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Clinical features of generalized lipodystrophy in Turkey: A cohort analysis

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Abstract

Aim: To describe the Turkish generalized lipodystrophy (GL) cohort with the frequency of each complication and the death rate during the period of the follow-up.

Methods: This study reports on 72 patients with GL (47 families) registered at different centres in Turkey that cover all regions of the country. The mean ± SD follow-up was 86 ± 78 months.

Results: The Kaplan–Meier estimate of the median time to diagnosis of diabetes and/or prediabetes was 16 years. Hyperglycaemia was not controlled in 37 of 45 patients (82.2%) with diabetes. Hypertriglyceridaemia developed in 65 patients (90.3%). The Kaplan–Meier estimate of the median time to diagnosis of hypertriglyceridaemia was 14 years. Hypertriglyceridaemia was severe (≥ 500 mg/dl) in 38 patients (52.8%). Seven (9.7%) patients suffered from pancreatitis. The Kaplan–Meier estimate of the median time to diagnosis of hepatic steatosis was 15 years. Liver disease progressed to cirrhosis in nine patients (12.5%). Liver disease was more severe in congenital lipodystrophy type 2 (CGL2). Proteinuric chronic kidney disease (CKD) developed in 32 patients (44.4%) and cardiac disease in 23 patients (31.9%). Kaplan–Meier estimates of the median time to diagnosis of CKD and cardiac disease

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were 25 and 45 years, respectively. Females appeared to have a more severe metabolic disease, with an earlier onset of metabolic abnormalities. Ten patients died during the follow-up period. Causes of death were end-stage renal disease, sepsis (because of recurrent intestinal perforations, coronavirus disease, diabetic foot infection and following coronary artery bypass graft surgery), myocardial infarction, heart failure because of dilated cardiomyopathy, stroke, liver complications and angiosarcoma.

Conclusions: Standard treatment approaches have only a limited impact and do not prevent the development of severe metabolic abnormalities and early onset of organ complications in GL.

KEYWORDS
generalized lipodystrophy, metabolic disease, mortality, organ complications

1 | INTRODUCTION

Generalized lipodystrophy (GL) is an ultrarare disease characterized by a near total absence of adipose tissue. GL is traditionally divided into two main groups based on aetiology, congenital and acquired.[1] Classical forms of congenital generalized lipodystrophy (CGL) are caused by genetic mutations in the *AGPAT2* (CGL1), *BSCL2* (CGL2), *CAV1* (CGL3) and *CAVIN1/PTRF* (CGL4) genes.[2] Additional genes have also been associated with generalized adipose tissue loss.[3-6] These classical cases of CGL develop fat loss from birth. Consanguinity is a common finding. Patients with acquired generalized lipodystrophy (AGL), on the other hand, can develop adipose tissue loss at any time during life.[1] Although the exact mechanisms of adipose tissue loss are unknown in AGL, some patients may present with panniculitis preceding loss of fat, autoimmune diseases, or can be found positive for perilipin antibodies.[7]

The estimated prevalence of diagnosed GL was reported as less than one in a million.[8] Although the disease phenotype is obvious in many cases with GL, limited awareness of the disease by the general medical community may still lead to underdiagnosis of the disease. Also, establishing epidemiology by geography is limited, with possible higher prevalence rates in countries where consanguinity is more frequent.

Adipose tissue is a metabolically active organ of a complex network that regulates crucial biological functions. The adipose tissue not only stores excess energy in the human body but also secretes adipokines that regulate metabolic processes. Leptin is one of the important adipokines secreted by adipocytes that control food intake. Leptin regulates glucose and lipid metabolism through several mechanisms.[9] In the absence of leptin, patients with GL are probable to develop severe insulin resistance and end-organ complications.[10] Also, molecular aetiology can contribute to early morbidity and mortality in GL.[11]

A clear description of the natural history of GL is critical to estimate the disease burden. Previous clinical observations reveal that metabolic disease is difficult to control in GL.[12,13] Metreleptin, a recombinant analogue of human leptin, has been approved as an adjunct to diet as replacement therapy to treat the complications of leptin deficiency in patients with GL.[14,15] Also, there are several new drugs currently being studied for lipodystrophy treatment.[16] None of these lipodystrophy-specific treatments were available in Turkey until recently, which provided a unique opportunity to study the natural disease progression in those who were not treated with lipodystrophy-specific approaches. Previously, we reported on our nationwide data from 33 patients with CGL.[17] As time passed, our registry has been supplemented with the addition of new patients. Also, we had the opportunity to follow our existing patients for a longer period of time. In this study, we report the description of the Turkish GL cohort with the frequency of each complication and the death rate during the period of the follow-up.

2 | MATERIALS AND METHODS

2.1 | Study cohort

Subjects were registered over a period of 20 years (from January 2003 to August 2022) at different centres in Turkey that cover all regions. A total of 79 patients from 49 families were registered. Seven patients were excluded because of a lack of follow-up data for at least 4 months. Data were analysed from 72 patients (from 47 independent families). The mean (standard deviation [SD]) follow-up was 86 ± 78 months. Ten patients died during the follow-up period.

GL was diagnosed clinically by treating physicians. Medical charts were reviewed, and the diagnosis of GL was confirmed by an expert (BA) in all cases. Clinical diagnosis of GL was supported by a fat loss pattern supporting near total fat loss, the timing of fat loss, medical history, family history, the presence of consanguinity (CGL cases), muscular appearance, prominent superficial veins, pseudoacromegaloid features and accompanying autoimmune features (AGL cases). Out of 67 patients with CGL, disease-causing mutations were documented in 66 subjects (98.5%). No DNA sample was available in one subject. This subject was classified as CGL based on fat loss that was apparent from birth. In five cases with AGL, the clinical diagnosis of AGL was supported by imaging. AGL was distinguished from CGL by the onset of fat loss later in life, the presence of additional autoimmune features, a lack of family history for GL and negative genetic testing for CGL.

The study protocol was reviewed and approved by the Dokuz Eylul University Ethics Committee. As per local regulations, no written consent was necessary for the use of retrospective anonymized (unidentifiable) data; however, all subjects gave written consent for genetic testing.

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2.2 | Genetic testing and imaging

DNA was isolated from peripheral blood cells and genetic analyses were conducted as described previously.[17] All genetic tests were completed at the Department of Medical Genetics, Ege University, Izmir, Turkey, except for the *CAV1* variant, which was identified in France as reported previously.[6]

Physical examination was sufficient to document the generalized fat loss in most patients. However, fat loss was further assessed in a subset of patients by whole-body magnetic resonance imaging (MRI) or dual-energy x-ray absorptiometry (DXA). Body composition scans were acquired on DXA systems manufactured by Hologic Horizon. The values for lean mass, fat mass and fat percentage for both the whole body and regional area were measured as per the manufacturer's protocol for body composition measures. Composition map colour-coded images were also reviewed visually. Whole-body MRI scans were acquired using a 1.5-T MRI system with a six multichannel body coil (Gyroscan Intera, release 8.1; Philips Medical Systems), as described previously.[18]

2.3 | Observational data collection

Data were collected prospectively at several centres using a common algorithm. Patients were examined at the time of diagnosis for GL, screened for metabolic abnormalities, tested for urine protein, then evaluated for liver disease by measuring serum alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transpeptidase (GGT) levels. Hepatic steatosis was assessed by high-resolution ultrasound with convex transducers (frequency bandwidth, 3–6 MHz). Fasting blood glucose, HbA1c, insulin, triglyceride, cholesterol levels, liver function tests and urine protein were measured by standardized methods with appropriate quality control and quality assurance procedures. The homeostasis model assessment (HOMA) score was calculated as fasting serum insulin (μIU/ml) × fasting plasma glucose (mmol/l)/22.5. Serum leptin and adiponectin levels were measured with ELISA.

Diabetes was defined according to the recommendations of the American Diabetes Association.[19] Hypertriglyceridaemia and low level of high-density lipoprotein (HDL) cholesterol were defined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines.[20] Age-specific thresholds were used for children and adolescents. Patients were assessed for gonadal functions, growth, puberty development (children) and end-organ complications. Further tests were ordered for end-organ complications based on the clinical decision of the treating physician. These clinical assessments were repeated annually. If the subject was not able to attend their follow-up visits (e.g. travel issues, restrictions during the coronavirus disease [COVID-19] pandemic), then tests were completed at their next follow-up visit.

2.4 | Retrospective chart review

Data for the current analysis were obtained through a retrospective review of medical charts and registry records. The final visit was the most recent clinical visit (leptin replacement-naïve patients) or the last visit before leptin replacement treatment initiation. Data collection tools included information regarding sex, lipodystrophy subtype, the age at diagnosis of GL, duration of follow-up, genetic testing, leptin level, the onset of diabetes and age at detection of components of metabolic disease (e.g. low HDL cholesterol, high triglycerides, hepatic steatosis, elevated liver enzymes), age at the final visit, weight and height at the final visit, laboratory assessments at the final visit, presence of acanthosis nigricans, hypertension, bone cysts, polycystic ovaries in women of reproductive age, the presence of end-organ complications (e.g. retinopathy, pancreatitis, chronic kidney disease [CKD], cirrhosis, ischaemic heart disease, major cardiovascular events, arrhythmias, cardiomyopathy, stroke, peripheral artery disease, neuropathy, diabetic foot ulcers, amputations, transplant history), age at the first cardiac event, medications (including daily insulin dose) and mortality (including causes of death in deceased patients). The cause of death was identified through hospital records at different centres and/or death certificates, and all cases were reviewed by one of the authors (BA) to establish the cause of death.

2.5 | Statistical analysis

Statistical analysis was performed using Statistical Package of Social Science (SPSS Inc.), version 22.0, for Windows. Data are expressed as mean ± SD, median (25th-75th percentiles) and range. Depending on data distribution, the Student *t*-test or Mann–Whitney U test was used for the comparison of variables. Categorical variables were compared by the χ^2 test. Time to a diagnosis of diabetes and/or prediabetes, hypertriglyceridaemia and hepatic steatosis from birth was plotted using Kaplan–Meier curves. These graphs were plotted for the whole study population and separately for different subtypes of GL. Also, Kaplan–Meier curves were generated to show the time to a diagnosis of CKD and cardiac disease. Finally, Kaplan–Meier curves were generated to describe the overall survival analysis. A log-rank test was conducted to compare time to a specific diagnosis between males and females. A *P* value of less than .05 was accepted as statistically significant.

3 | RESULTS

3.1 | Subjects and aetiology of GL

Data from 72 subjects were analysed (45 females and 27 males, mean age: 22 ± 16 years, ranging from 5 months to 76 years; Table 1). The mean body mass index was 19.06 ± 3.87 kg/m^2. Out of 72 patients, 67 had CGL (43 females and 24 males, mean age: 22 ± 15 years, ranging from 5 months to 67 years). Pathological genetic variants were documented in 66 of 67 subjects with CGL (98.5%). Variants were detected in *AGPAT2* (n = 28; 18 females, 10 males; mean age: 27 ± 17 years, range: 4 months-67 years), *BSCL2* (n = 19; eight females, 11 males; mean age: 16 ± 12 years, range: 8 months-41 years), *CAV1* (n = 4; three females, one male; mean age: 12 ± 8 years, range: 18 months-19 years), *CAVIN1/PTRF* (n = 7; six females, one male; mean age: 12 ± 8 years, range:

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TABLE 1 Demographics and laboratory assessments of patients with GL

		Mean	SD	Median	25th Percentile	75th Percentile	Minimum	Maximum
Age (y)		22	16	19	12	30	5 mo	76
Gender	Female: 45 (62.5%)							
	Male: 27 (37.5%)							
BMI (kg/m^2)		19.06	3.87	19.67	15.80	21.78	11.52	28.40
Age at diagnosis (y)		16	15	12	5	24	At birth	76
Age at first admission (y)		12	12	10	2	16	At birth	76
Follow up (mo)		86	78	72	19	132	4	384
GL subtype	AGL: 5 (6.9%)							
	CGL: 67 (93.1%)							
Leptin (ng/ml)		0.49	0.63	0.38	0.10	0.71	undetectable	4.30
Adiponectin (μg/ml)[a]		5.73	8.12	1.50	0.44	7.08	0.08	29.60
Insulin (μIU/ml)		48.79	64.08	26.30	12.40	55.00	3.00	300.00
HOMA-IR		18.47	24.50	8.54	2.72	24.06	0.84	113.28
Glucose (mg/dl)		157	97	114	88	190	72	540
HbA1c (%)		7.8	2.5	7.3	5.4	9.9	4.6	13.7
Triglyceride (mg/dl)		771	902	540	235	986	55	5033
Total cholesterol (mg/dl)		180	78	163	130	218	67	570
LDL cholesterol (mg/dl)		89	37	91	65	108	17	163
HDL cholesterol (mg/dl)		26	9	26	21	33	6	47
ALT (IU/L)		62	56	39	24	81	11	309
AST (IU/L)		52	45	40	22	70	10	267
GGT (IU/L)		65	63	43	21	80	9	326
Creatinine (mg/dl)		1.00	1.60	0.54	0.40	0.70	0.13	8.20
Urine protein (mg/day)		1911.21	3959.50	347.00	79.58	1375.00	0.18	15 200.00

Note: Data are presented as mean ± standard deviation (SD); median (25-75 percentiles); and range (minimum and maximum values). Categorical values are presented as n (%).

Abbreviations: AGL, acquired generalized lipodystrophy; *AGPAT2*, 1-acylglycerol3-phosphate O-acyltransferase 2; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; *BSCL2*, Berardinelli Seip congenital lipodystrophy 2; *CAV1*, caveolin 1; *CAVIN1*, caveolae associated protein 1; CGL, congenital generalized lipodystrophy; GGT, gamma glutamyl transferase; GL, generalized lipodystrophy; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; *LMNA*, lamin A/C; LDL, low density lipoprotein; *TYMP*, thymidine phosphorylase.

[a]Measured in a subset of subjects (n = 31).

30 months-22 years), *LMNA* (n = 6; all females; mean age: 28 ± 9 years, range: 12-40 years) and *TYMP* (an 18-year-old female and a 33-year-old male) genes. All variants are shown in Table 2. No DNA sample was available in one subject with CGL. Five patients had AGL (two females, three males; mean age: 33 ± 27 years, range: 14-76 years). AGL developed because of nivolumab in a 76-year-old male with metastatic lung cancer. The other four cases with AGL were far younger (mean age: 22 ± 12 years, ranging from 14 to 42 years). One of these cases had autoimmune diabetes and autoimmune hepatitis with accompanying low complement 4 (C4) levels. Another case had pancytopenia, possibly of autoimmune origin.

3.2 | First admission and diagnosis

The first clinic admission because of symptoms of GL occurred at an average age of 12 ± 12 (range: birth-76) years in the overall cohort (mean ± SD: 11 ± 10 years in CGL; 22 ± 31 years in AGL). However, the diagnosis of GL could be established at an average age of 16 ± 15 (range: birth-76) years, with an average delay of 52 ± 104 months between the first hospital admission for symptoms and making the diagnosis (mean ± SD: 50 ± 97 months in CGL, 86 ± 181 months in AGL). The main drivers that lead to diagnosis were physical appearance and metabolic abnormalities in both types, family history and consanguinity in CGL, and accompanying autoimmunity in AGL.

3.3 | Laboratory assessments

Laboratory assessments of patients with GL are shown in Table 1. As expected, leptin levels were low. Fasting insulin was elevated along with high HOMA-IR. Fasting glucose, HbA1c and triglycerides were elevated, and HDL cholesterol was low. Liver enzymes and GGT were moderately elevated.

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TABLE 2 Pathogenic variants detected in patients with CGL

Variant	n (total = 66)
AGPAT2	28
c.646A > T, p.(K216X)	1
c.316 + 1G > T, IVS2 + 1G > T	2
c.662-2A > C, IVS5-2 A > C	1
c.144C > A, p.(C48X)	5
c.538_539delGA, p.(D180PfsX5)	2
c.514G > A, p.(E172K)	4
c.685G > T, p.(E229X)	7
c.202C > T, p.(R68X)	3
c.268delC, p.(R90VfsX15)	1
c.667_705delinsCTGCG, p.(V223LfsX19)	2
BSCL2	19
c.316 + 2 T > C, IVS2 + 2 T > C	1
c.630 + 1G > A, IVS4 + 1G > A	4
c.766-3C > G, IVS6-3 C > G	3
c.62 A > T/c.133G > A, p.(Q21L)/p.(G45S)	2
c.88C > T, p.(Q30X)	1
c.280C > T, p.(Q94X)	4
c.465_468delGACT, p.(T156Rfs*8)	2
c.631delG, p.(V211X)	2
CAV1	4
c.237_238del, p.(H79Qfs*3)	4
CAVIN1/PTRF	7
c.1061_1091del, p.(E354Gfs*?)	1
c.481_482insGTGA, p.(K161SfsX51)	1
c.698del, p.(K233RfsX42)	1
c.214C > T, p.(Q72X)	1
c.259C > T, p.(Q87X)	3
LMNA	6
c.14565A > G)/wild, p.(K486E)	1
c.29C > T/wild, p.(T10I)	1
c.1745G > A, p. (R582H)	4
TYMP	2
c.1040 T > C, p.(L347P)	1
c.382C > T, p.(P131L)	1

Note: No DNA sample was available in one subject.
Abbreviations: *AGPAT2*, 1-acylglycerol3-phosphate O-acyltransferase 2; *BSCL2*, Berardinelli Seip congenital lipodystrophy 2; *CAV1*, caveolin 1; *CAVIN1*, caveolae associated protein 1; CGL, congenital generalized lipodystrophy; *LMNA*, lamin A/C; *TYMP*, thymidine phosphorylase.

3.4 | Diabetes and insulin resistance

Overall, 45 patients developed diabetes (62.5%) and two additional patients developed prediabetes (2.8%) during the follow-up period (Table 3). The age at the onset of diabetes was 18 ± 8 years, ranging from 3 to 45 years. The Kaplan–Meier estimate of the median time to diagnosis of diabetes and/or prediabetes was 16 years in the overall cohort (Figure 1A), 17 years in patients with CGL and 12 years in patients with AGL ($P > .05$). The Kaplan–Meier curves for GL subtypes are shown in Figure 1B. Diabetes developed at a mean age of 21 ± 9 years in patients with CGL1 and 16 ± 9 years in CGL2 ($P > .05$). Diabetes developed in a female patient with CGL3 at the age of 16 years. Her HbA1c level increased to 11.2% at the age of 19 years. Among seven patients with CGL4, diabetes developed in a female patient at the age of 20 years and quickly progressed to poorly controlled diabetes with a final HbA1c of 9.3%, despite treatment with insulin. Prediabetes was detected in a 12-year-old female with CGL4. Out of six patients with *LMNA*-associated GL, five females developed diabetes at a mean age of 13 ± 3 years. One of the two patients with *TYMP* variants developed diabetes at the age of 15 years.

Among 44 patients with diabetes, glycaemic levels were uncontrolled (≥ 7%) in 37 (82.2%), despite treatment with diet, oral antidiabetics and/or insulin. Among them, 23 patients had an HbA1c of 8.5% or higher (51.1%). Metformin was the most commonly used antidiabetic agent, followed by insulin (Table 4). The mean age at the onset of insulin treatment was 20 ± 10 years. The average daily insulin dose was 103 ± 76 units. Acanthosis nigricans was observed in 41 patients (56.9%). Twenty-three of 28 female patients of reproductive age (82.1%) had polycystic ovaries.

3.5 | Lipid abnormalities and pancreatitis

Low HDL cholesterol was almost a universal finding that was detected in 69 patients (95.8%). Hypertriglyceridaemia developed in 65 patients (90.3%) at a mean age of 13 ± 10 years. Thirty-eight patients (52.8%) had triglyceride levels exceeding 500 mg/dl, despite diet and/or treatment with lipid-lowering agents (Table 2). The most commonly used lipid-lowering agents were fibrates followed by fish oil and statins (Table 4). The Kaplan–Meier estimate of the median time to diagnosis of hypertriglyceridaemia was 13.6 years in the overall cohort (Figure 1C), 14 years in patients with CGL and 12 years in patients with AGL ($P > .05$). The GL subtypes are presented in Figure 1D.

Seven patients (9.7%) experienced episodes of acute pancreatitis (Table 3). Hypertriglyceridaemia developed earlier in patients with CGL2 compared with those with CGL1 (9 ± 10 vs. 17 ± 11 years, $P = .008$). One of four patients with CGL3 developed severely elevated triglyceride levels (5033 mg/dl). Hypertriglyceridaemia was detected in five of seven subjects with CGL4. Triglyceride levels were more than 1000 mg/dl in two of those subjects. All six females with *LMNA*-associated GL had high triglyceride levels that developed at a mean age of 18 ± 7 years. Both patients with *TYMP* variants had high triglyceride levels.

3.6 | Hepatic disease

Despite treatment with standard therapies, most patients developed end-organ abnormalities (Table 3). Hepatic steatosis was detected in

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TABLE 3 Metabolic abnormalities and organ complications in patients with GL

	All patients (n = 72)		AGL (n = 5)		CGL1 (n = 28)		CGL2 (n = 19)		CGL3 (n = 4)		CGL4 (n = 7)		*LMNA* (n = 6)		*TYMP* (n = 2)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Diabetes	45	62.5	0	0.0	7	25.0	8	42.1	3	75.0	5	71.4	1	16.7	1	50.0
Prediabetes	2	2.8	4	80.0	21	75.0	11	57.9	1	25.0	1	14.3	5	83.3	1	50.0
Acanthosis	41	56.9	1	20.0	0	0.0	0	0.0	0	0.0	1	14.3	0	0.0	0	0.0
Polycystic ovaries[a]	23	82.1	2	40.0	10	35.7	4	21.1	3	75.0	6	85.7	4	66.7	1	50.0
Retinopathy	11	15.3	5	100.0	21	75.0	17	89.5	3	75.0	7	100.0	6	100.0	2	100.0
Neuropathy	13	18.1	0	0.0	7	2.0	2	10.5	1	25.0	0	0.0	0	0.0	0	0.0
Low HDL cholesterol	69	95.8	5	100.0	20	71.4	16	84.2	4	100.0	7	100.0	6	100.0	0	0.0
Hypertriglyceridaemia	65	90.3	0	0.0	8	28.6	3	15.8	0	0.0	0	0.0	0	0.0	2	100.0
Triglyceride > 500 mg/dl	38	52.8	0	0.0	2	7.1	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0
Pancreatitis	7	9.7	5	100.0	26	92.9	18	94.7	4	100.0	7	100.0	6	100.0	2	100.0
Hepatic steatosis	64	88.9	1	20.0	3	10.7	0	0.0	1	25.0	2	28.6	0	0.0	0	0.0
Elevated liver enzymes	41	56.9	4	80.0	25	89.3	19	100.0	3	75.0	5	71.4	6	100.0	2	100.0
Cirrhosis	9	12.5	2	40.0	12	42.9	10	52.6	3	75.0	5	71.4	1	16.7	1	50.0
Chronic kidney disease	32	44.4	3	60.0	16	57.1	9	47.4	1	25.0	2	28.6	5	83.3	1	50.0
Hypertension	11	15.3	4	80.0	25	89.3	19	100.0	4	100.0	7	100.0	4	66.7	2	100.0
Cardiovascular disease	23	31.9	3	60.0	20	71.4	16	84.2	4	100.0	0	0.0	3	50.0	2	100.0
Coronary artery disease	8	11.1	2	40.0	8	28.6	3	15.8	0	0.0	7	100.0	3	50.0	0	0.0
Myocardial infarction	3	4.2	5	100.0	21	75.0	19	100.0	4	100.0	7	100.0	5	83.3	2	100.0
Cardiomyopathy	10	13.9	0	0.0	7	25.0	0	0.0	0	0.0	0	0.0	1	16.7	0	0.0
Arrhythmia	8	11.1	5	100.0	25	89.3	19	100.0	4	100.0	7	100.0	6	100.0	2	100.0
Peripheral artery disease	3	4.2	0	0.0	3	10.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Foot ulcer	7	9.7	3	60.0	26	92.9	17	89.5	4	100.0	5	71.4	4	66.7	2	100.0
Amputation	4	5.6	2	40.0	2	7.1	2	10.5	0	0.0	2	28.6	2	33.3	0	0.0
Bone cysts[b]	18	40.1	5	100.0	28	100.0	18	94.7	4	100.0	0	0.0	6	100.0	2	100.0
Scoliosis	6	8.3	0	0.0	0	0.0	1	5.3	0	0.0	7	100.0	0	0.0	0	0.0

Note: Data are presented as n (%).

Abbreviations: AGL, acquired generalized lipodystrophy; CGL, congenital generalized lipodystrophy; ESRD, end-stage renal disease; GL, generalized lipodystrophy; HDL, high density lipoprotein; *LMNA*, lamin A/C; *TYMP*, thymidine phosphorylase.

[a]Evaluated in 28 females of reproductive age.

[b]Imaging available in 44 patients; not all patients screened by using whole-body MRI and/or a skeletal survey. No DNA sample was available in one subject with CGL.

64 patients (88.9%) with a mean age at diagnosis of 13 ± 9 years. Forty-one patients (56.9%) had elevated liver function tests (LFTs). In some patients, elevated LFTs detected earlier than hepatic steatosis were found upon imaging, reflecting infrequent routine ultrasound follow-ups for hepatic disease in many of our patients. The liver disease progressed to cirrhosis in nine patients (12.5%). Of those, two patients had AGL, one had CGL1 and six had CGL2. A girl with CGL2 received a liver transplant at the age of 12 years.

The Kaplan–Meier estimate of the median time to diagnosis of hepatic steatosis was 15 years in the overall cohort (Figure 2A), 14 years in patients with CGL and 12 years in patients with AGL ($P > .05$). GL subtypes are presented in Figure 2B. Patients with CGL2 had more severe hepatic disease with a significantly earlier onset compared with CGL1 (9 ± 8 vs. 16 ± 7, $P = .002$) years. Hepatic steatosis was detected in one of four patients with CGL3, five of seven with CGL4 and all patients with *LMNA*-associated GL and *TYMP* variants.

3.7 | Kidney disease

In addition to the classical triad of poorly controlled diabetes, lipid abnormalities causing pancreatitis and liver disease, patients with GL developed additional co-morbidities and end-organ complications. The kidney was a frequently affected organ. Kidney disease was characterized by proteinuria progressing to kidney failure. Mean urinary protein excretion was 1911 ± 3960 (range: 0.18-15 200) mg/day.

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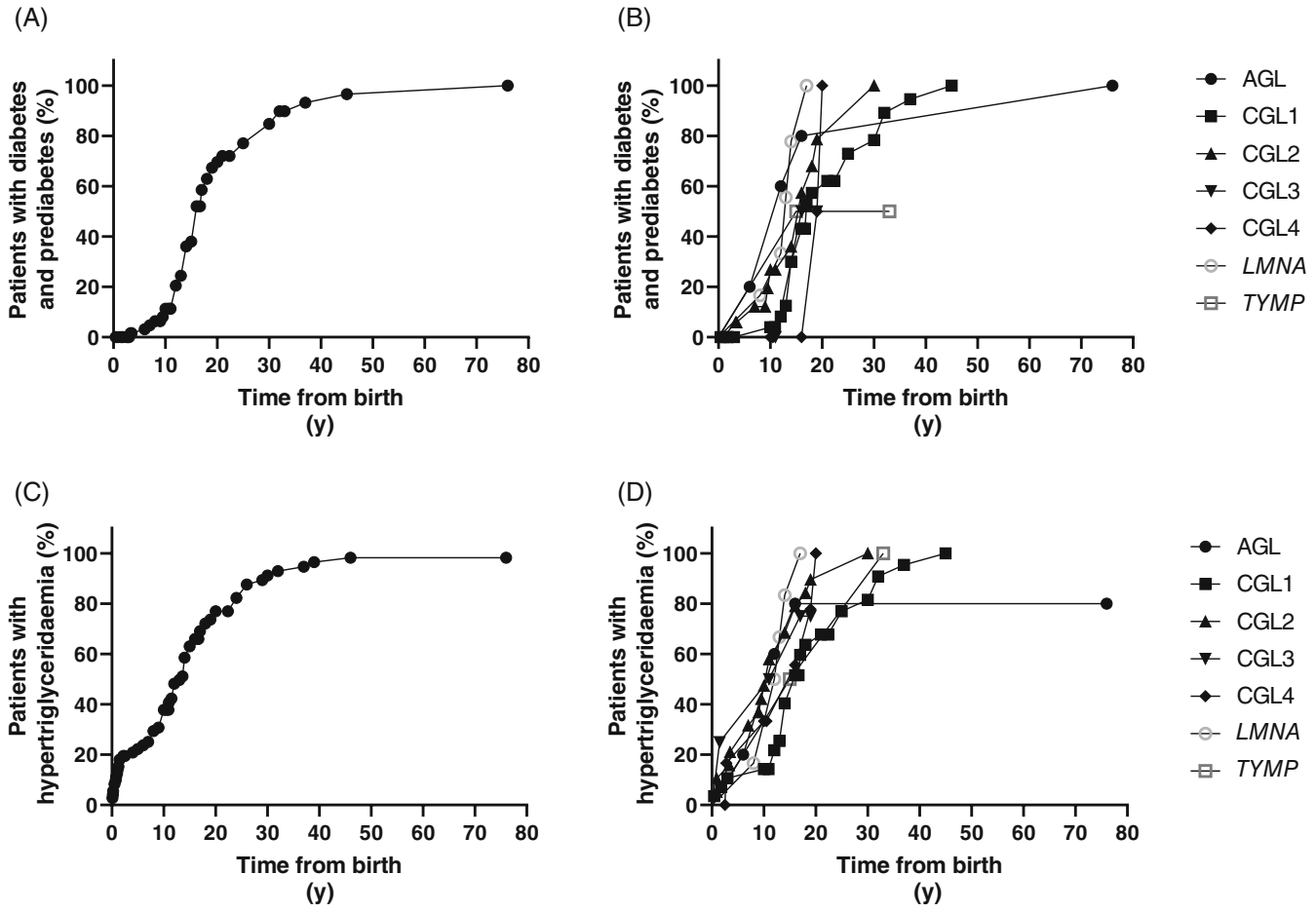


FIGURE 1 Time to diabetes/prediabetes, A, In the whole study cohort, and B, Stratified by molecular aetiology. Time to hypertriglyceridaemia, C, In the whole study cohort, and D, Stratified by molecular aetiology. Number of subjects (n): 72; AGL: 5 (6.9%), CGL1 (*AGPAT2*): 28 (38.9%), CGL2 (*BSCL2*): 19 (26.4%), CGL3 (*CAV1*): 4 (5.6%), CGL4 (*CAVIN1*): 7 (9.7%), *LMNA*: 6 (8.3%), *TYMP*: 2 (2.8%), no DNA sample available: 1 (1.4%). AGL, acquired generalized lipodystrophy; CGL, congenital generalized lipodystrophy

TABLE 4 Medications used in leptin naïve patients with GL

	n	%
Antidiabetic agents	44	61.1%
Insulin	31	43.1%
Metformin	37	51.4%
DDP4i	2	2.8%
TZDs	9	12.5%
Lipid-lowering agents	46	63.9%
Statins	8	11.1%
Fibrates	39	54.2%
Fish oil	14	19.4%
ACEi/AT2 antagonists	18	25.0%
Beta blockers	10	13.9%
Calcium channel blockers	3	4.2%
Antiplatelet agents	8	11.1%

Note: Data are presented as n (%).
Abbreviations: ACEi; angiotensin-converting-enzyme inhibitor; AT2, angiotensin II receptor type 2; DDP4i, dipeptidyl peptidase-4 inhibitor; GL, generalized lipodystrophy; TZD; thiazolidinedione.

CKD developed in 32 individuals (44.4%) during the follow-up. Six patients (8.3%) developed end-stage renal disease (ESRD). Of note, CKD was observed in the absence of longstanding diabetes in some patients, and most patients with CKD had no diabetic retinopathy. The mean age at diagnosis of CKD was 25 ± 11 years. The Kaplan–Meier estimate of median time to diagnosis of CKD was 25 years in the overall cohort (Figure 2C). GL subtypes are shown in Figure 2D.

3.8 | Cardiovascular disease

Cardiovascular disease was common in GL, developing in 31.9% of cases (Table 3). Hypertension was detected in 15.3% of patients. Coronary artery disease developed in nine patients. All these patients had diabetes and it was poorly controlled in many of them (eight patients had HbA1c ≥ 7% at the final visit). Cardiomyopathy was detected in 10 patients (13.9%); however, it should be noted that regular echocardiogram surveillance was not performed, and echocardiogram reports were not available for some patients. Cardiac arrhythmias were detected predominantly in patients with

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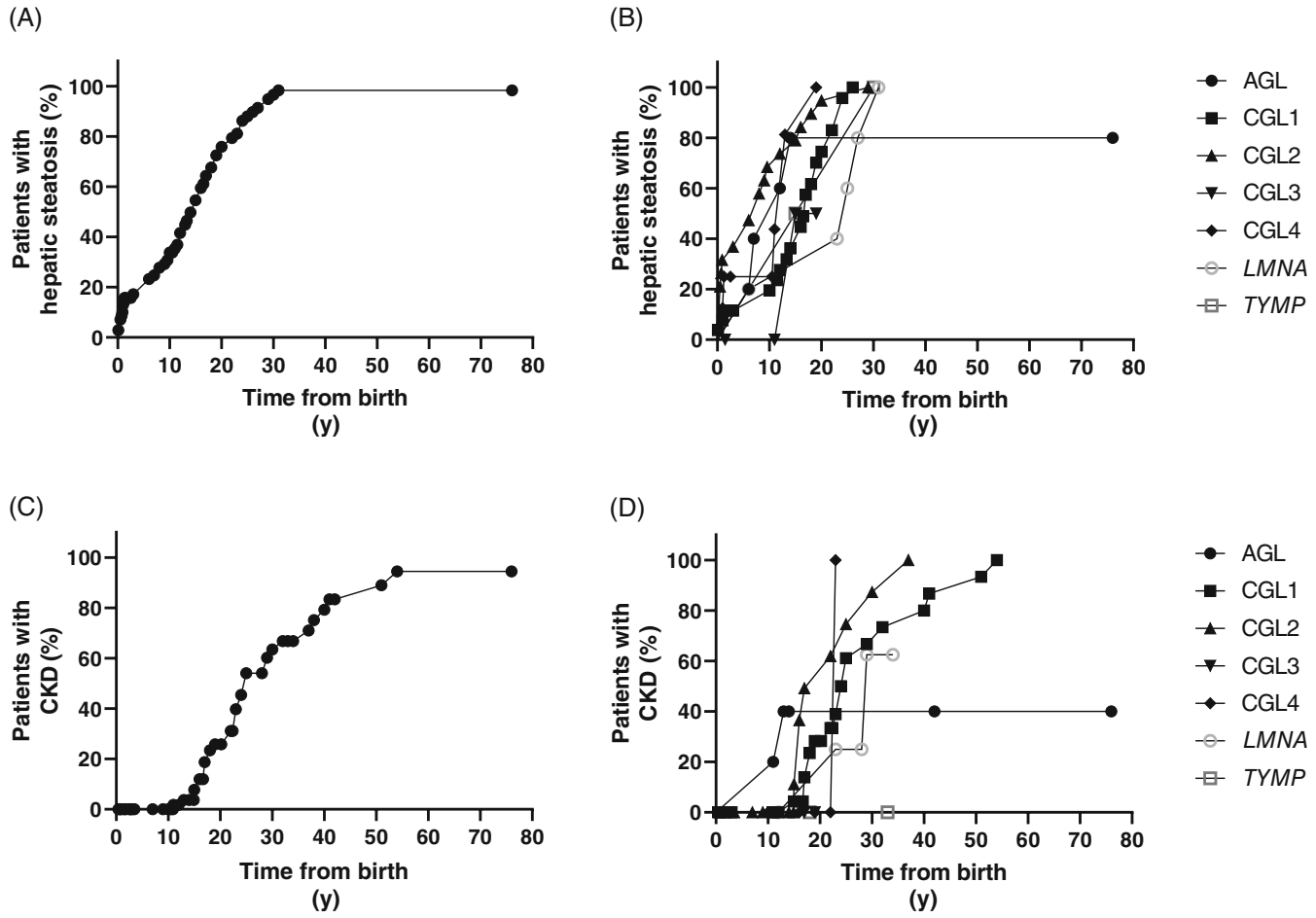


FIGURE 2 Time to hepatic steatosis, A, In the whole study cohort, and B, Stratified by molecular aetiology. Time to chronic kidney disease, C, In the whole study cohort, and D, Stratified by molecular aetiology. Number of subjects (n): 72; AGL: 5 (6.9%), CGL1 (*AGPAT2*): 28 (38.9%), CGL2 (*BSCL2*): 19 (26.4%), CGL3 (*CAV1*): 4 (5.6%), CGL4 (*CAVIN1*): 7 (9.7%), *LMNA*: 6 (8.3%), *TYMP*: 2 (2.8%), no DNA sample available: 1 (1.4%). AGL, acquired generalized lipodystrophy; CGL, congenital generalized lipodystrophy; CKD, chronic kidney disease

CGL4. All seven patients with CGL4 had cardiac arrhythmia as a cardinal component of the disease.

The mean age at diagnosis of cardiac disease was 18 ± 15 years. Cardiac disease was diagnosed earlier in patients with CGL4 (mean age at onset: 9 ± 6 years). Also, patients with *LMNA* variants had a comparatively young onset of cardiac disease (mean age at onset: 14 ± 12 years). The Kaplan–Meier estimate of median time to diagnosis of cardiac disease was 45 years in the overall cohort (Figure 3A). Patients with CGL4 had a significantly shorter median time-to-diagnosis of cardiac disease compared with two comparatively common subtypes of CGL (9 vs. 45 years, $P < .001$; Figure 3B).

3.9 | Additional organ complications

We observed additional co-morbidities during follow-up (Table 3). Diabetic foot ulcers developed in a subset of patients because of neuropathy and peripheral artery disease. Retinopathy was detected in 11 patients (15.3%) with diabetes. Bone cysts were predominantly detected in patients with CGL1 (14 patients), although a subset of patients with other subtypes (three with CGL2 and one with *LMNA*-associated GL) had bone cysts. Scoliosis was detected in four patients with CGL4, one with CGL1 and one patient with *LMNA*-associated GL. Gastrointestinal dysmotility was observed in patients with CGL3 and CGL4. Pyloric stenosis was detected in CGL4. Two patients with CGL3 had achalasia. Also, atypical retinitis pigmentosa was found in a patient with CGL3. Some of these findings were reported previously.[17,21-25]

3.10 | Sex effect

In general, females experienced more severe metabolic disease than males. Because GL has a multiple aetiology, we studied the effect of sex in our largest datasets of monogenic GL, CGL1 (18 females, mean age: 27 ± 16 years vs. 10 males, mean age 26 ± 19 years, $P > .05$). Females with CGL1 had significantly higher HbA1c (9.9% ± 2.3% vs. 6.8% ± 1.7%, $P = .002$), higher fasting insulin (63.74 ± 78.01 vs. 20.03 ± 16.20 μIU/ml, $P = .050$), higher HOMA-IR (26.78 ± 31.78 vs. 7.57 ± 9.22, $P = .016$) and

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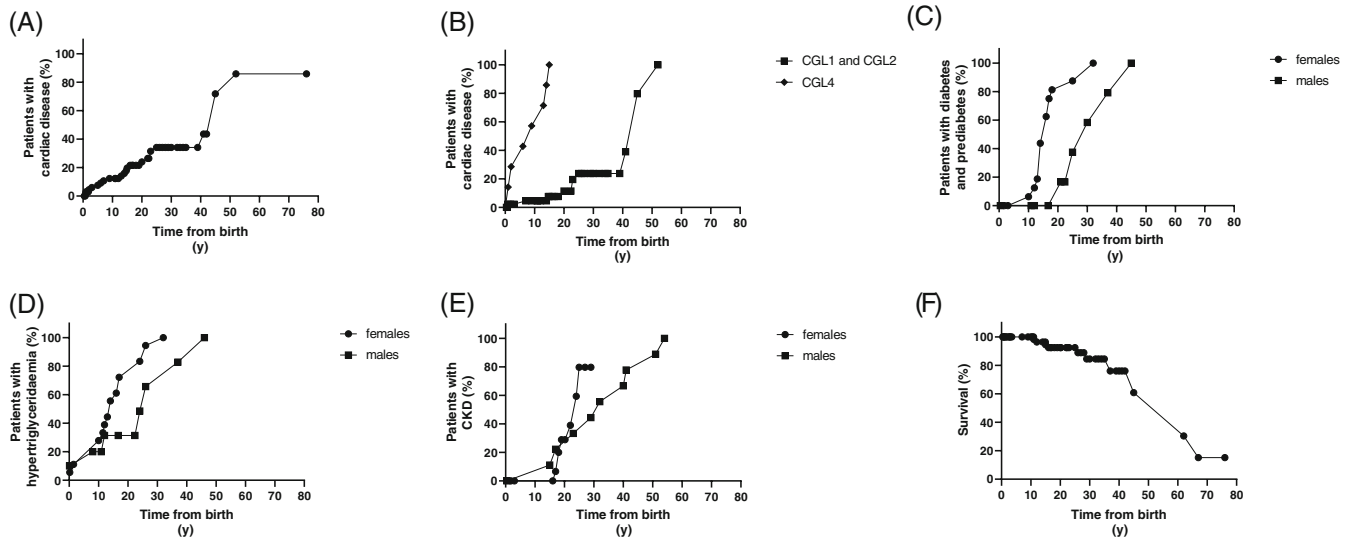


FIGURE 3 Time to cardiac disease, A, In the whole study cohort, and B, CGL1 and CGL2 versus CGL4. C, Time to diabetes/prediabetes in CGL1 (males vs. females). D, Time to hypertriglyceridaemia in CGL1 (males vs. females). E, Time to chronic kidney disease in CGL1 (males vs. females). F, Overall survival in the whole study cohort. Number of subjects (n): 72; AGL: 5 (6.9%), CGL1 (*AGPAT2*): 28 (38.9%), CGL2 (*BSCL2*): 19 (26.4%), CGL3 (*CAV1*): 4 (5.6%), CGL4 (*CAVIN1*): 7 (9.7%), *LMNA*: 6 (8.3%), *TYMP*: 2 (2.8%), no DNA sample available: 1 (1.4%). AGL, acquired generalized lipodystrophy. CGL, congenital generalized lipodystrophy; CKD, chronic kidney disease

triglycerides (1115 ± 1042 vs. 277 ± 199 mg/dl, $P = .001$) and an earlier onset of diabetes (18 ± 7 vs. 32 ± 10 years, $P = .004$). Among patients with CGL1, Kaplan–Meier estimates of the median time to diagnosis of diabetes and/or prediabetes was 16 years in females and 30 years in males ($P = .004$; Figure 3C). Also, females had a significantly shorter median time to diagnosis of hypertriglyceridaemia compared with males (14 vs. 26 years, $P = .046$; Figure 3D). The median time to CKD was shorter in females than males (24 vs. 32 years), but the difference was not statistically significant ($P = .180$; Figure 3E).

3.11 | Mortality

Ten patients (three males and seven females) died during the follow-up period (Table 5). The mean age at death was 35 ± 21 (range: 11-67) years. Sepsis, ESRD, cardiovascular disease and liver disease were the leading causes. Among three males, two had CGL1 that was complicated with ESRD and coronary artery disease. These two patients died after coronary artery bypass graft procedures. Another boy with CGL2 died at the age of 11 years because of liver-related complications. Among seven females, four had CGL1. Three of them died because of organ complications of GL (myocardial infarction, stroke and ESRD and diabetic foot ulcer complicated with sepsis). Another patient with CGL1 died because of a malignant tumour. A girl with CGL4 died at the age of 15 years because of sepsis from recurrent intestinal perforations that developed as a result of intestinal dysmotility. The Kaplan–Meier estimate of the median time to death was 62 years in the overall sample (Figure 3F).

4 | DISCUSSION

This is the most comprehensive nationwide study reported to date of the disease burden in GL in the absence of lipodystrophy-specific treatments that can correct underlying leptin deficiency. Nationwide disease registries are a useful platform to improve knowledge of a disease's natural history, which is usually very limited in most rare diseases. It is crucial to understand the timing of the development of metabolic disease components in GL and how the metabolic disease evolves over time. Also, the effect of additional clinical features because of specific molecular aetiology is of interest as these may contribute to the disease burden. Although the inclusion of standard care may alter some manifestations of the disease in our setting, we believe our dataset still provides valuable information. Findings resulting from clinical observations in 72 affected individuals from 47 independent families reveal a high disease burden in GL. Standard care in GL that aims to maintain metabolic control by using similar approaches to common diseases appears to have limited impact and does not prevent the development of end-organ complications.

Diabetes developed in childhood or early adulthood and was poorly controlled in most patients with GL, despite treatment with antidiabetics including insulin. Similarly, the baseline mean HbA1c was 8.6% in the National Institute of Health (NIH) GL cohort, despite 80.3% of the patients being on antidiabetic medications.[26] It should be noted, however, that most patients included in the NIH studies were referred for leptin treatment, making it probable that these patients had more severe clinical presentations. Our study, on the other hand, registered all GL patients nationwide, covering early and late phases of lipodystrophy and all spectrums of metabolic disease, better reflecting the natural history of disease in a diverse population.

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TABLE 5 Causes of mortality in patients with GL

Age at death (y)	Gender	Genetic variant	Cause of death
37	Female	*AGPAT2*; c.662-2A > C, IVS5-2 A > C	ESRD, sepsis after COVID
62	Female	*AGPAT2*; c.202C > T, p.(R68X)	Myocardial infarction
67	Male	*AGPAT2*; c.202C > T, p.(R68X)	ESRD, died after CABG
45	Male	*AGPAT2*; c.685G > T, p.(E229X)	ESRD, sepsis after CABG
62	Female	*AGPAT2*; c.685G > T, p.(E229X)	Stroke after CABG
29	Female	*AGPAT2*; c.514G > A, p.(E172K)	Angiosarcoma
26	Female	*BSCL2*; c.280C > T, p.(Q94X)	ESRD, sepsis after diabetic foot
11	Male	*BSCL2*; c.766-3C > G, IVS6-3 C > G	Liver complications
15	Female	Acquired GL	Sepsis, multiorgan failure
16	Female	*CAVIN1*; c.259C > T, p.(Q87X)	Sepsis because of recurrent intestinal perforations
12	Female	*LMNA*; c.29C > T/wild, p.(T10I)	Dilated cardiomyopathy, heart failure

Abbreviations: *AGPAT2*, 1-acylglycerol3-phosphate O-acyltransferase 2; *BSCL2*, Berardinelli Seip congenital lipodystrophy 2; CABG, coronary artery bypass graft; COVID, coronavirus disease; ESRD, end-stage renal disease; GL, generalized lipodystrophy; *LMNA*, lamin A/C.

Lipid abnormalities are commonly detected in patients with lipodystrophy. In our cohort, the most common lipid abnormalities were low HDL cholesterol followed by elevated triglycerides. Hypertriglyceridaemia was detected in childhood in most cases. Triglycerides were severely elevated in a subset of patients, despite the use of conventional lipid-lowering agents predisposing patients to episodes of acute pancreatitis that was observed in a subset of patients. Near-total loss of adipose storage capacity in GL leads to ectopic deposition of lipids in the liver, which contributes to the development of severe insulin resistance. Liver disease is prevalent among patients with GL, and non-alcoholic fatty liver disease (NAFLD) can progress to steatohepatitis and cirrhosis at comparatively young ages.[27] Our findings suggest that hepatic disease is present from the early stages of GL and is a major factor contributing to the development of severe insulin resistance.

Despite the use of the best clinical practice available, uncontrolled metabolic disease led to end-organ complications in the absence of lipodystrophy-specific treatments in our patients. Also, our findings suggest that molecular aetiology plays a significant role in phenotypic expression and the development of organ abnormalities. Patients with CGL2 had more severe liver disease, characterized by earlier onset and progression to end-stage liver disease in the early years of life. Also, the onset of hypertriglyceridaemia was earlier in patients with CGL2. Cardiovascular complications appear to occur with increased frequency and comparatively early onset in patients with GL. Almost all patients with ischaemic heart disease had poorly controlled diabetes. In addition to poor glycaemic control, elevated triglyceride levels are a known risk factor for cardiovascular disease.[28] Besides, molecular aetiology is important for cardiac disease monitoring and some subtypes of GL may present with distinctive clinical features. Cardiac disease develops early in patients with CGL4. Life-threatening arrhythmias are probable to develop during childhood with CGL4,[29,30] regardless of metabolic disease. Patients with CGL4 are homozygous for highly deleterious mutations in the *CAVIN1/PTRF* gene resulting in a generalized lack of caveolae, a cell surface organelle involved in lipid regulation and endocytosis.[31] The pathophysiology of cardiac arrhythmia in patients with CGL4 is poorly understood. *CAVIN1/PTRF* is a protein that maintains caveola formation and function, which protects cells against mechanical stress and is involved in signal transduction.[32] *CAVIN1/PTRF*-deficient mice are known to develop progressive cardiomyopathy and fibrosis around cardiomyocytes.[33] Postmortem findings in a patient with CGL4 reported fibro-fatty infiltration of the right ventricle, which may contribute to the development of arrhythmias.[34] Also, cardiac disease developed comparatively early in patients with *LMNA* variants.[30,35,36] GL was caused by the homozygous R582H *LMNA* variant in four of six patients with *LMNA*-associated GL. A paediatric case with the *LMNA* T10I variant developed severe cardiac disease and died because of heart failure secondary to cardiomyopathy at the age of 12 years. Proteinuric nephropathy was a frequent finding and developed at comparatively young ages. Proteinuric kidney disease progressed to ESRD and required dialysis and/or kidney transplant in a subset of patients. Similarly, a high incidence of proteinuric nephropathy was reported at baseline in patients with GL treated with metreleptin at the NIH.[37] In this cohort with more severe metabolic disease, around 20% of patients presented with nephrotic range proteinuria.[38] In our cohort, additional co-morbidities included retinopathy, neuropathy, peripheral artery disease, diabetic foot ulcers, amputations, gastrointestinal abnormalities, bone abnormalities and gastrointestinal dysmotility. Gastrointestinal abnormalities were observed in both CGL3 and CGL4, in which caveolae functions are affected. CGL3 is a very rare subtype of GL. In addition to metabolic disease, Karhan et al.[25] recently described dysphagia caused by achalasia, retinitis pigmentosa, and evidence for increased oxidative stress and premature senescence in a Turkish family with CGL3. This report includes longer follow-up data on this interesting family. Finally, the *TYMP* gene has recently been associated with a generalized loss of adipose tissue, insulin-resistant diabetes, oxidative stress, altered mitochondrial functions and promoted cellular senescence.[6] In addition to the

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previously reported Turkish family, an additional case is now reported in our GL cohort.

CGL is an autosomal recessive disease in most cases. Our clinical observations and MRI assessments reveal that both affected men and women have similar body fat distribution, causing near total loss of body fat in GL, although slight variations exist based on molecular aetiology (not different in males vs. females). However, our cohort is enriched in women and, similarly to our findings, more cases reported in the literature with GL have been women.[10,11,13] Several potential factors may have caused this skewed sex distribution. It appears to be difficult to identify men affected with lipodystrophy. Many men with GL are perceived as healthy athletic people and remain undiagnosed unless they develop severe metabolic abnormalities. As shown in our study, metabolic abnormalities develop later in men compared with women, presumably leaving some males with genetic variants clinically undiagnosed for years. Family screening can also be challenging in autosomal recessive diseases because many of the pedigrees are large as a result of complex consanguinity. In these families, affected people can be found not only in the index family, but also in far relatives of the index case. Sociocultural and economic dimensions affect family-screening strategies, especially in developing countries such as Turkey. There are variations across different layers of society, and acceptance rates can be low in those sociocultural areas where most of our patients are based on our observations over several years, there is a wide range of responses to genetic testing when offered to family members. Family members usually view genetic testing as leading to better care and, when people look healthy, they find genetic screening cumbersome.

A sex effect had been previously proposed by several groups in lipodystrophy.[39,40] To investigate this observation in the context of GL, we compared disease severity between females and males. To avoid the confounding effect of molecular aetiology, this observation was tested in the largest subgroup of GL in our cohort, within the CGL1 subgroup. In line with previous observations, females had a more severe metabolic disease that was characterized by an earlier onset of metabolic abnormalities and worse metabolic tests at the final visit. The underlying mechanism of the differential effect of gender on phenotypic expression is still unclear. Adipose tissue biology and metabolism are different across genders. Physiologically, women have more subcutaneous adipose tissue than men and secrete larger amounts of leptin.[41] Subcutaneous adipose tissue expresses large amounts of oestrogen receptors that promote adipocyte differentiation when interacting with oestrogen.[42] Also, adipose tissue distribution shows anatomical differences in males and females.[41] During periods of positive energy balance, women can store larger amounts of fat in adipose tissue compared with men, preferably in the lower body.[42,43] These observations suggest that adipose tissue has crucial roles in women and fat loss-induced insulin resistance can be more disturbing in women than in men. In our study, severe metabolic abnormalities mostly developed around adolescence in patients with GL and progressed further in young adulthood. Insulin sensitivity is known to decrease during puberty, a period of physiological change in human life characterized by the activation of gonad hormones and changes in body composition.[44] Leptin is an important regulator of puberty development.[45] The underlying mechanism of pubertal changes in metabolic health and the impact of sex on changes in insulin sensitivity and lipid metabolism have been poorly understood; however, changes in pancreatic beta cell function and glucose metabolism, increase in growth hormone and insulin-like growth factor, and changes in physical activity (greater in girls than boys), probably play a significant role. Altogether, these findings can support sex dichotomy and puberty development, leading to critical differences in adipose tissue biology[43]; however, the pathophysiological basis of this observation remains largely unexplained in GL at this time.

Although limited, our data suggest a significant impact of GL on survival. The mean age at death was 35 years, and there were patients who died in childhood. The Kaplan–Meier estimate of the median time-to-death was 62 years. Mortality was mainly caused by organ complications frequently complicated with severe infections. In one of the earliest studies, van Maldergem et al.[46] reported a mean age at death of 26 years in patients with CGL2. Later, Lima et al.[47] reported a mean age of death of 27 years in patients with CGL from rural Brazil. Life expectancy was 63 years in this Brazilian cohort of CGL. Causes of death were infections, liver disease, acute pancreatitis, renal failure and sudden death/myocardial infarction. In a review of paediatric age onset lipodystrophy cases, Gupta et al.[48] reported that the mean patient age of death was 32 years in AGL and 12.5 years in CGL, with death occurring as young as 0.4 years. Our group highlighted cardiovascular complications as a cause of death, especially in patients with CGL1.[17] Finally, in parallel to our findings from the current study, the international natural history study[10] revealed cardiovascular events, cerebrovascular disease, infections, acute pancreatitis, and liver- and kidney-related complications as important causes of mortality in patients with GL. The Kaplan–Meier estimate of the mean time to death was 51 years among patients with GL in the international natural history study. Despite variation across studies, probably because of the small number of deaths analysed, there is no doubt that GL shortens life expectancy among people affected with the disease when it is not treated with lipodystrophy-specific medications.

There were several limitations to our study. Clinical assessments were undertaken by multiple physicians at different locations. However, all centres followed a common algorithm and medical records were retrospectively reviewed by a single expert. Also, the application of standardized medical data collection tools across these multiple centres has helped us easily harmonize these clinical observations. Hyperphagia is a core finding in GL.[13] Uncontrolled appetite plays a central role in the development and worsening of metabolic abnormalities in GL. Although frequently noted in medical charts, there was no dedicated assessment of hyperphagia to monitor its severity in patients with GL from our cohort. Although metabolic data were collected comprehensively, adiponectin levels were available only in a subset of patients and free fatty acid levels were not studied. Cardiomyopathy is a comparatively common finding among subjects with GL that can lead to significant morbidity and early mortality. Lupsa et al.[49] reported evidence of left ventricular hypertrophy in 58% of patients with CGL and 46% of patients with AGL. Van Maldergem

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et al.[46] reported that patients with CGL have died as early as the age of 19 months because of complications of cardiomyopathy. Our data regarding cardiomyopathy are limited to clinical symptoms reported in medical charts and these findings were only recorded if cardiomyopathy was confirmed by an echocardiogram assessment in a subset of subjects. Thus, the prevalence of cardiomyopathy may be underestimated in our sample. In addition, our report lacks data regarding valvular heart disease. Also, we did not perform a dedicated assessment for skeletal muscular hypertrophy, although it was noted in the medical charts of all affected subjects. Emerging evidence suggests that patients' quality of life is severely affected by lipodystrophy.[50] One crucial missing piece of evidence is that our study did not assess patients' perspectives, although our medical notes frequently recorded a wide range of additional symptoms. We must also acknowledge that our study does not add any novelty concerning the clinical presentation or the description of a new strategy for the diagnosis of GL; however, this large nationwide study covers crucial areas such as the aetiology of GL, complications and mortality, and can help clinicians to better understand this rare disease. Finally, our study does not provide a clear assessment regarding disease progression in childhood; however, an international multicentre retrospective analysis to address that aim is underway.

In conclusion, our results reveal a significant disease burden in people living with GL. Metabolic disease in GL has an early onset, is highly distressing, and leads to further, potentially life-threatening, complications. Standard care in GL, without leptin replacement, is far from controlling metabolic disease that remains largely uncontrolled and progresses to end-organ abnormalities at comparatively young ages.

AUTHOR CONTRIBUTIONS

BA designed the study and performed data analysis. IYS and BA created the tables and figures. CA performed radiological studies. TA, HO and IJ performed genetic studies. EAO provided oversight in the execution of the study and manuscript writing. All the other authors contributed to data collection and analysis. All the authors read and approved the final version of the manuscript. BA is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All the authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

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CONFLICT OF INTEREST

BA attended advisory board meetings organized by Amryt Pharmaceuticals and Regeneron Pharmaceuticals and has served as a consultant and/or received honoraria as a speaker from Third Rock Ventures, Amryt, Regeneron, AstraZeneca, Lilly, MSD, Novartis, Novo Nordisk, Boehringer-Ingelheim, Servier and Sanofi-Aventis. EAO reports the following conflicts: grant support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Ionis Pharmaceuticals, Akcea Therapeutics, Gemphire Therapeutics, GI Dynamics (current) and AstraZeneca (past 2 years); consultant or advisor: AstraZeneca, Thera Therapeutics and BMS (past), Aegerion Pharmaceuticals (now Amryt Pharmaceuticals) and Regeneron Pharmaceuticals (current); drug support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Akcea Therapeutics and Rhythm Pharmaceuticals (all current); other support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals) and Regeneron Pharmaceuticals (current). CV received honoraria as a speaker from Amryt Pharmaceuticals, Ipsen, Lilly and Sanofi, and institutional research fundings from Aegerion Pharmaceuticals (now Amryt Pharmaceuticals). The other authors report no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15061.

DATA AVAILABILITY STATEMENT

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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