Supplement information

Details about three fatty acid oxidation pathways occurring in man

Alpha oxidation

Definition: Oxidation of the alpha carbon of the fatty acid, chain shortened by 1 carbon atom.

Localization: Peroxisomes[1]

Substrates: Phytanic acid, 3-methyl fatty acids and their alcohol and aldehyde derivatives, metabolites of farnesol, geranylgeraniol, and dolichols[2, 3].

Steps in the pathway: Activation requires ATP and CoA. Hydroxylation requires iron, ascorbate and alpha-keto-glutarate as cofactors and secondary substrates. Lysis requires thymine pyrophosphate and magnesium ions. Dehydrogenation requires NADP. End products are transported into mitochondria for further oxidation.

Enzymes and genes involved: Very long-chain acyl-CoA synthetase (E.C. 6.2.1.-) (*SLC27A2*, GeneID: 11001)[4], phytanoyl-CoA dioxygenase (E.C. 1.14.11.18, *PHYH*, GeneID: 5264), 2-hydrosyphytanoyl-coA lyase (E.C. 4.1.-.-, *HACL1*, GeneID: 26061), and aldehyde dehydrogenase (E.C. 1.2.1.3, *ALDH3A2*, GeneID: 224).

Disorders associated: Zellweger syndrome including RCDP type 1, where PTS2 receptor is defective and PHYH is unable to enter peroxisomes, and Refsum's disease.

Special features/ purpose: At the sub cellular level, the activation step can occur in the mitochondrion, endoplasmic reticulum, and peroxisome. Formic acid is the main byproduct of this pathway as opposed to carbon dioxide. Phytanic acid usually undergoes alpha oxidation; however, under conditions of enzyme deficiency, it undergoes omega oxidation and 3-methyladipic acid is produced as the end product[5].

Omega oxidation

Definition: Oxidation of omega carbon of the fatty acid for generation of mono- and di-carboxylic acids. No chain shortening occurs.

Localization: Fatty acid shuttles between cytosol and microsomes before entering the peroxisomes[6].

Substrates: Long and very long chain fatty acids.

Steps in the pathway: Hydroxylation requires NADPH and molecular oxygen as cofactors. Oxidation and dehydrogenation require both NAD and NADPH. Activation requires ATP.

Enzymes and genes involved: Leukotriene-B(4) 20-monooxygenase (E.C. 1.14.13.30, *CYP4F2*, GeneID: 8529, and *CYP4F3*, GeneID: 4051)[7-9], alcohol dehydrogenase class-3 (E.C. 1.1.1.1, *ADH5*, GeneID: 128), fatty aldehyde dehydrogenase (E.C. 1.2.1.3, *ALDH3A2*,

GeneID: 224), and microsomal long-chain-fatty-acid--CoA ligase 5 (E.C. 6.2.1.3, *ACSL5*, GeneID: 51703).

Special features/ purpose: The first step and the last step occur in the microsomes, whereas the remaining steps occur in cytosol. Although a minor pathway for fatty acid oxidation (accounting for 5-10% of total fatty acid oxidation), this is now being studied as a rescue pathway in order to compensate for various other genetic disorders of fatty acid oxidation, particularly for beta oxidation defects[1].

Peroxisomal beta oxidation

Definition: Oxidation of the beta carbon of the fatty acyl CoA molecule.

Localization: Peroxisome

Substrates: Bile acid intermediates, very long chain fatty acids (VLCFA), dicarboxylic fatty acids, xenobiotics, epoxy fatty acid, poly unsaturated fatty acids (PUFA), prostaglandins, pristanic acid, leukotrienes, thromboxane, very long chain-PUFA, dicarboxylic PUFA [2].

Enzymes and steps in the pathway: AcylcoA oxidase (E.C. 1.3.3.6) (desaturation), enoylcoA hydratase (E.C. 4.2.1.17) (hydration), 3-hydroxyacylcoA dehydrogenase (E.C. 1.1.1.35) (dehydrogenation), and 3- oxoacylcoA thiolase (E.C. 2.3.1.16) (thiolytic cleavage). Hydratase and dehydrogenase activities are displayed by LBP and DBP proteins[10].

Auxiliary enzymes and genes involved: 2,4-dienoyl-CoA reductase (E.C. 1.3.1.34, *DECR2*, GeneID: 26063), Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase (E.C. 5.3.3.-, *ECH1*, GeneID: 1891), and 3,2-trans-enoyl-CoA isomerase (E.C. 5.3.3.8), *PECI*, GeneID: 10455).

Transporters associated: ATP-binding cassette sub-family D member 1, 2, 3, carnitine o-octanoyltransferase, acylcarnitine carrier protein, and carnitine o-acetyltransferase.

Disorders associated: X-ALD, ACOX1 deficiency, DBP deficiency, racemase deficiency, SCPX deficiency, and BAAT deficiency[2].

Special features/ purpose: Peroxisomal beta oxidation is mainly used as a chain shortening pathway, and very long fatty acids (e.g., C26 and C24) are exclusive to this mechanism. The peroxisomal enzymes can shorten chains up to C8 or C6. Thereafter, they are handled by the mitochondrion. Due to the absence of dehydrogenation in the initial step, amount of energy produced is relatively lower.

Mitochondrial beta oxidation

Definition: Involves carnitine shuttle transport prior to the beta oxidation in the mitochondrial matrix.

Localization: Mitochondrial matrix

Substrates: Long, medium, short chain fatty acids, low activity for VLCFA, and optimal for plamitic acid and apha tocopherol[11, 12].

Enzymes and steps in the pathway: Acyl-CoA dehydrogenase (E.C. 1.3.99.-, 1.3.99.3 or 1.3.99.2) (desaturation), mitochondrial trifunctional protein (E.C. 4.2.1.17, E.C.1.1.1.211 and E.C. 2.3.1.16) (hydration and dehydrogenation), Enoyl-CoA hydratase (E.C. 4.2.1.17) (hydration), 3-hydroxy acyl-CoA dehydrogenase (E.C.1.1.1.35) (dehydrogenation), 3-ketoacyl-CoA thiolase (E.C. 2.3.1.16) (thiolytic cleavage). All of these enzymes have chain length specificity[13].

Auxiliary enzymes and genes involved: 2,4-dienoyl-CoA reductase (E.C. 1.3.1.34, *DECR1*, GeneID: 1666), 3,2-trans-enoyl-CoA isomerase (E.C. 5.3.3.8, *DCI*, GeneID: 1632).

Transporters associated: Carnitine shuttle system.

Disorders associated: Systemic carnitine deficiency, carnitine cycle disorder, ACADVL deficiency, ACADM deficiency, ACADS deficiency, HADHA deficiency, HADHB deficiency, SCHAD deficiency, ACAA2 deficiency, DECR1 deficiency, dicarboxylicaciduria etc.[14, 15].

Special features/ purpose: Energy production. There are two dehydrogenation steps involved, one requires FAD and the other one requires NAD as cofactor, which produce higher amount of ATP via oxidative phosphorylation.

Reversible version of the Recon 1 reactions

64 reactions of the carnitine shuttle system, consisting CPT-1 enzyme (*CPT1A* or *CPT1B* or *CPT1C*, GeneID:1374 or GeneID:1375 or GeneID:126129, E.C. 2.3.1.21), the carnitine/acylcarnitine translocase protein (*SLC25A20*, GeneID: 788) and the carnitine O-palmitoyltransferase 2 (*CPT2*, GeneID: 1376, E.C. 2.3.1.21) were added along with their Recon 1 counterparts, i.e., these reactions existed in Recon 1 in irreversible form and were made reversible in the Recon1_AC/FAO module. The Recon 1 reaction abbreviation and the new reaction abbreviation with modified reaction directionality have been shown in supplement table S9. As mentioned in the main text that carnitine shuttle system is reversible, hence, these refinements were made.

Figures

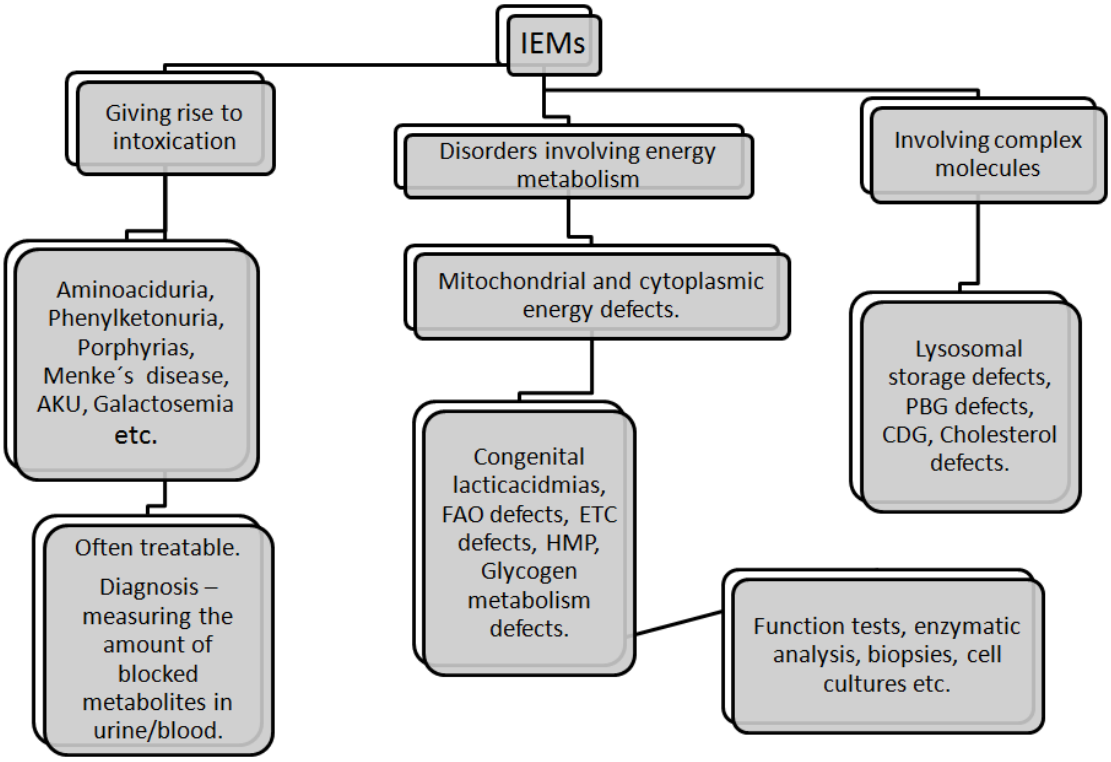

Figure S1: Classification scheme for IEMs. Derived from [16] .

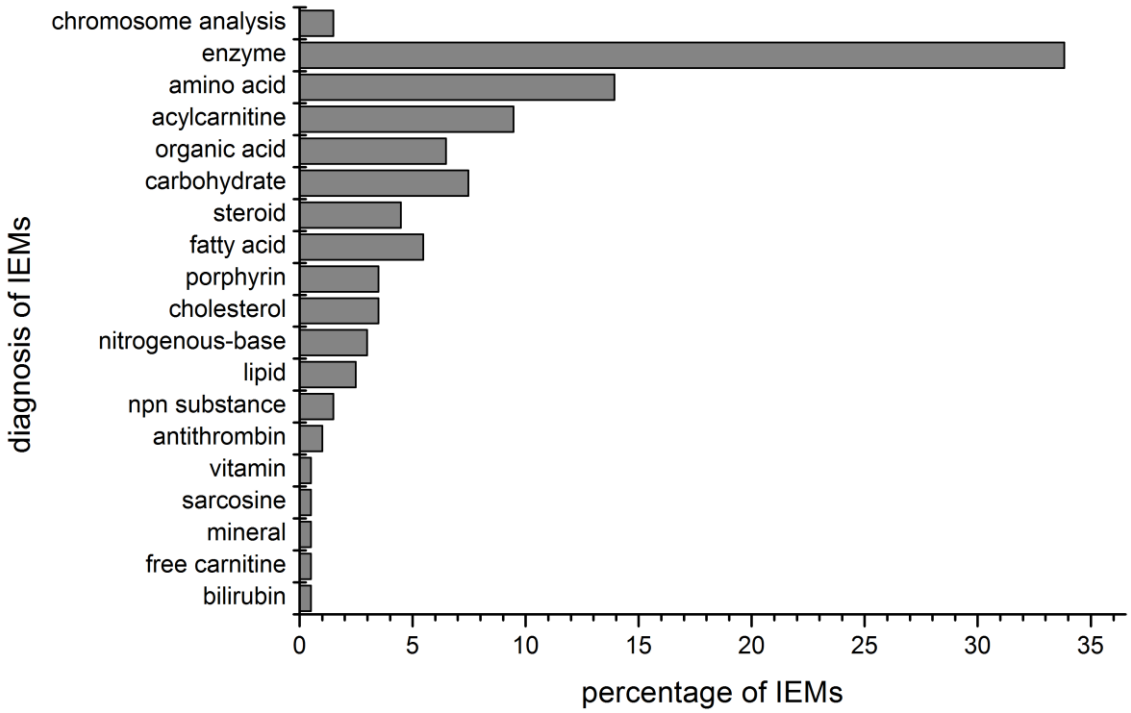

Figure S2: Different biomarkers used in the diagnosis of IEMs. The chromosome analysis refers to identification of specific mutations. Enzyme refers to enzyme assays. 17 different metabolic biomarkers have been shown. Certain tests are routinely done, such as the estimation of blood urea nitrogen [17] and lactate /pyruvate ratios. These tests provide information regarding the physiological condition of the infant, such as acid-base imbalance. These compounds have been included under the class of organic acid, since, succinate-CoA ligase deficiency (OMIM: 612073), lactic acidosis fatal/infantile (OMIM: 245400), and renal tubular acidosis (OMIM: 602722) depends on blood pyruvate and lactate estimation as diagnostic tool. *Abbreviations used:* npn: non-protein nitrogenous substance and nitrogen base (usually adenine, thymine, uracil and their derivates).

Tables

Table S1: Analytes measured under the newborn screening panel at Landspítali (National hospital of Iceland,) along with their concentration ranges. Three tests measure the sum of different amino acids, i.e., XLeu/Ile/HOPro, XLeu/Phe and XLeu/Ala, since the mass spectra of these amino acids cannot be distinguished. The newborn screening program generally looks for significantly elevated levels of analytes; however, both low and high values are of importance in case of C:16, C18:2, C18:1 and C18 acylcarnitines.

Analytes measured	**Chemical name**	**Metabolite abbreviation used in Recon 1**	**Metabolite HMDB ID**	**Chemical formula**	**Concentration range in whole blood (µmol/L) (<7 days) [18]**	**Concentration range in (µmol/L) (Iceland data)**	**World wide data [19]**
Acylcarnitines							
C0	Free carnitine	crn	HMDB00062	C7H15NO3		5.78-63.44	11-59
C2	Acetyl carnitine	acrn	HMDB00201	C9H17NO4		7.28-91.45	10-52
C3	Propionyl carnitine	pcrn	HMDB00824	C10H19NO4		0.22-6.88	0.57-4.74
C5:1	Tiglyl carnitine	c51crn	HMDB02366	C12H21NO4	0.00-0.04	0.00-0.38	0.001-0.080
C4	Butyryl carnitine	c4crn	HMDB02013	C11H21NO4	0.06-0.45	0.03-1.02	0.080-0.75
C5	Isovaleryl carnitine	ivcrn	HMDB00688	C12H23NO4	0.06-0.37	0.03-0.58	0.050-0.39
C6	Hexanoyl carnitine	c6crn	HMDB00705	C13H25NO4	0.01-0.13	0.02-0.24	0.020-0.18
C8:1	Octenoyl carnitine	c81crn		C15H27NO4		0.00-0.42	
C8	Octanoyl carnitine	c8crn	HMDB00791	C15H29NO4		0.00-0.23	0.020-0.21
C10:2	Decadienoyl carnitine	decdicrn		C17H29NO4		0.00-0.1	0.001-0.08
C10:1	Decenoyl carnitine	c101crn		C17H31NO4		0.00-0.23	0.020-0.18
C10	Decanoyl carnitine	c10crn	HMDB00651	C17H33NO4		0.03-0.30	0.022-0.26
C12:1	Dodecenoyl carnitine	ddece1crn		C19H35NO4		0.00-0.29	0.010-0.27
C12	Lauroyl carnitine	ddeccrn	HMDB02250	C19H37NO4		0.01-0.45	0.040-0.41
C14:2	Tetradecadienoyl carnitine	tetdec2crn		C21H37NO4		0.00-0.10	0.010-0.090
C14:1	Tetradecenoyl carnitine	tetdece1crn		C21H39NO4		0.00-0.37	0.030-0.37
C14	Myristoyl carnitine	tdcrn	HMDB05066	C21H41NO4		0.05-0.67	0.071-0.50
C16:1	Palmitoleoyl carnitine	hdcecrn or hdd2crn		C23H43NO4		0.02-0.53	

C16 (L/H)	Palmitoyl carnitine	pmtcrn	HMDB00222	C23H45NO4		0.35-7.87	0.80-6.0
C18:2 (L/H)	Linoelaidyl carnitine	lneldccrn	HMDB06461	C25H45NO4		0.04-0.65	0.060-0.60
C18:1 (L/H)	Elaidic carnitine	elaidcrn	HMDB06464	C25H47NO4		0.32-3.12	0.49-2.5
C18 (L/H)	Stearoyl carnitine	stcrn	HMDB00848	C25H49NO4		0.28-2.33	0.31-1.7
Hydroxyacylcarnitines							
C4-OH	3-hydroxy butyryl carnitine	3bcrn		C11H21NO5	0.01-0.12	0.03-0.51	0.050-0.49 (derivatized)
C5-OH	3-hydroxy-isovaleryl carnitine	3ivcrn		C12H23NO5	0.01-0.07	0.05-1.72	0.060-0.38 (derivatized)
C16-OH	3-hydroxyhexadecanoylcarnitine	3hexdcrn	HMDB13336	C23H45NO5	0.00-0.09	0.00-0.11	0.010-0.08
C18:2-OH	3-hydroxyoctadecadienoylcarnitine	3octdec2crn		C25H45NO5	0.00-0.03	0.00-0.07	
C18:1-OH	3-hydroxy-octadecenoyl carnitine	3octdece1crn		C25H47NO5	0.00-0.02	0.00-0.07	0.010-0.070
C18-OH	3-hydroxyoctadecanoyl carnitine	3octdeccrn		C25H49NO5	0.00-0.02	0.00-0.08	0.001-0.060
C14-OH	3-hydroxy-tetradecanoyl carnitine	3tdcrn		C21H41NO5	0.00-0.03	0.00-0.15	
C16:1-OH	3-hydroxyhexadecenoylcarnitine	3hdececrn		C23H43NO5	0.00-0.77	0.01-0.17	0.011-0.13
Dicarboxylic acylcarnitines							
C3DC	Malonyl carnitine	c3dc	HMDB02095	C10H16NO6	0.01-0.08	0.00-0.21	
C4DC	Succinyl carnitine	c4dc		C11H18NO6	0.00-0.04	0.06-1.64	
C6DC	Adipoyl carnitine	c6dc		C13H22NO6		0.00-0.24	0.022-0.17
C8DC	Suberyl carnitine	c8dc		C15H26NO6		0.00-0.19	
C10DC	Sebacoyl carnitine	c10dc		C17H30NO6		0.00-0.82	
C5DC	Glutaryl carnitine	c5dc	HMDB13130	C12H20NO6	0.00-0.05	0.01-0.15	
Amino acids							
Standard amino acids							
Glycine	Glycine	gly	HMDB00123	C2H5NO2		166.76-908.74	185-767

Arg	Arginine	arg_L	HMDB00517	C6H15N4O2		1.66-43.76	2.3-32
Ala	Alanine	ala_L	HMDB00161	C3H7NO2		82.02-456.42	117-507
Val	Valine	val_L	HMDB00883	C5H11NO2		57.99-259.1	57-212
Pro	Proline	pro_L	HMDB00162	C5H9NO2		109.76-917.61	
His	Histidine	his_L	HMDB00177	C6H9N3O2		14.56-207.01	
Met	Methionine	met_L	HMDB00696	C5H11NO2S		5.85-56.6	11-44
Serine	Serine	ser_L	HMDB00187	C3H7NO3		39.24-436.64	
Threonine	Threonine	thr_L	HMDB00167	C4H9NO3		10.6-86.05	
Tyr	Tyrosine	tyr_L	HMDB00158	C9H11NO3		23.78-435.06	34-207
Tryptophan	Tryptophan	trp_L	HMDB00929	C11H12N2O2		7.72-35.59	
Asparticac	Aspartic acid	asp_L	HMDB00191	C4H6NO4		23.54-217.16	
Glutamic ac	Glutamic acid	glu_L	HMDB00148	C5H8NO4		215.08-786.54	158-551
Lysine	Lysine	lys_L	HMDB00182	C6H15N2O2		69.71-456.79	
Tests measuring sum of amino acids							
XLeu/Ala (ath)2	Sum of leucine, isoleucine and hydroxyproline/ Alanine	leu_L, ile_L, 4hpro_LT/ ala_L				0.33-1.79	
XLeu/Phe (ath)	Sum of leucine, isoleucine and hydroxyproline/ Phenylalanine	leu_L, ile_L, 4hpro_LT/ phe_L				1.86-7.19	
XLeu/Ile/HOpro	Sum of leucine, isoleucine and hydroxyproline	leu_L, ile_L, 4hpro_LT				81.75-318.73	
Non-standard amino acids							
Cit	Citrulline	citr_L	HMDB00904	C6H13N3O3		3.52-33.1	6.0-28
Ornithine	Ornithine	orn	HMDB00214	C5H13N2O2		23.73-316.26	
Methylhistidin	Methyl-histidine					19.77-2.68	
ASA	Arginino-succinate	argsuc	HMDB00052	C10H17N4O6		0.00-2.06	0.04-0.66
Phenylketonuria specific tests							
Phe Int Test	Phenylalanine					7.7E+07-3.76E+0	

	internal standard test					8	
Phe-skimun	Statistical test, Phenylketonuria specific test					30.46-97.39	
Phe-monitoring	Phenylketonuria specific test					23.93-76.52	
Ratios of amino acids							
Phe/Tyr (ath)	Phenylalanine/Tyrosine	phe_L/ tyr_L				0.12-1.77	
Met/Phe (ath)	Methionine/Phenylalanine	met_L/ phe_L				0.13-1.59	
Val/Phe (ath)	Valine/Phenylalanine	val_L/phe_L				1.48-6.46	
Ratios of acylcarnitines							
C3/C16	Propionyl carnitine/ Palmitoyl carnitine	pcrn/pmtcrn				0.15-2.46	
C3/C2	Propionyl carnitine/ Acetyl carnitine	pcrn/ acrn				0.02-0.19	
C4/C2	Butyryl carnitine/ Acetyl carnitine	c4crn/ acrn				0.00-0.05	
C4/C3	Butyryl carnitine/ Propionyl carnitine	c4crn/ pcrn				0.03-0.79	
C5/C0	Isovaleryl carnitine/ Free carnitine	ivcrn/crn				0.00-0.03	
C5/C2	Isovaleryl carnitine/ Acetyl carnitine	ivcrn/acrn				0.00-0.02	
C5/C3	Isovaleryl carnitine/ Propionyl carnitine	ivcrn/pcrn				0.02-0.44	
C5-OH/C0	3-hydroxy-isovaleryl carnitine/ Free carnitine	3ivcrn/crn				0.00-0.05	
C5-OH/C8	3-hydroxy-isovaleryl carnitine/Octanoyl	3ivcrn/ c8crn				0.00-25.67	

	carnitine						
C8/C10	Octanoyl carnitine/ Decanoyl carnitine	c8crn/ c10crn				0.00-1.91	
C8/C2	Octanoyl carnitine/ Acetyl carnitine	c8crn/acrn				0.00-0.01	
C3DC/ C10	Malonyl carnitine/ Decanoyl carnitine	c3dc/ c10crn				0.00-3.02	
C14:1/C 12:1	Tetradecen oyl carnitine/ Dodeceno yl carnitine	tetdece1crn/ ddece1crn				0.00-16.1	
C14:1/C 16	Tetradecen oyl carnitine/ Palmitoyl carnitine	tetdece1crn/ pmtcrn				0.00-0.1	
C14:1/C 2	Tetradecen oyl carnitine/ Acetyl carnitine	tetdece1crn/ acrn				0.00-0.01	
C14:1/C 4	Tetradecen oyl carnitine/ Butyryl carnitine	tetdece1crn/c 4crn				0.00-1.64	
C5DC/ C16	Glutaryl carnitine/ Palmitoyl carnitine	c5dc/ pmtcrn				0.00-0.08	
C5DC/ C5-OH	Glutaryl carnitine/3 -hydroxy- isovaleryl carnitine	c5dc/3ivcrn				0.02-0.91	
C5DC/ C8	Glutaryl carnitine/ Octanoyl carnitine	c5dc/ c8crn				0.00-2.48	
C16-OH/C16	3-hydroxyhe xadecanoy lcarnitine/ Palmitoyl carnitine	3hexdcrn/pmt crn				0.00-0.06	

Table S4: Distribution of 235 IEMs based on their mode of inheritance. Majority of the IEMs have an autosomal recessive mode of inheritance.

Mode of inheritance	Number of IEMs
Autosomal recessive	163
X-linked pattern	16
Autosomal dominant	11
Autosomal recessive or autosomal dominant	7
X-linked or autosomal dominant	2

Table S5: Different phenotypic forms observed for the 235 IEMs. However, there can be various mutations in a gene, leading to a broader variety of phenotypic characteristics.

Phenotypic forms	Nubeer of IEMs
2 phenotypic forms	**39**
3 phenotypic forms	**33**
4 phenotypic forms	**9**
5 phenotypic forms	**5**
6 phenotypic forms	**1**
7 phenotypic forms	**3**

Table S6: Different therapeutic measures used for treatment of IEMs.

Therapeutic measures	Number of IEMs
Gene therapy	1
Enzyme therapy	6
Organ transplantation	24
No treatment	27
Medications	50
Diet	54

Table S7: Databases referred during the acylcarnitine reconstruction.

Databases	Type of information extracted
EntrezGene[20] (http://www.ncbi.nlm.nih.gov/gene) **HUGO Gene nomenclature committee (http://www.genenames.org/)** **Ensembl (http://www.ensembl.org/index.html)** **GeneCards (http://www.genecards.org/)**	Genome annotation
UniProt[21] (http://www.uniprot.org/)	Protein localization
BRENDA[22] (http://www.brenda-enzymes.org/) **ExPasy[23] (http://expasy.org/)**	Enzyme and E.C number
HMDB[24] (http://www.hmdb.ca/) **KEGG[25] (http://www.genome.jp/kegg/)** **BiGG[26] (http://bigg.ucsd.edu/)** **PubChem[20] (http://pubchem.ncbi.nlm.nih.gov/)** **ChEBI[27] (http://www.ebi.ac.uk/chebi/)**	Metabolite information

Table S8: Databases used to compile the compendium of IEMs

Databases	Web address
Genetics home reference	http://ghr.nlm.nih.gov/
Gene reviews	http://www.ncbi.nlm.nih.gov/books/NBK1116/
Metabolic and genetic information centre	http://www.metagene.de/
Human metabolome database	http://www.hmdb.ca/
Orphanet	http://www.orpha.net/consor/cgi-bin/Disease.php
Online mendelian inheritance in man	http://www.ncbi.nlm.nih.gov/omim
The online metabolic and molecular bases of inherited disease	http://www.ommbid.com/
EntrezGene	http://www.ncbi.nlm.nih.gov/gene
UniProt	http://www.uniprot.org/

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