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High incidence of cholesterol gallstone disease in type 1 Gaucher disease: characterizing the biliary phenotype of type 1 Gaucher disease

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Abstract

Background—In Gaucher disease (GD), lysosomal glucocerebrosidase deficiency results in glucosylceramide accumulation in macrophage lysosomes. Hepatocytes do not accumulate glucosylceramide due in part to biliary secretion. Although gallstones (GS) occur in type 1 Gaucher disease (GD1), the chemical nature of stones, their association with metabolic

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parameters, and whether bile composition is altered are not understood. We assessed the prevalence of GS, their chemical composition, biliary lipids, and associated metabolic factors.

Methods—The study cohort comprised 417 patients comprehensively evaluated for GD1 severity. Ascertainment of GS, fasting lipoprotein profile, and bile lipid analyses were performed.

Results—The prevalence of GS in GD1 was 32%. Compared with men, the prevalence of GS was higher in women, increasing from 4.2% and 11.8% at age 20–29 years to 71% and 60% at age >70 years, respectively. Patients with GS were more likely to be asplenic ($p<0.0001$), older ($p<0.0001$), have higher low-density lipoprotein (LDL) cholesterol ($p=0.002$), and more severe GD1 disease compared with those without GS. On multiple logistic regression analysis, factors associated with GS were age ($p<0.001$), female sex ($p=0.03$), and splenectomy ($p=0.005$). Compared with the general population, prevalence of GS was ~5-fold higher. Bile lipid analyses revealed cholesterol stones in five patients and pigment stones in one. Bile lipid composition was abnormal and contained glucosylceramide.

Conclusions—Our results point to a metabolic syndrome in GD1 consisting of a propensity to cholesterol GS, low high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and body mass index (BMI) associated with abnormal biliary lipid secretion.

Introduction

The incidence of cholesterol gallstone disease (CGD) in the Western world is ~15% and is endemic among Hispanics and American Indians (Shaffer 2006). Age, female sex, obesity, and type 2 diabetes are well-known risk factors for CGD (Shaffer 2006). These factors are the key components of the metabolic syndrome due to insulin resistance (Eckel et al. 2005). In fact, induction of selective insulin resistance in the liver of a mouse model resulted in CGD (Biddinger et al. 2008). Additional insights implicate the role of infection, chronic inflammation, and immune dysfunction, especially involving T lymphocytes, in the development of cholesterol gallstones (Maurer et al. 2009). There has been intense interest in the delineation of genetic susceptibility factors for CGD following the demonstration of striking ethnic differences and phenotypic concordance in identical twins but not in dizygotic twins (Hoblinger and Lammert 2008). This led to an extensive inventory of candidate genes for human CGD. These candidate genes involve bile lipid secretion (i.e., ABC G5/G8, ABCB4, and ABCB11), lipoprotein metabolism, and bile acid synthesis. An example of a monogenic disorder that results in CGD phenotype is mutations in ABCB4 (MDR3 or progressive familial intrahepatic cholestasis type 3) (Hoblinger and Lammert 2008).

An inborn error of metabolism that is associated with gallstones as well as chronic systemic inflammation, T cell dysfunction, and insulin resistance is type 1 Gaucher disease (GD1), the most common lysosomal storage disorder (Cox and Schofield 1997). In GD, lysosomal glucocerebrosidase (GCase) deficiency due to mutations in the acid β -glucosidase (*GBA1*) gene leads to glucocerebroside (GlcCer) accumulation in mononuclear phagocyte lysosomes and a complex multisystemic phenotype involving the liver, spleen, bone marrow, skeleton, and lungs. In rare neuronopathic forms of the disease, neurodegenerative disease occurs (Beutler 2006). Chronic inflammation and immune dysfunction, notably T-cell dysfunction, are known pathophysiologic features of the disease (Lacerda et al. 1999; Allen et al. 1997). The overall prevalence of GD1 is 1:40,000, but in the Ashkenazi Jews, it is ~1:550 (Grabowski 2005).

Abnormal metabolism of glycolipids and plasma lipoproteins may a priori contribute to cholesterol gallstones in GD. GlcCer storage material accumulates in tissue macrophages but not in other cells, although lysosomal GCase is deficient in all cell types in GD.

Presumably, the residual GCase activity is sufficient to hydrolyze endogenously generated GlcCer in parenchymal cells, but the storage material accumulates in the macrophages due to high turnover of exogenous complex glycolipids arising from senescent cell membranes (Beutler 2001). It has been suggested that GlcCer does not accumulate overtly in hepatocytes due to efficient biliary secretion of GlcCer (Pentchev et al. 1981; Tokoro et al. 1987). Whether the presence of glucosylceramide and related lipids alter bile lithogenicity is not known. Metabolism of plasma lipoproteins is also abnormal in GD: hypercatabolism of apolipoproteins (apo) B and A1 was found to underlie low low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels characteristic of GD (Le et al. 1988). It was suggested that increased lipoprotein catabolism was mediated via the activated macrophage system. However, the question of whether lipid flux through the hepatocyte is also abnormal has not been investigated (Le et al. 1988; Lorberboym et al. 1997; Ginsberg et al. 1984). Finally, recent data suggest impaired insulin signaling in GD1 due to enrichment of lipid rafts harboring insulin receptors, with GM3 arising from accumulation of its precursor, GlcCer (Langeveld and Aerts 2009; Langeveld et al. 2008).

Gallstones (GS) have been recognized as a manifestation of GD1 by few other authors (Ben Harosh-Katz et al. 2004; Rosenbaum and Sidransky 2002). However, the metabolic factors associated with GS in GD1 are not known, nor is the chemical composition of stones or bile. Thus, the pathophysiology of cholelithiasis in GD1 remains unknown. A priori, there are several factors in GD1 that could promote cholesterol GS in GD1, namely, insulin resistance (Langeveld et al. 2008), chronic inflammation (Allen et al. 1997), T-cell dysfunction (Lacerda et al. 1999), and low HDL cholesterol (de Fost et al. 2009), which may reflect enhanced reverse cholesterol transport. However, it should be kept in mind that GD1 may be associated with shortened red cell span, which could contribute to formation of pigment stones (Hughes et al. 2007). We undertook a cross-sectional study to explore the prevalence and metabolic predictors of GS in a large cohort of well-characterized GD1 patients.

Patients and methods

The study population consisted of 417 consecutive patients (>10 years) with GD1 who presented to a tertiary referral clinic and its collaborating clinic, encompassing the States of New York, Connecticut, and New Jersey, USA (Table 1). All patients received the standard of care for GD1. The study was approved by the Institutional Review Boards of Yale University School of Medicine and New York University School of Medicine. Between 1996 and 2008, consecutive patients with GD1 (confirmed by low, <10% of normal, leukocyte GBA1 activity and *GBA1* genotyping) underwent comprehensive evaluation of severity of GD in all disease compartments (Charrow et al. 1998). The majority of our patients were of Ashkenazi Jewish ancestry (95%), and therefore, N370S was most common disease allele. The database captured information on presence of GS (by imaging or history of cholecystectomy for symptomatic GS), age of onset of GD symptoms, gender, genotype, body mass index (BMI), HDL, LDL, and triglycerides.

Data to determine overall severity of GD1 and of individual disease components were obtained at the time of the first visit (Charrow et al. 1998). These include bone disease score (Hermann Score) (Hermann et al. 1997), overall Severity Score Index (SSI) (Zimran et al. 1992), spleen status (intact or removed), liver volume, and spleen volume. Spleen and liver volumes were determined by magnetic resonance imaging (MRI) (Barton et al. 1991). Results were expressed as multiples of normal (x N): normal liver volume was 2.5% body weight and normal spleen volume 0.2% body weight (Barton et al. 1991). Gallbladder contents were analyzed in six GD1 patients, and five other patients (one man and four women, aged 33–70 years) not affected by GD and who underwent cholecystectomy for symptomatic GS. Gallbladder bile was cultured for aerobic and anaerobic bacteria and then

stored at -80°C . GS were harvested and stored in distilled water at 4°C . Biliary bile salt concentrations and species were analyzed by high-performance liquid chromatography (HPLC) (VanPatten et al. 2001). Phospholipid concentrations in bile were determined by an inorganic phosphorous procedure (VanPatten et al. 2001). Bile salt hydrophobic index was determined according to Heuman (1989). Molecular species of phosphatidylcholines were quantified by HPLC and phosphatidylcholine hydrophobic index calculated as described previously (Hay et al. 1993). Following organic extraction, GS cholesterol contents were determined enzymatically. Complex and free glycosphingolipids were quantified in bile of four GD1 patients and four non-GD patients using liquid chromatography-tandem mass spectroscopy (LC-MS/MS). All experiments were performed using an Applied Biosystems ABI 3000 tandem mass spectrometer operated in the positive ion mode in conjunction with a Perkin Elmer series 200 autosampler and HPLC pumps. Quantitation was facilitated by comparing peak areas of known internal standards to that of the analyte. Details of this procedure has been published previously (Sullards 2000; Sullards and Merrill 2001). The results are expressed as fold change compared with control biles.

Statistical analysis

Mean [standard deviation (SD)] is provided for continuous variables, and proportion is provided for categorical variables. Univariate and multivariate logistic regression models were performed to evaluate predictors of GS. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Two hundred fifty-three of the 417 individuals had complete data available on all predictors. Multiple imputation was performed using sequential generalized regression (IVEware: Survey Methodology Program at the University of Michigan's Survey Research Center, Institute for Social Research: <http://www.isr.umich.edu/src/smp/ive/>) to accommodate missing data. The assumption of this multiple imputation process is that data are missing at random, i.e., missing observations may be dependent on observed data but are conditionally independent of unobserved values (Allison 2001). Mean and SDs for the various GD parameters in the two groups were similar between the complete and imputed data set (Table 1). $P < 0.05$ was considered statistically significant for all analyses.

Results

Four hundred and seventeen patients were included in the analysis (Table 1). The overall prevalence of GS in GD1 patients was 32%. Age of patient ranged from 10 to 89 years, mean $\pm$ SD, 52.7 ± 18.9 years. Patients were characterized by low BMI, HDL cholesterol and LDL cholesterol. Twenty-four percent of patients were asplenic. All patients harbored at least one N370S GBA1 allele, with 53% of patients being N370S homozygous. Compared with male patients, the prevalence of GS was higher in female patients ($p = 0.03$). The prevalence of GS increased with age from 4.2% and 11.8% in the 20- to 29-years-old group to 71% and 60% at age >70 years, respectively (Table 2). The rates of cholecystectomy for symptomatic GS also increased in each decade interval, with 42.5% of all patients aged ≥ 20 years with GS having had prior cholecystectomy for symptomatic GS disease (data not shown). Compared with GD patients without GS, patients with GS were older ($p < 0.0001$), more likely to have undergone prior splenectomy ($p < 0.0001$), have higher BMI ($p = 0.005$), have higher total and LDL cholesterol ($p = 0.005$ and 0.02 , respectively), have higher bone disease score ($p = 0.01$), and higher overall SSI ($p = 0.01$) (Table 3). There was a trend toward higher triglycerides among GD patients with GS ($p = 0.06$). Interestingly, patients with GS tended to have a later age of GD diagnosis ($p = 0.01$) and later age of onset of GD-related symptoms ($p = 0.02$). It should be noted that there was no difference in hemoglobin levels in patients with GS compared with those without GS (Table 3). Finally, univariate and multiple logistic regression models were performed on complete (Table 4) and imputed (Table 5)

cases. In complete cases, the univariate model showed significant association of GS disease with older age ($p < 0.0001$), higher BMI ($p = 0.04$), higher LDL cholesterol ($p = 0.05$), and several indicators of more severe GD, i.e., greater hepatomegaly ($p = 0.005$), bone disease score ($p = 0.01$), and SSI ($p = 0.01$) (Table 4). By multiple logistic regression model after imputation, there was highly significant association of GS and older age ($p < 0.0001$), female sex ($p = 0.03$), and splenectomy ($p = 0.005$) (Table 5). Interestingly, in complete cases, multiple logistic regression suggested patients with GS tended to have lower HLD cholesterol compared with those without GS ($p = 0.05$) (Table 4).

The baseline characteristics of GD1 patients are notable for two metabolic traits: low BMI and low serum cholesterol. Sixty-two percent of male and 71% of female GD1 patients had normal or low BMI; i.e., ≤ 25 . The prevalence of obesity (BMI ≥ 30) was extremely low in the GD1 population: 7% of male and 10% of female patients. However, even within the context of overall low BMI, patients with GS had higher BMI compared with those without GS on univariate model (Fig. 1). However, on multiple logistic regression, there was no relationship of BMI with GS (Fig. 1). Secondly, GD1 patients had marked hypocholesterolemia (Table 1). Low total cholesterol was largely due to markedly low HDL cholesterol, which was consistently below the fifth percentile in each decade interval (data not shown). In contrast, whereas LDL cholesterol was low, it was consistently above the fifth percentile for normal population (data not shown).

The degree of hypolipidemia in GD was related to severity of GD1. This was illustrated by negative Pearson's correlation coefficient between spleen volume as multiples of normal and HDL cholesterol ($r = -0.44$, $p < 0.001$) and LDL cholesterol ($r = -0.38$, $p < 0.001$). There was a weak but significant positive correlation between spleen volume and LDL/HDL cholesterol ratio ($r = 0.19$, $p = 0.01$), suggesting that perhaps reduction of HDL cholesterol is the more significant abnormality of the two lipoprotein classes. Compared with patients without GS, patients who developed GS had higher total and LDL cholesterol, but there was no difference in HDL cholesterol (Tables 3, 4, and 5). Overall, HDL cholesterol was markedly depressed in GD1 patients with GS as well as those without GS: mean HDL cholesterol in GD patients was 30–34 mg/dl compared with 5th–95th percentile for the general population (35–95 mg/dl) (Table 3).

We searched for surrogate markers of hemolysis to investigate whether this might contribute to GS through formation of pigment stones. These parameters were examined after stratification for spleen status because of major changes that occur in the asplenic state in hematologic parameters. There were no differences in hemoglobin, total bilirubin, and reticulocytes of patients who developed GS compared with those who did not (data not shown).

Increased prevalence of GS in asplenic patients

Prevalence of GS in asplenic patients was increased. The excess of GS in asplenic patients remained significant after controlling for age and gender by binary logistic regression (odds ratio 2.38, 95% CI 1.26–4.48, $p = 0.005$). Interestingly, asplenic patients also have higher rates of cholecystectomy for symptomatic GS than patients with intact spleen (25% vs 11%, $p = 0.002$, data not shown).

Bile lipid analysis

The lipid composition of GS and bile of six GD1 patients who underwent cholecystectomy for symptomatic GS are presented in Tables 6, 7, and 8. Gallstones in GD1 were composed predominantly of cholesterol in five patients (cholesterol content by weight 71–95%), and only one patient had pigment stones, i.e., 0.5% cholesterol by weight (Table 6, Fig. 1).

Interestingly, cholesterol composition by weight seems to increase with increasing SSI (Table 6). Bile lipid composition was determined in four patients (Table 7). In addition to concentrations of individual lipids, three indices are presented as standard measures for assessing the aggregative behavior of biliary lipids (Table 7). CSI compares the experimentally determined cholesterol solubility in bile to the equilibrium solubility of cholesterol in micellar particles that would be observed in model bile systems of the same lipid composition. The hydrophobic index of bile salts and phosphatidylcholines are calculated concentration-weighted average of hydrophobicities of individual bile salts or phosphatidylcholines present in the mixture. These indices allow the overall hydrophobicity of biliary bile salts or phosphatidylcholines to be represented by single values.

When compared with bile from cholesterol GS patients without GD, bile from GD1 patients with cholesterol GS generally displayed lower total lipid concentrations (Table 7). Moreover, values of cholesterol saturation index, bile salt hydrophobic index and phosphatidylcholine hydrophobic index that fell above or below the range for non-GD cholesterol GS patients (Table 7). These differences were most notable for patient 1, in whom bile was dilute as well as highly cholesterol supersaturated and contained very hydrophobic bile salts and phosphatidylcholine. GS in this patient was 72% by weight of cholesterol (Table 6) and on bile microscopy contained plentiful cholesterol crystals (Fig. 1, middle panel). Interestingly, patient 1 was the most severely affected by GD, reflected by bone marrow failure dependent on blood transfusions that was not responsive to enzyme replacement therapy (SSI 22). Corresponding values for the GD patient with pigment GS (patient 4, Table 6) were typical of those observed in patients with pigment GS without GD (total lipid concentration 10.1 ± 3.0 g/dl, cholesterol saturation index 0.96 ± 0.34 , hydrophobic index for bile salts 0.301 ± 0.078 , phosphatidylcholine hydrophobic index 0.069 ± 0.027).

Complex sphingolipid and sphingoid base analysis revealed normal pattern in three of the four patients, i.e., patients 2, 3, and 4 (Table 8). Interestingly, the most severely affected patient (1), who had the most dilute bile as well as most cholesterol supersaturated bile, showed a marked difference in complex sphingolipid concentration. Compared with non-GD control, the bile of patient 1 contained fourfold more abundant ceramide, 15-fold more abundant sphingomyelin, and 35-fold more abundant glucosylceramide (Table 8). Moreover, free sphingoid base analysis in patient 1 revealed 7.6-fold greater abundance of sphingosine-1-phosphate and 5.4-fold greater abundance of psychosine compared with controls. In other patients, sphingoid base analysis was similar to control bile.

Discussion

Our study reveals an unexpectedly high prevalence of CGD in GD1. The overall prevalence of GS in our study cohort was 32%, and in these patients, nearly 43% required cholecystectomy for symptomatic GS. Compared with the normal GS prevalence rates derived from a meta-analysis of European Caucasian populations (Martinez de Pancorbo et al. 1997), the overall prevalence of GS in GD1 is approximately fivefold greater. A previous study reported a GS prevalence rate of 25% in GD1, but the nature of GS or bile and associated metabolic factors were not investigated (Rosenbaum and Sidransky 2002). In a subset of patients in whom we were able to obtain gallbladder contents at clinically indicated cholecystectomy, we found the GS to be comprised predominantly of cholesterol (5/6 patients), at variance with the general perception that GS in GD1 represent pigment stones associated with apparently shortened red blood cell survival in this disorder (Hughes et al. 2007). Moreover, although the bile of CGD GD1 patients displayed lower total lipid concentrations, the relative lipid composition was altered. Despite this, we noticed the presence of highly cholesterol-supersaturated bile containing very hydrophobic bile salts and phosphatidylcholine.

Closely associated with propensity for CGD in GD is the highly abnormal lipoprotein profile. This abnormality spares triglyceride-rich lipoproteins, but both LDL and HDL cholesterol levels are extremely low in GD1 patients. Reduction of HDL cholesterol appears to be the key abnormality. Whereas there is evidence that hypercatabolism of LDL in GD may occur via the macrophage system, such evidence is lacking for HDL (Le et al. 1988; Lorberboym et al. 1997; Ginsberg et al. 1984). Turnover studies of radio-labeled HDL in GD1 showed an increased fractional catabolic rate of apoA1, which may reflect increased reverse cholesterol transport to result in enhanced biliary cholesterol secretion (Le et al. 1988; Thornton et al. 1981). In keeping with this concept, GS were predominantly composed of cholesterol in 5/6 patients, and in one patient with cholesterol GS, there was marked cholesterol supersaturation, emphasized by presence of abundant cholesterol crystals visualized on microscopy of bile (Fig. 1). Low HDL in GD1 most likely reflects increased reverse cholesterol transport (Thornton et al. 1981) rather than HDL deficiency per se. The latter seems unlikely, as low HDL in GD1 is not associated with increased risk of atherosclerosis (de Fost et al. 2009). The propensity for cholesterol GS in GD1 associated with low HDL is consistent with the known inverse relationship in the general population between HDL cholesterol and bile saturation index as well as risk of CGD (Thornton et al. 1981). The mechanism underlying this association is hepatic class B scavenger receptor (SR-B1)-mediated uptake of plasma HDL and subsequent increased biliary cholesterol secretion (Kozarsky et al. 1997). The basis for possible increased reverse cholesterol transport in GD1 is not known. Whether increased glucosylceramide content of HDL in GD1 alters reverse cholesterol transport via increased avidity with cognate receptors deserves further study (Clarke 1981). For example, increased levels of apoE-HDL in GD1 may mediate reverse cholesterol transport more efficiently (Le et al. 1988).

Another potential mechanism underlying increased incidence of CGD in GD1 might operate via insulin resistance (Langeveld et al. 2008). Recent studies have led to the concept that increasing cellular GlcCer in GD1 drives increased synthesis of GM3; increased GM3 in the lipid rafts surrounding the insulin receptor is envisioned to impair insulin signaling (Langeveld et al. 2008). Indeed, inhibitors of glycosphingolipid synthesis reduce cellular GM3 and concomitantly ameliorate insulin resistance (Langeveld and Aerts 2009). Therefore CGD in GD1 can be viewed as a manifestation of insulin resistance. Indeed, induction of liver-specific insulin resistance in a mouse model results directly in cholesterol cholelithiasis, attributed to increased biliary cholesterol secretion via enhanced expression of ABCG5/ABCG8 (Biddinger et al. 2008). Interestingly, genome-wide association studies (GWAS) for cholelithiasis genes implicated polymorphisms in ABCG5/ABCG8 as genetic risk factors for insulin resistance and cholesterol GS formation (Hoblinger and Lammert 2008; Rudkowska and Jones 2008). Therefore, it would be interesting to assess for hepatic expression of ABCG5/ABCG8 in GD.

Our study and another report (Ben Harosh-Katz et al. 2004) demonstrate that within high-risk GD1 patient population for GS, splenectomy confers the highest risk for GS. It is of interest to note an almost threefold increase of hepatic glucosylceramide and glucosylsphingosine and of GM3 occurs after splenectomy, presumably because after removal of the primary reservoir of storage cells, there is greater demand to alternative routes of deposition and excretion (Nilsson et al. 1985). It should be noted that systemic administration of GlcCer to mice results in liver swelling without overt accumulation of storage material (Datta and Radin 1988).

There are several shortcomings of our study. It is a cross-sectional observational study. Very few gallbladders were available for analysis. Thus, changes in bile lipid composition and cholesterol content of GS needs verification in a larger number of patients. Contribution by

low-level compensated hemolysis to formation of pigment stones cannot be absolutely ruled out.

In one of our four patients with CGS in whom bile was available for analysis, there were significant alternations in glycosphingolipids and sphingoid base composition. In this patient (1, Table 8), there was 22-fold elevation of glucosylceramide, fivefold increase of psychosine, 36-fold increase of sphingomyelin, and eightfold increase of sphingosine-1-phosphate. It is intriguing to consider how these glycosphingolipids enter bile. This observation clearly indicates that there is a pathway by which accumulating lysosomal lipids exit the lysosomal compartments (Elleder 2006). Increased levels of GlcCer and psychosine are expected due to the deficiency of lysosomal GlcCer activity. Striking increase of sphingomyelin and sphingosine-1-phosphate in bile was unexpected. One potential mechanism is via ceramide generation in the extralysosomal compartment by the action of GBA2 (neutral glucocerebrosidase or bile GBA1) (Yildiz et al. 2006; Boot et al. 2007). Accumulating ceramide in the extralysosomal compartment is then envisioned to drive increased formation of sphingomyelin and sphingosine and of sphingosine-1-phosphate, thereof. Whether MDR2 (ABCB4) or BSEP (ABCB11) bile transporters are involved in secretion of glycosphingolipids and sphingoid bases is an important topic for further investigation. With the newly available mouse models of GD, such studies are now feasible (Enquist et al. 2006; Xu et al. 2003). An area worthy of future investigation is whether the biliary secretion of GlcCer could be exploited for therapeutic purposes and whether this pathway contributes to modification of highly diverse Gaucher phenotypes (Boot et al. 2007).

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Fig. 1.

The spectrum of cholesterol gallstone disease in type 1 Gaucher disease. *Left panel* shows 72% cholesterol stones with a rim of calcium bilirubinate, *middle panel* shows plentiful cholesterol crystals on bile microscopy, and *right panel* depicts >95% cholesterol gallstones

Table 1

Characteristics of complete cases and imputed cases by using sequential generalized regression (IVEware)

Characteristics	Complete case	Imputation
	<i>N</i> =253	<i>N</i> =417
Age ^a	53.2 (18.4)	52.7 (18.9)
Gender ^a : Female %	52.2	53.2
Genotype ^a : N370S/other %	46.3	46.5
Body mass index	23.3 (4.3)	23.2 (4.5)
HDL	29.2 (13.9)	31.8 (16.3)
LDL	86.9 (36.5)	87.9 (37.1)
Triglycerides(Log ₁₀)	2.1 (0.2)	2.1 (0.2)
Liver volume(Log ₁₀)	3.3 (0.2)	3.3 (0.2)
Herman Score	2.6 (1.5)	2.5 (1.5)
Severity Score Index	7.2 (3.7)	7.0 (3.7)
Spleen status: total splenectomy %	22.5	23.8
Gallstone: yes %	31.2	31.9

Mean (SD) is provided for continuous variables, and proportion is provided for categorical variables

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *SD* standard deviation

^aVariables without missing data

Table 2

Prevalence of gall stones by age in decade intervals

Age group	Male (%)	Female (%)
<20	0	0
20–29	4.2	11.8
30–39	15.8	25.7
40–49	17.2	27.5
50–59	22.2	37.5
60–69	32.3	36.4
70–79	71.4	60.0
80+	56.3	55.6

Table 3
Comparison of patient characteristics according to presence of gallstones by symptoms vs patients without gallstones

Characteristics	Gallstone		P value
	Yes; symptomatic N=58	No N=284	
Age	65 (16.4)	48 (18.2)	<0.0001
Female	38 (65.5)	142 (50.0)	0.08
Ashkenazi heritage	41 (80.4)	225 (83.6)	0.26
Age at diagnosis of Gaucher disease	32 (20.9)	26 (17.8)	0.01
Age at onset symptom of Gaucher disease	29 (20.2)	22 (18.1)	0.02
Total splenectomy	32 (55.2)	44 (15.9)	<0.0001
Liver volume	2214 (828)	2,041 (853)	0.15
Liver volume	2268 (905)	2,543 (1,099)	0.43
Intact spleen	2131 (711)	1,939 (758)	0.31
Body mass index	24 (4.7)	23 (4.5)	0.01
Total cholesterol	158 (49.9)	146 (39.4)	0.005
High-density lipoprotein (HDL)	34 (21.0)	32 (16.1)	0.42
Low-density lipoprotein (LDL)	94 (39.1)	84 (34.0)	0.02
LDL:HDL	3.6 (1.5)	3.6 (6.1)	1.00
Triglycerides	148 (68.0)	152 (80.0)	0.06
Hemoglobin	12 (1.8)	13 (1.9)	0.22
Herman Score	3.0 (1.4)	2.4 (1.5)	0.01
Severity Score Index	7.5 (3.9)	6.5 (3.5)	0.01

For continuous variables, mean (SD) is provided. For categorical variables, N (%) is provided and p-value is based on chi-square test

Table 4

Logistic model for complete cases to determine risk factors associated with CGD

Characteristics	Univariate model		Multivariate model	
	OR(95% CI)*	P-value	OR(95% CI)	P-value
Age	1.05 (1.03–1.06)	<0.0001	1.05 (1.03–1.07)	<0.0001
Gender: Male vs Female	0.88 (0.51–1.49)	0.63	0.73 (0.39–1.40)	0.35
Genotype: N370S/Other vs N370S/N370S	1.12 (0.65–1.90)	0.69	1.18 (0.56–2.46)	0.66
Body mass index	1.07 (1.00–1.14)	0.04	1.03 (0.95–1.12)	0.47
HDL	0.99 (0.97–1.01)	0.21	0.97 (0.94–1.00)	0.05
LDL	1.01 (1.00–1.01)	0.05	1.00 (0.99–1.01)	0.50
Triglycerides(Log ₁₀)	2.16 (0.56–8.29)	0.26	0.54 (0.09–3.08)	0.49
Liver volume(Log ₁₀)	10.82 (2.06–56.89)	0.005	2.11 (0.17–25.97)	0.56
Hermann Score	1.25 (1.05–1.50)	0.01	0.76 (0.53–1.10)	0.14
Severity Score Index	1.10 (1.02–1.18)	0.01	1.12 (0.97–1.30)	0.12
Spleen status: Total splenectomy vs Other	3.02 (1.64–5.57)	0.0004	1.72 (0.78–3.79)	0.18

OR: odds ratio, CI is confidence interval

Table 5

Logistic regression model after imputation

Characteristics	Univariate model		Multivariate model	
	OR(95% CI)*	P-value	OR(95% CI)	P-value
Age	1.05 (1.03–1.06)	<0.0001	1.04 (1.02–1.06)	<0.0001
Gender: Male vs Female	0.66 (0.44–1.01)	0.05	0.58 (0.35–0.96)	0.03
Genotype: N370S/Other vs N370S/N370S	0.84 (0.56–1.27)	0.41	0.94 (0.54–1.65)	0.84
Body mass index	1.07 (1.02–1.13)	0.006	1.03 (0.97–1.10)	0.34
HDL	1.00 (0.99–1.01)	0.84	0.99 (0.97–1.01)	0.19
LDL	1.01 (1.00–1.02)	0.003	1.00 (0.99–1.01)	0.60
Triglycerides(Log ₁₀)	2.07 (0.76–5.66)	0.16	1.46 (0.42–5.04)	0.55
Liver volume(Log ₁₀)	3.13 (0.89–11.02)	0.07	0.80 (0.14–4.55)	0.65
Hermann Score	1.26 (1.09–1.46)	0.002	0.84 (0.63–1.14)	0.27
Severity Score Index	1.08 (1.02–1.14)	0.07	1.09 (0.96–1.23)	0.17
Spleen status: Total splenectomy vs Other	3.33 (2.07–5.36)	<0.0001	2.37 (1.30–4.34)	0.005

OR: odds ratio, CI is confidence interval

Table 6

Gallstone analysis for cholesterol content and bile lipid composition

Patient	Age	Genotype	Spleen status	SSI	Cholesterol content (mg)
1	72	N370S/N370S	Asplenic	22	92%
2	36	N370S/N370S	Asplenic	5	79%
3	73	N370S/N370S	Intact spleen	5	72%
4	32	N370S/other	Asplenic	4	0.5%
5	51	N370S/L444P	Asplenic	13	84%
6	56	N370S/N370S	Asplenic	14	95%

Table 7**Bile lipid composition**

Patient	Total lipid (g/dl)	Cholesterol (mM)	Phospholipid (mM)	Bile salts (mM)	CSI	BSHI	Total lipid concentrations g/dl
1	1.2	4.8	5.0	12	4.12	0.55	1.2
2	3.1	1.9	6.4	50.1	0.95	0.3	3.1
3	7.8	5.0	22.4	117	0.66	0.25	7.8
4	3.7	7.3	12.2	49	2.00	0.45	3.7
Normal control	7±3.5	17.5±3.6	56.4±5.6	172±14	nd	0.32	-
CGD control	9.2±5.2	14.1±1.4	37.6±3.4	114±15	1.34±0.51	0.38±	9.2±5.2

CSI cholesterol saturation index, BSHI bile salt hydrophobicity index

Complex and free sphigoid base analysis in bile of four type 1 Gaucher disease (GD1) patients. Results are expressed as fold change compared with non-GD control bile; mean of three replicates for all analyses

Table 8

Patient	GlcCer	Psychosine	Ceramide	Sphingomyelin	Sphingarin	Sphingosine	So-P	Phytosphinganine
1	21.1	5.4	1.5	36.5	2.2	0.1	7.6	0.9
2	0.8	1.1	0.6	0.3	1.0	0.2	1.3	0.9
3	0.7	1.0	1.3	0.4	0.7	0.4	0.5	1.1
4	0.8	3.5	0.8	0.3	1.1	0.2	0.6	0.7

GlcCer: glucosylceramide
Sa-P: Sphiganine-1-phosphate
So-P: Sphingosine-1-phosphate