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## **Clinical features of generalized lipodystrophy in Turkey: a cohort analysis**

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## Abstract

### Aims

Without access to leptin replacement, patients with generalized lipodystrophy (GL) are at high risk for severe metabolic disease. Although there is a well-established GL registry in Turkey since the early 2000s, leptin replacement treatment was not available until recently. This gave us a unique opportunity to describe the natural history of GL.

### Methods

This study reports on 72 patients with GL (47 families) who were registered at different centers in Turkey that cover all regions of the country. The mean  $\pm$  SD follow-up was  $86 \pm 78$  months.

### Results

Kaplan-Meier estimate of the median time to diagnosis of diabetes and/or prediabetes was 16 years. Hyperglycemia was not controlled in 37 of 45 patients (82.2%) with diabetes. Hypertriglyceridemia developed in 65 patients (90.3%). Kaplan-Meier estimate of the median time to diagnosis of hypertriglyceridemia was 14 years. Hypertriglyceridemia was severe ( $\geq 500$  mg/dL) in 38 patients (52.8%). Seven (9.7%) patients suffered from pancreatitis. Kaplan-Meier estimate of the median time to diagnosis of hepatic steatosis was 15 years. Liver disease progressed to cirrhosis in 9 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 were 25 years and 45 years, respectively. Females appeared to have a more severe metabolic disease with earlier onset of metabolic abnormalities. Ten patients died during the follow-up period. Causes of death were end-stage renal disease, sepsis (due to recurrent intestinal perforations, coronavirus disease, diabetic foot infection, and following coronary artery bypass graft surgery), myocardial infarction, heart failure due to 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.

### Abbreviations:

ACEi: Angiotensin-converting-enzyme inhibitor

AGL: Acquired generalized lipodystrophy

AGPAT2: 1-acylglycerol3-phosphate O-acyltransferase 2

ALT: Alanine aminotransferase  
AST: Aspartate aminotransferase  
AT2: Angiotensin II receptor type 2  
BMI: Body mass index  
*BSC2*: Berardinelli Seip congenital lipodystrophy 2  
CABG: Coronary artery bypass graft  
*CAV1*: Caveolin 1  
*CAVIN1*: Caveolae associated protein 1  
CGL: Congenital generalized lipodystrophy  
CKD: Chronic kidney disease  
COVID: Coronavirus disease  
C4: Complement 4  
DNA: Deoxyribonucleic acid  
DPP4i: Dipeptidyl peptidase 4 inhibitor  
DXA: Dual-energy x-ray absorptiometry  
ESRD: End-stage renal disease  
GGT: Gamma glutamyl transferase  
GL: Generalized lipodystrophy  
HbA1c: Hemoglobin A1c  
HDL: High density lipoprotein  
HOMA-IR: homeostatic model assessment for insulin resistance  
LDL: Low density lipoprotein  
LFT: Liver function tests  
*LMNA*: Lamin A/C  
MHz: Megahertz  
MRI: Magnetic resonance imaging  
NAFLD: Non-alcoholic fatty liver disease  
NCEP-ATP III: National Cholesterol Education Program Adult Treatment Panel III  
NIH: National Institute of Health  
*PTRF*: Polymerase I and transcript release factor  
SD: Standard deviation  
T: Tesla  
TYMP: Thymidine phosphorylase  
TZD: Thiazolidinedione

## 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 etiology, congenital and acquired <sup>1</sup>. 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 <sup>2</sup>. Additional genes have also been associated with generalized adipose tissue loss <sup>3-6</sup>. 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 <sup>1</sup>. 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 <sup>7</sup>.

The estimated prevalence of diagnosed GL was reported as less than one in a million <sup>8</sup>. 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 <sup>9</sup>. In the absence of leptin, patients with GL are likely to develop severe insulin resistance and end-organ complications <sup>10</sup>. Also, molecular etiology can contribute to early morbidity and mortality in GL <sup>11</sup>.

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 <sup>12,13</sup>. Metreleptin, a recombinant analog 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 <sup>14,15</sup>. Also, there are several new drugs currently being studied for lipodystrophy treatment <sup>16</sup>. 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 <sup>17</sup>. 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 (between January 2003 and August 2022) at different centers in Turkey that cover all regions. A total of 79 patients from 49 families were registered. Seven patients were excluded due to a lack of follow-up data for at least four months. Data were analyzed from 72 patients (from 47 independent families). The

mean (standard deviation, SD) follow-up was  $86 \pm 78$  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, 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 5 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, 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. 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.

## **2.2 Genetic testing and imaging**

DNA was isolated from peripheral blood cells and genetic analyses were conducted as described previously<sup>17</sup>. 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<sup>6</sup>.

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 (MRI) imaging 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 percent for both the whole body and regional area were measured per the manufacturer's protocol for body composition measures. Composition map color-coded images were also reviewed visually. Whole-body MRI scans were acquired using a 1.5 Tesla MRI system with a six multichannel body coil (Gyroscan Intera, release 8.1; Philips Medical Systems) as described previously<sup>18</sup>.

## **2.3 Observational data collection**

Data were collected prospectively at several centers using a common algorithm. Patients were examined at the time of diagnosis for GL, screened for metabolic abnormalities, tested for urine protein, and evaluated for liver disease by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and  $\gamma$ -glutamyl transpeptidase (GGT) levels. Hepatic steatosis was assessed by high-resolution ultrasound with convex transducers (frequency bandwidth, 3–6 MHz). Fasting blood glucose, hemoglobin A1c (HbA1c), insulin, triglyceride, cholesterol levels, liver parameters, and urine protein were measured by standardized methods with appropriate quality control and quality assurance procedures. Homeostasis model assessment (HOMA) score was calculated as fasting

serum insulin ( $\mu\text{IU/ml}$ )  $\times$  fasting plasma glucose ( $\text{mmol/liter}$ )/22.5. Serum leptin and adiponectin levels were measured with ELISA.

Diabetes was defined according to the recommendations of the American Diabetes Association<sup>19</sup>. Hypertriglyceridemia 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<sup>20</sup>. 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), 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 at reproductive age, presence of end-organ complications (e.g., retinopathy, pancreatitis, chronic kidney disease (CKD), cirrhosis, ischemic 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 centers 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  $\pm$  standard deviation (SD), median (25<sup>th</sup>-75<sup>th</sup> 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  $\chi^2$  test. Time to a diagnosis of diabetes and/or prediabetes, hypertriglyceridemia, 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. Log-rank test was conducted to compare time to a specific diagnosis between males and females. A p-value less than 0.05 was accepted as statistically significant.

### 3. Results

#### 3.1 Subjects and etiology of GL

Data from 72 subjects were analyzed (45 females and 27 males, mean age:  $22 \pm 16$  years, ranging from 5 months to 76 years; Table 1). Mean body mass index (BMI) was  $19.06 \pm 3.87$  kg/m<sup>2</sup>. Out of 72 patients, 67 had CGL (43 females and 24 males, mean age:  $22 \pm 15$  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:  $27 \pm 17$  years, range: 4 months - 67 years), *BSCL2* (n = 19; 8 females, 11 males; mean age:  $16 \pm 12$  years, range: 8 months - 41 years), *CAV1* (n = 4; 3 females, 1 male; mean age:  $12 \pm 8$  years, range: 18 months - 19 years), *CAVIN1/PTRF* (n = 7; 6 females, 1 male; mean age:  $12 \pm 8$  years, range: 30 months - 22 years), *LMNA* (n = 6; all females; mean age:  $28 \pm 9$  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 (2 females, 3 males; mean age:  $33 \pm 27$  years, range: 14 - 76 years). AGL developed due to nivolumab in a 76-year-old male with metastatic lung cancer. The other four cases with AGL were far younger (mean age:  $22 \pm 12$  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 due to symptoms of GL occurred at an average age of  $12 \pm 12$  years (range: birth - 76 years) in the overall cohort (mean  $\pm$  SD:  $11 \pm 10$  years in CGL;  $22 \pm 31$  years in AGL). However, the diagnosis of GL could be established at an average age of  $16 \pm 15$  years (range: birth - 76 years), with an average delay of  $52 \pm 104$  months between the first hospital admission for symptoms and making the diagnosis (mean  $\pm$  SD:  $50 \pm 97$  months in CGL,  $86 \pm 181$  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.

#### 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 onset of diabetes was  $18 \pm 8$  years, ranging from 3 to 45 years. 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 ( $p > 0.05$ ). Kaplan-Meier



curves for GL subtypes are shown in Figure 1B. Diabetes developed at a mean age of  $21 \pm 9$  years in patients with CGL1 and  $16 \pm 9$  years in CGL2 ( $p > 0.05$ ). Diabetes developed in a female patient with CGL3 at the age of 16. Her HbA1c level rose to 11.2% at the age of 19. Among 7 patients with CGL4, diabetes developed in a female patient at the age of 20 and quickly progressed to poorly controlled diabetes with a final HbA1c of 9.3% despite treatment with insulin. Prediabetes was detected in another 12-year-old female with CGL4. Out of 6 patients with *LMNA*-associated GL, 5 females developed diabetes at a mean age of  $13 \pm 3$  years. One of the two patients with *TYMP* variants developed diabetes at the age of 15.

Among 44 patients with diabetes, glycemic levels were uncontrolled ( $\geq 7\%$ ) in 37 (82.2%) despite treatment with diet, oral antidiabetics, and/or insulin. Among them, 23 patients had HbA1c  $\geq 8.5\%$  (51.1%). Metformin was mostly used antidiabetic agent followed by insulin (Table 4). The mean age at the onset of insulin treatment was  $20 \pm 10$  years. The average daily insulin dose was  $103 \pm 76$  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 which was detected in 69 patients (95.8%). Hypertriglyceridemia developed in 65 patients (90.3%) at a mean age of  $13 \pm 10$  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). Kaplan-Meier estimate of the median time to diagnosis of hypertriglyceridemia was 13.6 years in the overall cohort (Figure 1C), 14 years in patients with CGL, and 12 years in patients with AGL ( $p > 0.05$ ). GL subtypes are presented in Figure 1D.

Seven patients (9.7%) suffered from episodes of acute pancreatitis (Table 3). Hypertriglyceridemia developed earlier in patients with CGL2 compared to those with CGL1 ( $9 \pm 10$  years vs  $17 \pm 11$  years,  $p = 0.008$ ). One of four patients with CGL3 developed severely elevated triglyceride levels (5033 mg/dL). Hypertriglyceridemia was detected in 5 of 7 subjects with CGL4. Triglyceride levels were more than 1000 mg/dL in 2 of them. All six females with *LMNA*-associated GL had high triglyceride levels that developed at a mean age of  $18 \pm 7$  years. Both patients with *TYMP* variants had high triglycerides.

### **3.6 Hepatic disease**

Despite treatment with standard therapies, most patients developed end-organ abnormalities (Table 3). Hepatic steatosis was detected in 64 patients (88.9%) with a mean age at diagnosis of  $13 \pm 9$  years. Forty-one patients (56.9%) had elevated liver function tests (LFTs). In some patients, elevated LFTs detected earlier than hepatic steatosis was found on imaging reflecting infrequent routine ultrasound follow-ups for hepatic disease in many of our patients. The liver disease progressed to cirrhosis in 9 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 age of 12.

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 ( $p > 0.05$ ). GL subtypes are presented in Figure 2B. Patients with CGL2 had more severe hepatic disease with a significantly earlier onset compared to CGL1 ( $9 \pm 8$  vs.  $16 \pm 7$ ;  $p = 0.02$ ). 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 comorbidities 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  $1911 \pm 3960$  mg/day (range: 0.18 – 15200 mg/day). 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  $25 \pm 11$  years. 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  $HbA1c \geq 7\%$  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 CGL4. All 7 patients with CGL4 had cardiac arrhythmia as a cardinal component of the disease.

The mean age at diagnosis of cardiac disease was  $18 \pm 15$  years. Cardiac disease was diagnosed earlier in patients with CGL4 (mean age at onset:  $9 \pm 6$  years). Also, patients with *LMNA* variants had a relatively young onset of cardiac disease (mean age at onset:  $14 \pm 12$  years). 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 to two relatively common subtypes of CGL (9 years vs. 45 years,  $p < 0.001$ , Figure 3B).

### **3.9 Additional organ complications**

We observed additional comorbidities during follow-up (Table 3). Diabetic foot ulcers developed in a subset of patients due to 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<sup>17,21-25</sup>.

### **3.10 Sex effect**

In general, females had a more severe metabolic disease than males. Because GL has multiple etiology, we studied the effect of sex in our largest datasets of monogenic GL, CGL1 (18 females, mean age:  $27 \pm 16$  years vs. 10 males, mean age  $26 \pm 19$  years,  $p > 0.05$ ). Females with CGL1 had significantly higher HbA1c ( $9.9 \pm 2.3\%$  vs.  $6.8 \pm 1.7\%$ ,  $p = 0.002$ ), higher fasting insulin ( $63.74 \pm 78.01$   $\mu\text{IU/mL}$  vs.  $20.03 \pm 16.20$   $\mu\text{IU/mL}$ ,  $p = 0.050$ ), higher HOMA-IR ( $26.78 \pm 31.78$  vs.  $7.57 \pm 9.22$ ,  $p = 0.016$ ) and triglycerides ( $1115 \pm 1042$  mg/dL vs.  $277 \pm 199$  mg/dL,  $p = 0.001$ ), and earlier onset of diabetes ( $18 \pm 7$  years vs.  $32 \pm 10$  years,  $p = 0.004$ ). Among patients with CGL1, Kaplan-Meier estimates the median time to diagnosis of diabetes and/or prediabetes was 16 years in females and 30 years in males ( $p = 0.004$ , Figure 3C). Also, females had a significantly shorter median time to diagnosis of hypertriglyceridemia compared to males (14 years vs. 26 years,  $p = 0.046$ , Figure 3D). The median time to CKD was shorter in females than males (24 years vs. 32 years), but the difference was not statistically significant ( $p = 0.180$ , Figure 3E).

### **3.11 Mortality**

Ten patients (3 males and 7 females) died during the follow-up period (Table 5). The mean age at death was  $35 \pm 21$  years (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 (CABG) procedures. Another boy with CGL2 died at age of 11 due to liver related complications. Among 7 females, 4 had CGL1. Three of them died due to 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 tumor. A girl with CGL4 died at age of 15 because of sepsis due to recurrent intestinal perforations that developed due to intestinal dysmotility. 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 due to specific molecular etiology is of interest as they 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 from 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 anti-diabetic medications<sup>26</sup>. It should be noted, however, that most patients included in the NIH studies were referred for leptin treatment, making it likely 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. 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. Hypertriglyceridemia 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 which 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 relatively young ages<sup>27</sup>. 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 etiology plays a significant role in phenotypic expression and 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 hypertriglyceridemia was earlier in patients with CGL2. Cardiovascular complications seem to occur with increased frequency and relatively early onset in patients with GL. Almost all patients with ischemic heart disease had poorly controlled diabetes. In addition to poor glycemic control, elevated triglyceride levels are a known risk factor for cardiovascular disease<sup>28</sup>. Besides, molecular etiology 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 likely to develop during childhood with CGL4<sup>29,30</sup>, 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<sup>31</sup>. 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<sup>32</sup>. *CAVIN1/PTRF* deficient mice are known to develop progressive cardiomyopathy and fibrosis around cardiomyocytes<sup>33</sup>. Postmortem findings in a patient with CGL4 reported fibro-fatty infiltration of the right ventricle which may contribute to the development of arrhythmias<sup>34</sup>. Also, cardiac disease developed relatively early in patients with *LMNA* variants<sup>30,35,36</sup>. GL was caused by the homozygous R582H *LMNA* variant in 4 of 6 patients with *LMNA*-associated GL. A pediatric case with the *LMNA* T10I variant developed severe cardiac disease and died due to heart failure secondary to cardiomyopathy at age 12.

Proteinuric nephropathy was a frequent finding and developed at relatively 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<sup>37</sup>. In this cohort with more severe metabolic disease, around 20% of patients presented with nephrotic range proteinuria<sup>38</sup>. In our cohort, additional comorbidities included retinopathy, neuropathy, peripheral artery disease, diabetic foot ulcers, amputations, gastrointestinal abnormalities, bone abnormalities, and gastrointestinal dysmotility. Gastrointestinal abnormalities were observed both in CGL3 and CGL4 in which caveola functions are affected. CGL3 is a very rare subtype of GL. In addition to metabolic disease, Karhan et al.<sup>25</sup> recently described dysphagia due to 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 been recently associated with generalized loss of adipose tissue, insulin-resistant diabetes, oxidative stress, altered mitochondrial functions, and promoted cellular senescence<sup>6</sup>. In addition to the 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 etiology (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<sup>10,11,13</sup>. Several potential factors may have caused this skewed sex distribution. It seems relatively 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 relatively later in men compared to women, presumably leaving some males with genetic variants clinically undiagnosed for years. Family screening can be also challenging in autosomal recessive diseases because many of the pedigrees are large due to 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 sociocultural areas from which most of our patients are. Based on our observations over 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<sup>39,40</sup>. To investigate this observation in the context of GL, we compared disease severity between females and males. To avoid the confounding effect of molecular etiology, 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 worst 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<sup>41</sup>. Subcutaneous adipose tissue expresses large amounts of estrogen receptors that promote adipocyte differentiation when interacting with estrogen

<sup>42</sup>. Also, adipose tissue distribution shows anatomical differences in males and females <sup>41</sup>. During periods of positive energy balance, women can store larger amounts of fat in adipose tissue as compared with men, preferably in the lower body <sup>42,43</sup>. 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 physiologic change in human life characterized by the activation of gonad hormones and changes in body composition <sup>44</sup>. Leptin is an important regulator of puberty development <sup>45</sup>. 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 (IGF-1), and changes in physical activity (greater in girls than boys) are likely to play a significant role. Altogether, these findings can support sex dichotomy and puberty development leading to critical differences in adipose tissue biology <sup>43</sup>; however, the pathophysiological basis of this observation largely remains 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. 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. <sup>46</sup> reported a mean age at death of 26 years in patients with CGL2. Later, Lima et al. <sup>47</sup> 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 pediatric age onset lipodystrophy cases, Gupta et al. <sup>48</sup> 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. Later, our group highlighted cardiovascular complications as a cause of death, especially in patients with CGL1 <sup>17</sup>. Finally, in parallel to our findings from the current study, the international natural history study <sup>10</sup> revealed cardiovascular events, cerebrovascular disease, infections, acute pancreatitis, and liver and kidney-related complications as important causes of mortality in patients with GL. 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 likely due to the small number of deaths analyzed, there is no doubt that GL shortens life expectancy among people affected with the disease when not treated with lipodystrophy-specific medications.

There were several limitations of our study. Clinical assessments were undertaken by multiple physicians at different locations. However, all centers 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 centers has helped us easily harmonize these clinical observations. Hyperphagia is a core finding in GL <sup>13</sup>. 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 relatively common finding among subjects with GL that can lead to significant morbidity and

early mortality. Lupsa et al. <sup>49</sup> reported evidence of left ventricular hypertrophy in 58% of patients with CGL and 46% of patients with AGL. Van Maldergem et al. <sup>46</sup> reported that patients with CGL have died as early as age 19 months due to complications of cardiomyopathy. Our data regarding cardiomyopathy is 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 <sup>50</sup>. One crucial missing piece is that our study did not assess patient perspective 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 etiology of GL, complications, and mortality, and can help clinicians better understand this rare disease. Finally, our study does not provide a clear assessment regarding disease progression in childhood; however, an international multicenter retrospective analysis to 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 relatively young ages.

### **Author contributions**

BA designed the study and performed data analysis. IYS and BA created 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 other authors contributed to data collection and analysis. All 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 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), AstraZeneca (past two years). Consultant or Advisor: AstraZeneca, Thera Therapeutics, and BMS (past), Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Regeneron Pharmaceuticals (current). Drug support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Akcea Therapeutics, Rhythm Pharmaceuticals (all current). Other support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), 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). Other authors report no conflicts of interest.

**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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## Tables

**Table 1:** Demographics and laboratory assessments of patients with GL

	Mean	SD	Median	25th Percentile	75th Percentile	Minimum	Maximum
Age (years)	22	16	19	12	30	5 months	76
Gender	Female: 45 (62.5%)						
	Male: 27 (37.5%)						
BMI (kg/m <sup>2</sup> )	19.06	3.87	19.67	15.80	21.78	11.52	28.40
Age at diagnosis (years)	16	15	12	5	24	At birth	76
Age at first admission (years)	12	12	10	2	16	At birth	76
Follow up (months)	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 (ug/mL)	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	15200.00

Data are presented as mean standard  $\pm$  deviation (SD); median (25-75 percentiles); and range (minimum and maximum values). Categorical values are presented as n (%). \*measured in a subset of subjects (n = 31).

Abbreviations: AGL; acquired generalized lipodystrophy, *AGPAT2*; 1-acylglycerol3-phosphate O-acyltransferase 2, ALT; alanine aminotransferase, AST; aspartate aminotransferase, BMI; body mass index, *BSCCL2*; 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.

**Table 2:** Pathogenic variants detected in patients with CGL

Variant	n (total = 66)
<i>AGPAT2</i>	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
<i>BSCL2</i>	19
c.316+2T>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
<i>CAV1</i>	4
c.237_238del, p.(H79Qfs*3)	4
<i>CAVIN1/PTRF</i>	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
<i>LMNA</i>	6
c.14565A>G) / wild, p.(K486E)	1
c.29C>T / wild, p.(T10I)	1
c.1745G>A, p. (R582H)	4
<i>TYMP</i>	2
c.1040T>C, p.(L347P)	1
c.382C>T, p.(P131L)	1

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.

**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*	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
Hypertriglyceridemia	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#	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

Data are presented as n (%). \*Evaluated in 28 females of reproductive age. #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.

Abbreviations: AGL; acquired generalized lipodystrophy, CABG; coronary artery bypass graft, CGL; congenital generalized lipodystrophy, eGFR; estimated glomerular filtration rate, ESRD; end-stage renal disease, GL; generalized lipodystrophy, HDL; high density lipoprotein, LMNA; lamin A/C, TYMP; thymidine phosphorylase.

**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%

Data are presented as n (%). ACEi; angiotensin-converting-enzyme inhibitors, AT2; Angiotensin II receptor type 2, DDP4i; dipeptidyl peptidase 4 inhibitors, TZD; thiazolidinediones.

**Table 5:** Causes of mortality in patients with GL

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

Abbreviations: *AGPAT2*; 1-acylglycerol3-phosphate O-acyltransferase 2, *BSCL2*; Berardinelli Seip congenital lipodystrophy 2, *CAV1*; caveolin 1, CABG; coronary artery bypass graft, *CAVIN1*; caveolae associated protein 1, COVID; coronavirus disease, ESRD; end-stage renal disease, GL; generalized lipodystrophy, *LMNA*; lamin A/C.



## Figure legend

### Figure 1.

Time to diabetes/prediabetes (A) in the whole study cohort, (B) stratified by molecular etiology. Time to hypertriglyceridemia (C) in the whole study cohort, (D) stratified by molecular etiology. 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%).

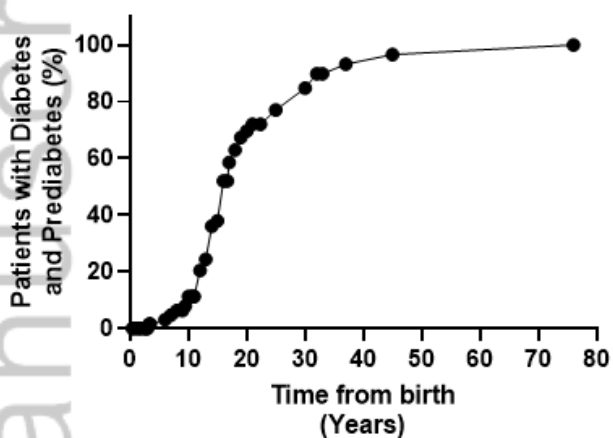
### Figure 2.

Time to hepatic steatosis (A) in the whole study cohort, (B) stratified by molecular etiology. Time to chronic kidney disease (C) in the whole study cohort, (D) stratified by molecular etiology. 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%).

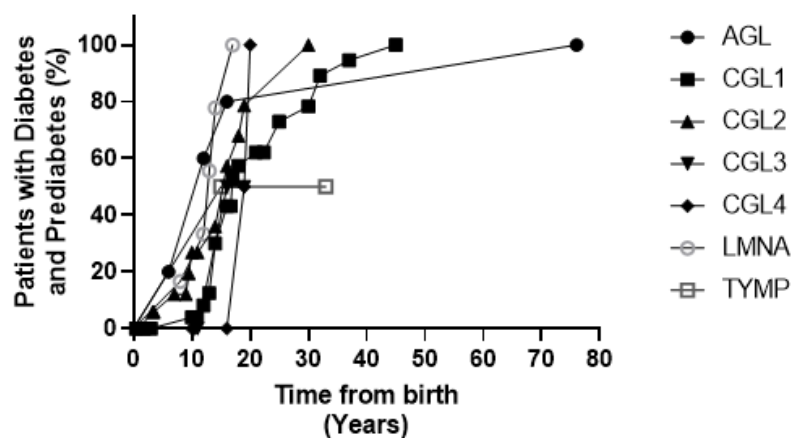
### Figure 3.

Time to cardiac disease (A) in the whole study cohort, (B) CGL1 and CGL2 vs. CGL4. Time to diabetes/prediabetes (C) in CGL1 (males vs. females). Time to hypertriglyceridemia (D) in CGL1 (males vs. females). Time to chronic kidney disease (E) in CGL1 (males vs. females). Overall survival (F) 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%).

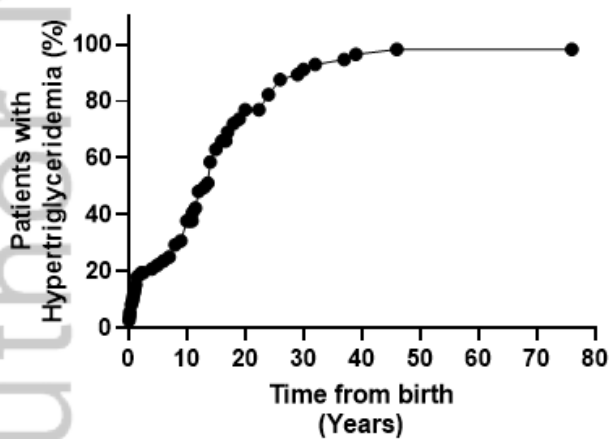
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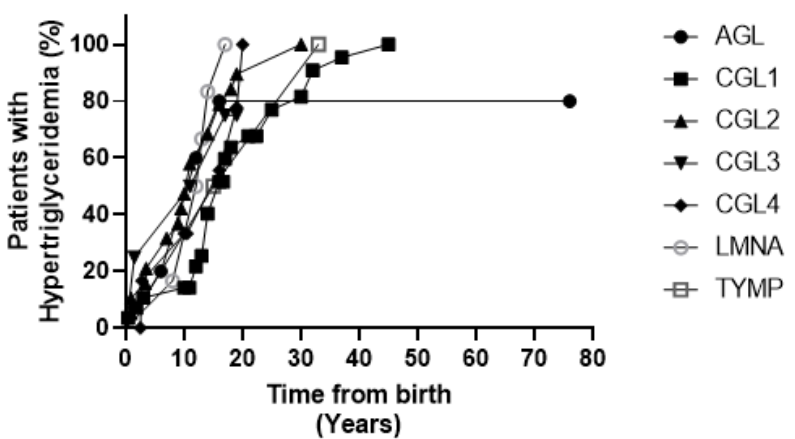
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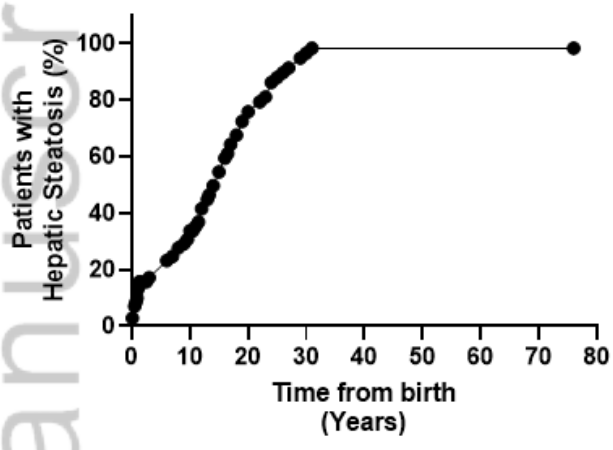
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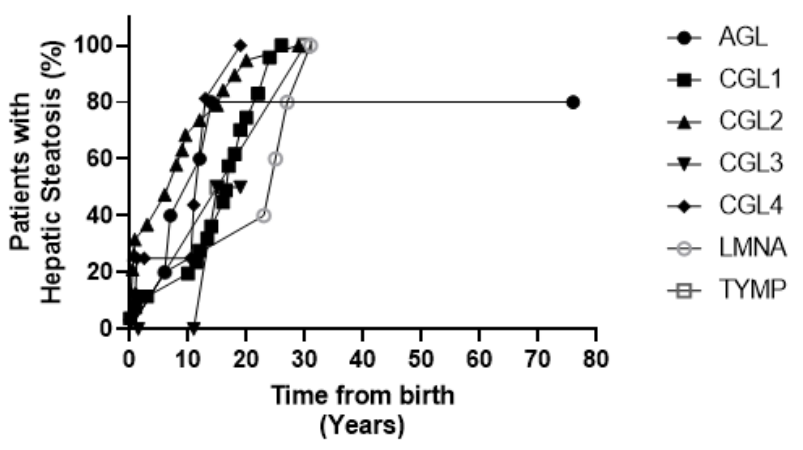
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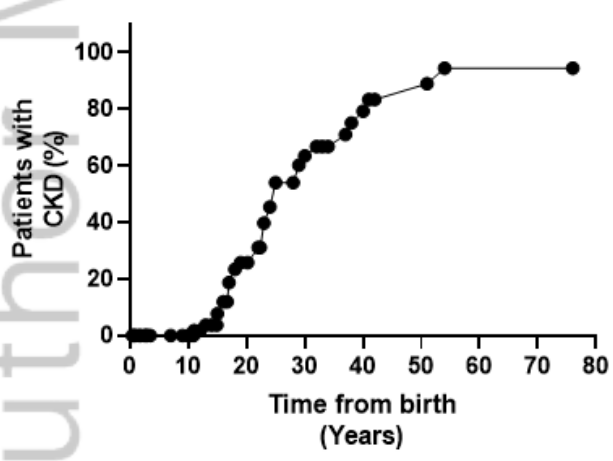
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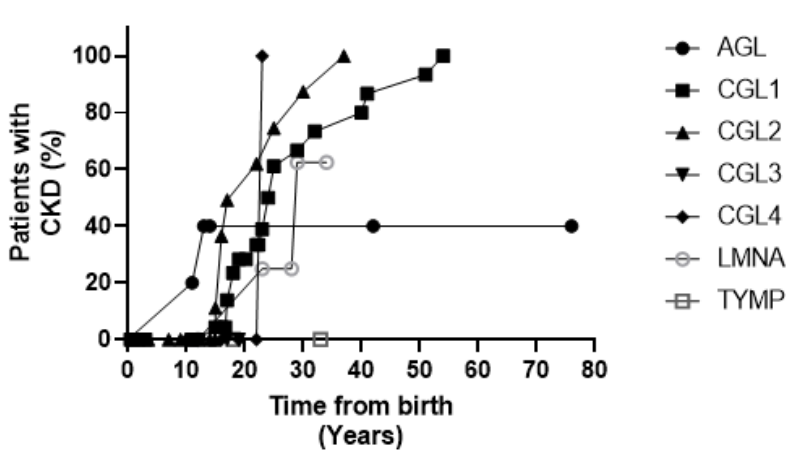
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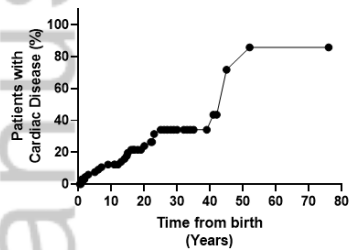
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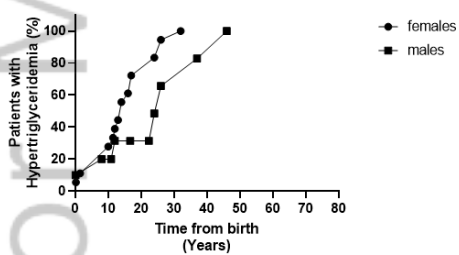
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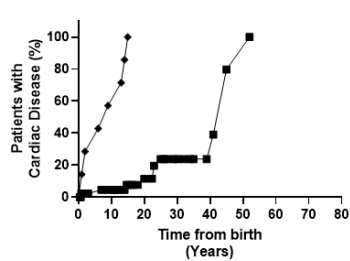
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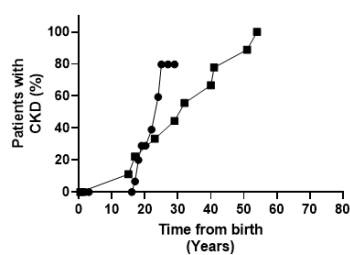
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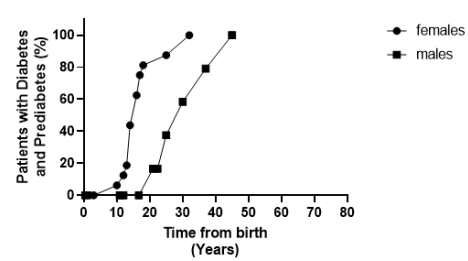
B.



E.



C.



F.

