glucosamine 3-O-sulfotransferase	Chondroitin/heparan sulfate biosynthesis
LTA4H	Leukotriene A-4 hydrolase	Eicosanoid Metabolism
PGISr	Prostaglandin I2 synthase	Eicosanoid Metabolism
DHFR*	dihydrofolate reductase	Folate Metabolism
MTHFD*	methylenetetrahydrofolate dehydrogenase (NADP)	Folate Metabolism
HAS1	hyaluronan synthase	Hyaluronan Metabolism
HAS2	hyaluronan synthase	Hyaluronan Metabolism
S23T3g	beta-galactoside alpha-2,3-sialyltransferase (complex N-glycan)	Keratan sulfate biosynthesis
HSD17B3r	testicular 17-beta-hydroxysteroid dehydrogenase	Steroid Metabolism
PYDXDH	pyridoxal dehydrogenase	Vitamin B6 Metabolism
S23T3g	beta-galactoside alpha-2,3-sialyltransferase (T antigen)	O-Glycan biosynthesis
MTHFC*	methenyltetrahydrofolate cyclohydrolase	Folate Metabolism
34HPLFM	3-(4-hydroxyphenyl-)lactate formation	Ubiquinone Biosynthesis

NOTE: This is a list of significantly enhanced RECON 1 enzyme reactions (without exchange, transport or biomass-growth related reactions) in the *Oat3* knockout.
*Enhanced reactions related to folate metabolism (DHFR, MTHFD and MTHFC) are highlighted in Fig. 2.

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TABLE 5
Mass Spectrometry Features Highly Accumulated in the Plasma of *Oat3* Deficient Mice

Name	Fold Change	p-value	m/z	Retention Time	Putative Associated Molecule
M301T2_5	17.7	0.0045	301.0746	1.59	Sulfaquinoxaline and others
M457T9_1	13.8	0.0125	457.1144	9.37	**Epicatechin 3-O-(3-O-methylgallate) and others**
M573T10	9.9	0.0075	573.1626	10.4	**Licuroside and 3 others**
M441T11_1	8.3	0.0325	441.1205	11.27	**Pongamoside**

NOTE. Flavonoid metabolites are in bold

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TABLE 6
Known OAT3 Metabolites

Metabolite	Assay	Reference
Pomgamoside A	Up in Oat3KO plasma	This study
9-amino-nonanoic acid	Up in Oat3KO plasma	This study
Flavin mononucleotide	Up in Oat3KO plasma	Vallon, 2008
Thymidine	Up in Oat3KO plasma	Vallon, 2008
Urate	Down in Oat3KO urine	Eraly, 2007
Dideoxycytidine	mOat3, X. laevis	Truon, 2008
17b-estradiol-D-17bglucuronide	mOat3, LLC-PK1	Nagata, 2002
Estrone sulfate	hOAT3, X. laevis	Windass, 2007
Homovanillic acid	rOat3, X. laevis	Mori,2003
3-Carboxy-4-methyl-5-propyl-2-furanpropionate	hOAT3, HEK293	Deguchi,2004
Indoxyl sulfate	hOAT3, HEK293	Deguchi, 2004
Prostaglandin E2	hOAT3, S2 segment	Kimura, 2002
Prostaglandin F2a	hOAT3, S2 segment	Kimura, 2002
Dehydroepiandrosterone sulfate	hOAT3, HEK293	Ueo, 2005
Genistein-7-O-glucuronide	hOAT3, HEK293	Wong, 2011
Glycitein-7-O-glucuronide	hOAT3, HEK293	Wong, 2011
Quercetin-3′-O-glucuronide	hOAT3, HEK293	Wong, 2011

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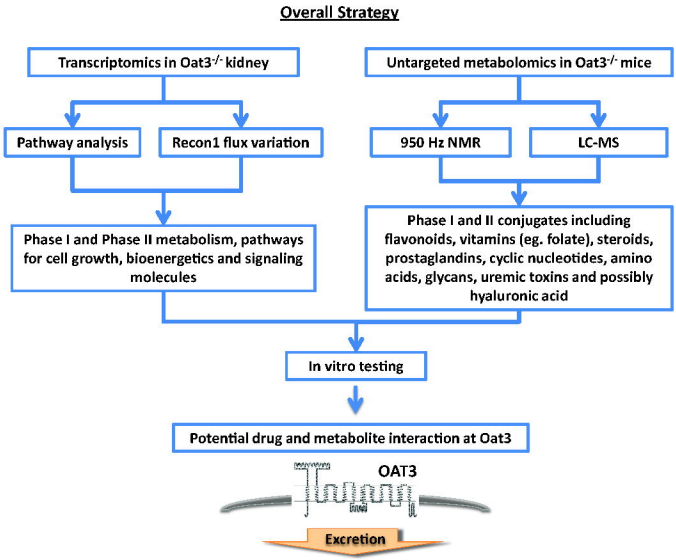
Overall Strategy
Transcriptomics in Oat3-/- kidney
Untargeted metabolomics in Oat3-/- mice
Pathway analysis
Recon1 flux variation
950 Hz NMR
LC-MS
Phase I and Phase II metabolism, pathways for cell growth, bioenergetics and signaling molecules
Phase I and II conjugates including flavonoids, vitamins (eg. folate), steroids, prostaglandins, cyclic nucleotides, amino acids, glycans, uremic toxins and possibly hyaluronic acid
In vitro testing
Potential drug and metabolite interaction at Oat3
OAT3
Excretion

Figure 1.

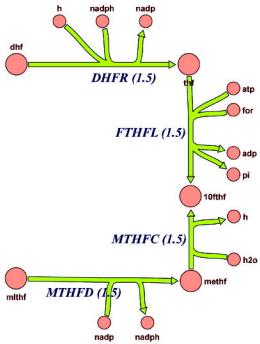
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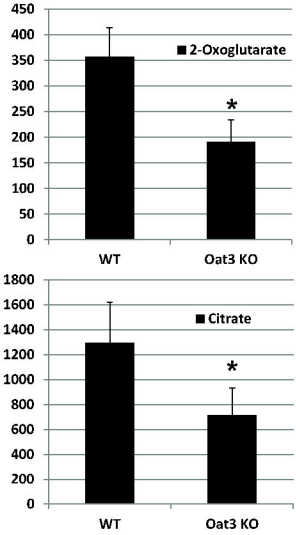
Figure 2.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0
2-Oxoglutarate
*
WT
Oat3 KO
40
35
30
25
20
15
10
5
0
4-Hydroxyphenylacetate
*
WT
Oat3 KO
1800
1600
1400
1200
1000
800
600
400
200
0
Citrate
*
WT
Oat3 KO
20
18
16
14
12
10
8
6
4
2
0
Valine
*
WT
Oat3 KO

Figure 3.

A. List of significantly altered (>2.5X) natural metabolites in the plasma of Oat3 deficient mice.

Feature	m/z	Fold Change Oat3KO/Wt	Molecule
M441T11_1	441.1205	**8.3**	Pongamoside A
M174T10_2	174.1496	**7.2**	9-amino-nonanoic acid
M310T13_1	310.1137	**6.9**	9-O-Acetylneuraminic acid
M457T11_1	457.0867	**6.5**	2-Amino-3-hydroxy-5-nitrobenzophenone glucuronide
M454T13_1	454.1812	**4.6**	1-Hydroxyfluphenazine
M175T3_2	175.0242	**3.9**	Dehydroascorbic acid
M241T9	241.0885	**3.5**	2-Oxo-9-methylthiononanoic acid
M320T10_1	320.0954	**2.7**	7-Methylguanosine
M415T14_3	415.3282	**0.4**	Palmitoyl Serotonin
M195T14	195.0385	**0.2**	Hydantoin-5-propionic acid
M366T17	366.3406	**0.1**	Behenic acid(d3)

B.

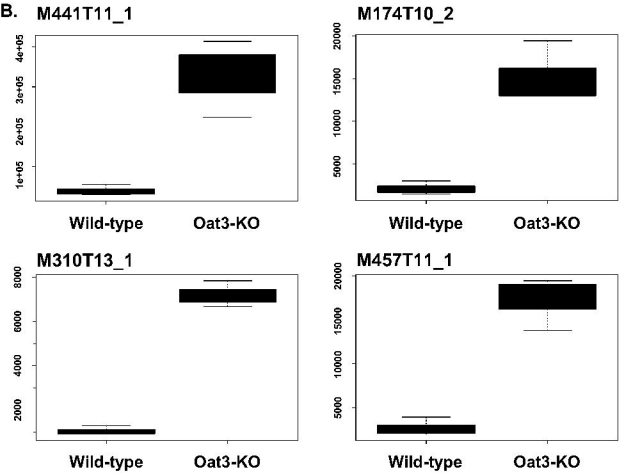


Figure 4.

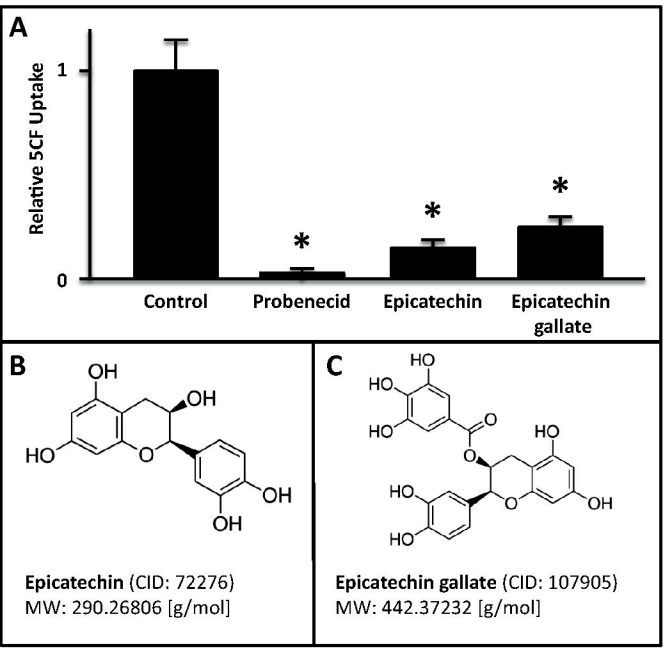

A
Relative 5CF Uptake
1
0
*
*
*
Control
Probenecid
Epicatechin
Epicatechin gallate
B
OH
OH
HO
O
OH
OH
Epicatechin (CID: 72276)
MW: 290.26806 [g/mol]
C
HO
HO
HO
O
O
HO
HO
O
OH
HO
Epicatechin gallate (CID: 107905)
MW: 442.37232 [g/mol]

Figure 5.

	OAT3 Substrate
Cellular Metabolite	Homovanillic acid
Pharmaceuticals	Zidovudine, Pravastatin
Toxin	Indoxyl sulfate
Prostaglandins	Prostaglandin E2, F2
Steroid/Flavonoid	ES, DHES, Quercetin-3′-O-glucuronide

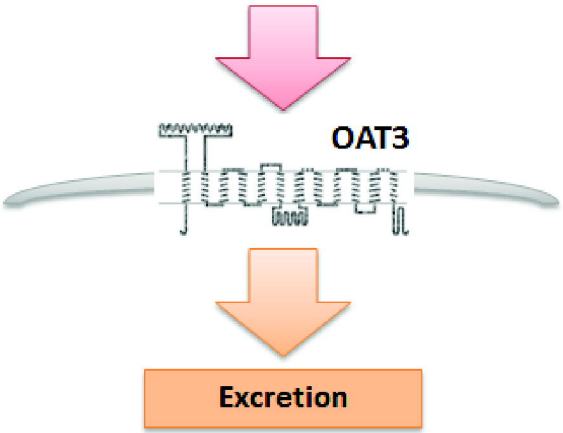
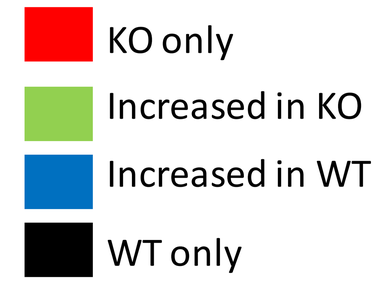


Figure 6.

MULTISPECIFIC DRUG TRANSPORTER *Slc22a8* (*Oat3*) REGULATES MULTIPLE METABOLIC AND SIGNALING PATHWAYS. Wei Wu, Neema Jamshidi, Satish A. Eraly, Henry C. Liu, Kevin T. Bush, Bernhard O. Palsson, and Sanjay K. Nigam. Drug Metabolism and Disposition.

Supplemental Table 5. List of some significantly altered Mass-spect features in the plasma of *Oat3* deficient mice.

Feature	Fold change	p-value	m/z	Ret. time	Molecule
M441T11_1	**8.3**	0.0325	441.1205	11.27	**Pongamoside A**
M174T10_2	**7.2**	0.0218	174.1496	10.07	**9-amino-nonanoic acid**
M310T13_1	**6.9**	0.0012	310.1137	12.77	**9-O-Acetylneuraminic acid**
M457T11_1	**6.5**	0.0081	457.0867	11.25	**2-Amino-3-hydroxy-5-nitrobenzophenone glucuronide**
M454T13_1	**4.6**	7E-05	454.1812	13.25	**7-Hydroxyfluphenazine**
M175T3_2	**3.9**	3E-05	175.0242	2.62	**Dehydroascorbic acid**
M531T11	**3.5**	0.0042	531.0891	11.24	**Fluorescein monoglucuronide**
M241T9	**3.5**	0.0102	241.0885	9.35	**2-Oxo-9-methylthiononanoic acid**
M320T10_1	**2.7**	0.0099	320.0954	9.84	**1-Methylguanosine**
M477T16	**2.3**	0.0211	477.1686	16.43	**3-Hydroxypromazine glucuronide**
M342T2_6	**2.2**	0.0202	342.2013	1.63	**Dihydrotetrabenazine**
M637T19_2	**2.0**	0.0351	636.6307	18.64	**Tricosanamide**
M320T16	**2.0**	0.0189	320.1871	15.65	**Chloroquine**
M286T2_7	**2.1**	0.0118	286.1598	1.53	**N-Tritylaziridine**
M529T3_4	**2.1**	0.0081	529.282	2.88	**3β-ACETOXYDEOXODIHYDROGEDUNIN**
M281T16_1	**2.1**	0.0229	281.1422	16.18	**N-HISTIDYL-2-AMINONAPHTHALENE (βNA)**
M302T2_4	**2.2**	0.0218	302.124	2.12	**Desmethylondansetron**
M659T3	**2.2**	0.0315	659.002	2.63	**Isoscutellarein 4'-methyl ether 8-(2'',4''-disulfatoglucuronide)**
M238T4	**2.3**	0.0434	238.0716	4.29	**KINETIN**
M285T2_4	**2.3**	0.0039	285.1572	1.53	**Tropicamide**
M300T13_2	**2.3**	0.042	300.1496	12.79	**Metochlopramide**
M230T11_4	**2.4**	0.0259	230.0808	11.33	**FENAMISAL**
M230T2_3	**2.4**	0.0092	230.0985	2.12	**MIGLITO**
M545T3_2	**2.5**	0.0001	545.1329	2.74	**CAY10406**
M252T2_4	**2.5**	0.031	252.0802	2.11	**H-9**
M415T14_3	**2.7**	0.0025	415.3282	13.76	**Palmitoyl Serotonin**
M366T18_1	**3.3**	0.0336	366.3408	17.65	**Behenic acid(d3)**
M195T14	**4.7**	0.0005	195.0385	14.4	**Hydantoin-5-propionic acid**
M366T17	**10.9**	8E-05	366.3406	17.28	**Behenic acid(d3)**
M366T18_2	**18.6**	0.001	366.3404	17.88	**Behenic acid(d3)**
Note: Up or Down in Oat3 deficient plasma.					



MULTISPECIFIC DRUG TRANSPORTER *Slc22a8* (*Oat3*) REGULATES MULTIPLE METABOLIC PATHWAYS. Wei Wu, Neema Jamshidi, Satish A. Eraly, Henry C. Liu, Kevin T. Bush, Bernhard O. Palsson and Sanjay K. Nigam. *DRUG METABOLISM AND DISPOSITION*.

Supplemental Table 1. Unique_changes_WT_KO

Reaction	Span	WT vs KO	Name	Equation	Subsystem
3MOBt2im	500000	WT Unique	3-methyl-2-oxobutanoat	3mob[c] + h	Transport, Mitochondrial
3MOPt2im	500000	WT Unique	3-Methyl-2-oxopentanoa	3mop[c] + h	Transport, Mitochondrial
4MOPt2im	500000	WT Unique	4-methyl-2-oxopentanoa	4mop[c] + h	Transport, Mitochondrial
4PYRDX	1	WT Unique	4-Pyridoxal secretion	4pyrdx[c] +	Transport, Extracellular
ARACHDt2	1000000	WT Unique	fatty acid transport via di	arachd[e] <=	Transport, Extracellular
ARACHt	1000000	WT Unique	fatty acid transport via di	arach[e] <=>	Transport, Extracellular
ATPasel	12.86041	WT Unique	V-type ATPase, H+ transp	atp[c] + (3)	Transport, Lysosomal
CHOLATEt2	258.922917	WT Unique	cholate transport via sod	cholate[e] +	Transport, Extracellular
CHOLATEt3	258.922917	WT Unique	ABC bile acid transporter	atp[c] + cho	Transport, Extracellular
DOPAt4(2)r	500000	WT Unique	Dopamine reversible trar	dopa[e] + (2	Transport, Extracellular
EX_4pyrdx(e)	1	WT Unique	4-Pyridoxate Demand		Transport
EX_ha(e)	8.319895	WT Unique	Hyaluronan Demand		Transport
EX_ha_pre1(e)	16.639789	WT Unique	Hyaluronan biosynthesis, precursor 1		Transport
FATP4t	1000000	WT Unique	fatty acid electroneutral	arach[c] + n	Transport, Extracellular
FATP5t	1000000	WT Unique	fatty acid electroneutral	arachd[c] +	Transport, Extracellular
GLCAASE8ly	25.72082	WT Unique	beta-glucuronidase, lysos	[l] : h2o + h	Hyaluronan Metabolism
GLCAASE9ly	25.72082	WT Unique	beta-glucuronidase, lysos	[l] : (2) h2o	Hyaluronan Metabolism
GLCURtly	51.441639	WT Unique	glucuronate transport int	glcur[c] + h[	Transport, Lysosomal
HAS1	26.452804	WT Unique	hyaluronan synthase	uacgam[c] +	Hyaluronan Metabolism
HAS2	25.72082	WT Unique	hyaluronan synthase	ha_pre1[e]	Hyaluronan Metabolism
HAtly	25.72082	WT Unique	hyaluronan transport, ex	ha[e] --> ha	Transport, Lysosomal
HMGCOASim	293.497396	WT Unique	Hydroxymethylglutaryl C	[m] : aacoa	Cholesterol Metabolism
ILETA	500000	WT Unique	isoleucine transaminase	[c] : akg + il	Valine, Leucine, and Isoleucine Metabolism
ILETAm	500000	WT Unique	isoleucine transaminase,	[m] : akg + i	Valine, Leucine, and Isoleucine Metabolism
ILEt5m	500000	WT Unique	Isoleucine mitochondrial	ile-L[c] <==>	Transport, Mitochondrial
LEUTA	500000	WT Unique	leucine transaminase	[c] : akg + le	Valine, Leucine, and Isoleucine Metabolism
LEUTAm	500000	WT Unique	leucine transaminase, mi	[m] : akg + l	Valine, Leucine, and Isoleucine Metabolism
LEUt5m	500000	WT Unique	leucine mitochondrial tra	leu-L[c] <==	Transport, Mitochondrial
MALTe	1	WT Unique	alpha-glucosidase, extrac	[e] : h2o + r	Starch and Sucrose Metabolism
NACHEX27ly	25.72082	WT Unique	beta-N-acetylhexosamini	[l] : h2o + h	Hyaluronan Metabolism
NRPPHRt4(2)r	500000	WT Unique	Norepinephrine reversibl	(2) na1[e] +	Transport, Extracellular
PYDXDH	1	WT Unique	pyridoxal dehydrogenase	[c] : h2o + o	Vitamin B6 Metabolism

TCHOLAt	258.922917	WT Unique	taurocholate transport vi	hco3[c] + tc	Transport, Extracellular
TCHOLAt3	258.922917	WT Unique	ABC bile acid transporter	atp[c] + h2c	Transport, Extracellular
TRPt	1	WT Unique	L-tryptophan transport	trp-L[e] <==	Transport, Extracellular
VALTA	500000	WT Unique	valine transaminase	[c] : akg + v	Valine, Leucine, and Isoleucine Metabolism
VALTAm	500000	WT Unique	valine transaminase, mit	[m] : akg + \	Valine, Leucine, and Isoleucine Metabolism
VALt5m	500000	WT Unique	Valine reversible mitoch	val-L[c] <==	Transport, Mitochondrial
sink_3mob(c)	1	WT Unique	3-Methyl-2-oxobutanoate	Demand	Transport
sink_3mop	1	WT Unique	(S)-3-Methyl-2-oxopentanoate	Dema	Transport
sink_4mop	1	WT Unique	4-Methyl-2-oxopentanoate	Demand	Transport
AHCYStr	3	KO Unique	S-Adenosyl-L-homocystei	ahcys[c] <=	Transport, Endoplasmic Reticular
AMETr	3	KO Unique	S-Adenosyl-L-methionine	amet[c] <==	Transport, Endoplasmic Reticular
AMETt2m	3	KO Unique	S-Adenosyl-L-methionine	ahcys[m] +	Transport, Mitochondrial
ARGt4	1	KO Unique	L-arginine transport in vi	arg-L[e] + n	Transport, Extracellular
ASPCTr	49.980399	KO Unique	aspartate carbamoyltran	[c] : asp-L +	Pyrimidine Biosynthesis
BTNt3i	259.722917	KO Unique	Biotin transport via sodiu	atp[c] + btn	Transport, Extracellular
CBPS	49.980399	KO Unique	carbamoyl-phosphate sy	[c] : (2) atp	Pyrimidine Biosynthesis
CYSt4	500000	KO Unique	L-cysteine reversible tran	cys-L[e] + n	Transport, Extracellular
CYTDn	65.005729	KO Unique	cytidine deaminase, nucl	[n] : cytd + l	Nucleotides
CYTDtn	65.005729	KO Unique	cytidine transport in nuc	cytd[c] <==	Transport, Nuclear
DHORD9	49.980399	KO Unique	dihydoorotic acid dehydr	dhor-S[c] +	Pyrimidine Biosynthesis
DHORTS	49.980399	KO Unique	dihydroorotase	[c] : dhor-S	Pyrimidine Biosynthesis
EX_tyr-L(e)	1	KO Unique	L-tyrosine transport in via sodium syr		L-tyrosine transport in via sodium symport
GHMT2r	29.264607	KO Unique	glycine hydroxymethyltra	[c] : ser-L +	Glycine, Serine, and Threonine Metabolism
GLCtly	2	KO Unique	glucose efflux from lysos	glc-D[l] --> g	Transport, Lysosomal
GMPS2	86.907639	KO Unique	GMP synthase	[c] : atp + gl	Nucleotides
ILEt4	500000	KO Unique	L-isoleucine transport in	ile-L[e] + na	Transport, Extracellular
IMPD	86.907639	KO Unique	IMP dehydrogenase	[c] : h2o + i	Nucleotides
MALTly	1	KO Unique	alpha-glucosidase, lysoso	[l] : h2o + m	Starch and Sucrose Metabolism
MALTt1r	1	KO Unique	maltose transport (unipo	malt[e] <==	Transport, Extracellular
NH4tn	65.005729	KO Unique	ammonia nuclear transpo	nh4[c] <==>	Transport, Nuclear
OMPDC	45.991031	KO Unique	orotidine-5'-phosphate d	[c] : h + oro	Pyrimidine Biosynthesis
ORPT	45.991031	KO Unique	orotate phosphoribosyltr	[c] : orot5p	Pyrimidine Biosynthesis
PCFLOPm	1	KO Unique	phosphatidylcholine flipp	atp[c] + h2c	Transport, Mitochondrial
PETOHMm_hs	1	KO Unique	phosphatidylethanolamir	[m] : (3) am	Glycerophospholipid Metabolism

PETOHMr_hs	1	KO Unique	phosphatidylethanolamir	[r] : (3) ame	Glycerophospholipid Metabolism
PE_HSter	1	KO Unique	phosphatidylethanolamir	pe_hs[c] <=	Transport, Endoplasmic Reticular
PGCD	40.05	KO Unique	phosphoglycerate dehyd	[c] : 3pg + n	Glycine, Serine, and Threonine Metabolism
PSERT	40.05	KO Unique	phosphoserine transamir	[c] : 3php +	Glycine, Serine, and Threonine Metabolism
PSP_L	40.05	KO Unique	phosphoserine phosphat	[c] : h2o + p	Glycine, Serine, and Threonine Metabolism
TRPt4	1	KO Unique	L-tryptophan transport ir	na1[e] + trp	Transport, Extracellular
TYRt4	1	KO Unique	L-tyrosine transport in vi	na1[e] + tyr	Transport, Extracellular
Uritn	65.005729	KO Unique	uridine transport in nucl	uri[c] <==>	Transport, Nuclear
VALt4	500000	KO Unique	L-valine transport in via s	na1[e] + val	Transport, Extracellular
sink_orot(c)	20.025	KO Unique	Orotate demand		Transport

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Supplemental Table 2. Min_max_span_tx

Reactions	minWT	maxWT	spanWT	minKO	maxKO	spanKO	spanKO/spanWT	Name	Equation	Subsystem
DM_datp(m)	0	3.3	3.3	0	9.45	9.45	2.863636364	Transport/Demand		Transport/Demand
DM_datp(n)	0	3.3	3.3	0	9.45	9.45	2.863636364	Transport/Demand		Transport/Demand
DM_dctp(m)	0	1.3	1.3	0	13.35	13.35	10.26923077	Transport/Demand		Transport/Demand
DM_dctp(n)	0	1.3	1.3	0	13.35	13.35	10.26923077	Transport/Demand		Transport/Demand
DM_dgtp(m)	0	0.15	0.15	0	9.45	9.45	63	Transport/Demand		Transport/Demand
DM_dgtp(n)	0	0.15	0.15	0	9.45	9.45	63	Transport/Demand		Transport/Demand
DM_dttp(m)	0	1.3	1.3	0	14.91	14.913	11.47183154	Transport/Demand		Transport/Demand
DM_dttp(n)	0	1.3	1.3	0	15.67	15.666	12.05089846	Transport/Demand		Transport/Demand
DM_ethamp(r)	0	1	1	0	37.31	37.309	37.308694	Transport/Demand		Transport/Demand
EX_2hb(e)	0	2	2	0	3	3	1.5	Transport/Demand		Transport/Demand
EX_2mcit(e)	0	2	2	0	3	3	1.5	Transport/Demand		Transport/Demand
EX_35cgmp(e)	0	0.15	0.15	0	9.45	9.45	63	Transport/Demand		Transport/Demand
EX_3aib-D(e)	0	1.3	1.3	0	27.65	27.649	21.26809769	Transport/Demand		Transport/Demand
EX_4hphac(e)	0	1	1	0	2	2	2	Transport/Demand		Transport/Demand
EX_adn(e)	0	3.3	3.3	0	9.45	9.45	2.863636364	Transport/Demand		Transport/Demand
EX_camp(e)	0	3.3	3.3	0	9.45	9.45	2.863636364	Transport/Demand		Transport/Demand
EX_chol(e)	-1	0	1	-1	0.333	1.3333	1.333333	Transport/Demand		Transport/Demand
EX_cytd(e)	0	1.3	1.3	0	13.35	13.35	10.26923077	Transport/Demand		Transport/Demand
EX_dad-2(e)	0	3.3	3.3	0	9.45	9.45	2.863636364	Transport/Demand		Transport/Demand
EX_dcyt(e)	0	1.3	1.3	0	13.35	13.35	10.26923077	Transport/Demand		Transport/Demand
EX_dgsn(e)	0	0.15	0.15	0	9.45	9.45	63	Transport/Demand		Transport/Demand
EX_din(e)	0	3.3	3.3	0	11.81	11.813	3.579545455	Transport/Demand		Transport/Demand
EX_duri(e)	0	1.3	1.3	0	19.74	19.744	15.18796385	Transport/Demand		Transport/Demand
EX_for(e)	0	2	2	0	3	3	1.5	Transport/Demand		Transport/Demand
EX_gly(e)	-1	1	2	-1	19.53	20.525	10.2625	Transport/Demand		Transport/Demand
EX_gsn(e)	0	0.15	0.15	0	9.45	9.45	63	Transport/Demand		Transport/Demand
EX_ile-L(e)	-1	0	1	-0.57	0	0.5684	0.568421	Transport/Demand		Transport/Demand
EX_ins(e)	0	3.3	3.3	0	11.81	11.813	3.579545455	Transport/Demand		Transport/Demand
EX_lpchol_hs(e	0	1	1	0	1.333	1.3333	1.333333	Transport/Demand		Transport/Demand
EX_meoh(e)	0	2	2	0	3	3	1.5	Transport/Demand		Transport/Demand
EX_pchol_hs(e)	0	1	1	0	1.333	1.3333	1.333333	Transport/Demand		Transport/Demand
EX_pe_hs(e)	0	1	1	0	4.5	4.5	4.5	Transport/Demand		Transport/Demand
EX_pi(e)	-21.3	6.6	27.92	-51.8	6.6	58.364	2.090738431	Transport/Demand		Transport/Demand
EX_ps_hs(e)	0	1	1	0	4.5	4.5	4.5	Transport/Demand		Transport/Demand
EX_ser-L(e)	-1	0	1	-1	40.05	41.05	41.05	Transport/Demand		Transport/Demand
EX_spc_hs(e)	0	1	1	0	1.333	1.3333	1.333333	Transport/Demand		Transport/Demand
EX_sph1p(e)	0	1	1	0	9.255	9.2546	9.254618	Transport/Demand		Transport/Demand
EX_sphs1p(e)	0	1	1	0	9.09	9.0903	9.090335	Transport/Demand		Transport/Demand
EX_tymsf(e)	0	1	1	0	2	2	2	Transport/Demand		Transport/Demand
EX_urate(e)	0	3.3	3.3	0	11.81	11.813	3.579545455	Transport/Demand		Transport/Demand
EX_uri(e)	0	1.3	1.3	0	20.03	20.025	15.40384615	Transport/Demand		Transport/Demand
EX_val-L(e)	-1	0	1	-0.73	0	0.7298	0.729825	Transport/Demand		Transport/Demand
sink_34hpl(m)	0	1	1	0	2	2	2	Transport/Demand		Transport/Demand
sink_34hpp	0	1	1	0	2	2	2	Transport/Demand		Transport/Demand
sink_4hphac(c)	0	1	1	0	2	2	2	Transport/Demand		Transport/Demand
sink_nadp(c)	0	0	0	0	0	0	#DIV/0!	Transport/Demand		Transport/Demand

Supplemental Table 3. Min_max_span_internal

Reactions	minWT	maxWT	spanW	minKO	maxKO	spanKC	spanKO,	Name	Equation	Subsystem
2HBO	-2	0	2	-3	0	3	1.5	2-Hydroxybut	[c] : 2hb + na	Propanoate Metabolism
2HBt2	-2	0	2	-3	0	3	1.5	2-hydroxybut	2hb[e] + h[e]	Transport, Extracellular
2MCITt	0	2	2	0	3	3	1.5	2-methylcitra	2mcit[c] <==>	Transport, Extracellular
34HPLFM	0	1	1	0	2	2	2	3-(4-hydroxy	[m] : 34hpp +	Ubiquinone Biosynthesis
3DSPHR	0	1	1	0	37.54	37.54	37.536	3-Dehydrospl	[c] : 3dsphgn	Sphingolipid Metabolism
4HOXPACDOX(N/	0	1	1	0	2	2	2	4-Hydroxyph	[c] : 4hoxpac	Tyrosine metabolism
AACTOOR	0	2	2	0	24.58	24.58	12.291	Aminoaceton	[c] : aact + h2	Gly, Ser, Thr Metabolism
AACTtm	0	2	2	0	24.58	24.58	12.291	Aminoaceton	aact[m] --> a	Transport, Mitochondrial
ACACt2m	-217	260.7	478.1	-219	149.7	368.6	0.771	Acetoacetate	acac[c] + h[c]	Transport, Mitochondrial
ACACtx	-254	0	254.4	-130	0	129.9	0.5105	acetoacetate	acac[c] <==>	Transport, Peroxisomal
ACGAMtly	0	56.44	56.44	0	5	5	0.0886	N-acetyl-gluc	acgam[l] --> a	Transport, Lysosomal
ADK3m	0	4.6	4.6	0	16.41	16.41	3.5666	adentylate ki	[m] : amp + g	Nucleotides
ADNK1m	0	4.6	4.6	0	16.41	16.41	3.5666	adenosine kir	[m] : adn + at	Nucleotides
ADNtm	0	4.6	4.6	0	16.41	16.41	3.5666	adenosine fa	adn[c] <==> a	Transport, Mitochondrial
ADSL2	0	2	2	0	10.51	10.51	5.2563	adenylosucci	[c] : 25aics -->	IMP Biosynthesis
AHC	0	2	2	0	3	3	1.5	adenosylhom	[c] : ahcys + h	Methionine Metabolism
AICART	0	2	2	0	10.51	10.51	5.2563	phosphoribos	[c] : 10fthf + a	IMP Biosynthesis
AIRCr	0	2	2	0	10.51	10.51	5.2563	phosphoribos	[c] : air + co2	IMP Biosynthesis
AKGMALtm	-708	5E+05	5E+05	-710	170	879.9	0.0018	alpha-ketogl	akg[m] + mal	Transport, Mitochondrial
ALCD1	-2	0	2	-3	0	3	1.5	alcohol dehy	[c] : meoh + r	Miscellaneous
AOBUTDsm	0	2	2	0	24.58	24.58	12.291	L-2-amino-3-(	[m] : 2aobut	Gly, Ser, Thr Metabolism
BETALDHxm	0	1	1	0	2	2	2	betaine-aldeh	[m] : betald +	Gly, Ser, Thr Metabolism
BHMT	0	1	1	0	2	2	2	betaine-hom	[c] : glyb + hc	Gly, Ser, Thr Metabolism
BPNT	0	1	1	0	2	2	2	3',5'-bisphos	[c] : h2o + pa	Nucleotides
BUP2	0	1.3	1.3	0	27.65	27.65	21.268	beta-ureidop	[c] : 3uib + (2	Pyrimidine Catabolism
CAMPt	0	3.3	3.3	0	9.45	9.45	2.8636	cAMP transp	atp[c] + camp	Transport, Extracellular
CGMPt	0	0.15	0.15	0	9.45	9.45	63	cGMP transp	35cgmp[c] + a	Transport, Extracellular
CHLtm	0	1	1	0	2	2	2	choline trans	chol[c] <==> c	Transport, Mitochondrial
CHOLD2m	0	1	1	0	2	2	2	choline dehy	[m] : chol + fa	Gly, Ser, Thr Metabolism
CHOLtu	0	1	1	-0.33	1	1.333	1.3333	Choline unipo	chol[e] <==> c	Transport, Extracellular
CLFORtex	0	1	1	0	1.5	1.5	1.5	chloride trans	cl[e] + (2) for	Transport, Extracellular
COAtm	-108	234.8	342.7	-108	0	108.2	0.3158	CoA transpor	coa[c] <==> c	Transport, Mitochondrial
COAtp	-254	0	254.4	-130	0	129.9	0.5105	coenzyme A t	coa[c] <==> c	Transport, Peroxisomal
CRNCARtp	0	254.4	254.4	0	129.9	129.9	0.5105	carnitine-ace	acrn[x] + crn[	Transport, Peroxisomal
CSNATp	0	254.4	254.4	0	129.9	129.9	0.5105	carnitine O-a	[x] : accoa + c	Fatty Acid Metabolism
CYTDt	-1.15	0.15	1.3	-13.2	0.15	13.35	10.269	cytidine facil	cytd[e] <==>	Transport, Extracellular
D-3AIBt	0	1.3	1.3	0	27.65	27.65	21.268	D-3-amino-is	3aib-D[c] -->	Transport, Extracellular
DATPtn	0	3.3	3.3	0	9.45	9.45	2.8636	dATP diffusio	datp[c] <==>	Transport, Nuclear
DCTPtn	0	1.3	1.3	0	13.35	13.35	10.269	dCTP diffusio	dctp[c] <==>	Transport, Nuclear
DCYTt	-1.3	0	1.3	-13.4	0	13.35	10.269	deoxycytidine	dcyt[e] <==>	Transport, Extracellular
DGK1	-4.6	0	4.6	-16.5	0	16.46	3.5779	deoxyguanyla	[c] : atp + dgr	Nucleotides
DGK2m	0	4.6	4.6	0	16.33	16.33	3.5507	deoxyguanyla	[m] : datp + d	Nucleotides
DGNSKm	0	4.6	4.6	0	16.33	16.33	3.5507	deoxyguanos	[m] : atp + dg	Nucleotides
DGSNt	-0.15	0	0.15	-9.45	0	9.45	63	deoyguanosir	dgsn[e] <==>	Transport, Extracellular
DGSNtm	0	4.6	4.6	0	16.33	16.33	3.5507	deoyguanosir	dgsn[c] <==>	Transport, Mitochondrial
DGTPtn	0	0.15	0.15	0	9.45	9.45	63	dGTP diffusio	dgtp[c] <==>	Transport, Nuclear
DHCRD1	0	1	1	0	37.31	37.31	37.309	dihydroceran	[c] : dhcrm_h	Sphingolipid Metabolism
DHFR	0	1.3	1.3	0	27.65	27.65	21.268	dihydrofolate	[c] : dhf + h +	Folate Metabolism
DHPM1	0	1.3	1.3	0	47.17	47.17	36.287	dihydropyrim	[c] : 56dura +	Pyrimidine Catabolism
DHPM2	0	1.3	1.3	0	27.65	27.65	21.268	dihydropyrim	[c] : 56dthm -	Pyrimidine Catabolism
DMGDHm	0	1	1	0	2	2	2	dimethylglyci	[m] : dmgly +	Gly, Ser, Thr Metabolism
DMGtm	0	1	1	0	2	2	2	dimethylglyci	dmgly[c] <==:	Transport, Mitochondrial
DNDPt12m	0	4.6	4.6	0	16.41	16.41	3.5666	dADP transpo	adp[m] + dad	Transport, Mitochondrial
DNDPt13m	0	4.6	4.6	0	16.41	16.41	3.5666	dADP transpo	atp[m] + dad	Transport, Mitochondrial

DNDPt18m	0	4.6	4.6	0	16.41	16.41	3.5666	dUDP transpo	adp[m] + dud	Transport, Mitochondrial
DNDPt19m	0	4.6	4.6	0	16.41	16.41	3.5666	dUDP transpo	atp[m] + dud	Transport, Mitochondrial
DNDPt1m	0	3.95	3.95	0	10.27	10.27	2.5995	dATP transpo	adp[m] + dat	Transport, Mitochondrial
DNDPt20m	0	4.6	4.6	0	16.41	16.41	3.5666	dTDP transpo	atp[m] + dtd	Transport, Mitochondrial
DNDPt21m	0	4.6	4.6	0	16.41	16.41	3.5666	dTDP transpo	adp[m] + dtd	Transport, Mitochondrial
DNDPt2m	0	3.95	3.95	0	10.27	10.27	2.5995	dATP transpo	atp[m] + dat	Transport, Mitochondrial
DNDPt30m	0	4.6	4.6	0	16.41	16.41	3.5666	dCDP transpo	adp[m] + dcd	Transport, Mitochondrial
DNDPt31m	0	4.6	4.6	0	16.41	16.41	3.5666	dCDP transpo	atp[m] + dcd	Transport, Mitochondrial
DNDPt32m	0	4.6	4.6	0	16.41	16.41	3.5666	dGDP transpo	atp[m] + dgd	Transport, Mitochondrial
DNDPt3m	0	7.9	7.9	0	20.48	20.48	2.5925	dATP transpo	datp[c] + dcd	Transport, Mitochondrial
DNDPt44m	0	1.3	1.3	0	14.91	14.91	11.472	dTTP transpo	atp[m] + dttp	Transport, Mitochondrial
DNDPt45m	0	1.3	1.3	0	14.91	14.91	11.472	dTTP transpo	adp[m] + dtt	Transport, Mitochondrial
DNDPt46m	0	1.3	1.3	0	14.91	14.91	11.472	dTTP transpo	dttp[c] + dud	Transport, Mitochondrial
DNDPt47m	0	1.3	1.3	0	14.91	14.91	11.472	dTTP transpo	dtdp[m] + dtt	Transport, Mitochondrial
DNDPt48m	0	1.3	1.3	0	14.91	14.91	11.472	dTTP transpo	dgdp[m] + dt	Transport, Mitochondrial
DNDPt49m	0	1.3	1.3	0	14.91	14.91	11.472	dTTP transpo	dadp[m] + dt	Transport, Mitochondrial
DNDPt4m	0	7.9	7.9	0	20.48	20.48	2.5925	dATP transpo	datp[c] + dud	Transport, Mitochondrial
DNDPt50m	0	1.3	1.3	0	14.91	14.91	11.472	dTTP transpo	dcdp[m] + dt	Transport, Mitochondrial
DNDPt51m	0	1.3	1.3	0	13.35	13.35	10.269	dCTP transpo	dcdp[m] + dc	Transport, Mitochondrial
DNDPt52m	0	1.3	1.3	0	13.35	13.35	10.269	dCTP transpo	dctp[c] + dud	Transport, Mitochondrial
DNDPt53m	0	1.3	1.3	0	13.35	13.35	10.269	dCTP transpo	dctp[c] + dgd	Transport, Mitochondrial
DNDPt54m	0	1.3	1.3	0	13.35	13.35	10.269	dCTP transpo	dadp[m] + dc	Transport, Mitochondrial
DNDPt55m	0	1.3	1.3	0	13.35	13.35	10.269	dCTP transpo	adp[m] + dct	Transport, Mitochondrial
DNDPt56m	0	1.3	1.3	0	13.35	13.35	10.269	dCTP transpo	atp[m] + dctp	Transport, Mitochondrial
DNDPt57m	0	0.15	0.15	0	9.45	9.45	63	dGTP transpo	atp[m] + dgtp	Transport, Mitochondrial
DNDPt58m	0	0.15	0.15	0	9.45	9.45	63	dGTP transpo	adp[m] + dgt	Transport, Mitochondrial
DNDPt59m	0	0.15	0.15	0	9.45	9.45	63	dGTP transpo	dadp[m] + dg	Transport, Mitochondrial
DNDPt5m	0	7.9	7.9	0	20.48	20.48	2.5925	dATP transpo	datp[c] + dtd	Transport, Mitochondrial
DNDPt60m	0	0.15	0.15	0	9.45	9.45	63	dGTP transpo	dgtp[c] + dud	Transport, Mitochondrial
DNDPt61m	0	0.15	0.15	0	9.45	9.45	63	dGTP transpo	dgtp[c] + dtd	Transport, Mitochondrial
DNDPt62m	0	0.15	0.15	0	9.45	9.45	63	dGTP transpo	dgdp[m] + dg	Transport, Mitochondrial
DNDPt63m	0	0.15	0.15	0	9.45	9.45	63	dGTP transpo	dcdp[m] + dg	Transport, Mitochondrial
DNDPt6m	0	7.9	7.9	0	20.48	20.48	2.5925	dATP transpo	datp[c] + dgd	Transport, Mitochondrial
DNDPt7m	0	7.9	7.9	0	20.48	20.48	2.5925	dATP transpo	dadp[m] + da	Transport, Mitochondrial
DOPAtu	#####	388.4	5E+05	0	389.6	389.6	0.0008	Dopamine un	dopa[e] <==>	Transport, Extracellular
DSAT	0	1	1	0	37.31	37.31	37.309	dihydrosphin	[c] : Rtotalco	Sphingolipid Metabolism
DTMPK	0	1.3	1.3	0	15.67	15.67	12.051	dTMP kinase	[c] : atp + dtn	Nucleotides
DTTPtn	0	1.3	1.3	0	15.67	15.67	12.051	dTTP diffusio	dttp[c] <==>	Transport, Nuclear
DURAD	-1.3	0	1.3	-47.2	0	47.17	36.287	dihydrouracil	[c] : 56dura +	Pyrimidine Catabolism
DURAD2	-1.3	0	1.3	-27.6	0	27.65	21.268	dihydrothymi	[c] : 56dthm -	Pyrimidine Catabolism
DURIPP	-1.3	0	1.3	-27.8	0	27.78	21.371	deoxyuridine	[c] : duri + pi	Nucleotides
DURIt	-1.3	0	1.3	-19.7	0	19.74	15.188	deoxyuridine	duri[e] <==>	Transport, Extracellular
FACOAL160i	0	22.48	22.48	0	38.31	38.31	1.7043	C160 fatty ac	[c] : atp + coa	Fatty acid activation
FALDH	-1	1	2	-1	2	3	1.5	formaldehyd	[c] : fald + gtl	Tyrosine metabolism
FALDtm	0	1	1	0	2	2	2	formaldehyd	fald[m] <==>	Transport, Mitochondrial
FORt2m	-2	0	2	-3	0	3	1.5	formate mito	for[m] <==> f	Transport, Mitochondrial
FTHFL	0	2	2	0	3	3	1.5	formate-tetra	[c] : atp + for	Folate Metabolism
FTHFLm	0	2	2	0	3	3	1.5	formate-tetra	[m] : atp + fo	Folate Metabolism
GARFT	0	2	2	0	10.51	10.51	5.2563	phosphoribos	[c] : 10fthf + g	IMP Biosynthesis
GLUPRT	0	2	2	0	10.51	10.51	5.2563	glutamine ph	[c] : gln-L + h2	IMP Biosynthesis
GLUt2m	-562	5E+05	5E+05	-565	160.6	725.3	0.0014	L-glutamate r	glu-L[c] + h[c]	Transport, Mitochondrial
GLYATm	0	2	2	0	24.58	24.58	12.291	glycine C-ace	[m] : accoa +	Gly, Ser, Thr Metabolism
GLYBtm	0	1	1	0	2	2	2	Glycine betai	glyb[m] <==>	Transport, Mitochondrial
GLYOp	0	2	2	0	26.22	26.22	13.112	glycine oxida	[x] : gly + h2o	Gly, Ser, Thr Metabolism
GLYtm	-3	1	4	-3	24.58	27.58	6.8953	glycine passiv	gly[c] <==> gl	Transport, Mitochondrial
GLYtp	0	2	2	0	26.22	26.22	13.112	glycine passiv	gly[c] <==> gl	Transport, Peroxisomal

GMPR	0	0.15	0.15	0	86.92	86.92	579.45	GMP reducta	[c] : gmp + (2 Nucleotides
GSNt	0	0.15	0.15	-9.3	0.15	9.45	63	guanosine fa	gsn[e] <==> g Transport, Extracellular
GUAD	0	0.15	0.15	0	11.81	11.81	78.75	guanine dean	[c] : gua + h + Purine Catabolism
H2Otly	0	110.9	110.9	0	9	9	0.0812	H2O transpor	h2o[c] <==> h Transport, Lysosomal
HDCAter	0	1	1	0	37.31	37.31	37.309	palmitate ER	hdca[r] --> hc Transport, ER
HMGCOAtm	-235	107.9	342.7	0	108.2	108.2	0.3158	Hydroxymeth	hmgcoa[c] <= Transport, Mitochondrial
HMGCOAtx	0	254.4	254.4	0	129.9	129.9	0.5105	Hydroxymeth	hmgcoa[c] <= Transport, Peroxisomal
HMGLm	0	296.8	296.8	0	108.2	108.2	0.3646	hydroxymeth	[m] : hmgcoa Cholesterol Metabolism
HMGLx	0	254.4	254.4	0	129.9	129.9	0.5105	hydroxymeth	[x] : hmgcoa · Cholesterol Metabolism
HPACtr	0	1	1	0	2	2	2	hydroxyphen	4hphac[c] <= Transport, Extracellular
HXANtx	0	3.3	3.3	0	11.81	11.81	3.5795	hypoxanthine	hxan[c] --> hx Transport, Peroxisomal
ILEtec	0	1	1	#####	0.568	5E+05	500001	L-isoleucine t	ile-L[e] <==> i Transport, Extracellular
IMPC	-2	0	2	-10.5	0	10.51	5.2563	IMP cyclohyd	[c] : h2o + im IMP Biosynthesis
MCITS	0	2	2	0	3	3	1.5	2-methylcitra	[c] : h2o + oa Propanoate Metabolism
MEOHt2	-2	0	2	-3	0	3	1.5	Methanol dif	meoh[e] <==> Transport, Extracellular
METAT	0	2	2	0	3	3	1.5	methionine a	[c] : atp + h2c Methionine Metabolism
MTHFC	-1.3	2	3.3	-5	20.36	25.36	7.6843	methenyltetr	[c] : h2o + me Folate Metabolism
MTHFD	-1.3	0	1.3	-7	18.53	25.53	19.635	methylenetet	[c] : mlthf + n Folate Metabolism
NDPK4	0	1.3	1.3	0	15.67	15.67	12.051	nucleoside-di	[c] : atp + dtd Nucleotides
NRPPHRtu	#####	388.4	5E+05	0	389.6	389.6	0.0008	Norepinephri	nrpphr[e] <= Transport, Extracellular
NTD8	0	4.6	4.6	0	16.46	16.46	3.5779	5'-nucleotida	[c] : dgmp + h Nucleotides
OBDHc	0	2	2	0	3	3	1.5	2-Oxobutano	[c] : 2obut + (Gly, Ser, Thr Metabolism
OCOAT1m	-221	289.9	511.3	-223	150.4	373.4	0.7301	3-oxoacid Co	[m] : acac + s Val, Leu, ileu Metabolism
PIt2m_2	#####	540	5E+05	-236	542	777.9	0.0016	PIt2m_2	(2) h[c] + pi[c Oxidative Phosphorylation
PIt9	-3	24.83	27.83	-3	54.19	57.19	2.0554	phosphate tr	(2) na1[e] + p Transport, Extracellular
PRAGSr	0	2	2	0	10.51	10.51	5.2563	phosphoribos	[c] : atp + gly IMP Biosynthesis
PRAIS	0	2	2	0	10.51	10.51	5.2563	phosphoribos	[c] : atp + fpr IMP Biosynthesis
PRASCS	0	2	2	0	10.51	10.51	5.2563	phosphoribos	[c] : 5aizc + a IMP Biosynthesis
PRFGS	0	2	2	0	10.51	10.51	5.2563	phosphoribos	[c] : atp + fga IMP Biosynthesis
PSDm_hs	0	1	1	0	5.5	5.5	5.5	Phosphatidyl	[m] : h + ps_h Glycerophospholipid Met.
PSFLIPm	0	1	1	0	5.5	5.5	5.5	phosphatidyl	atp[c] + h2o[(Transport, Mitochondrial
PYNP2r	0	1.3	1.3	0	47.29	47.29	36.378	pyrimidine-nu	[c] : pi + uri <= Pyrimidine Catabolism
RNDR2	0	0.15	0.15	0	9.45	9.45	63	ribonucleosid	[c] : gdp + trd Nucleotides
SADT	0	1	1	0	2	2	2	sulfate adeny	[c] : atp + h + Nucleotides
SERPT	0	1	1	0	37.54	37.54	37.536	serine C-palm	[c] : h + pmtc Sphingolipid Metabolism
SFGTH	-1	1	2	-1	2	3	1.5	S-Formylglut	[c] : Sfglutth - Tyrosine metabolism
SGPL12r	0	1	1	0	37.31	37.31	37.309	Sphingosine-	[r] : h2o + spl Sphingolipid Metabolism
SLCBK1	0	1	1	0	9.255	9.255	9.2546	sphingolipid l	[c] : atp + sph Sphingolipid Metabolism
SMPD4	0	1	1	0	37.31	37.31	37.309	sphingomyeli	[c] : h2o + sp Sphingolipid Metabolism
SMS	0	1	1	0	37.31	37.31	37.309	Sphingomyeli	[c] : crm_hs + Sphingolipid Metabolism
SPC_HSt	0	1	1	0	1.333	1.333	1.3333	sphingosylph	spc_hs[c] <== Transport, Extracellular
SPH1Pte	0	1	1	0	9.255	9.255	9.2546	sph1p transp	sph1p[c] <== Transport, Extracellular
SPHK21c	0	1	1	0	37.31	37.31	37.309	sphingosine k	[c] : atp + sph Sphingolipid Metabolism
SPHMDAc	0	1	1	0	37.31	37.31	37.309	sphingomyeli	[c] : h2o + spl Sphingolipid Metabolism
SPHS1Pte	0	1	1	0	9.09	9.09	9.0903	sphingosine-1	sphs1p[c] <== Transport, Extracellular
SPHS1Ptr	0	1	1	0	37.31	37.31	37.309	sphingosine-1	sphs1p[c] <== Transport, ER
TMDPP	0	1.3	1.3	0	27.65	27.65	21.268	thymidine ph	[c] : pi + thym Pyrimidine Catabolism
TMDS	0	1.3	1.3	0	27.65	27.65	21.268	thymidylate s	[c] : dump + r Nucleotides
TYMSFt	0	1	1	0	2	2	2	Tyramine O-s	tymsf[c] --> ty Transport, Extracellular
TYMSULT	0	1	1	0	2	2	2	Tyramine Sul	[c] : paps + ty Tyrosine metabolism
TYRCBOX	0	1	1	0	2	2	2	L-Tyrosine ca	[c] : h + tyr-L Tyrosine metabolism
TYROXDAc	0	1	1	0	2	2	2	Tyramine:oxy	[c] : h2o + o2 Tyrosine metabolism
UPPN	0	1.3	1.3	0	47.17	47.17	36.287	b-ureidoprop	[c] : cala + (2) Pyrimidine Catabolism
URATEt	0	3.3	3.3	0	11.81	11.81	3.5795	urate export	urate[c] --> u Transport, Extracellular
URATEtx	0	3.3	3.3	0	11.81	11.81	3.5795	urate export	urate[x] --> u Transport, Peroxisomal
URIt	-0.15	1.15	1.3	-18.9	1.15	20.03	15.404	uridine facila	uri[e] <==> ur Transport, Extracellular

VALtec	0	1	1	#####	0.73	5E+05	500001	L-valine trans	val-L[e] <==> Transport, Extracellular
XANtx	0	0.15	0.15	0	11.81	11.81	78.75	xanthine diffu	xan[c] --> xan Transport, Peroxisomal
XAO2x	0	3.3	3.3	0	11.81	11.81	3.5795	xanthine oxid	[x] : h2o + hx Purine Catabolism
XAOx	0	3.3	3.3	0	11.81	11.81	3.5795	xanthine oxi	[x] : h2o + o2 Purine Catabolism

MULTISPECIFIC DRUG TRANSPORTER Slc22a8 (Oat3) REGULATES MULTIPLE METABOLIC PATHWAYS.

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DRUG METABOLISM AND DISPOSITION.

Supplemental Table 4. Core_differences

AHCYStr	KO	S-Adenosyl	ahcys[c] <= Transport, Endoplasmic Reticular
AMETr	KO	S-Adenosyl	amet[c] <= Transport, Endoplasmic Reticular
AMETt2m	KO	S-Adenosyl	ahcys[m] + Transport, Mitochondrial
ARGt4	KO	L-arginine t	arg-L[e] + r Transport, Extracellular
BTNt3i	KO	Biotin tran	atp[c] + bti Transport, Extracellular
CYSt4	KO	L-cysteine	cys-L[e] + r Transport, Extracellular
CYTDn	KO	cytidine de	[n] : cytd + Nucleotides
CYTDtn	KO	cytidine tr	cytd[c] <==> Transport, Nuclear
ILEt4	KO	L-isoleucin	ile-L[e] + n Transport, Extracellular
PCFLOPm	KO	phosphatic	atp[c] + h2 Transport, Mitochondrial
PETOHMm_hs	KO	phosphatic	[m] : (3) an Glycerophospholipid Metabolism
PETOHMr_hs	KO	phosphatic	[r] : (3) am Glycerophospholipid Metabolism
Uritn	KO	uridine tra	uri[c] <==> Transport, Nuclear
VALt4	KO	L-valine tra	na1[e] + va Transport, Extracellular
3MOPt2im	WT	3-Methyl-2	3mop[c] + Transport, Mitochondrial
4PYRDX	WT	4-Pyridoxa	4pyrdx[c] + Transport, Extracellular
ARACHDt2	WT	fatty acid t	arachd[e] < Transport, Extracellular
ARACHt	WT	fatty acid t	arach[e] <= Transport, Extracellular
CHOLATEt2	WT	cholate tra	cholate[e] Transport, Extracellular
CHOLATEt3	WT	ABC bile ac	atp[c] + ch Transport, Extracellular
DOPAt4(2)r	WT	Dopamine	dopa[e] + (Transport, Extracellular
EX_4pyrdx(e)	WT	4-Pyridoxate	Demand Transport, Extracellular
FATP4t	WT	fatty acid e	arach[c] + Transport, Extracellular
FATP5t	WT	fatty acid e	arachd[c] + Transport, Extracellular
HMGCOASim	WT	Hydroxym	[m] : aacoa Cholesterol Metabolism
ILETA	WT	isoleucine	[c] : akg + i Valine, Leucine, and Isoleucine Metabolism
NRPPHRt4(2)r	WT	Norepinep	(2) na1[e] Transport, Extracellular
PYDXDH	WT	pyridoxal d	[c] : h2o + Vitamin B6 Metabolism
TCHOLAt	WT	taurochola	hco3[c] + t Transport, Extracellular
TCHOLAt3	WT	ABC bile ac	atp[c] + h2 Transport, Extracellular
VALTA	WT	valine tran	[c] : akg + \ Valine, Leucine, and Isoleucine Metabolism
sink_3mob(c)	WT	3-Methyl-2-oxobutan	Transport, Extracellular
sink_3mop	WT	(S)-3-Methyl-2-oxope	Transport, Extracellular

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