Opening a window on lysosomal acid lipase deficiency: new biochemical and molecular insights

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Supplementary Table 1. LAL enzyme activity with respect to sample type, age, and gender in fibroblasts, liver specimens, amniocytes, and chorionic villus sampling.

Sample type		**Number of patients with LAL-D** mean age at diagnosis, years±SD	**LAL activity value in confirmed LAL-D patients** mean±SD pmol/min/mg protein
WBS (n=625) (normal values >25 pmol/min/mg protein)		37 (23.6±25.4)	5.90±4.27
Age (years±SD)	25.1±22.9		
Males tested	351		
Females tested	251		
Gender unknown tested	23		
Fibroblasts (n=30) (normal range 100-290 pmol/min/mg protein)		7 (12.91±14.73)	5.74±0.71
Age (years±SD)	12.94±17.29		
Males tested	13		
Females tested	2		
Gender unknown tested	15		
Liver (n=4) (normal range 20-200 pmol/min/mg protein)		0	N/A
Age (years±SD)	7.06±5.9		
Males tested	0		
Females tested	4		
Gender unknown tested	0		
Amniocytes (n=13) (normal range for fetal cells 40-160 pmol/min/mg protein)	N/A	3 (N/A)	1.4±0.75
CVS (n=9) (normal range for fetal cells 40-160 pmol/min/mg protein)	N/A	0	N/A

N/A: Not applicable; WBC: white blood cell, CVS: chorionic villus sampling; LAL: lysosomal acid lipase activity, LAL-D: acid lipase activity deficiency.

Supplementary Table 2. The two novel mutations, p.S103R and p.A286P, with *in-silico* predictions of pathogenicity by SIFT (Sorting Intolerant From Tolerant), Polyphen2, LRT (likelihood ratio test), Mutation Taster, and CADD (Combined Annotation Dependent Depletion) are shown. The variants, p.S103R and p.A286P, are not present in 1000Genomes, and the mutation p.A286P is not present in ExAc, while the mutation p.S103R in 1/121,406 alleles (www.ExAc.broadinstitute.org).

Chr	AA Change	SIFT score	SIFT pred	Polyphen2 HDIV score	Polyphen2 HDIV pred	LRT pred	Mutation Taster score	Mutation Taster pred	CADD phred
10:90988076G>T	LIPA:NM_000235:exon4:c.C309A:p.S103R,LIPA:NM_001127605:exon4:c.C309A:p.S103R	0	D	1.0	D	D	1.000	D	18.45
10:90982306C>G	LIPA:NM_001288979:exon6:c.G508C:p.A170P, LIPA:NM_000235:exon8:c.G856C:p.A286P, LIPA:NM_001127605:exon8:c.G856C:p.A286P	0.01	D	0.996	D	D	1.000	D	26.8

SIFT scores < 0.05 mutation predicted to be deleterious; PolyPhen-2 scores closer to 1 indicate that the amino acid substitution is deleterious. D: deleterious. Chr: chromosome, AA: amino acid.

Supplementary Table 3. All *LIPA* pathogenic variants reported to date. Novel mutations reported in this study are in bold.

	Reference	**c.DNA**	**Protein**	exon/intron
1	Lin et al. 2015	c.57_60delTGAG		exon2
2	Stein et al., 2007	c.67G>A, c.260G>T	p.Gly23Arg, p.Gly87Val	exon2, exon4
3	Wiebusch et al., 1996	c.67G>A, c.894G>A	p.Gly23Arg, p.Ser275_Gln298del	exon2, exon8
4	ExAc (LOF)	c.111+1G>A		intron2
5	Fujiyama et al., 1996		p.Tyr43Ter	exon3
6	Lohse et al., 1999	c.119_126del	p.Tyr43Isofs*6	exon3
7	Lohse et al., 1999	c.120_127delTATCTCTT	p.Ile41Leufs*5	exon3
8	ExAc (LOF)		p.Ser42LeufsTer6	exon3
9	ExAc (LOF)	c.132G>A	p.Trp44Ter	exon3
10	Elleder et al., 1999	c.170A>T	p.Asp57Val	exon3
11	Redonnet-Vernhet et al., 1997	c.193C>T	p.Arg65Ter	exon3
12	Fasano et al., 2012	c.229+1G>A	IVS3 ds G-A +1	intron3
13	Pisciotta et al., 2017	c.229+3A > C		intron3
14	Pisciotta et al., 2009	c.230_231ins35;2323_245del	p.Pro78Alafs*5	intron3
15	Pagani 1998	c.254A>G	p.Gln85Arg	exon4
16	Santillán-Hernández et al., 2015	c.253C>A	p.Gln85Lys	exon4
17	CLINVAR (mutations not previously described)	c.253C>T	p.Gln85Ter	exon4
18	Reynders et al., 2016	c.256C>T	p.His86Tyr	exon4
19	Pagani 1996	c.260G>T	p.Gly87Val	exon4
20	Elleder et al., 1999	c.283T>A	p.Trp95Arg	exon4
21	Santillán-Hernández et al., 2015	c.294C>G	p.Asn98Lys	exon4
22	**Cappuccio, Donti et al., 2018**	**c.309C>A**	**p.Ser103Arg**	**exon4**
23	Huang et al., 2012	c.317_318insT	p.I107Hfs*4	exon4
24	Anderson et al., 1999	c.347G>A	p.Trp116Ter	exon4
25	ExAc (LOF)	c.351delinsCCG	p.Met117IlefsTer45	exon4

26	Hooper et al., 2008	c.356A>G	p.Asn119ser	exon4
27	Gramatges et a al., 2009	c.361A>G	p.Arg121Gly	exon4
28	Reynders et al., 2016	c.377C>T	p.Ser126Phe	exon4
29	Ries et al., 1998	c.386A>C	p.His129Pro	exon4
30	Ries et al., 1998	c.386A>G	p.His129Arg	exon4
31	Anderson et al., 1999	c.397_398delTC	p.Val134Phefs*4	exon4
32	ExAc (LOF)	c.401delinsGT	p.Val134GlyfsTer5	exon4
33	Seedorf et al., 1999	c.398delC	p.Ser133Ter	exon4
34	Lohse et al., 1999	c.414dupA	p.Phe139Isofs*7	exon4
35	Reynders et al., 2016	c.417C>A	p.Phe139Leu	exon4
36	Fasano et al., 2012	c.419G>A	p.Trp140Ter	exon4
37	Maciejko et al., 2017	c.428+1G>A	IVS4 ds G-A +1	intron4
38	Elleder et al., 1999	c.435T>A	p.Asp145Glu	exon5
39	Lee et al., 2011	c.482delA	p.Asn161Ifs*18	exon5
40	ExAc (LOF)		p.Asn161IlefsTer19	exon5
41	Chora et al., 2017	c.528C>T	p.Gly176Gly	exon5
42	Reynders et al., 2016	c.526G>A	p.Gly176Ser	exon5
43	Reynders et al., 2016	c.538+5G>A	IVS5 ds G-A +5	intron5
44	Chora et al., 2017	c.538+6T>C	IVS5 ds T-C +6	intron5
45	ExAc (LOF)	c.565G>T	p.Glu189Ter	exon6
46	Anderson et al., 1994	c.594dupT	p.Ala199Cysfs*13	exon6
47	Malsen et al., 1993	c.599T>C	p.Leu200Pro	exon6
48	Klima et al., 1993		p.Leu200Pro, p.Phe208Leu, p.Gly266Ter	exon6-7
49	Pagani 1996	c.605C>T	p.Pro202Leu	exon6
50	Kuranobu et al., 2015	c.607G>C	p.Val203Leu	exon6
51	Elleder et al., 2000	c.635delC	p.Pro212Leufs*5	exon6
52	Pisciotta et al., 2009	c.652C>T	p.Arg218Ter	exon6
53	Chora et al., 2017	c.676-23T>C	IVS6 as T-C -23	intron6
54	Pagani et al., 1996	c.676-2A>G	IVS6 as A-G -2	intron6
55	ExAc (LOF)	c.676-2A>T		intron6

56	Anderson et al., 1999	c.684delT	p.Phe228Leufs*13	exon7
57	Krivit et al., 2000	c.724delT	Trp242Glyfs*12	exon7
58	Kuranobu et al., 2015	c.791T>C	p.Leu264Pro	exon7
59	Aslanidis et al., 1996	c.796G>T	p.Gly266Ter	exon7
60	Kojima et al., 2013		p.Asn271His	exon7
61	Yang et al., 2009	c.822+1G>A	IVS7 ds G-A +1	intron7
62	Reynders et al., 2016	c.822+1G>C	IVS7 ds G-C +1	intron7
63	Ghosh et al., 2017	c.824C>T	p.Ser275Phe	exon8
64	Chora et al., 2017	c.846A>G	p.Thr282Thr	exon8
65	Pagani 1998	c.863C>T	p.Thr288Ile	exon8
66	**Cappuccio, Donti et al. 2018**	**c.856G>C**	**p.Ala286Pro**	**exon8**
67	Anderson et al., 1999	c.866C>G	p.Ser289Cys	exon8
68	Pagani et al., 1996	c.881T>C	p.Leu294Ser	exon8
69	Pagani et al., 1994	c.883C>T	p.His295Tyr	exon8
70	Chora et al., 2017	c.891C>T	p.Ser297Ser	exon8
71	Gómez-Nájera et al., 2015	c.894G>C	p.Gln298His	exon8
72	Klima et al., 1993	c.894G>A	IVS8 ds G-A -1	exon8
73	Ries et al., 1996	c.892C>T	p.Gln298Ter	exon8
74	Aslanidis et al., 1996	c.894+1G>A	IVS8 ds G-A +1	intron8
75	ExAc (LOF)		p.Val300AsnfsTer30	exon9
76	Reynders et al., 2016	c.931G>A	p.Gly311Arg	exon9
77	ExAc (LOF)		p.Tyr317Ter	exon9
78	Sjouke et al., 2016	c.966+3A>T	IVS9 ds A-T +3	intron9
79	Seedorf 1999		p.Tyr324Ter	exon10
80	Ameis et al., 1995	c.967_968delAG	p.Ser323Leufs*44	exon10
81	Reynders et al., 2016	c.974C>T	p.Pro325Leu	exon10
82	Anderson et al., 1999	c.980delC	p.Thr327Asnfs*4	exon10
83	Anderson et al., 1999	c.1024G>A	p.Gly342Arg	exon10
84	Lohse et al., 1999	c.1024G>T	p.Gly342Trp	exon10
85	Lohse et al., 2000	c.1028delG	p.Gly343Valfs*15	exon10
86	Pisciotta et al., 2017	c.1033G > A	p.Asp345Asn	exon10

87	Sjouke el al., 2015	c.1067T>G	p. Leu356Ter	exon10
88	Seedorf 1999	c.1070T>C	p.Leu357Pro	exon10
89	ExAc (LOF)		p.Pro370ArgfsTer25	exon10
90	Chora et al., 2017	c.1113A>G	p. Glu371Glu	exon10
91	Chora et al., 2017	c.1188G>A	p.Arg396Arg	exon10
	Gross rearrangements			
1	Lee et al., 2011	at least part of ex. 4		
2	Ghosh et al., 2017	entire *LIPA* gene		
3	Anderson et al., 1999	*LIPA* ex. 4	r.230_428del	
4	Pullinger et al., 2015	incl. *LIPA* ex. 4		
5	Ries et al., 1998	*LIPA* intr. 1 to ex. 4		
6	Lee et al., 2011	*LIPA* 5'Ex 4 del		
7	Pisciotta et al., 2009	c.229-33_c.230dup, c.232_245del	p.Gly77fs*5	
8	Ries et al., 1998	c.64-?_428+?del	exon2_4 del	