## SUPPORTING INFORMATION

### Table S1. Genes associated with hyperammonemia

Square brackets enclose protein function and disease, with the disease underlined.

| | |
|---|---|
| Urea cycle genes | |
| ALDH18A1 | aldehyde dehydrogenase 18 member 1 [ornithine, arginine, proline biosynthesis, cutis laxa type IIIA] |
| ARG1 | arginase, liver [argininemia] |
| ASS1 | argininosuccinate synthase 1 [citrullinemia type I] |
| ASL | argininosuccinate lyase [argininosuccinic acidemia] |
| CPS1 | carbamoyl phosphate synthase 1, mitochondrial |
| GLUL | glutamate-ammonia ligase [synthesis of glutamine from glutamate, congenital glutamine deficiency] |
| NAGS | N-acetylglutamate synthase |
| ORNT1 | SLC25A15, solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15 [hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome] |
| ORNT2 | SLC25A2, solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 2 |
| ORNT3 | SLC25A29, solute carrier family 25 (mitochondrial carnitine/acylcarnitine carrier protein CACL) member 29 |
| OTC | ornithine transcarbamylase |
| SLC7A7 | solute carrier family 7 (cationic amino acid transporter, y+ system) member 7 [Arg, Lys, ornithine transport in kidney and small intestine, lysinuric protein intolerance] |
| SLC25A13 | solute carrier family 25 member 13 (citrin) [exchange of Asp for Glu across inner mitochondrial membrane, citrullinemia type II] |
| Fatty acid oxidation genes | |
| ACADVL | acyl-CoA dehydrogenase, very long chain |
| ACADM | acyl-CoA dehydrogenase, C-4 to C-12 straight chain |
| CPT1A | carnitine palmitoyltransferase 1A (liver) |
| CPT2 | carnitine palmitoyltransferase 2 |
| ETFA | electron-transfer-flavoprotein, alpha polypeptide [glutaric acidemia IIA] |
| ETFB | electron-transfer-flavoprotein, beta polypeptide [glutaric acidemia IIB] |
| ETFDH | electron-transferring-flavoprotein dehydrogenase [glutaric acidemia IIC] |
| HADHA | hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, alpha subunit |
| HADHB | hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, beta subunit |
| HLCS | holocarboxylase synthase (biotin- (propionyl-CoA-carboxylase (ATP-hydrolysing)) ligase) [gluconeogenesis, branched chain amino acid catabolism] |
| HMGCL | 3-hydroxymethyl-3-methylglutaryl-CoA lyase [final step in leucine degradation] |
| IVD | isovaleryl-CoA dehydrogenase [valine, leucine, isoleucine degradation, isovaleric acidemia] |
| LMBRD1 | LMBR1 domain containing 1 [cobalamin transporter, homocystinuria-megaloblastic anemia type F] |
| MCCC1 | methylcrotonoyl-CoA carboxylase 1 (alpha) [leucine catabolism] |
| MCCC2 | methylcrotonoyl-CoA carboxylase 2 (beta) [leucine catabolism] |
| MLYCD | malonyl-CoA-decarboxylase [stimulates fatty acid oxidation by converting malonyl-CoA to acetyl-CoA, [combined malonic and methylmalonic acidemia] |
| MMAA | methylmalonic acidemia (cobalamin deficiency) cblA type [methylmalonic acidemia] |
| MMAB | methylmalonic acidemia (cobalamin deficiency) cblB type [methylmalonic acidemia] |
| MMACHC | methylmalonic acidemia (cobalamin deficiency) cblC type, with homocystinuria [methylmalonic acidemia] |
| MMADHC | methylmalonic acidemia (cobalamin deficiency) cblD type, with homocystinuria [methylmalonic acidemia] |
| MUT | methylmalonyl CoA mutase [isomerization of methylmalonyl-CoA to succinyl-CoA, methylmalonic acidemia] |
| PCCA | propionyl CoA carboxylase, alpha polypeptide [propionic acidemia] |
| PCCB | propionyl CoA carboxylase, beta polypeptide [propionic acidemia] |
| SLC25A20 | solute carrier family 25 member 20 [carnitine/acylcarnitine translocase deficiency] |
| SLC22A5 | solute carrier family 22 (organic cation/carnitine transporter) member 5 [carnitine deficiency] |
| Other anaplerosis genes | |
| DLAT | dihydrolipoamide S-acetyltransferase [in mitochondrial complex that converts pyruvate to acetyl-CoA] |
| GLUD1 | glutamate dehydrogenase 1 [hyperinsulinism-hyperammonemia syndrome] |
| PC | pyruvate carboxylase [mitochondrial pyruvate oxidation to oxaloacetate] |
| PDHA1 | pyruvate dehydrogenase (lipoamide) alpha 1 [in mitochondrial complex that converts pyruvate to acetyl-CoA] |
| TUFM | Tu translation elongation factor, mitochondrial [protein translation in mitochondria, combined oxidative phosphorylation deficiency] |

**Table S2. Patient missense and splice site mutations**

For the 43 hyperammonemia genes plus DPYD, we obtained allele frequencies and disease associations from the SNP database https://www.ncbi.nlm.nih.gov/snp. Abbreviations: NA, not applicable; NV, normal variant based on SIFT and PolyPhen2 predictions and high allele frequency; Ref DNA, reference DNA sequence; SD, splice donor site.

| *Gene* | *SNP ID* | *Ref DNA* | *Patient DNA* | *Mutation* | *SIFT* | *PolyPhen2* | *Allele freq* | *Conclusion* |
|---|---|---|---|---|---|---|---|---|
| CPS1 | rs1047891 | C | C/A | T1406N | tolerated | benign | 0.288 | NV[A] |
| ORNT1 | rs17849654 | A | A/T | I254L | tolerated | benign | 0.323 | NV |
| ORNT2 | rs3749780 | G | G/A | V226I | tolerated | benign | 0.128 | NV |
| | rs10075302 | G | G/T | G159C | damaging | possibly damaging | 0.043 | damaging[B] |
| MMAB | rs9593 | T | T/A | M239K | tolerated | benign | 0.487 | NV |
| LMBRD1 | rs12648 | A | A/T | D469E | tolerated | benign | 0.385 | NV |
| HLCS | rs61732502 | G | G/A | V96I | tolerated | benign | 0.058 | NV |
| CPT2 | rs1799821 | G | G/A | V368I | tolerated | benign | 0.413 | NV |
| | rs776754218 | C | C/T | P520L | tolerated | benign | 0.00005 | benign[C] |
| MUT | rs8589 | A | A/G | I671V | tolerated | benign | 0.451 | NV |
| | rs2229385 | G | G/A | A499T | tolerated | benign | 0.086 | benign[D] |
| ETFA | rs1801591 | C | C/T | T171I | damaging | damaging | 0.051 | damaging[E] |
| ETFDH | rs11559290 | C | C/T | T31I | tolerated | benign | 0.327 | NV |
| SLC7A7 | rs8018462 | A | G/G | SD-2 | NA | NA | 0.418 | benign[F] |
| DPYD | rs1801159 | A | A/G | I543V | tolerated | benign | 0.185 | NV[G] |
| | rs1801265 | T | C/C | C29R | tolerated | benign | 0.260 | NV[G] |

Notes

A T1406N has no effect on plasma arginine concentrations[1].

B G159C shows decreased activity in cells transfected with a cDNA expression vector[2].

C P520L is predicted to preserve protein function and is not among the 64 mutations found in neonatal or severe infantile carnitine palmitoyltransferase II deficiency[3].

D A499T confers normal enzymatic activity[4].

E T171I affects thermal stability of the ETF enzyme and is over-represented among patients with very-long-chain acyl-CoA dehydrogenase deficiency[5].

F The splice donor SD-2 polymorphism had no effect on RNA, as determined by RNA-sequence analysis of 12 acute myelogenous leukemias (Fig. 2E).

G Patient had normal enzymatic activity (Fig. 2B).

**Table S3. Patient nonsense mutations**

A nonsense mutation was deemed homozygous if we observed multiple homozygous reads at both the mutation site and adjacent SNPs (in parentheses). Other nonsense mutations were heterozygous.

| *Gene* | *Mutation* | *Homozygous reads* | *Protein* | *Function* |
|---|---|---|---|---|
| PDE4DIP | p.R785X | | phosphodiesterase 4D interacting protein | anchors cAMP PDE4D to Golgi |
| CPN2 | p.Q509X | 12 (61) | carboxypeptidase N subunit 2 | carboxypeptidase |
| SLC6A18 | p.Y319X | | solute carrier family 6 member 18 | Na-dependent neutral a.a. transporter, role in iminoglycinuria & hyperglycinuria |
| ZNF117 | p.R428X | | zinc finger protein 117 | possible transcriptional regulator |
| FSCN3 | p.R423X | | fascin-3 | actin bundling protein |
| LPL | p.S474X | | lipoprotein lipase precursor | triglyceride hydrolysis |
| VPS13B | p.Y413X | | vacuolar protein sorting-assoc protein 13B | vesicle-mediated transport |
| IFNE | p.Q71X | | interferon epsilon precursor | interferon |
| CTBP2 | p.Q445X | | C-terminal-binding protein 2 | corepressor of transcriptional regulators |
| OR1B1 | p.R192X | 20 (145) | olfactory receptor 1B1 | olfactory receptor |
| OR10X1 | p.W66X | 47 (118) | olfactory receptor 10X1 | olfactory receptor |
| OR51Q1 | p.R236X | | olfactory receptor 51Q1 | olfactory receptor |
| OR4X2 | p.Y27X | | olfactory receptor 4X2 | olfactory receptor |
| OR4X1 | p.Y273X | 108 (71) | olfactory receptor 4X1 | olfactory receptor |
| OR4C3 | p.W174X | | olfactory receptor 4C3 | olfactory receptor |
| OR4A15 | p.Q256X | | olfactory receptor 4A15 | olfactory receptor |
| OR4C16 | p.Q17X | | olfactory receptor 4C16 | olfactory receptor |
| OR5AR1 | p.Q19X | | olfactory receptor 5AR1 | olfactory receptor |
| OR10V1 | p.Q123X | | olfactory receptor 10V1 | olfactory receptor |
| SLC22A10 | p.W96X | | solute carrier family 22 member 10 | organic anion transporter |
| KLRK1 | p.W74X | | NKG2-D type II integral membrane protein | receptor on NK cells |
| HEBP1 | p.Q147X | | heme-binding protein 1 | removes free porphyrinogens |
| ACSM2A | p.R115X | | acyl-coA synthetase medium chain member 2A | medium chain fatty acid:CoA ligase |
| TM4SF5 | p.S160X | | transmembrane 4 L6 family member 5 | tetraspannin protein, cell proliferation |
| MAP2K3 | p.Q73X | | dual specificity MAP kinase kinase 3 | component of the MAP kinase cascade |
| HAP1 | p.W104X | | huntingtin-assoc protein 1 isoform 3 | associates with huntingtin |
| CDC27 | p.R631X | | cell division cycle protein 27 homolog | anaphase promoting complex component |
| SERPINB7 | p.R266X | | serpin peptidase inhib, clade B member 7 | inhibits lysine-specific proteases |
| RHPN2 | p.Q378X | | rhophilin-2 | organization of actin cytoskeleton |
| USP29 | p.Y913X | 129 | ubiquitin carboxyl-terminal hydrolase 29 | de-ubiquitinating (DUB) enzyme |
| WFDC8 | p.R5X | | WAP four-disulfide core dom protein 8 | protease inhibitor |
| UBE2NL | p.L89X | | putative ubiq-conjug enzyme E2 N-like | 91% identical to UBE2N, DNA repair |
| NEIL1 | p.R325X | | endonuclease VIII-like 1 | DNA glycoslylase in BER |
| ZACN | p.Q281X | 55 (101) | Zn-activated ligand-gated ion channel | ? |
| ZC3H3 | p.S880X | 6 (21) | Zn finger CCCH dom-containing protein 3 | ? |
| MAGEE2 | p.E120X | 33 (106) | melanoma-associated antigen E2 | ? |
| MAGEB16 | p.R272X | | melanoma-associated antigen B16 | ? |
| C5orf49 | p.R72X | | hypothetical protein LOC134121 | ? |
| UNC93A | p.W151X | | protein unc-93 homolog A isoform 2 | ? |
| CC2D2B | p.Q320X | | protein CC2D2B isoform 2 | ? |
| PRAMEF2 | p.E211X | | PRAME family member 2 | ? |
| MOBKL2C | p.R24X | | mps1 binder kin. activator-like 2C isoform | ? |
| SPATA8 | p.R34X | | spermatogenesis-associated protein 8 | ? |
| TPTE | p.R211X | | putative Tyr-protein phosphatase TPTE | ? |
| C1orf227 | p.R37X | | hypothetical protein LOC149643 | ? |
| VWA3B | p.E219X | | von Willebrand factor A domain-containing | ? |
| SERHL2 | p.Q212X | | serine hydrolase-like protein 2 | ? |
| FTHL17 | p.E148X | | ferritin heavy polypeptide-like 17 | ? |

**Table S4. Patient invariant splice site mutations**
The table shows genes with mutations in the splice donor site (SD) invariant GT, or splice acceptor site (SA) invariant AG. An invariant splice site mutation was deemed homozygous if we observed multiple homozygous reads at both the mutation site and adjacent SNPs (in parentheses). Other invariant splice site mutations were heterozygous.

| *Gene* | *Mutation* | *Homozygous reads* | *Protein* |
|---|---|---|---|
| TCTEX1D1 | SD1 | | tctex1 domain-containing protein 1 |
| HTR3D | SD1 | 65 (65) | 5-hydroxytryptamine receptor 3D isoform 3 |
| WDR67 | SD1 | | WD repeat-containing protein 67 isoform 1 |
| C9orf43 | SD1 | | hypothetical protein LOC257169 |
| NUCB2 | SD1 | 74 | nucleobindin-2 precursor |
| GSTT2 | SD1 | | glutathione S-transferase theta-2 |
| SAA1 | SD2 | 3 (115) | serum amyloid A protein preproprotein |
| AGL | SA-2 | | glycogen debranching enzyme isoform 1 |
| ZFP91 | SA-2 | | zinc finger protein 91 homolog isoform 2 |
| GREB1 | SA-1 | | protein GREB1 isoform c |
| MUC7 | SA-1 | | mucin-7 precursor |
| XRCC4 | SA-1 | 14 (24) | DNA repair protein XRCC4 isoform 2 |
| OAS1 | SA-1 | 43 (86) | 2'-5'-oligoadenylate synthase 1 isoform 1 |
| C13orf26 | SA-1 | | hypothetical protein LOC122046 |
| C17orf57 | SA-1 | | EF-hand dom-containing protein C17orf57 |
| LILRA2 | SA-1 | | leukocyte Ig-like rec subfamily |

**Table S5. Patient insertion/deletion mutations**
The table shows insertions or deletions (indels) sequenced more than once. Indels for CLCA4, SMARCA2, and ATN1 occur in repeated amino acid sequences, and are therefore presumed to be polymorphisms.

| *Gene* | *Mutation* | *Reads* | *Protein* |
|---|---|---|---|
| CLCA4 | 2 a.a. del: (PT)5 > (PT)4 | 2 | Calcium-activated chloride channel regulator 4 precursor |
| OR7C2 | frameshift | 2 | Olfactory receptor 7C2 |
| C21orf6 | frameshift | 3 | Uncharacterized protein C21orf62 |
| GPATCH4 | frameshift | 3 | G patch domain-containing protein 4 |
| SMARCA2 | 1 a.a. del: (Q)23 > (Q)22 | 4 | Probable global transcription activator SNF2L2 |
| ATN1 | 5 a.a. del: (Q)19 > (Q)14 | 2 | Atrophin-1 (Dentatorubral-pallidoluysian atrophy) |
| ALMS1 | L525_T527del/insP | 2 | Alstrom syndrome protein 1 |

**Table S6. Population deleterious SNPs among 43 hyperammonemia genes**
We obtained data from the SNP database https://www.ncbi.nlm.nih.gov/snp. For each gene, the table shows the SNP with the highest allele frequency of the SNPs deemed pathogenic by the 1000 Genomes phase 3 genotype data from 2500 worldwide individuals, as released in the May 2013 dataset. The table is restricted to the 16 genes with pathogenic SNPs with global mean allele frequencies of at least 0.0004, which corresponds to 2 observations in 1088 individuals (or 2176 chromosomes).

| *Gene* | *Chromosome* | *SNP id* | *Protein change* | *Allele frequency* |
|---|---|---|---|---|
| ACADM | 1 | rs147559466 | E43K | 0.0020 |
| ACADVL | 17 | rs28934585 | P65L | 0.0391 |
| ASL | 22 | rs28941471 | R190Q | 0.0010 |
| ASS1 | 9 | rs35269064 | R108L | 0.0050 |
| CPS1 | 2 | rs200214298 | G169R | 0.0004 |
| CPT1A | 11 | rs2229738 | A275T | 0.0238 |
| CPT2 | 1 | rs1871748 | S565C | 0.0016 |
| IVD | 15 | rs28940889 | A284V | 0.0004 |
| MCCC2 | 5 | rs119103219 | E99Q | 0.0004 |
| | | rs150591260 | V339M | 0.0004 |
| MMAB | 12 | rs35648932 | A135T | 0.0030 |
| MMACHC | 1 | rs398124292 | frameshift | 0.0010 |
| MUT | 6 | rs547709692 | T297I | 0.0022 |
| ORNT1 | 13 | rs141028076 | intron variant | 0.0010 |
| OTC | X | rs1800328 | Q270R | 0.0167 |
| PDHA1 | X | rs2229137 | M282L | 0.0495 |
| SLC22A5 | 5 | rs11568514 | Y473D | 0.0016 |

**Table S7. Population frequency of pathogenic SNPs**
The sum of the allele frequencies for the 16 genes in Table S6 was 0.149. Five additional genes had pathogenic SNPs with global mean allele frequencies of 0.0002, providing a total contribution of 0.001. The remaining 21 genes did not have pathogenic SNPs observed in the 1000 Genomes phase 3 genotype data. Thus, the average number of deleterious SNPs in the global population was estimated to be 0.150. This number was used as the parameter $\lambda$ for the Poisson distribution, which estimates the probability for n pathogenic SNPs, $P(n) = (\lambda^n/n!) \exp(-\lambda)$. Shown are the estimated fraction of the population carrying: zero, P(0); one or more, P(≥1); and two or more, P(≥2) deleterious SNPs.

| *Sum of allele frequencies* | *P(0)* | *P(≥1)* | *P(≥2)* |
|---|---|---|---|
| 0.150 | 0.861 | 0.139 | 0.010 |

## Materials and Methods

*DPYD analysis*

DPYD enzymatic activity was measured in peripheral blood lymphocytes from the patient and an age-matched healthy control. Samples were harvested at the same time, and shipped on dry ice. The laboratory of Dr. Robert Diasio (Mayo Clinic, Rochester, MN) measured DPYD enzymatic activity and performed DPYD mutation analysis.

*Exome sequencing*

After obtaining informed consent from the Stanford University Administrative Panel for the Protection of Human Subjects, the patient's exome was analyzed using biotinylated RNA baits to capture exome DNA[6], and then sequenced to 84-fold coverage, with a range of 6 to 498 reads (Hudson-Alpha Institute, Huntsville, AL). To determine if amino acid substitutions affected protein function, we applied two algorithms. SIFT (Sorting Tolerant From Intolerant) assumes that important positions in the amino acid sequence of a protein have been conserved during evolution, and predicts the effects of substitutions at each position in the amino acid sequence[7]. PolyPhen-2 (Polymorphism Phenotyping version 2) uses sequence-based and structure-based algorithms to predict the functional importance of an amino acid substitution[8]. Allele frequencies and other information for specific genes were obtained from GeneCards (http://www.genecards.org/).

*RNA sequence analysis*

To determine whether the SD-2 splice donor site SNP (A/C)**A**G|GUPuAGU > (A/C)**G**G|GUPuAGU at position 1083 affects RNA levels, we analyzed published RNAseq data from 12 acute myelogenous leukemias heterozygous for SNPs in at least one of five positions (Fig. 2E): SRR061899, SRR061823, SRR061886, SRR061900, SRR061757, SRR054844, SRR061824, SRR061898, SRR061897, SRR061758, SRR061885, SRR054845, respectively (http://0-www.ncbi.nlm.nih.gov.elis.tmu.edu.tw/Traces/sra/sra.cgi?view=search_obj).

*Measurement of plasma ammonia levels*

To measure plasma ammonia levels, Stanford Health Care Clinical Laboratory placed the blood sample on ice, and analyzed the sample within 15 minutes. Sample handling is important, because samples not placed on ice, or analyzed after a longer delay yield falsely elevated plasma ammonia levels[9, 10].

## Supporting Information References


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