The global prevalence and genetic spectrum of lysosomal acid lipase deficiency: an ultra-rare mimic of NAFLD

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List of abbreviations:

LAL-D, lysosomal acid lipase deficiency; NAFLD, non-alcoholic fatty liver disease; LAL, lysosomal acid lipase, CESD, cholesteryl ester storage disease; E8SJM, exon 8 splice junction mutation; AXIS, Appraisal tool for cross-sectional studies; RoB, Risk of bias; Het, heterozygote carrier of *LIPA* mutation; hom, homozygous patient affected with LAL-D; CAD, coronary artery disease; WD, Wolman disease; ACMG, American College of Medical Genetics and Genomics; ExAC, Exome Aggregation Consortium; ESP, Exome Sequencing Project; 1000G, 1000 Genomes Project; gnomAD, Genome Aggregation Database; CI, confidence interval; AF, allele frequency; AFR, African / African American; ASJ, Ashkenazi Jewish; EAS, East Asian, FIN, Finnish; NFE, Non-Finnish European; LAT, Latino; OTH, Other; SAS, South Asian.

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Conflict of interest

The authors have no conflict of interest to declare.

ABSTRACT

Background & Aims: Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive condition that may present in a mild form (cholesteryl ester storage disease (CESD)), which mimics non-alcoholic fatty liver disease (NAFLD). It has been suggested that CESD may affect 1 in 40,000 and is under-diagnosed in NAFLD clinics. Therefore, we aimed to estimate the prevalence of LAL-D using analysis of genetic variation in *LIPA*.

Methods: MEDLINE and EMBASE were systematically searched for previously reported disease variants and prevalence estimates. Previous prevalence estimates were meta-analysed. Disease variants in *LIPA* were annotated with allele frequencies from gnomAD and combined with unreported major functional variants found in humans. Pooled ethnicity-specific prevalences for LAL-D and CESD were calculated using the Hardy-Weinberg equation.

Results: Meta-analysis of existing genetic studies estimated the prevalence of LAL-D as 1 per 160,000 (95% CI 1 per 65,025-761,652) using the allele frequency of c.894G>A in *LIPA*. 98 previously reported disease variants in LIPA were identified, of which 32/98 were present in gnomAD, gave a prevalence of 1 per 307,482 (95% CI 257,672 – 366,865). Wolman disease was associated with more loss-of-function variants than CESD. When this was combined with 22 previously unreported major functional variants in *LIPA* identified in humans the pooled prevalence of LAL-D was 1 per 177,452 (95% CI 149,467 – 210,683) with a carrier frequency of 1 per 421. Prevalence is lowest in those of East Asian, South Asian, and Finnish ancestry.

Conclusion: Using 120 disease variants in *LIPA*, these data can reassure clinicians that LAL-D is an ultra-rare disorder. Given the therapeutic capability of sebelipase alpha, investigation for LAL-D might be included in second-line metabolic screening in NAFLD.

Word count: 273

LAY SUMMARY

Lysosomal Acid Lipase Deficiency (LAL-D) is a rare genetic condition that can cause severe liver disease but it is difficult to diagnose and sometimes can look like simple fatty liver. It wasn't clear how common LAL-D was and whether many cases were being missed. To study this, we searched for all genetic mutations that could cause LAL-D, calculated how common those mutations were, and added them up. This let us estimate that LAL-D affects roughly 1 in 175,000 people. We conclude that LAL-D is a very rare condition but it is treatable so may be included in a 'second-line' of tests for causes of fatty liver.

INTRODUCTION

Lysosomal Acid Lipase Deficiency (LAL-D) is an autosomal recessive disorder caused by mutations in *LIPA* that manifests as a spectrum of liver disease and dyslipidaemia[1,2]. It is regarded as a rare disorder however recognition of more mild forms of the condition have led to the suggestions that it may represent a significant proportion of patients presenting with non-alcoholic fatty liver disease (NAFLD)[3,4]. Emerging data have also reported reduced lysosomal acid lipase (LAL) activity in association with more advanced NAFLD[5–8]. Clarity on the prevalence of LAL-D, and its distribution across ethnicities, is needed to determine whether LAL activity testing should be a routine part of NAFLD clinics.

LIPA encodes the lipase A protein, which functions to catalyse the hydrolysis of cholesteryl esters and triglycerides in the lysosome[9,10]. Loss of function mutations are associated with the accumulation of cholesteryl esters in lysosomes in hepatocytes, macrophages, and the spleen. LAL-D describes a spectrum of clinical disease where its most severe form is Wolman disease (with <1% LIPA activity), presenting with acute liver failure at under 4 months of age, microvesicular steatosis, hepatosplenomegaly, failure to thrive, and adrenal calcification. Without treatment, Wolman disease is fatal at under a year but enzyme replacement therapy significantly improves prognosis. Cholesteryl ester storage disease (CESD) is used to refer to a more mild form of the disorder, characterised by 1-5% LIPA activity[11,12]. It presents insidiously with elevated aminotransferases, hepatic steatosis, dyslipidaemia, gradual decline in hepatic function, and premature atherosclerosis. These conditions are difficult to diagnose; it is unclear how many children die of Wolman's disease undiagnosed and there is currently no consensus on routine testing for *LIPA* activity in adults presenting with NAFLD.

The true prevalence of LAL-D (both CESD and Wolman disease) is not known. The most common LIPA variant associated with CESD is a splice-junction mutation in exon 8 (rs116928232: c.894G>A, p.S275_Q298del), referred to as E8SJM[13], which has been used to derive an estimated prevalence

as high as 1 per 40,000. This is much higher than clinical observations, leading to the suggestion that LAL-D is underdiagnosed and may be unmasked in patients presenting with NAFLD[3,14–16].

It is also not yet established whether heterozygous carriers of pathogenic *LIPA* variants are at increased risk of progressive liver disease in combination with other insults to the hepatic parenchyma. Data are conflicting as to whether the heterozygous carrier state influences circulating lipid profile[17,18].

Based on these data, we hypothesised that the clinical prevalence of LAL-D would be lower than the estimated genetic prevalence and that there may be a minority of NAFLD patients with undiagnosed LAL-D. Therefore, in order to address this and estimate the potential impact of under-diagnosis we aimed to calculate the population prevalence of LAL-D by: 1. Meta-analysis of previous prevalence estimates; 2. Aggregation of population allele frequencies for previously reported pathogenic *LIPA* variants; and 3. Identification of previously unreported major functional *LIPA* variants from next generation sequencing data.

METHODS

Systematic review of previous prevalence estimates

NCBI Pubmed/MEDLINE and EMBASE were systematically searched for all articles related to LIPA, Wolman disease (WD), CESD, or LAL-D (see Supplementary Methods for full search term used). The search was finalised on 12/07/2018. Two reviewers (AC & JPM) independently screened abstracts to assess relevance. Any disagreements were resolved through discussion with a third reviewer. Inclusion criteria were: reporting an original estimate of prevalence or incidence or pathogenic allele frequency for LAL-D, CESD, or Wolman disease, determined by either genetically or by identification of clinical cases. Reviews, commentaries and editorials reporting non-original data; and *in vitro* or non-human studies were excluded.

Included articles were first grouped into those reporting the prevalence of LAL-D, CESD, or WD, in the general population and those reporting the prevalence in specific patient cohorts. Genetic studies (reporting prevalence derived from LIPA sequencing or pathogenic allele frequency) and epidemiological studies (reporting identification of clinical cases) were assessed separately. It was also recorded whether studies referred to the prevalence of LAL-D, CESD, and/or Wolman Disease. Three prevalence estimates from genetic studies of population cohorts were suitable for meta-analysis using an inverse variance model with a double arcsin transformation[19]. Variance was defined as p[1–p]/POP, with POP representing the sample size for genetic studies. Included studies were assessed for risk of bias using the Appraisal tool for Cross-Sectional Studies (AXIS). Meta-analysis of clinical/epidemiological studies was used to derive a pooled prevalence at birth. Meta-analysis of genetic studies gave an overall mutant allele frequency, which was used to calculate a prevalence of LAL-D (at birth) via the Hardy Weinberg equation.

Identification of previously reported LIPA variants

A systematic search was performed to identify all previously reported pathogenic variants in LIPA. The same search of Pubmed/MEDLINE and EMBASE (as described above) was used. Two independent reviewers screened abstracts to determine suitability for inclusion and disagreements were resolved through discussion with a third reviewer. Inclusion criteria were describing variants in LIPA in humans associated with a loss of function and/or LAL-D disease spectrum. Articles not in English and *in vitro* or non-human studies were excluded. Variants reported as disease-causing, pathogenic, probably pathogenic, or harmful were extracted.

In addition, ClinVar (www.ncbi.nlm.nih.gov/clinvar/, accessed 12/07/2018) was screened for LIPA variants with published reports of pathogenicity and these were added to our list. Mutations derived from personal communications and without a linked publication were excluded.
Variants were annotated as being associated with Wolman disease, CESD, or LAL-D (if both, or not otherwise stated).

Variants were classified on a case by case basis into 'pathogenic' and 'likely pathogenic' according to ACMG criteria[20]. Variants classified as 'benign', 'likely benign', or of 'uncertain significance' were excluded. (Likely) Pathogenic variants for Wolman disease were those found in patients with: hepatic steatosis, raised aminotransferases, reduced LAL activity, and fatal under age 1 (excepting of cases in a trial of sebelipase alpha); and for CESD as: the presence of hepatic steatosis with dyslipidaemia with reduced LAL activity, with onset over 1 year of age. In addition to ACMG criteria, variants were classified as 'likely pathogenic' if there was no evidence of reduced LAL activity in the affected patients or when there were conflicting reports regarding individual variants.

Identification of previously unreported major functional variants in LIPA

Ensembl (http://www.ensembl.org/, accessed 12/07/2018) for LIPA transcript ENST00000336233.9 was used to search for all *LIPA* variants identified in humans via the Exome Aggregation Consortium

(ExAC) [21], Exome Sequencing Project (ESP), or 1000 Genomes Project (1000G) [22]. Those with predicted major consequences on protein structure and function were extracted, including: frameshift, premature stop codon, initiator codon, splice donor, splice acceptor, and start loss variants. The Genome Aggregation Database (gnomAD, gnomad.broadinstitute.org/, accessed 12/07/2018) was also searched and all duplicates were removed. Coverage was assessed in gnomAD variants using specific site quality metrics. Non-PASS filter variants were excluded. This list of major functional variants was compared with previously reported *LIPA* variants found in patients to identify unreported variants predicted to affect LAL activity. Finally, variants underwent frequency filtering[23].

Annotation of variants with allele frequencies and functional predictions

Coding sequence nucleotide changes for each identified variant were converted to HGVS format (hg38) using Mutalyzer (https://mutalyzer.nl/). The Ensembl Variant Effect Predictor (http://www.ensembl.org/Tools/VEP), was used to annotate variants with SIFT/PolyPhen-2 *in silico* predictions of pathogenicity and population allele frequencies from the 1000G, ESP, ExAC, and gnomAD data sets. Where variants had been identified in more than one dataset the larger cohort was used.

LIPA structural information and modelling

Data on the domain structure and active site positions for LIPA was extracted from UniProtKB (https://uniprot.org) for P38571[24]. Modelling was extracted from ExPasy SWISS-MODEL (https://swissmodel.expasy.org) for P38571, based upon the gastric triacylglycerol lipase[10,25,26]. Detail on the exon structure of LIPA was also retrieved from neXtProt (https://nextprot.org)[27].

Prevalence estimation for CESD, Wolman disease, and LAL-D

We performed a prevalence estimation without allele frequency filtering in an attempt to identify the maximum genetic prevalence of LAL-D. Total allele frequencies were used to calculate estimations of global population prevalence of homozygous and heterozygous LAL-D genotypes using the Hardy-Weinberg equation. This was performed for: previously reported pathogenic variants (separately for CESD and Wolman), previously reported pathogenic plus possibly-pathogenic variants, and then previously reported plus unreported major functional variants.

In addition, we estimated the prevalence across 8 different ethnicities. 95% confidence intervals (CI) were calculated for the total allele frequencies using the Wilson score method across a binomial distribution where more than 5 alleles had been identified.

Software

Meta-analysis was performed using the Meta-XL add-in for Microsoft Excel (www.epigear.com) and GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com (see Supplementary CTAT Table).

RESULTS

Meta-analysis of previous prevalence estimates

1926 abstracts were identified by systematic search, of which 194 studies were included (Supplementary Figure 1). 9 reported the prevalence of WD or CESD in the general population (Table 1) of which 6/9 used clinically identified cases and 3/9 used genetic sequencing of *LIPA*. Studies were generally of high quality and low risk of bias (Table 1 & Supplementary Figure 2).

Epidemiological studies were retrospective in nature and predominantly identified patients with WD from national or regional referral centres for inherited metabolic disease. Prevalences ranged from 1 per 165,530 to 1 per 909,091 (Supplementary Figure 3). Meta-analysis of prevalence estimates using random effects model gave a prevalence of 1 per 451,116 (95% CI 282,022 – 840,092) with a carrier frequency of 1 per 336 (95% CI 266-458).

All genetic studies used sequencing for c.894G>A in *LIPA* to generate a pathogenic allele frequency (AF). Disease prevalence estimates were then calculated using the proportion of pathogenic *LIPA* variants comprised by c.894G>A (estimated 50-60%, where Scott et al. calculated 95% CI 51-69%)[4,34].

Meta-analysis of c.894G>A genetic studies using a random effects model gave a pooled AF of 0.0015 (95% CI 0.001-0.002, Figure 1). Assuming that c.894G>A accounts for 60% (95% CI: 51%-69%)[34] of all CESD mutations, this corresponds to a prevalence for CESD of 1 per 160,000 (95% CI: 1 per 65,025-761,652) and a carrier frequency of 1 per 400 (95% CI: 1 per 255-873).

Study	Cohort assessed	Diagnostic method	Population (n)	Cases (n)	Reported prevalence	Comments	Quality (AXIS tool)
Clinical studies							
Giugliani et al., 2017 (24) [Brazil]	General population	Clinical diagnosis lysosomal acid lipase deficiency	41,719,041 births (part of study period)	10 patients	0.011 per 100,000	Birth data only available for part of the study period. May be an under-estimation.	High quality, low RoB
Moammar et al., 2010 (25) [Saudi Arabia]	General population	Clinical diagnosis Wolman disease	165,530	1 patient	Not stated	Only 1 case identified. No calculation of prevalence.	High quality, low RoB
Poupětová et al., 2010 (26) [Czech Republic]	General population	Clinical diagnosis Wolman disease or CESD	5,480,885 births	15 patients	1 per 365,392 hom affected; 1 per 302 het carrier	Majority of patients had juvenile CESD. No confidence intervals calculated for CESD.	High quality, low RoB
Dionisi-Vici et al., 2002 (27) [Italy]	General population	Clinical diagnosis Wolman disease / CESD	7,13,959 births	9 patients	Not stated	No calculation of prevalence.	High quality, low RoB
Applegarth et al., 2000 (28) [Canada]	General population	Clinical diagnosis Wolman disease	1,035,816 births	6 patients	Not stated	No calculation of prevalence. Stringent diagnostic criteria used.	High quality, low RoB
Meikle *et al.*, 1999 (29) [Australia]	General population	Clinical diagnosis Wolman disease	4,222,323 births	8 patients	1 per 528,000 hom affected; 1 per 363 het carrier	WD only, not CESD/LAL-D. Australian population ancestry.	High quality, very low RoB
Genetic studies							
Scott *et al.*, 2013 (30) [United States]	General population	LIPA sequencing for c.894G>A	33,156 alleles	53 alleles	0.8 per 100,000	Estimated c.894G>A accounted for 60% of LAL-D. Multiple genetic ancestries included.	High quality, low RoB
Stitziel *et al.*, 2013 (18) [United States]	i. General population with lipid profile data ii. General population with CAD data	LIPA sequencing for c.894G>A	i. 26,388 alleles ii. 54,944 alleles	i. 42 het ii. 60 het	Not stated	No association with lipid profile or CAD in heterozygous state. European ancestry population.	High quality, low RoB
Muntoni *et al.*, 2007 (4) [Germany]	General population	LIPA sequencing for c.894G>A	4,046 alleles	10 alleles	1 per 40,000 hom affected; 1 in 200 het carrier	Estimated c.894G>A accounted for 50% of LAL-D. European ancestry population.	High quality, low RoB

Table 1. Epidemiological and genetic studies estimating the prevalence of lysosomal acid lipase deficiency. AXIS, Appraisal tool for cross-sectional studies; RoB, Risk of bias; Het, heterozygote carrier of *LIPA* mutation; hom, homozygous patient affected with LAL-D; CAD, coronary artery disease.

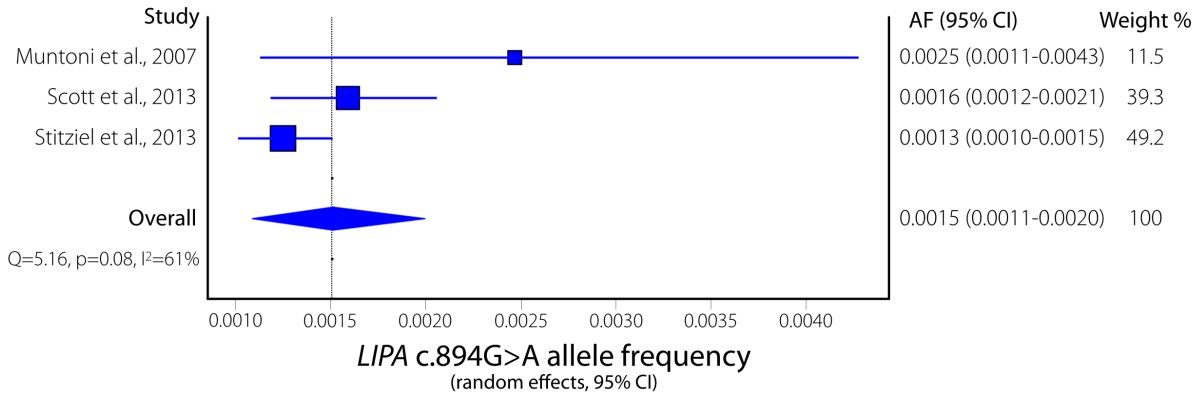

Figure 1. Meta-analysis of genetic studies estimating the prevalence of LAL-D using c.894G>A allele frequency.

We also identified 8 studies reporting the prevalence of CESD in specific patient populations: 5 in cohorts of patients with dyslipidaemia (n=4,193 patients) and 3 in cohorts with liver disease (n=430, Supplementary Table 1). 2/8 (25%) studies used *LIPA* sequencing and 6/8 assessed LAL activity. These data were not suitable for meta-analysis due to significant patient and methodological heterogeneity.

In light of these wide confidence intervals and assumption of pathogenic contribution we then proceeded to attempt to obtain a more accurate estimate of global LAL-D prevalence, using publicly available sequencing data.

Identification and analysis of previously reported LIPA variants

After removal of duplicates and benign variants (Supplementary Figure 4), 98 disease-causing variants in *LIPA* were identified from publications (Supplementary Table 2). 23/98 (23%) had been associated with WD only, 55/98 (56%) with CESD only), and the remainder with either both or not stated. 36/98 (37%) of variants were classified as pathogenic and the remaining 62/98 (63%) likely pathogenic. The exonic distribution along *LIPA* of protein-coding variants and relationship to the active site is shown in Figure 2. The most common mutation consequence was missense, accounting

for 43% (42/98) of all disease variants (Figure 3A). However, this was not evenly distributed across the LAL-D spectrum: Wolman disease was associated with a higher proportion of frameshift mutations and premature stop variants (Figure 3C), compared to CESD (Figure 3B).

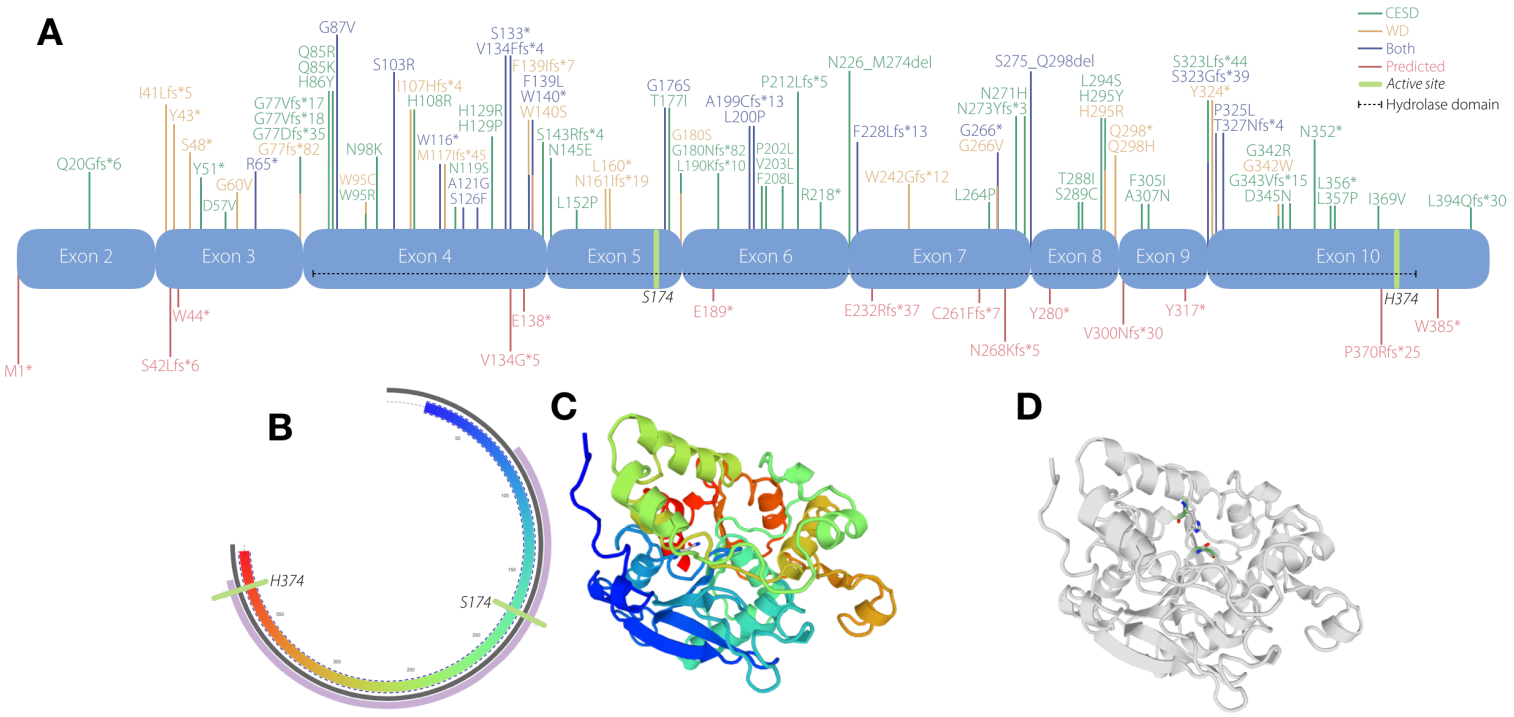

Figure 2. The exonic distribution of previously reported protein coding disease mutations and unreported major functional variants in *LIPA* (A). A modelled structure of lysosomal acid lipase (B & C) with its active site highlighted (D).

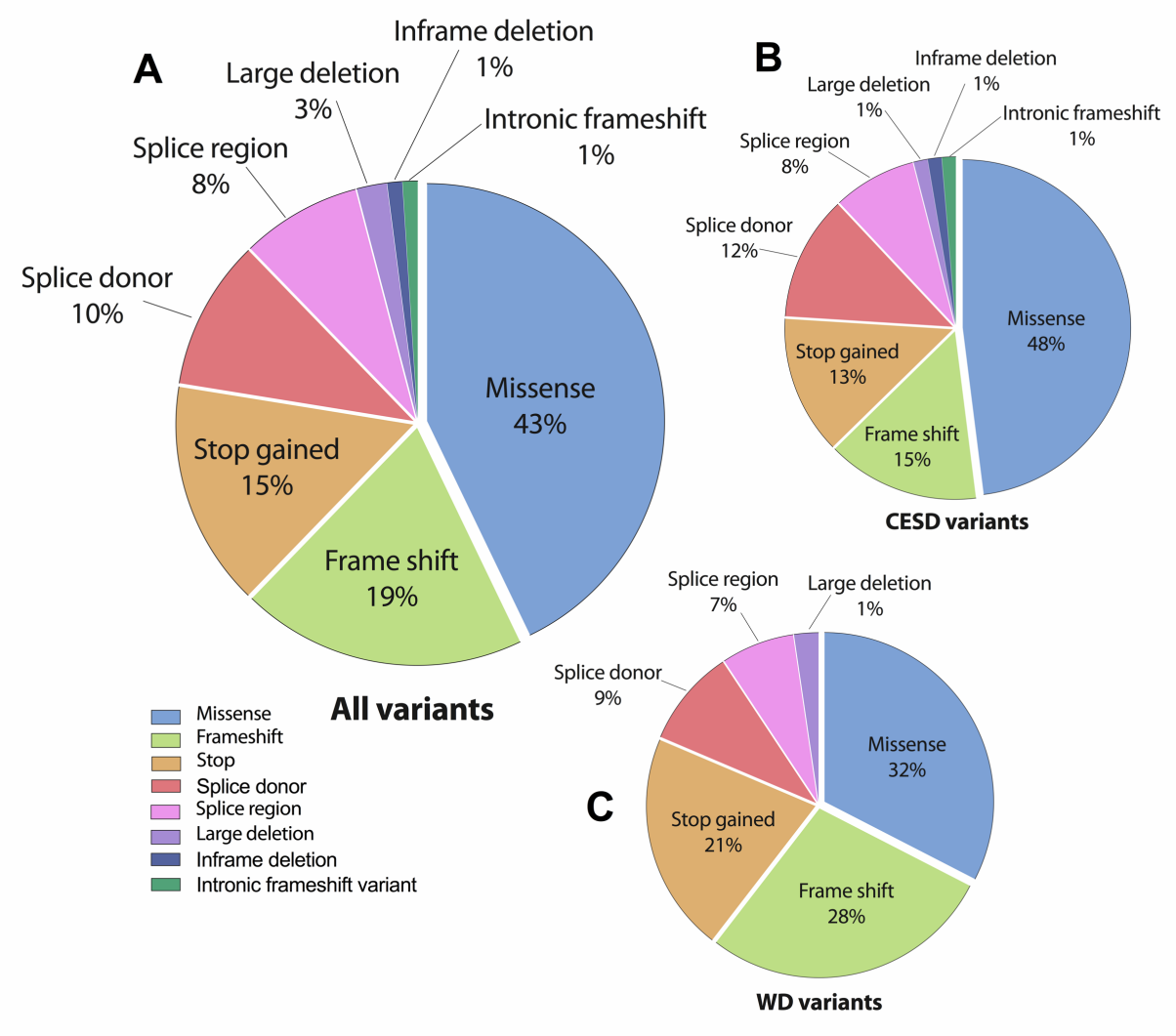

Figure 3. Functional classification of disease variants in LIPA (A), those associated with only CESD (B) and those with WD (C).

32/98 (33%) of disease variants had been identified in population databases (Table 2); all variants found in ESP, ExAC, or 1000G were also found in gnomAD and therefore only gnomAD allele frequencies (AF) are reported. The most frequently reported allele (E8SJM, c.894G>A) had the highest AF of 0.0089 overall in the gnomAD data set and was notably not found in any individuals of East Asian ancestry. The 66 variants not identified in gnomAD were in regions with >60x mean depth coverage per base (Supplementary Figure 5).

Genomic position	Nucleotide change	Amino acid change	Consequence	rsID	Clinical presentation	gnomAD AF
10:89214921-89214921	c.1107A>C	p.Ile369Val	Missense variant	rs768436255	2	0.000004
10:89214958-89214958	c.1070T>C	p.Leu357Pro	Missense variant	rs772684869	2	0.000040
10:89215000-89215000	c.1028delG	p.Gly343Valfs*15	Frameshift variant	rs750301834	2	0.000007
10:89215004-89215004	c.1024G>A	p.Gly342Arg	Missense variant	rs776472526	2	0.000011
10:89215054-89215054	c.974C>T	p.Pro325Leu	Missense variant		3	0.000008
10:89215936-89215936	c.966+3A>T	p.(=)	Splice donor variant	rs201242614	2	0.000141
10:89215984-89215984	c.920C>A	p.Ala307Asp	Missense variant	rs754964952	2	0.000020
10:89222511-89222511	c.894G>A	p.Ser275_Gln298del	Splice region inframe deletion	rs116928232	3	0.000892
10:89222511-89222511	c.894G>C	p.Gln298His	Splice region missense variant	rs116928232	1	0.000012
10:89222514-89222514	c.891C>T	p.(=)	Splice region synonymous variant	rs145066614	2	0.000271
10:89222524-89222524	c.881T>C	p.Leu294Ser	Missense variant	rs756310979	2	0.000004
10:89222542-89222542	c.863C>T	p.Thr288Ile	Missense variant		2	0.000004
10:89223683-89223683	c.822+1G>A	p.?	Splice donor variant		2	0.000032
10:89223710-89223710	c.796G>T	p.Gly266*	Stop gained	rs267607218	3	0.000007
10:89223822-89223822	c.684delT	p.Phe228Leufs*13	Frameshift variant	rs770074196	3	0.000012
10:89225093-89225093	c.676-23T>C	p.?	Splice region variant	rs140488274	2	0.000148
10:89225115-89225115	c.652C>T	p.Arg218*	Stop gained	rs771330022	2	0.000004
10:89225145-89225145	c.622T>C	p.Phe208Leu	Missense variant	rs148713974	2	0.000022
10:89225168-89225168	c.599T>C	p.Leu200Pro	Missense variant	rs121965086	3	0.000012
10:89225173-89225174	c.594dupT	p.Ala199Cysfs*13	Frameshift variant	rs780495201	3	0.000014
10:89226895-89226895	c.538+6T>C	p.?	Splice donor variant	rs772236690	2	0.000016
10:89226978-89226978	c.455T>C	p.Leu152Pro	Missense variant	rs748267444	2	0.000004
10:89228230-89228230	c.398delC	p.Ser133*	Frameshift variant	rs756016704	1	0.000020
10:89228242-89228242	c.386A>G	p.His129Arg	Missense variant		2	0.000016
10:89228277-89228278	c.350_351insCC	p.Met117Ilefs*45	Frameshift variant	rs753796180	1	0.000007
10:89228319-89228319	c.309C>A	p.Ser103Arg	Missense variant	rs766364179	3	0.000004
10:89228328-89228328	c.229+3A>C	p.?	Splice region variant	rs750405436	2	0.000008
10:89228334-89228334	c.294C>G	p.Asn98Lys	Missense variant	rs767688436	2	0.000012
10:89228368-89228368	c.260G>T	p.Gly87Val	Missense variant	rs587778878	3	0.000008
10:89228372-89228372	c.256C>T	p.His86Tyr	Missense variant	rs749180806	2	0.000011
10:89228375-89228375	c.253C>A	p.Gln85Lys	Missense variant	rs797045094	2	0.000004
10:89245712-89245712	c.193C>T	p.Arg65*	Stop gained	rs779712562	3	0.000025
					LAL-D (WD +CESD) Combined AF	**0.00180**
					95% CI	**(0.00165-0.00197)**
					WD Combined AF	**0.00102**
					95% CI	**(0.00091 - 0.0012)**
					CESD Combined AF	**0.00176**
					95% CI	**(0.0016 - 0.0019)**

Table 2. Previously identified LIPA disease variants present in gnomAD with global allele frequencies and combined allele frequencies for each clinical condition. AF, allele frequency.

Identification and analysis of previously unreported LIPA variants

In order to account for genetic variants that have not yet been identified in patients with LAL-D, we examined all variants that been identified in humans that were predicted to have a significant functional impact on LAL activity (Supplementary Figure 6). 22 previously unreported major functional LIPA variants were identified (Table 3, Figure 2, & Supplementary Table 3). The most common functional classification was splice donor or acceptor in 8/22 (36%).

Genomic position	Nucleotide change	Amino acid change	Consequence	rsID	gnomAD AF
10:89214874-89214874	c.1154G>A	p.Trp385*	Stop gained		0.000004
10:89214919-89214919	c.1109delC	p.Pro370Argfs*25	Frameshift variant	rs762074434	0.000004
10:89215953-89215953	c.951T>A	p.Tyr317*	Stop gained	rs760413481	0.000004
10:89216003-89216006	c.898_901delGTTA	p.Val300Asnfs*30	Frameshift variant	rs754498326	0.000004
10:89216010-89216010	c.895-1G>A	p.?	Splice acceptor		0.000032
10:89222565-89222565	c.840T>G	p.Tyr280*	Stop gained	rs139691556	0.000077
10:89223702-89223702	c.804delT	p.Asn268Lysfs*5	Frameshift variant		0.000004
10:89223725-89223726	c.780_781delCT	p.Cys261Phefs*7	Frameshift variant		0.000004
10:89223813-89223813	c.693dupA	p.Glu232Argfs*37	Frameshift variant		0.000004
10:89223832-89223832	c.676-2A>T	p.?	Splice acceptor	rs747508159	0.000004
10:89225202-89225202	c.565G>T	p.Glu189*	Stop gained	rs749421449	0.000004
10:89228216-89228216	c.412G>T	p.Glu138*	Stop gained		0.000004
10:89228228-89228228	c.400dupG	p.Val134Glyfs*5	Frameshift variant	rs760472104	0.000004
10:89245755-89245756	c.149_150delTG	p.?	Splice donor		0.000032
10:89245773-89245773	c.132G>A	p.Trp44*	Stop gained	rs181646633	0.000008
10:89245779-89245780	c.125_126delCT	p.Ser42Leufs*6	Frameshift variant	rs759603689	0.000008
10:89247537-89247537	c.111+1G>A	p.?	Splice donor	rs762960877	0.000008
10:89247597-89247597	c.52T>C	p.?	Splice donor		0.000097
10:89247598-89247598	c.51G>A	p.?	Splice donor		0.000032
10:89247648-89247648	c.1A>C	p.Met1?	Start lost	rs767039444	0.000004
10:89251735-89251735	c.-2+2T>A	p.?	Splice donor		0.000065
10:89251736-89251736	c.-2+1G>A	p.?	Splice donor		0.000162
				Combined AF	**0.00057**
				95% CI	**(0.0004-0.0008)**

Table 3. Previously unreported variants in LIPA predicted to have major functional effects found in gnomAD. AF, allele frequency.

c.894G>A accounted for 49% of the pooled allele frequency for all 98 previously reported disease-causing variants and 38% of the pooled allele frequency when including all 120 variants. Top 4 most frequent LIPA variants (c.894G>A, c.891C>T, c.676-23T>C, and c.966+3A>T) accounted for 81% of the total allele frequency for previously reported variants and 61%, when including unreported variants.

Estimation of population prevalence of WD, CESD, and LAL-D

Pooling of the allele frequencies for all previously reported disease variants gave a global mutant AF of 0.0018 (95% CI 0.0017 - 0.002) for LAL-D (Table 4 & Figure 4), which is equivalent to a prevalence at birth of LAL-D as 1 per 307,482 (95% CI 257,672 – 366,865). When combined with unreported major functional variants, the pooled AF increased to 0.0024 (95% CI 0.0022 – 0.0026), giving a prevalence at birth of LAL-D as 1 per 177,452 (95% CI 149,467 – 210,683) and a heterozygous carrier rate of 1 per 421 (95% CI 387 – 459). Analysis by ethnicity demonstrated a higher prevalence in those of non-Finnish European ancestry: 1 per 103,286 (95% CI 83,142 – 128, 321), whilst the lowest prevalence of LAL-D in those of East Asian, Finnish, South Asian, and Ashkenazi Jewish ancestry (Figure 4 & Supplementary Table 4).

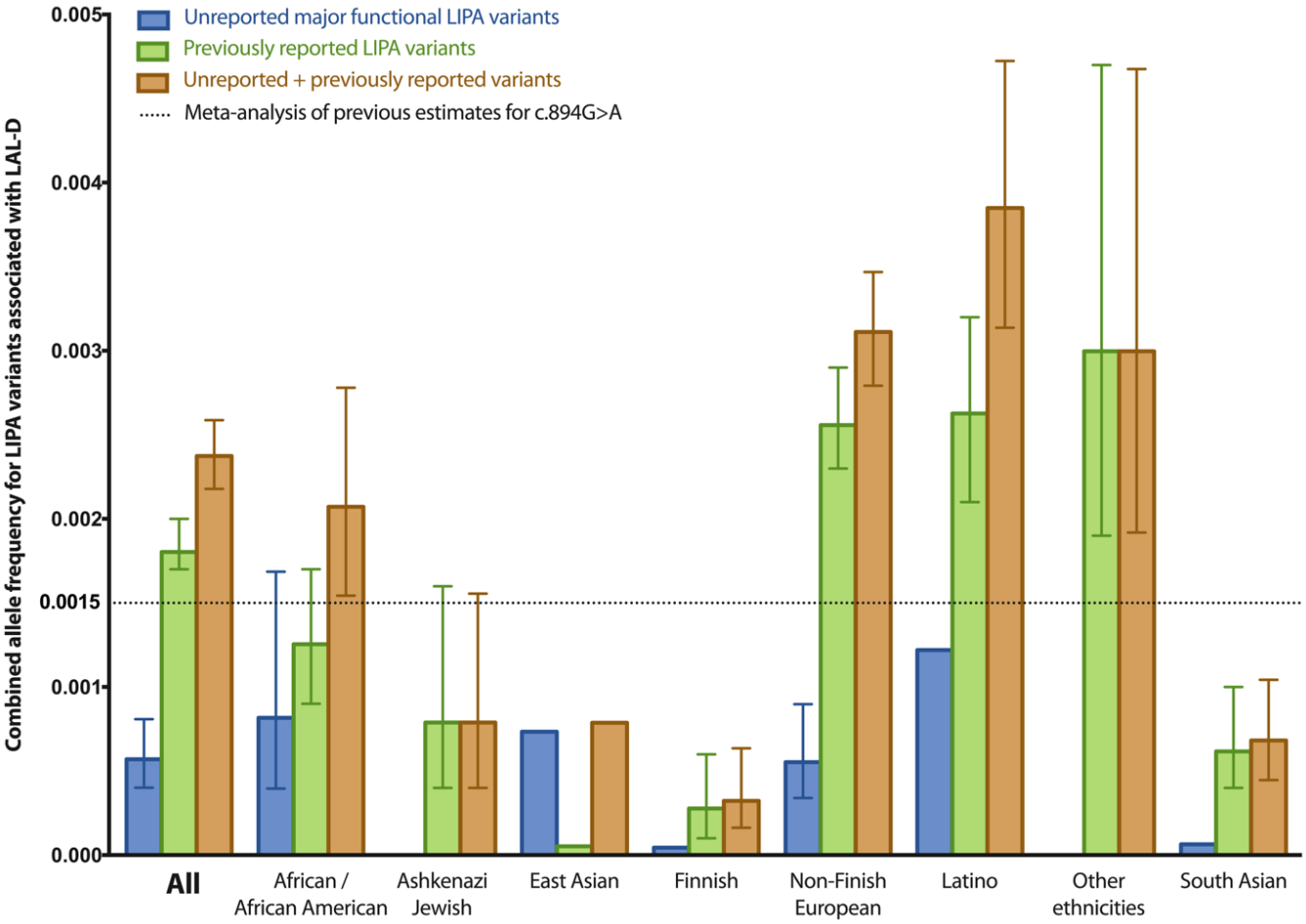

Figure 4. The pooled allele frequency for disease variants in LIPA from unreported major functional variants (blue), previously reported disease variants (green), and combined (orange) for 8 genetic ancestries. These are compared to the meta-analysed genetic prevalence of c.894G>A (dotted line).

	WD		CESD		**LAL-D (WD +CESD)**	
	Previously reported variants	**Previously reported + unreported variants**	**Previously reported variants**	**Previously reported + unreported variants**	**Previously reported variants**	**Previously reported + unreported variants**
Combined AF	0.00102	0.001593882	0.0018	0.0023	**0.001803393**	**0.002374**
95% CI	(0.0009 - 0.0012)	(0.0014 - 0.0018)	(0.0016 - 0.0019)	(0.0021 - 0.0025)	**(0.0017 - 0.002)**	**(0.0022 - 0.0026)**
Het carrier (1 per ...)	977	627	556	435	**555**	**421**
95% CI	(869 - 1099)	(561 - 701)	(519 - 620)	(393 - 467)	**(508 - 606)**	**(387 - 459)**
Hom affected (1 per ...)	954,812	393,630	321,489	183,543	**307,482**	**177,452**
95% CI	(754,660 – 1,208,088)	(314,937 – 492,010)	(269,021 – 384,354)	(154,440 – 218,135)	**(257,672 – 366,865)**	**(149,467 – 210,683)**

Table 4. Prevalence of LAL-D, WD, and CESD estimated from pooled allele frequencies of previously reported variants and combined with unreported variants. Het, heterozygote carrier of *LIPA* mutation; hom, homozygous patient affected with LAL-D.

DISCUSSION

LAL-D (MIM #278000) is recognised as a treatable[35] monogenic condition that may masquerade as NAFLD or present with cryptogenic cirrhosis. Recent data has led to suggestions that it may be under-diagnosed. In this study we sought to estimate the prevalence of LAL-D using accurate population sequencing data and deepen our understanding of the genetic variation of *LIPA*. Using next generation sequencing data, we found LAL-D to affect 1 per 177,452 across the global population, which is significantly lower than previous estimates that have been up to 1 per 40,000. The main strength of this study is use of 120 *LIPA* mutants from a population of >150,000 for our prevalence estimate, whereas previous studies have focus on c.894G>A (E8SJM) [4,34]. In doing so, we have also produced a curated list of disease variants in *LIPA* that may prove useful for further *in vitro* studies or targeted sequencing in patients. The majority of these are extremely rare, with 66 of 98 not being identified in any population sequencing cohort, giving an allele frequency of less than $6.7x10^{-6}$.

Using existing data, we have produced prevalence estimates for CESD and WD separately from LAL-D, however there is conflicting data over the consistency of genotype-phenotype correlations[36–38]. These estimates are limited by the difficulty in establishing and reporting clinical diagnoses. The key differentiating factor is residual activity in LAL, which reliably separates the two conditions[12]. Although, data in this study supports the notion that WD is more associated with major functional variants (stop gained or splice site variants). Our estimate of WD prevalence from genetic variants (1 per 393,630) is slightly higher than the prevalence derived from meta-analysis of previous studies (1 per 451,116, (95% CI 282,022 – 840,092)). Given the challenge in diagnosing LAL-D, the disparity between these clinical epidemiological data and the genetic prevalence are most likely to represent under-diagnosis. The uncertainty associated with these calculations are reflected in their relatively wide confidence intervals.

It is not possible to state whether CESD is under-diagnosed as there are no clinical studies assessing the prevalence of CESD in the general population. However, targeted testing of LAL activity in >1800 patients with dyslipidaemia and raised aminotransferases found no affected patients[39].

LIPA is a highly conserved gene and its hydrolase domain is similar to other lipases[10]. Protein-coding disease variants were found to cluster particularly in exons 4 and 10, in addition to splice junctions. The active site codons were not found to be affected directly by any point mutations however previous modelling has demonstrated how adjacent variants disrupt LAL activity[40,41]. Though this methodology has been used to estimate the prevalence of other monogenic disorders[42], it is based upon the assumptions of the Hardy-Weinberg principle and there is no *in vitro* data to support many of the variants. These limitations would be likely to result in an over-estimation of the prevalence of LAL-D in these results. Our overall calculated prevalence is also limited by an inability to differentiate between WD and CESD in newly discovered variants.

LAL-D is associated with a high proportion of (intronic) splice-site variants. gnomAD has deep coverage at splice sites (Supplementary Figure 5) however this highlights the need for deep exome or whole genome sequencing to diagnose affected patients. This may be a limitation to incorporating genetic diagnosis into clinical practice.

This updated prevalence estimate has implications for the diagnosis of LAL-D and exclusion of secondary metabolic causes of NAFLD. EASL-EASD-EASO guidelines recommended that Wilson disease and alpha-1-antitrypsin deficiency should be tested at an 'extended investigation' stage[43]. These data suggest that LAL-D is significantly less common than these two conditions and therefore could be included in a 'second-line' of metabolic tests along with other rare mimics of NAFLD (Supplementary Figure 7 & Supplementary Table 5). A consensus is needed on whether to include

LAL-D screening in in patients with atypical features of NAFLD for example those who are especially young, with a significant family history, low BMI, or without insulin resistance.

It remains to be established whether heterozygotes have a clinically manifest phenotype and whether 1-5% LAL activity would accelerate concomitant NAFLD, viral hepatitis, or any other hepatic parenchymal disease. The currently presented data would suggest that the heterozygote carrier rate is much lower than that for other monogenic liver diseases that may influence NAFLD progression, such as HFE hereditary haemochromatosis (1 in 8-10)[44] or alpha-1-antitrypsin deficiency (1 in 15-20)[45].

In conclusion, through comprehensive analysis of genetic variation in *LIPA* we have expanded our recognition of disease-causing mutants to 120 variants. LAL-D is an ultra-rare disease, even in its late-onset form as CESD, and these data can help reassure clinicians that LAL-D is unlikely to represent significant proportion of patients presenting with NAFLD. A consensus is needed for when testing LAL activity should be performed in patients with dyslipidaemia or hepatic steatosis.

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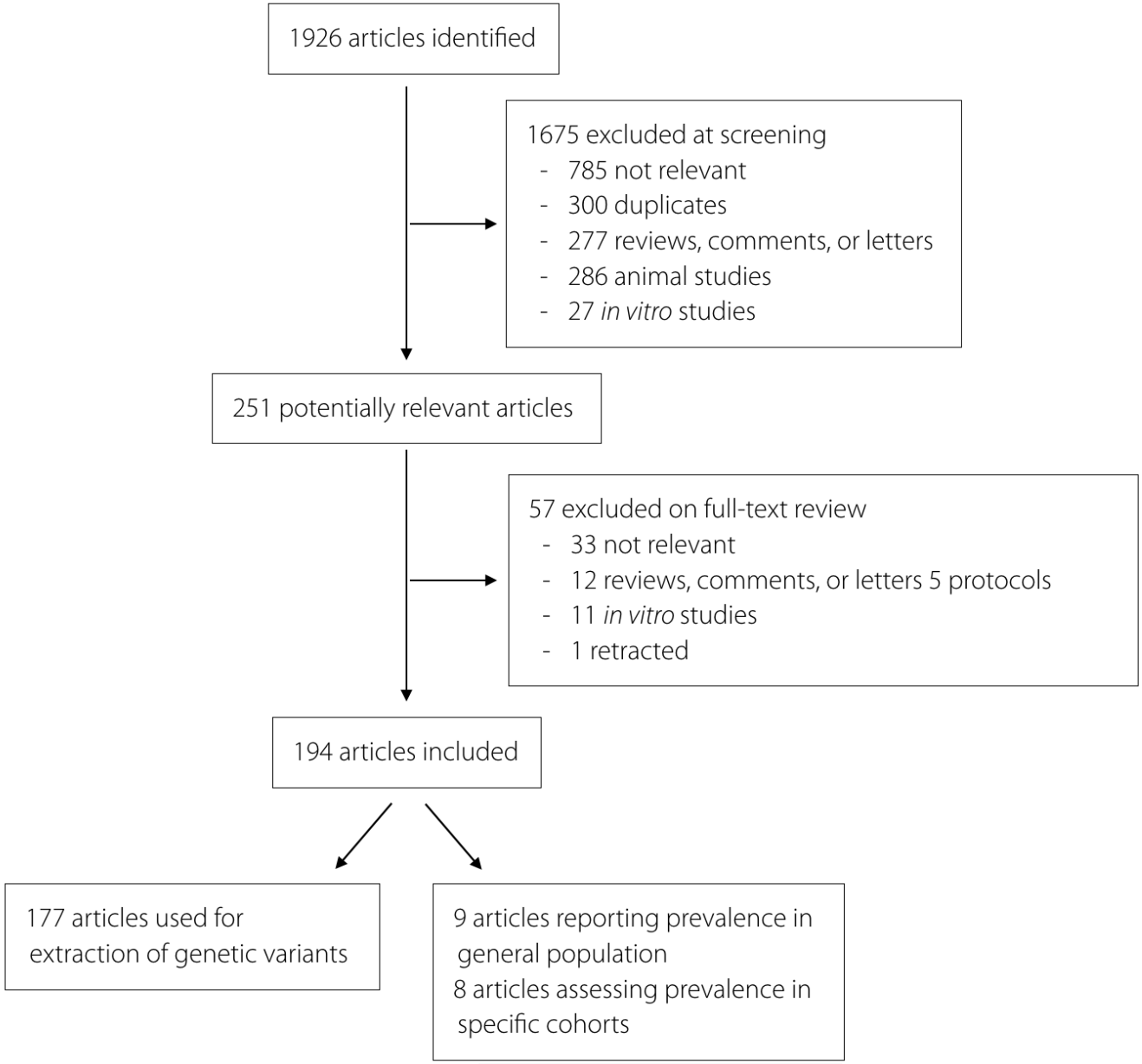
Supplementary methods

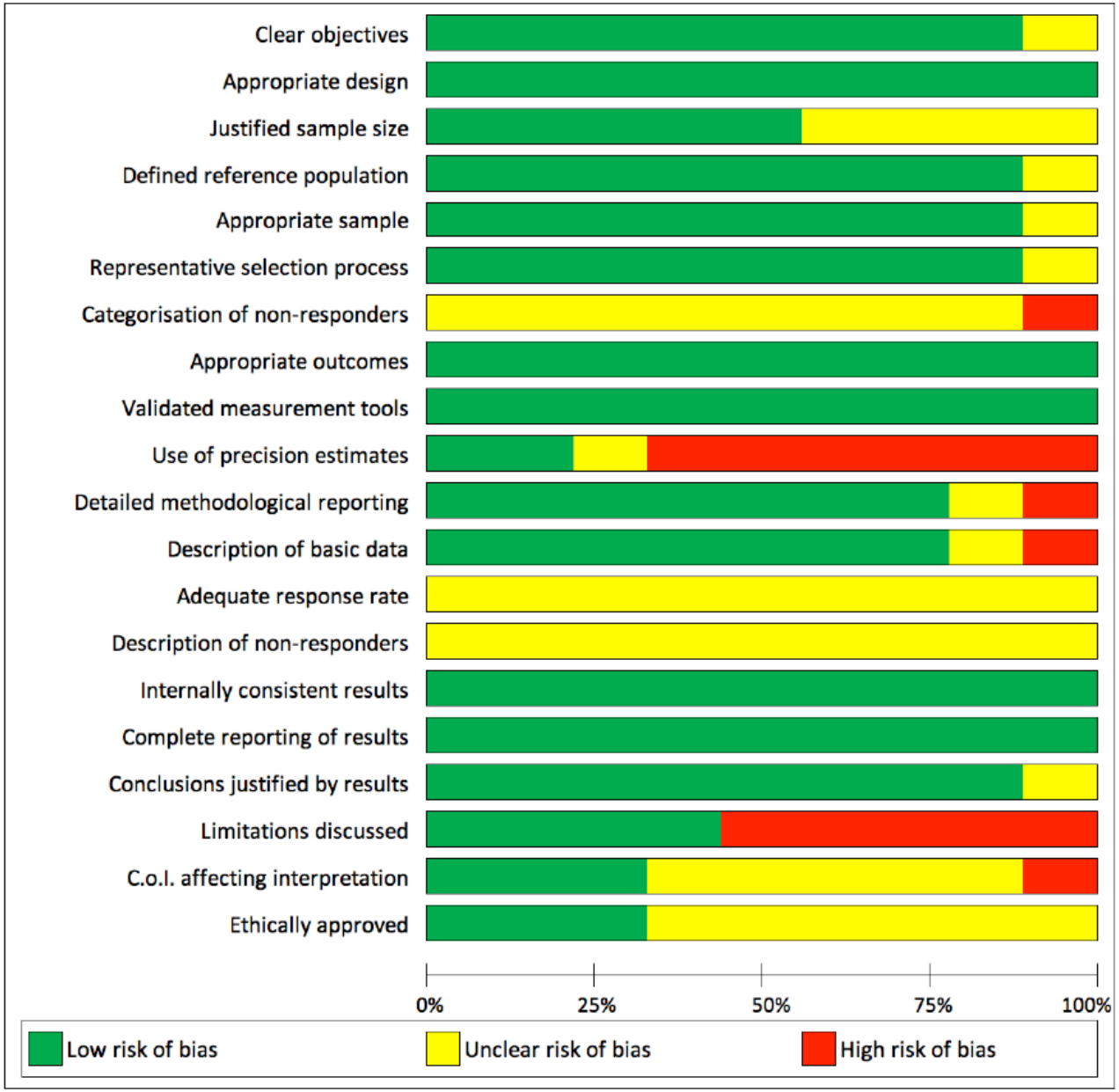
Full search term
(("lysosomal storage*") AND ("frequency" OR "prevalence")) OR ("lysosomal acid lipase deficiency" OR "Wolman disease" OR "cholesteryl ester storage disease" OR "LAL deficiency")

Search completed on 14/07/2018.

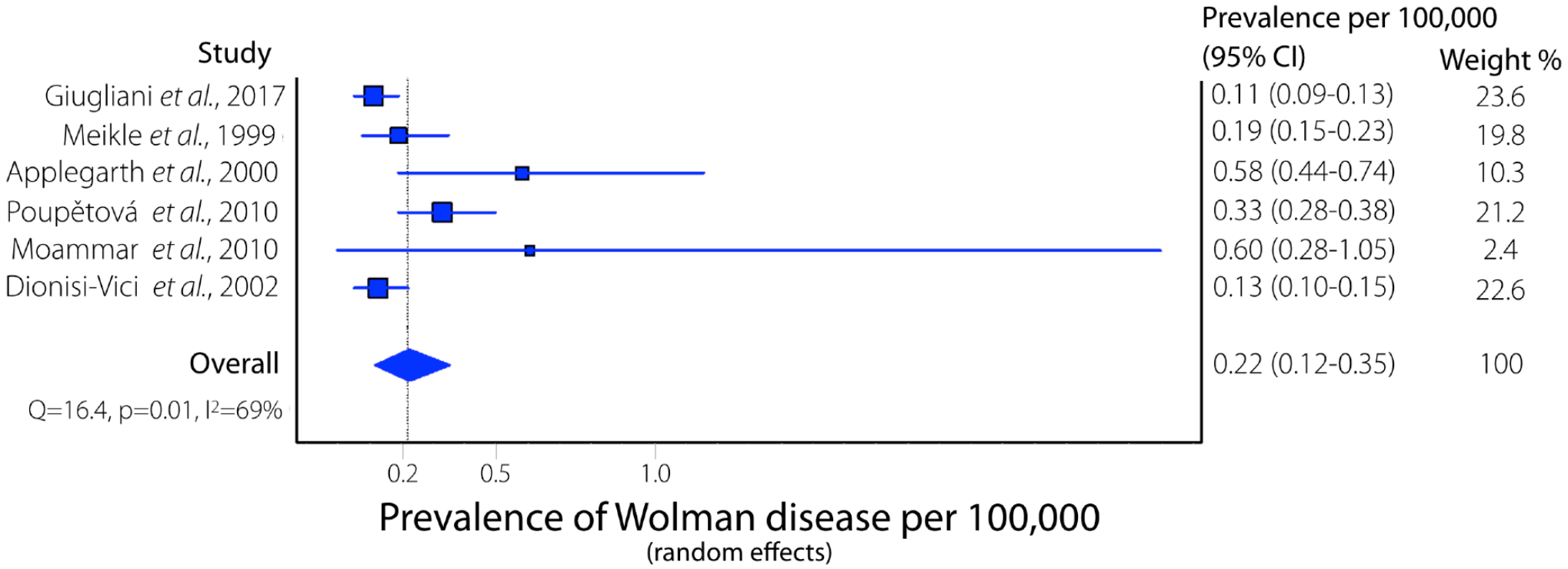
Supplementary figures



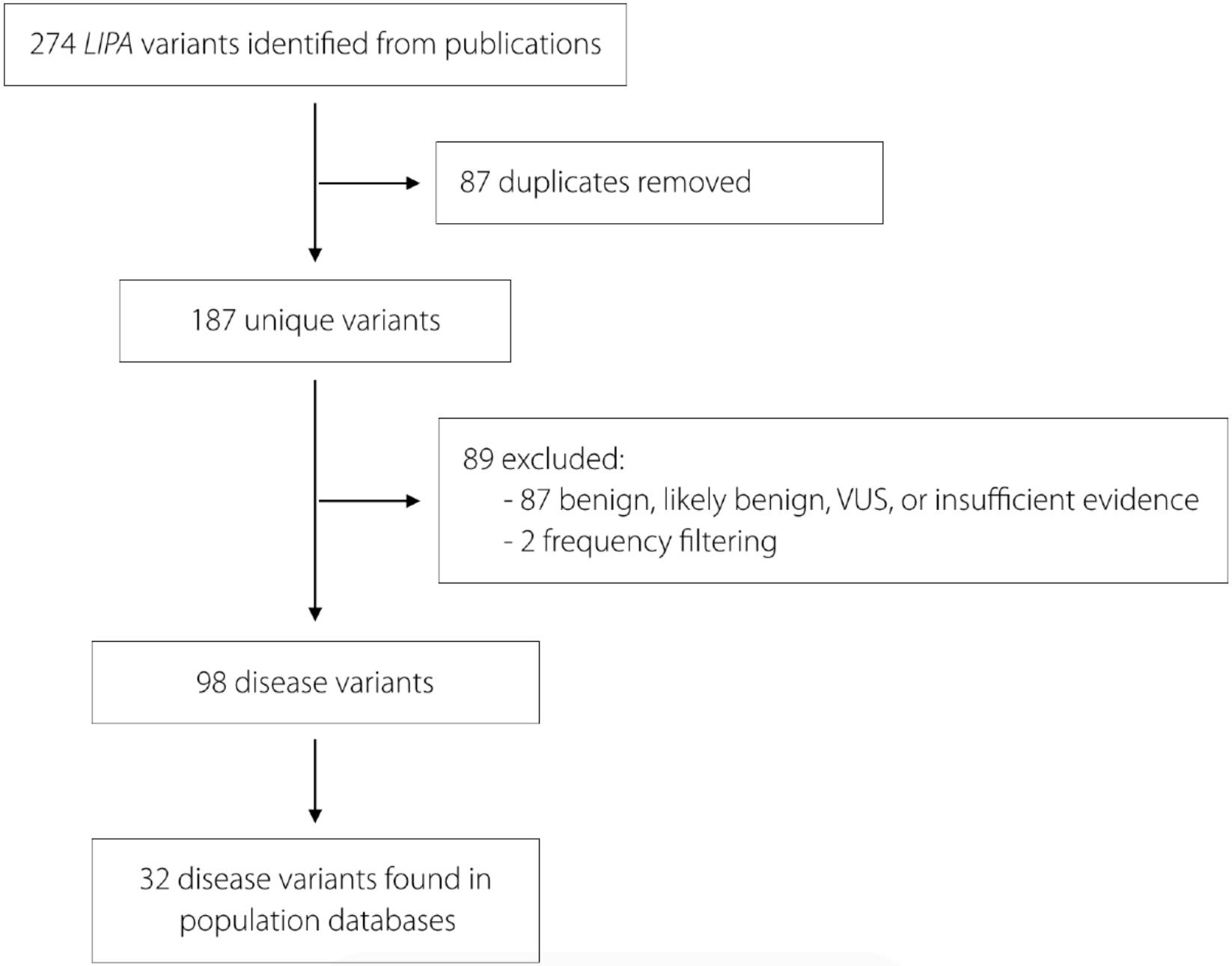
Supplementary figure 1. Inclusion-exclusion flowchart for identification of articles reporting prevalence of LAL-D and genetic variants in *LIPA*.



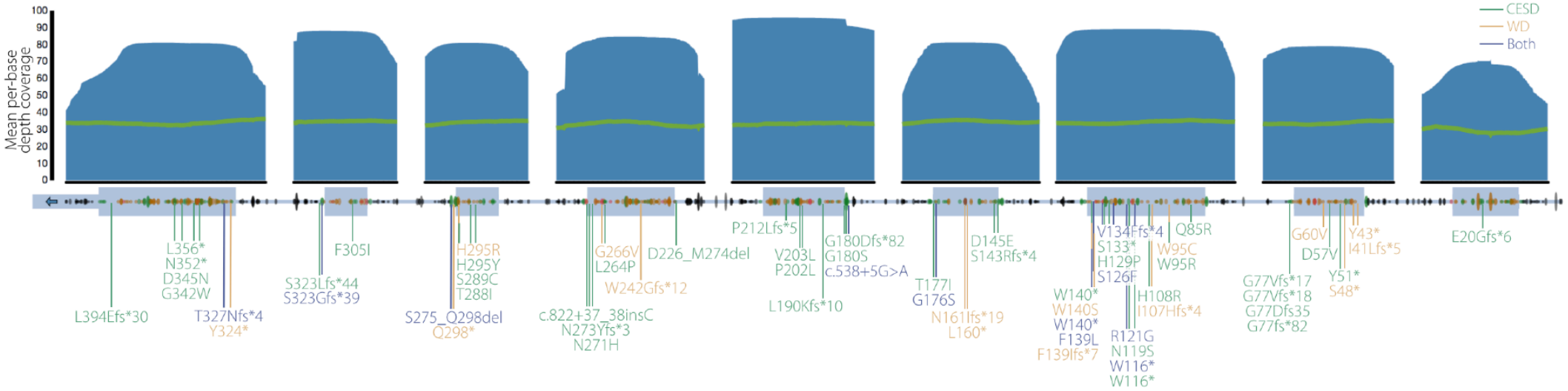
Supplementary figure 2. Risk of bias chart illustrating the results from the AXIS (appraisal of cross-sectional studies) tool for studies in Table 1.



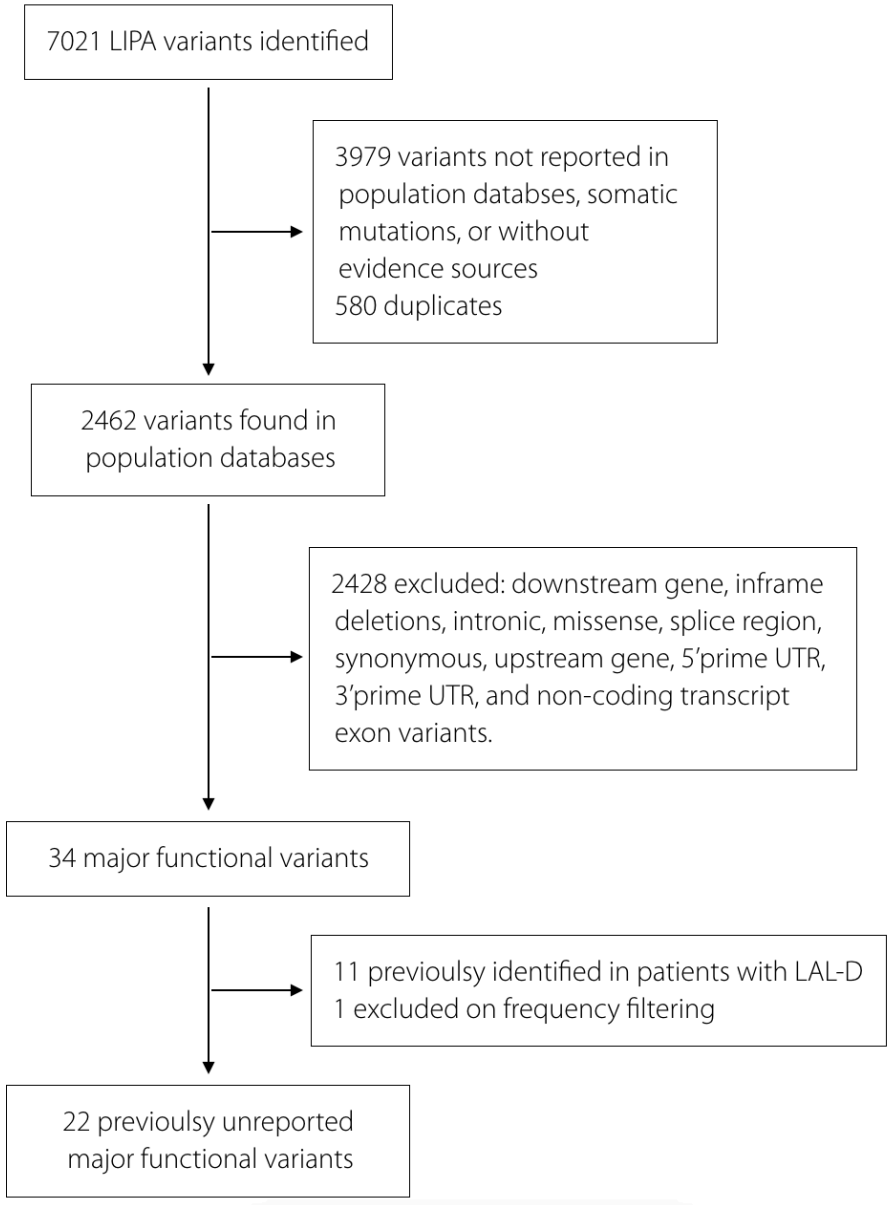
Supplementary figure 3. Meta-analysis of epidemiological studies estimating the prevalence of Wolman disease.



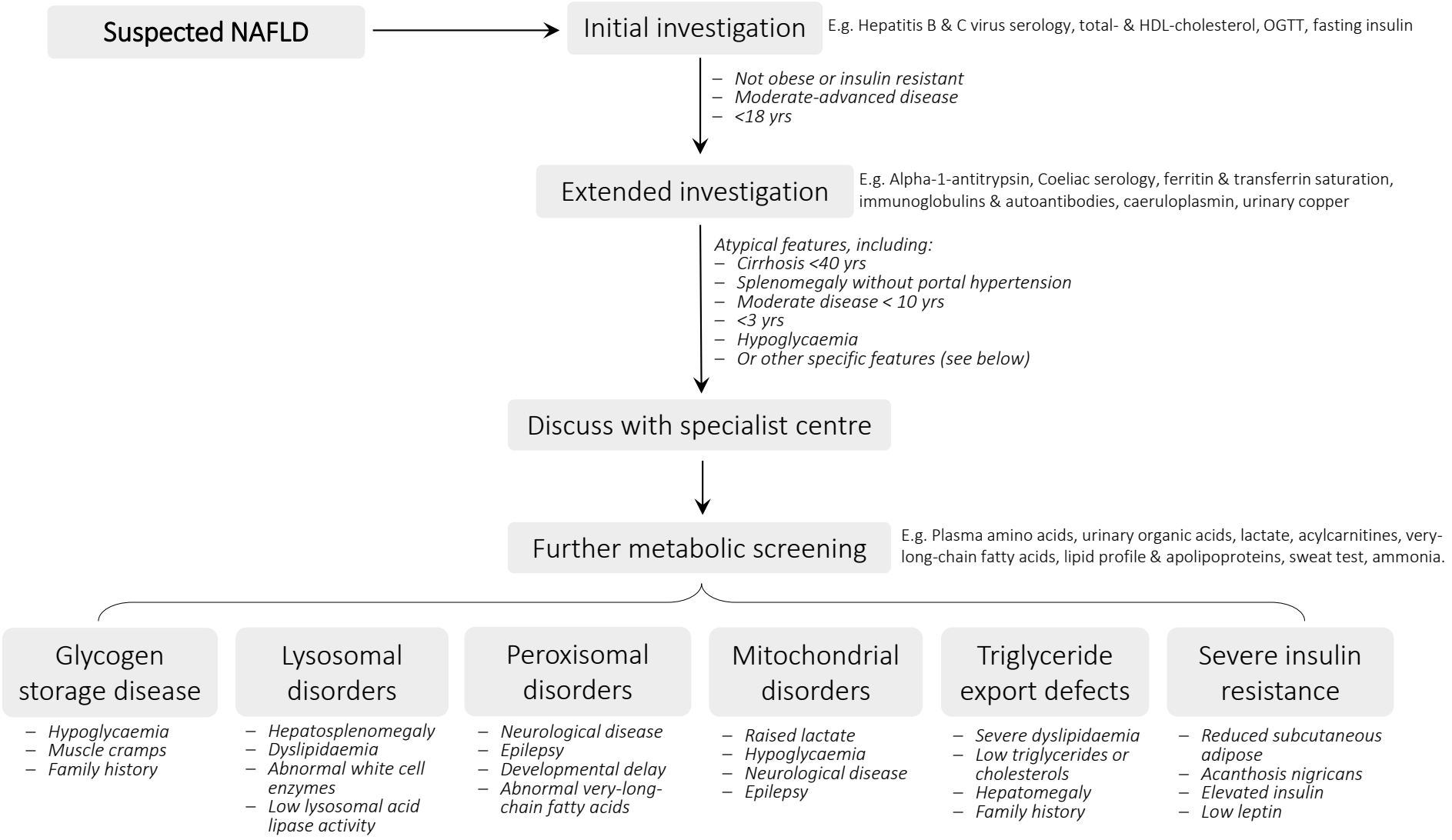
Supplementary figure 4. Inclusion-exclusion flow chart for identification of genetic variants in *LIPA* previously reported to be associated with LAL-D.



Supplementary figure 5. *LIPA* coverage plot from gnomAD illustrating the mean per-base depth coverage and the position of all disease causing variants that had not been identified in gnomAD.



Supplementary figure 6. Inclusion-exclusion flow chart for identification of previously unreported major functional variants in *LIPA* found in population sequencing databases.



Supplementary figure 7. A suggested flow-chart for investigation of metabolic disorders that may mimic NAFLD. Testing for LAL-D may feature as part of a further line of metabolic screening tests after discussion with an inherited metabolic disease specialist.

Study	Cohort assessed	Diagnostic method	Population (n)	Cases (n)	Prevalence	Comments
Dyslipidaemia						
Reynolds *et al.*, 2018 (54) [United Kingdom]	Patients with HDL <33mg/dL & AST/ALT >60 IU/L	LAL activity on DBS	1,825 patients	0 affected	Carrier <1 in 91	LIPA sequencing in 6 patients with cryptogenic cirrhosis found 2 with unclassified variants.
Sjouke *et al.*, 2016 (14) [Netherlands]	Familial hypercholesterolaemia	LIPA sequencing	276 patients	6 het 0 hom	Not stated. Greater than expected for population.	6 patients with pathogenic or potentially pathogenic. LAL activity not assessed.
Ashfield-Watt *et al.*, 2016 (55) [United Kingdom]	Familial hypercholesterolaemia	LIPA sequencing for c.894G>A	545 patients	3 het 0 hom	1 in 182 het carrier	LAL activity not assessed.
Pullinger *et al.*, 2015 (28) [United States]	Dyslipidaemia	LIPA sequencing for c.894G>A, then full sequencing.	1,357 patients	5 het 1 hom	Not stated.	LAL activity not assessed.
Coates et al., 1986 (56) [United States]	Premature CVD +/- hyperlipidaemia, plus controls	LAL activity in leukocytes	190 patients 209 controls	21 patients & 6 controls suspected heterozygotes	Higher in pa tient population than controls (11% vs 5%)	Included CESD patients and obligate heterozygotes for validation. No genetic analysis.
Liver disease						
Selvakumar et al., 2016 (57) [Italy]	Children with NAFLD	LAL activity on DBS	168 patients	0 affected	Not stated.	Lower LAL activity in children with significant fibrosis. No genetic analysis.
Shteyer et al, 2016 (58) [Israel]	i. Microvesicular steatosis ii. Cryptogenic cirrhosis iii. Adult NAFLD	LAL activity on DBS	i. 9 patients ii. 4 patients iii. 9 patients	0 affected	Not stated.	Changes in LAL activity (within the normal range) associated with markers of liver disease. No genetic analysis.
Baratta et al., 2015 (59) [Italy]	Adults with NAFLD, plus controls	LAL activity on DBS	240 patients 100 controls	0 affected	Not stated.	Lower LAL activity in NAFLD and NASH (within the normal range). No genetic analysis.

Supplementary table 1. Clinical studies assessing the prevalence of LAL-D in cohorts of patients with dyslipidaemia and liver disease. NASH, non-alcoholic steatohepatitis.

							gnomAD allele frequencies									
Genomic position	Nucleotide change	Amino acid change	Consequence	rsID	WD / CESD	Classification	Global	AFR	ASJ	EAS	FIN	NFE	LAT	OTH	SAS	Example reference
10:89214844-89214848	c.1180_1184delCTAAT	p.Leu394Glufs*30	Frameshift variant		CESD	2										(1)
10:89214921-89214921	c.1107A>C	p.Ile369Val	Missense variant	rs768436255	CESD	1	0.000004061	0.00006534								(2)
10:89214958-89214958	c.1070T>C	p.Leu357Pro	Missense variant	rs772684869	CESD	1	0.00003968				0.00003877	0.00007892				(3)
10:89214961-89214961	c.1067T>G	p.Leu356*	Stop gained		CESD	1										(4)
10:89214971-89214973	c.1055_1057delACG	p.Asn352*	Inframe deletion	rs767207643	CESD	1										(5)
10:89214995-89214995	c.1033G>A	p.Asp345Asn	Missense variant		CESD	1										(6)
10:89215000-89215000	c.1028delG	p.Gly343Valfs*15	Frameshift variant	rs750301834	CESD	1	0.000007216					0.00001579				(7)
10:89215004-89215004	c.1024G>A	p.Gly342Arg	Missense variant	rs776472526	WD	1	0.00001082					0.000007893	0.00002906		0.00003249	(8)
10:89215004-89215004	c.1024G>T	p.Gly342Trp	Missense variant		CESD	1										(9)
10:89215048-89215048	c.980delC	p.Thr327Asnfs*4	Frameshift variant		LAL-D	1										(10)
10:89215054-89215054	c.974C>T	p.Pro325Leu	Missense variant		LAL-D	2	0.000008126					0.00001792				(11)
10:89215056-89215056	c.972T>A	p.Tyr324*	Stop gained		WD	1										(12)
10:89215060-89215060	c.967_968delAG	p.Ser323Leufs*44	Splice region frameshift variant	rs917089035	CESD	1										(13)
10:89215936-89215936	c.966+3A>T	p.(=)	Splice donor variant	rs201242614	CESD	2	0.0001407	0.0001666				0.0001894	0.0002615	0.0003094		(14)
10:89215936-89215936	c.966+2A>T	p.Ser323Glyfs*39	Splice donor frameshift variant		LAL-D	2										(15)
10:89215984-89215984	c.920C>A	p.Ala307Asp	Missense variant	rs754964952	CESD	1	0.00002031					0.00003582			0.00003249	(16)
10:89215991-89215991	c.913T>A	p.Phe305Ile	Missense variant		CESD	2										(11)
10:89222511-89222511	c.894G>A	p.Ser275_Gln298del	Splice region inframe deletion	rs116928232	WD	1	0.000892	0.0002081	0.0007885		0.0001939	0.001297	0.001134	0.001392	0.0005523	(17)
10:89222511-89222511	c.894G>C	p.Gln298His	Splice region missense variant	rs116928232	LAL-D	2	0.0000122					0.00001795	0.00002979			(8)
10:89222511-89222511	c.894+1G>A	p.Ser275_Gln298del	Splice donor inframe deletion		LAL-D	1										(17)
10:89222513-89222513	c.892C>T	p.Gln298*	Splice region stop gained		WD	1										(18)
10:89222514-89222514	c.891C>T	p.(=)	Splice region synonymous variant	rs145066614	CESD	2	0.0002709	0.0001665				0.0004667	0.0002907	0.0003096		(19)
10:89222521-89222521	c.884A>G	p.His295Arg	Missense variant		WD	1										(20)

10:89222522-89222522	c.883C>T	p.His295Tyr	Missense variant		CESD	1										(2)
10:89222524-89222524	c.881T>C	p.Leu294Ser	Missense variant	rs756310979	CESD	2	0.000004065					0.00000897				(21)
10:89222539-89222539	c.866C>G	p.Ser289Cys	Missense variant		CESD	2										(10)
10:89222542-89222542	c.863C>T	p.Thr288Ile	Missense variant		CESD	1	0.000004066				0.00004486					(12)
10:89223683-89223683	c.822+1G>A (IVS7)	p.?	Splice donor variant		WD	2	0.00003231					0.00006662				(22)
10:89223683-89223683	c.822+37_38insC	p.?	Splice donor variant		CESD	1										(23)
10:89223688-89223687	c.817_818delAA	p.Asn273Tyrfs*3	Frameshift variant		CESD	1										(4)
10:89223695-89223695	c.811A>C	p.Asn271His	Missense variant		CESD	1										(24)
10:89223709-89223709	c.797G>T	p.Gly266Val	Missense variant		WD	2										(20)
10:89223710-89223710	c.796G>T	p.Gly266*	Stop gained	rs267607218	LAL-D	1	0.000007241					0.00001587				(25)
10:89223715-89223715	c.791T>C	p.Leu264Pro	Missense variant		CESD	1										(26)
10:89223782-89223782	c.724delT	p.Trp242Glyfs*12	Frameshift variant		WD	2										(27)
10:89223822-89223822	c.684delT	p.Phe228Leufs*13	Frameshift variant	rs770074196	LAL-D	1	0.00001221	0.00006567				0.00001796				(28)
10:89225093-89225093	c.676-2A>G	p.Asp226_Met274del	Splice region inframe deletion		CESD	1										(20)
10:89225093-89225093	c.676-23T>C	p.?	Splice region variant	rs140488274	CESD	2	0.0001484	0.00008356				0.0001427	0.0004945	0.0006209		(19)
10:89225115-89225115	c.652C>T	p.Arg218*	Stop gained	rs771330022	CESD	1	0.000004061					0.000008954				(29)
10:89225132-89225132	c.635delC	p.Pro212Leufs*5	Frameshift variant		CESD	1										(30)
10:89225140-89225199	c.568_627del60insAAATTTTC	p.Leu190Lysfs*10	Frameshift variant		CESD	1										(31)
10:89225145-89225145	c.622T>C	p.Phe208Leu	Missense variant	rs148713974	CESD	1	0.00002164	0.0002497								(17)
10:89225160-89225160	c.607G>C	p.Val203Leu	Missense variant		CESD	1										(26)
10:89225162-89225162	c.605C>T	p.Pro202Leu	Missense variant		CESD	1										(32)
10:89225168-89225168	c.599T>C	p.Leu200Pro	Missense variant	rs121965086	LAL-D	1	0.00001218					0.00002686				(33)
10:89225173-89225174	c.594dupT	p.Ala199Cysfs*13	Frameshift variant	rs780495201	LAL-D	1	0.00001446	0.00004178				0.00002373				(34)
10:89225228-89225228	c.539-2A>C	p.Gly180Aspfs*82 (IVS5)	Splice donor frameshift variant		CESD	2										(35)
10:89226895-89226895	c.538+6T>C	p.?	Splice donor variant	rs772236690	WD	2	0.00001625						0.00008936	0.000182		(19)
10:89226895-89226895	c.538G>A	p.Gly180Ser	Missense variant		CESD	2										(20)
10:89226895-89226895	c.538+5G>A	p.?	Splice donor variant		LAL-D	2										(11)
10:89226903-89226903	c.530C>T	p.Thr177Ile	Missense variant		CESD	1										(36)

10:89226907-89226907	c.526G>A	p.Gly176Ser	Missense variant		LAL-D	2										(11)
10:89226951-89226951	c.482delA	p.Asn161Ilefs*19	Frameshift variant	rs762559980	WD	1										(37)
10:89226956-89226956	c.477delT	p.Leu160*	Stop gained		WD	1										(23)
10:89226978-89226978	c.455T>C	p.Leu152Pro	Missense variant	rs748267444	CESD	2	0.000004062					0.000008957				(31)
10:89226998-89226998	c.435T>A	p.Asp145Glu	Missense variant		CESD	2										(7)
10:89227659-89227659	c.230-106_c.428+541del	p.Gly77Valfs*17	Intronic frameshift variant		CESD	1										(38)
10:89228199-89228199	c.428+1G>A	p.Ser143Argfs*4	Splice donor frameshift variant		CESD	2										(1)
10:89228200-89228398	c.230_428del (Ex4del)	p.Gly77Valfs*18	Frameshift variant		CESD	1										(10)
10:89228208-89228208	c.420G>A	p.Trp140*	Stop gained		CESD	2										(39)
10:89228209-89228209	c.419G>C	p.Trp140Ser	Missense variant		WD	2										(20)
10:89228209-89228209	c.419G>A	p.Trp140*	Stop gained		LAL-D	1										(40)
10:89228211-89228211	c.417C>A	p.Phe139Leu	Missense variant		LAL-D	2										(41)
10:89228214-89228214	c.414insA	p.Phe139Ilefs*7	Frameshift variant		WD	2										(42)
10:89228230-89228229	c.397_398delTC	p.Val134Phefs*4	Frameshift variant		LAL-D	1										(9)
10:89228230-89228230	c.398delC	p.Ser133*	Frameshift variant	rs756016704	WD	1	0.00002031					0.00003583	0.00002978			(22)
10:89228230-89228230	c.398C>A	p.Ser133*	Stop gained		CESD	1										(4)
10:89228242-89228242	c.386A>G	p.His129Arg	Missense variant		CESD	1	0.00001625						0.0001191			(5)
10:89228242-89228242	c.386A>C	p.His129Pro	Missense variant		CESD	1										(43)
10:89228251-89228251	c.377C>T	p.Ser126Phe	Missense variant		LAL-D	2										(11)
10:89228267-89228267	c.361A>G	p.Arg121Gly	Missense variant		LAL-D	2										(44)
10:89228272-89228272	c.356A>G	p.Asn119Ser	Missense variant		CESD	1										(45)
10:89228277-89228278	c.350_351insCC	p.Met117Ilefs*45	Frameshift variant	rs753796180	WD	1	0.000007215	0.00008323								(20)
10:89228280-89228280	c.348G>A	p.Trp116*	Stop gained		CESD	2										(39)
10:89228281-89228281	c.347G>A	p.Trp116*	Stop gained		LAL-D	2										(27)
10:89228303-89228303	c.325A>G	p.His108Arg	Missense variant		CESD	1										(46)
10:89228311-89228311	c.317insT	p.Ile107Hisfs*4	Frameshift variant		WD	1										(47)
10:89228319-89228319	c.309C>A	p.Ser103Arg	Missense variant	rs766364179	LAL-D	2	0.000004062					0.000008958				(11)
10:89228328-89228328	c.229+3A>C	p.?	Splice region variant	rs750405436	CESD	1	0.000008129					0.00001791				(6)

10:89228328-89228328	c.229+1G>A	p.Gly77Aspfs*35	Splice donor frameshift variant		CESD	1										(40)
10:89228334-89228334	c.294C>G	p.Asn98Lys	Missense variant	rs767688436	CESD	1	0.00001219						0.00008935			(36)
10:89228343-89228343	c.285G>T	p.Trp95Cys	Stop gained		WD	1										(42)
10:89228345-89228345	c.283T>A	p.Trp95Arg	Missense variant		CESD	2										(7)
10:89228368-89228368	c.260G>T	p.Gly87Val	Missense variant	rs587778878	LAL-D	2	0.000008126						0.00002978	0.000182		(48)
10:89228372-89228372	c.256C>T	p.His86Tyr	Missense variant	rs749180806	CESD	2	0.00001083	0.0001248								(5)
10:89228374-89228374	c.254A>G	p.Gln85Arg	Missense variant		CESD	1										(12)
10:89228375-89228375	c.253C>A	p.Gln85Lys	Missense variant	rs797045094	CESD	1	0.000004063						0.00002978			(36)
10:89228383-89228383	c.(230_231ins35;232_245del)	p.Gly77fs*82	Frameshift variant		WD	1										(29)
10: 89228???-89225???	5'Ex4del	p.?	Large deletion		WD	2										(37)
10:89245712-89245712	c.193C>T	p.Arg65*	Stop gained	rs779712562	LAL-D	1	0.00002526			0.00005301		0.00004738				(10)
10:89245724-89245724	c.181T>G	p.Gly60Val	Missense variant		WD	1										(30)
10:89245735-89245735	c.170A>T	p.Asp57Val	Missense variant		CESD	2										(7)
10:89245752-89245752	c.153C>A	p.Tyr51*	Stop gained		CESD	1										(49)
10:89245764-89245765	c.140_141insA	p.Ser48*	Stop gained		WD	2										(50)
10:89245776-89245776	c.129C>G	p.Tyr43*	Stop gained	rs121965087	WD	1										(51)
10:89245778-89245775	c.120_127del	p.Ile41Leufs*5	Frameshift variant		WD	1										(9)
10:89247585-89228200	c.64-?_428+?del	p.Ex2_4del	Large deletion		CESD	1										(52)
10:89247589-89247592	c.57_60delTGAG	p.Glu20Glyfs*6	Frameshift variant		CESD	1										(53)
				LAL-D (WD + CESD)		**Combined AF**	**0.00180**	0.00126	0.00079	0.00005	0.00028	0.00256	0.00263	0.00300	0.00062	
						AF 95% CI	**(0.0017-0.0020)**	(0.0009-0.0017)	(0.0004-0.0016)		(0.0001-0.0006)	(0.0023-0.0029)	(0.0021-0.0032)	(0.0019-0.0047)	(0.004-0.0010)	
				WD		**Combined AF**	**0.00102**	0.00040	0.00079	0.00005	0.00019	0.00151	0.00122	0.00157	0.00055	
						AF 95% CI	(0.00091 - 0.0012)	(0.00021 - 0.0008)	(0.0004 - 0.0016)		(0.00008 - 0.0005)	(0.00131 - 0.0017)	(0.0009 - 0.0017)	(0.00086 - 0.0029)	(0.00034 - 0.0009)	
				CESD		**Combined AF**	**0.00176**	0.00117	0.00079	0.00005	0.00028	0.00250	0.00257	0.00300	0.00062	
						AF 95% CI	(0.0016 - 0.0019)	(0.0008 - 0.0016)	(0.0004 - 0.0016)	(0.0001 - 0.0006)	(0.0022 - 0.0028)	(0.0021 - 0.0032)	(0.0019 - 0.0047)	(0.0004 - 0.001)	(0.0016 - 0.0019)	

Supplementary table 2. All previously reported disease variants in LIPA associated with LAL-D, CESD, or WD annotated with allele frequencies from gnomAD. AFR, African / African American; ASJ, Ashkenazi Jewish; EAS, East Asian, FIN, Finnish; NFE, Non-Finnish European; LAT, Latino; OTH, Other; SAS, South Asian.

Supplementary table 2 references

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Genomic position	Nucleotide change	Amino acid change	Consequence	rsID	Global	AFR	ASJ	EAS	FIN	NFE	LAT	OTH	SAS
10:89214874-89214874	c.1154G>A	p.Trp385*	Stop gained		0.000004						0.000030		
10:89214919-89214919	c.1109delC	p.Pro370Argfs*25	Frameshift variant	rs762074434	0.000004					0.000009			
10:89215953-89215953	c.951T>A	p.Tyr317*	Stop gained	rs760413481	0.000004								0.000032
10:89216003-89216006	c.898_901delGTTA	p.Val300Asnfs*30	Frameshift variant	rs754498326	0.000004					0.000009			
10:89216010-89216010	c.895-1G>A	p.?	Splice acceptor		0.000032			0.000618					
10:89222565-89222565	c.840T>G	p.Tyr280*	Stop gained	rs139691556	0.000077	0.000227							
10:89223702-89223702	c.804delT	p.Asn268Lysfs*5	Frameshift variant		0.000004			0.000058					
10:89223725-89223726	c.780_781delCT	p.Cys261Phefs*7	Frameshift variant		0.000004					0.000009			
10:89223813-89223813	c.693dupA	p.Glu232Argfs*37	Frameshift variant		0.000004					0.000009			
10:89223832-89223832	c.676-2A>T	p.?	Splice acceptor	rs747508159	0.000004								0.000033
10:89225202-89225202	c.565G>T	p.Glu189*	Stop gained	rs749421449	0.000004					0.000009			
10:89228216-89228216	c.412G>T	p.Glu138*	Stop gained		0.000004					0.000009			
10:89228228-89228228	c.400dupG	p.Val134Glyfs*5	Frameshift variant	rs760472104	0.000004				0.000045				
10:89245755-89245756	c.149_150delTG	p.?	Splice donor		0.000032					0.000067			
10:89245773-89245773	c.132G>A	p.Trp44*	Stop gained	rs181646633	0.000008	0.000131							
10:89245779-89245780	c.125_126delCT	p.Ser42Leufs*6	Frameshift variant	rs759603689	0.000008					0.000015			
10:89247537-89247537	c.111+1G>A	p.?	Splice donor	rs762960877	0.000008					0.000018			
10:89247597-89247597	c.52T>C	p.?	Splice donor		0.000097	0.000229				0.000067			
10:89247598-89247598	c.51G>A	p.?	Splice donor		0.000032						0.001193		
10:89247648-89247648	c.1A>C	p.Met1?	Start lost	rs767039444	0.000004			0.000058					
10:89251735-89251735	c.-2+2T>A	p.?	Splice donor		0.000065	0.000230							
10:89251736-89251736	c.-2+1G>A	p.?	Splice donor		0.000162					0.000334			
				Combined AF	**0.0006**	**0.0008**		**0.0007**		**0.0006**	**0.0012**		**0.0001**
				95% CI	(0.0004 - 0.0008)	(0.0004 - 0.0017)				(0.0003 - 0.0009)			

Supplementary table 3. Unreported major functional variants in *LIPA* identified in population sequencing databases, with multi-ancestry allele frequencies.

AFR, African / African American; ASJ, Ashkenazi Jewish; EAS, East Asian, FIN, Finnish; NFE, Non-Finnish European; LAT, Latino; OTH, Other; SAS, South Asian.

		Global	AFR	ASJ	EAS	FIN	NFE	LAT	OTH	SAS
WD	**Combined AF**	**0.0016**	0.0012	0.0008	0.0008	0.0002	0.0021	0.0024	0.0016	0.0006
	95% CI	**(0.0014 - 0.0018)**	(0.0007 - 0.002)	(0.0004 - 0.0016)	-	(0.0001 - 0.0005)	(0.0018 - 0.0024)	(0.0018 - 0.0033)	(0.0009 - 0.0029)	(0.0004 - 0.001)
	Het carrier (1 per ...)	**627**	822	1268	1270	4189	485	409	635	1620
	95% CI	**(561 - 701)**	(507 - 1336)	(643 - 2503)	-	(1920 - 9139)	(422 - 556)	(304 - 550)	(345 - 1169)	(1037 - 2530)
	Hom affected (1 per ...)	**954,812**	6,288,300	1,608,409	355,864,275	26,597,718	438,892	668,188	403,432	3,278,309
	95% CI	**(754,660 – 1,208,088)**	(1,741,461 – 22,714,660)	(414,840 – 62,62,519)	-	(4,854,203 – 145,755,408)	(329,419 – 584,786)	(363,317 – 1,229,235)	(119,257 – 1,366,727)	(1,278,534 – 8,407,444)
CESD	**Combined AF**	**0.0023**	0.0020	0.0008	0.0008	0.0003	0.0031	0.0038	0.0030	0.0007
	95% CI	**(0.0021 - 0.0025)**	(0.0015 - 0.0027)	(0.0004 - 0.0016)	-	(0.0002 - 0.0006)	(0.0027 - 0.0034)	(0.0031 - 0.0047)	(0.0019 - 0.0047)	(0.0004 - 0.001)
	Het carrier (1 per ...)	**428**	503	1268	1270	3102	327	264	334	1466
	95% CI	**(393 - 467)**	(372 - 679)	(643 - 2503)	-	(1572 - 6120)	(293 - 365)	(215 - 324)	(214 - 521)	(959 - 2241)
	Hom affected (1 per ...)	**183,543**	252,744	1,608,409	1,613,447	9,622,384	106,951	69,608	111,363	2,148,143
	95% CI	**(154,440 – 218,135)**	(138,464 – 461,513)	(413,401 – 6,262,519)	-	(2,471,435 – 37,453,801)	(86,004 – 133,004)	(46,125 – 105,079)	(45,747 – 271,522)	(919,422 – 5,020,491)
LAL-D (WD + CESD)	**Combined AF**	**0.0024**	0.0021	0.0008	0.0008	0.0003	0.0031	0.0038	0.0030	0.0007
	95% CI	**(0.0022 - 0.0026)**	(0.00154 - 0.0028)	(0.0004 - 0.0016)	-	(0.00016 - 0.0006)	(0.00279 - 0.0035)	(0.00314 - 0.0047)	(0.00192 - 0.0047)	(0.00045 - 0.001)
	Het carrier (1 per ...)	**421**	483	1268	1270	3102	321	260	334	1466
	95% CI	**(387 - 459)**	(360 - 648)	(643 - 2503)	-	(1572 - 6121)	(288 - 358)	(212 - 319)	(214 - 521)	(959 - 2241)
	Hom affected (1 per ...)	**177,452**	232,850	1,608,409	1,613,447	9,622,384	103,286	67,471	111,363	2,148,143
	95% CI	**(149,467 – 210,683)**	(129,332 – 419,377)	(413,422 – 6,262,519)	-	(2,471,746 – 37,472,145)	(83,142 – 128,321)	(44,810 – 10,1618)	(45,747 – 271,508)	(919,474 – 5,020,041)

Supplementary table 4. Prevalence of LAL-D, WD, and CESD estimated from pooled allele frequencies of previously reported variants and combined with unreported variants across 8 genetic ancestries. Het, heterozygote carrier of *LIPA* mutation; hom, homozygous patient affected with LAL-D. AFR, African / African American; ASJ, Ashkenazi Jewish; EAS, East Asian, FIN, Finnish; NFE, Non-Finnish European; LAT, Latino; OTH, Other; SAS, South Asian.

Condition	Gene	Estimated prevalence	Specific treatment available	1st line (or diagnostic) laboratory test	Estimated cost[1]
Lysosomal Acid Lipase deficiency[2]	*LIPA*	1 per 177,452	Yes: sebelipase alpha	Enzyme activity level on dried blood spot	£40
Hereditary haemochromatosis[3]	*HFE* (most)	1 per 200-500	Yes: venesection	Ferritin and transferring saturation	£11
Familial combined hyperlipidemia[4]	*USF1*	1 per 500-2000	Yes: high dose statins	Lipid profile	£2.90
Hypobetalipoproteinemia[5]	*APOB*	1 per 1000	Yes: high dose vitamins (plus diet)	Lipids, & vitamins, & blood smear	£15
Alpha 1 anti-trypsin deficiency[6]	*SERPINA1*	1 per 5-6,000 in Europe	No: symptomatic (for liver disease)	Serum alpha-1 antitrypsin level and phenotyping	£2.50 (level) £36 (phenotype)
Wilson disease[7]	*ATP7B*	1 per 10-20,000	Yes: copper chelation agents	Serum caeruloplasmin and urinary copper	£25
Gaucher's disease[8]	*GBA1*	1 per 40-60,000	Yes: enzyme-replacement therapy	White cell enzymes	£40
Glycogen storage diseases[9]	*PHKA2*, *PHKB*, *G6PC*, *AGL*, (various)	>1 per 100,000 (combined)	No: dietary management or transplantation	Clinical features plus genome sequencing	£800
Citrin deficiency[10]	*SLC25A13*	1 per 17,300-50,000 in East Asia	No: dietary management	Ammonia, citrulline, & arginine	£15
Abetalipoproteinemia[5]	*MTTP*	<1 per 100,000	Yes: high dose vitamins (plus diet)	Lipids and Apo-B-containing lipoproteins	£13
Niemann-Pick disease type C[11]	*NPC1/2*	1 per 100-150,000	Yes: substrate reduction therapy (miglustat)	"Flilipin staining" on cultured fibroblasts	£485
Congenital generalised lipodystrophy[12]	*AGPAT2*, *BSCL2*	1 per 10,000,000	Yes: leptin	Fasting (or post-prandial) leptin & insulin	£15
Weber-Christian disease[13]	Unknown	Unknown	No: symptomatic	Clinical features plus skin biopsy	£295

Supplementary table 5. Examples of inherited metabolic diseases that may mimic NAFLD[14]. Comparison is made to LAL-D in regards to prevalence, whether a specific treatment is available, and the cost of first-line screening tests.

Supplementary table 5references

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