QJM: An International Journal of Medicine, 2016, 449–452

doi: 10.1093/qjmed/hcw002
Advance Access Publication Date: 19 January 2016
Original paper

ORIGINAL PAPER

Residual enzymatic activity as a prognostic factor in patients with Gaucher disease type 1: correlation with Zimran and GAUSS-I index and the severity of bone disease

M.A. Torralba[1], S. Olivera[1], J.C. Bureo[2], J. Dalmau[3], R. Nuñez[4], P. León[5] and J. Villarrubia[6]

From the [1]Department of Internal Medicine, "Lozano Blesa" University Hospital, Zaragoza, Spain, [2]Department of Internal Medicine, "Infanta Cristina" University Hospital, Badajoz, Spain, [3]Department of Pediatrics, "La Fé" University Hospital, Valencia, Spain, [4]Department of Hematology, "Virgen Del Rocío" University Hospital, Sevilla, Spain, [5]Department of Hematology, "Dr Peset" University Hospital, Valencia, Spain and [6]Department of Hematology, "Ramón Y Cajal" University Hospital, Madrid, Spain

Address correspondence to M.A. Torralba, "Lozano Blesa" University Hospital, Avda. San Juan Bosco, 15; 50009 Zaragoza, Spain. email: mantorralba@gmail.com

Summary

Background: Gaucher disease (GD) is an autosomal recessive disorder produced by mutations in the glucocerebrosidase gene (GBA), causing storage of glucosylceramide in reticuloendothelial cells in multiple organs. Traditionally, the prediction of the phenotype based on the genotype has been reported to be limited.

Subjects and Methods: We investigated the correlation between the enzymatic residual activity (ERA) and the phenotype at diagnosis of the disease in 45 GD Spanish patients (44 with type I and 1 with type III GD). The genotype involved two of the following previously expressed proteins: *c.517A > C (T134P)*, 1%; *c.721G > A (G202R)*, 17%; *c.1090G > T (G325W)*, 13.9%; *c.1208G > A (S364N)*, 4.1%; *c.1226A > G (N370S)*, 17.8%; *c.1246G > A (G377S)*, 17.6%; *c.1289C > T (P391L)*, 8.5%; *c.1448T > C (L444P)*, 3%; and *c.1504C > T (R463C)*, 24.5%. Recombinant alleles, deletion of 55 bp in exon 9 and *84GG* mutation were considered as mutations with no residual enzymatic activity.

Results: The ERA showed a statistically significant correlation with chitotriosidase ($P < 0.001$), age ($P < 0.001$), spleen size ($P < 0.001$), 'Zimran's Severity Score Index' ($P < 0.01$) and the 'Gaucher Disease Severity Score Index—Type I' ($P < 0.0001$) at diagnosis of the disorder. Previous to any medical intervention, a comparison between the ERA and bone involvement, demonstrated a statistically significant relationship ($P < 0.01$) between the two variables.

Conclusions: This study data allowed us to define a new criterion for prognostic assessment of the disease at diagnosis, called Protein Severity Index, which expresses the theoretical severity of the genotype presented by patients, according to the corresponding ERA.

Received: 12 November 2015; **Revised (in revised form):** 5 December 2015

Introduction

Gaucher disease (GD) type I (OMIM # 230800) is a lysosomal disease inherited with an autosomal recessive pattern, encompassing the signs and symptoms occurring in patients with a congenital disorder in the glycolipid metabolism, leading to glycosylceramide accumulation. This substance derives from the cellular membrane of senescent leukocytes and it accumulates in lysosomes of tissue macrophages, due to the deficiency in the enzyme glycocerebrosidase or β-glycosidase (EC 3.2.1.45).[1] The gene codifying β-glycosidase is the glucocerebrosidase (GBA) gene, localized in chromosome 1 (q21–q31).[2] Mutations in GBA altering the stability of the enzyme or its active site are the main cause of GD. Up to date, more than 300 different mutations in GBA have been described and gathered in databases, such as The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff (http://www.hgmd.cf.ac.uk/ac/index.php). Out of these mutations, more than 80% are single nucleotide substitutions and the rest correspond to complex mutant alleles. The four mutant alleles *N370S (c.1226 A > G), L444P (c.1448 T > C), 84GG (c.84dupG)* and *ISV2 + 1 (c.115 + 1G > A)* are the most prevalent worldwide.[3] Traditionally, it has been claimed that it is not possible to foresee the severity of GD based only on the genotype; although, patients with a N370S allele are protected against neuropathic development of the disease, while homozygous patients for L444P probably do develop neuropathic pathology.[4]

In 2001, we published the characterization of 10 defective alleles of GBA with high prevalence in the Spanish population.[5] Fifteen years later, we plan to use the enzymatic residual activity (ERA) data obtained at the time—by means of expressing each allele individually—to study the putative correlation between the genotype and phenotype in a series of patients with predominately GD type I, in order to use this variable to guide genetic advice.

Materials and methods

We conducted a non-interventionist study, in which six physicians from five different University hospitals in Spain, located in the cities of Badajoz, Madrid, Seville, Valencia and Zaragoza, voluntarily participated and were submitted the clinical data. Every participating physician provided the first data registered in the clinical history of their patients with GD at the moment of diagnosis. The data provided included age, gender, genotype, hemoglobin and platelets values, liver and spleen size were measured in cm below costal rib margin, presence or absence of splenectomy, the activity of plasmatic chitotriosidase, and the Zimran's severity score index (SSI).[6] Since every patient (except one type III) had GD type I, the 'Gaucher Disease SSI—Type I' (GauSSI-I) was calculated.[7] Special attention was placed on the assessment of bone pathology, which was classified as follows: (i) no bone pathology, (ii) mild bone pathology (osteopenia/osteoporosis or bone pain with no other alteration) and (iii) severe bone pathology (any bone lesion other than osteopenia/osteoporosis or bone pain with no other alteration).

The residual enzyme activity in each patient was estimated by adding the activity obtained after individually expressing each of the two alleles responsible for the disease in COS-1 cells.[5] The residual activities corresponding to the mutant alleles have been previously published and are shown in Table 1.

After excluding those patients with a genotype containing an allele not previously expressed in COS-1 cells, the final number of patients was 45, and all of them had been diagnosed with GD years earlier by a reduced activity of acid β-glycosidase in leukocytes. Mutant recombinant alleles, the 55 bp deletion in exon 9 and the *84GG* mutation were considered as mutations with no residual enzymatic activity.

Statistics

The statistical analyses were conducted using the SPSS 20.0 software. Qualitative variables were expressed as percentages and quantitative variables by measures of central tendency and dispersion (mean, standard deviation and range). A 95% confidence interval was calculated for the means. The normality of the variables was assessed by the Kolmogorov–Smirnov test. The correlation between quantitative variables was performed using the Pearson correlation or Spearman rho, according to the normality of the variable. Both, the Zimran's SSI and the GauSSI-I were normalized for the maximum score of both indexes in our population (26 in the case of the Zimran's SSI, and 17 in the case of the GauSSI-I index) to perform correlation analyses with other variables. Significance was considered for correlations with $P < 0.05$. A comparison between the ERA and bone pathology was performed by means of the analysis of variance (ANOVA) test, and later on the *post hoc* analysis to assess the correlation between the ERA and bone pathology of any degree. Last, receiver operating characteristic (ROC) curves were performed to obtain the correlation of bone pathology with the different quantitative variables; when the correlation was significant, the optimal cut-off diagnostic point of such variable was obtained.

Table 1. Residual activities corresponding to expressed wild type and mutant alleles

Plasmid	Activity (nM/h/mg)	Corrected expression	%
GBA wild type	69.0	233.5	100
T134P	2.4	2.4	1
G202R	28.1	39.9	17
G325W	24.2	32.5	14
S364N	9.4	9.6	4
N370S	35.6	41.6	18
G377S	31.4	40.8	18
P391L	6.9	19.7	9
L444P	6.9	6.9	3
R463C	28.6	57.2	24

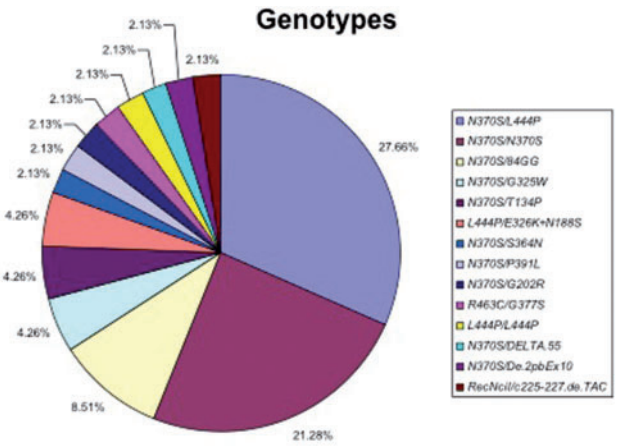

Figure 1. Genotypes of the studied population and ERA.

Table 2. Clinical and biological parameters of study patients

Genotype	N	Residual activity (%)	Type of Gaucher disease	Age at diagnosis years	Chitotriosidase (mM/ml·h)	CCL18/PARC (ng/ml)	Bone disease (% of patients)	Liver (cm) Average and (statistical range)	Spleen (cm) Average and (statistical range)	SSI Average and (statistical range)	GauSSI-I Average and (statistical range)
N370S/L444P	13	21	1	19.6 (SD 9.5; 10–41)	10 309 (7632; 1640–28 420)	489 (338; 176–859)	No 27.3%Mild 45.5%[a]Severe 27.3%	2.6 (2.3; 0–8)	12.5 (7.6; 0–25)	7.1 (2.4; 4–10)	7.55 (2.6; 2–12)
N370S/N370S	10	36	1	39.9 (SD 16.3; 21–68)	6687 (3934; 1060–12 503)	1285	No 80%Mild 10%Severe 10%	1.3 (1.6; 0–3)	1.4 (1.4; 0–4)	4.7 (2.3; 1–10)	3.9 (2; 1–7)
N370S/Del55	7	18	1	22 (SD 16.4; 5–53)	21 187 (11 412; 1123–33 582)	209 (223; 51–367)	No 14.3%Mild 71.4%Severe 14.3%	5.4 (9; 0–24)	15 (10.5; 0–30)	8.5 (2.3; 5–11)	9.29 (3.6; 5–16)
N370S/84GG	4	18	1	9 (SD 4.2; 4–14)	20 406 (1363; 7226–32 758)	573 (131; 381–676)	No 50%Severe 50%	2.8 (0.9; 2–4)	9.8 (3.9;6–14)	9 (3.2;6–13)	11.25 (5.1;6–17)
N370S/G325W	2	32	1	39.5 (SD 3.5; 37–42)	15 535 (49.5; 15 500–15 570)		Mild	2.5 (0.7; 2–3)	11.3 (10.3; 4–19)	9 (5.7; 5–13)	7
N370S/T134P	2	19	1	12.5 (SD 0.7; 12–13)	19 275 (1539; 18 187–20 364)	371	Mild 50%Severe 50%	2.5 (0.5; 2–3)	10 (4.2; 7–13)	9.5 (0.7; 9–10)	7.5 (0.7; 7–8)
L444P/L444P	1	6	3	3	9142		Severe 100%	0	7	26	–
N370S/Del 2pb Ex10	1	18	1	14	10 860		Severe 100%	0	16	9	10
N370S/G202R	1	35	1		7011		Not available				
N370S/P391L	1	26	1	25	5706		Severe 100%	17	11	12	8
N370S/S364N	1	22	1	51	7085		Severe 100%	5	7	11	6
R463C/G377S	1	41	1	1	17 461		Severe 100%	5	8	6	5
RecNcil/c225-227delTAC	1	0	1	12	37 132	3763	Severe 100%	4	14		10

[a]Mild bone disease (osteopenia or osteoporosis); severe bone disease (fractures or avascular necrosis or bone crisis or deformities or prosthesis).

Results

Out of the 45 patients with GD, 51% were males. Mean age at diagnosis was 23 years (range 1–68). The most frequent genotype was the *N370S/L444P* (28%), followed by *N370S/N370S* (21%) and *N370S/c.1263del55* (13%) (Figure 1). Clinical and biological parameters are shown in Table 2. At the moment of diagnosis, the mean residual activity was 21 (range 0–41). Thirty-nine point five percent of patients had no bone disease; 30% had mild disease and 30% severe disease (Figure 2).

When assessing the correlation of the residual enzymatic activity with the chitotriosidase, age at diagnosis and spleen size, a moderate correlation statistically significant ($P < 0.001$) was observed, in a way such that the lower residual activity, the greater were the chitotriosidase activity and the spleen size, and the earlier the age at diagnosis. And this is exactly the same thing happens with the Zimrańs SSI and the GAUSS-I score ($P < 0.001$). No significant correlation was observed between the residual enzymatic activity and hemoglobin, platelets values or liver size.

The Zimran's SSI and GauSSI-I were determined at diagnosis with a mean of 7.9 (range 1–26) and 7.44 (range 1–17), respectively. By means of the Spearman test, it was confirmed a great correlation between both severity indexes ($r = 0.691$, $P < 0.0001$). Both indexes were normalized as stated in methods for the following correlation analyses. The correlation between the Zimran's SSI and the residual enzymatic activity was moderate and statistically significant ($r = 0.548$, $P < 0.01$) and that between the GauSSI-I index and the residual enzymatic activity was strong ($r = 0.665$, $P < 0.0001$), in such a way that a lesser residual activity involves a more severe phenotype presented by patients at diagnosis. A subanalysis in non-pediatric patients (age 14 years or older) showed a similar moderate correlation between the Zimrańs SSI and the residual activity ($r = 0.478$, $P < 0.01$), and a stronger correlation between the GauSSI-I index and the residual activity ($r = 0.787$, $P < 0.0001$).

The ANOVA test showed a statistically significant ($P < 0.01$) association between the residual enzymatic activity and bone pathology (Levene statistic: 0.104, F: 9.152, $P = 0.001$). In the *post hoc* analysis (Scheffe test), a statistically significant difference of greater magnitude was observed in the correlation between the residual activity and the group with bone disease, whether it was mild or severe (Mild bone disease F:19.23, Severe bone disease F: 20.33, $P < 0.05$).

The correlation between bone disease and the different quantitative variables was obtained by means of ROC curves and

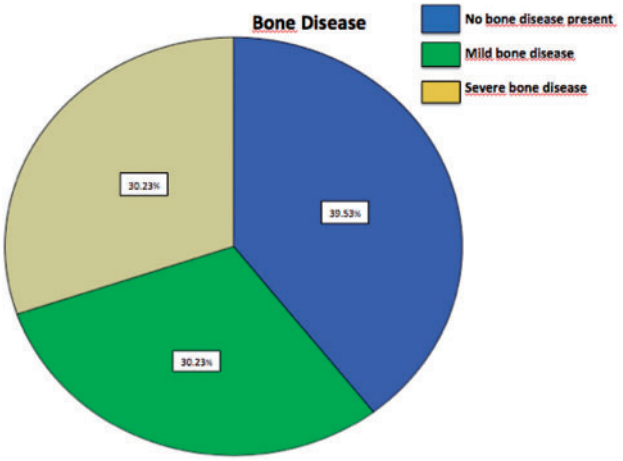

Figure 2. Patients percentage with different degree of bone disease.

were significant: Zimran's SSI (Area Under the Curve (AUC):0.872; 95% CI = 0.766–0.979); GauSSI-I (AUC:0.799; 95% CI = 0.666–0.931); Splenomegaly (AUC:0.721; 95% CI = 0.562–0.879) and Hepatomegaly (AUC:0.695; 95% CI = 0.523–0.867).

Discussion

Our study demonstrates that the residual activity obtained from these rare expressed mutant proteins justify a percentage of the clinical profile observed in these Spanish patients, before any intervention. However the clinical experience with GD and the results of genotype/phenotype correlation studies suggest that GBA deficiency is necessary but not sufficient to explain the ultimate clinical outcome.[8] In general, the great variability in systemic involvement in GD patients requires considering that there are other underlying mechanisms playing a very important role such as promoter mutations, environment, and other epigenetics and non-genetic causes.[9]

Despite the limitations of this study we consider it is important to communicate our findings to the Gaucher's scientific community. In the 1990s, the expression studies performed by Grabowski *et al.*[10] and Grace *et al.*[11] showed that the mutations involving exons 5, 8, 9 and 10 are the ones causing the greatest impact in the catalytic activity of the enzyme. Yet, there are many mutations described in the literature which have not been studied *in vitro*. Thus, it has not been possible to establish a complete classification of the alleles able to determine the severity of their phenotype according to their expression. In the studied patients sample, it is clear the statistical correlation between the residual enzymatic activity—calculated as the addition of the expression percentage of both alleles with respect to the normal protein expressed in mammalian cells—and determined biological and clinical parameters before any intervention. Particularly noticeable is the correlation at disease diagnosis, such as age, activity of plasmatic chitotriosidase, spleen size and Zimrań's SSI. Furthermore it is mainly illustrative the high correlation between the residual enzymatic activity and the GauSSI-I coefficient. The GauSSI-I index is the most valuable tool from a clinical and phenotypical point of view and for the first time, its correlation with the presence or absence of bone involvement has been shown. In addition, the current study shows that, at least in this series of patients the GauSSI-I and the Zimrań's SSI show a high correlation; thus, a more severe genotype—that with lower residual activity from the alleles responsible for the disease—causes a more severe phenotype and vice-versa.

The main limitation of the study is the individual assessment of the alleles; thus, we still need to find out what happens with the residual activity when both alleles are expressed at the same time.

In view of the report data, we can define a new criterion for prognostic evaluation at disease diagnosis, which we have called Protein Severity Index, which expresses the theoretical severity of the patient genotype, according to the corresponding residual enzymatic activity. Further studies with a high number of patients should be performed to establish the utility of this new prognosis index.

Conclusions

The study shows that the mutations found in this group of patients cause a decrease of the enzymatic activity, constituting the first link in the chain of events participating in the pathogenesis of GD, and explaining a significant amount of the final phenotype. In addition, this finding allows for the classification of the studied mutations according to their severity, with the corresponding implications from the point of view of genetic advice and disease prognosis.

Conflict of interest: Dr Torralba, Bureo, Dalmau, Núñez, León and Villarrubia are consultants for Genzyme Corporation and Shire Company and participate in advisory panels and conferences on lysosomal storage diseases. They have received research support from both companies, but the preparation of this case report was carried out entirely independently.

References

1. Brady RO, Kanfer JN, Bradley RM, Shapiro D. Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease. *J Clin Invest* 1966; **45**:1112–5.
2. Devine EA, Smith M, Arredondo-Vega FX, Shafit-Zagardo B, Desnick RJ. Regional assignment of the structural gene for human acid beta-glucosidase to q42 leads to qter on chromosome 1. *Cytogenet Cell Genet* 1982; **33**:340–4.
3. Horowitz M, Tzuri G, Eyal N, Berebi A, Kolodny EH, Brady RO, *et al*. Prevalence of nine mutations among Jewish and non-Jewish Gaucher disease patients. *Am J Hum Genet* 1993; **53**:921–30.
4. Dahl N, Lagerstrom M, Erikson A, Pettersson U. Gaucher disease type III (Norrbottnian type) is caused by a single mutation in exon 10 of the glucocerebrosidase gene. *Am J Hum Genet* 1990; **47**:275–8.
5. Torralba MA, Perez-Calvo JI, Pastores GM, Cenarro A, Giraldo P, Pocovi M. Identification and characterization of a novel mutation c.1090G > T (G325W) and nine common mutant alleles leading to Gaucher disease in Spanish patients. *Blood Cells Mol Dis* 2001; **27**:489–95.
6. Zimran A, Kay A, Gelbart T, Garver P, Thurston D, Saven A, *et al*. Gaucher disease. Clinical, laboratory, radiologic, and genetic features of 53 patients. *Medicine* 1992; **71**:337–53.
7. Di Rocco M, Giona F, Carubbi F, Linari S, Minichilli F, Brady RO, *et al*. A new severity score index for phenotypic classification and evaluation of responses to treatment in type I Gaucher disease. *Haematologica* 2008; **93**:1211–8.
8. Koprivica V, Stone DL, Park JK, Callahan M, Frisch A, Cohen IJ, *et al*. Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. *Am J Hum Genet* 2000; **66**:1777–86.
9. Goker-Alpan O, Hruska KS, Orvisky E, Kishnani PS, Stubblefield BK, Schiffmann R, *et al*. Divergent phenotypes in Gaucher disease implicate the role of modifiers. *J Med Genet* 2005; **42**:e37.
10. Grabowski GA, Gatt S, Horowitz M. Acid beta-glucosidase: enzymology and molecular biology of Gaucher disease. *Crit Rev Biochem Mol Biol* 1990; **25**:385–414.
11. Grace ME, Graves PN, Smith FI, Grabowski GA. Analyses of catalytic activity and inhibitor binding of human acid beta-glucosidase by site-directed mutagenesis. Identification of residues critical to catalysis and evidence for causality of two Ashkenazi Jewish Gaucher disease type 1 mutations. *J Biol Chem* 1990; **265**:6827–35.