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Chitotriosidase on treatment-naïve patients with Gaucher disease: A genotype vs phenotype study

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ABSTRACT

Background: Chitotriosidase (ChT) is used as a biomarker for the follow-up of patients with Gaucher disease (GD), once his activity is extremely elevated and declines during ERT. However, some variants in the *CHIT1* gene affect ChT activity.

Methods: To assess association between ChT genotype, and clinical/biochemical features of GD were performed *CHIT1* genotyping for: c.1049_1072dup24, p.Gly102Ser, p.Gly354Arg, c.1155_1156 + 2delGAGT, c.1156 + 5_1156 + 8delGTAA, p.Ala442Val/Gly and the rearrangement delE/I-10.

Results: Were evaluated 42 patients with GD from Southern Brazil. Pretreatment ChT activity was available for 32 patients. Allelic frequencies found for dup24, p.Gly102Ser and p.Ala442Gly were 0.14, 0.32 and 0.12, respectively. Only one patient presented reduced ChT activity (dup24 homozygous). Comparison between wild homozygous and heterozygous for dup24 showed that both differ in relation to the ChT activity before (15,230 vs 6936 nmol/h/mL, $p < .001$), but not after treatment (5212 vs 3045 nmol/h/mL, $p = .227$).

Conclusions: Pretreatment ChT activity was not correlated with clinical/biochemical features. There was a reduction of 63% in the ChT activity after 12 months on treatment ($p < .001$). There is no evidence that higher ChT levels are associated with a more severe symptomatology in untreated GD patients. The pretreatment ChT levels appear to be mainly dependent on the presence/absence of the dup24 allele.

1. Introduction

Gaucher disease (GD, MIM#230800) in an autosomal recessive inborn error of glycosphingolipid metabolism caused by the deficient activity of the acid β-glucosidase (GCase, EC 3.2.1.45). It is characterized by a buildup of glucosylceramide (GC, also known as glucocerebroside) in the macrophage lysosomes, which leads to dysfunction of many organs and systems, organomegaly, hematologic disorders, and skeletal anomalies being the most common. Some patients exhibit neurological symptoms [1]. The worldwide incidence of GD is approximately 1–2 per 100,000 live births. Although its distribution is panethnic, it is more prevalent in Ashkenazi Jews [2].

Two modalities of therapies are available for management of GD: enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) [3]. ERT uses recombinant forms of GCase, administered intravenously at varying doses (15–60 IU/kg/inf), depending on the patient's symptoms [4]. In SRT, glucosylceramide synthase inhibitors are used aiming at increasing the efficiency of residual GCase [5,6]. Studies have presented highly variable results in biomarkers analysis in treatment naïve GD patients and during therapies [7].

One parameter used to monitor GD treatment efficacy is the activity in plasma of chitinase 1 or chitotriosidase (ChT, EC 3.2.1.14), a human chitinase synthesized by activated macrophages. ChT is encoded by the *CHIT1* gene (NG_012867.1), located on chromosome 1q31-32 and comprising 12 exons [8]. ChT activity is extremely elevated in the plasma of patients with GD, and decline with therapy, but rises if treatment is discontinued [9–12]. Pretreatment ChT levels show correlation with some clinical parameters such as Hb and platelet levels, organ volumes and disease specific severity scores like SSI and QCSI, thus reflecting disease severity, but not presented correlation with

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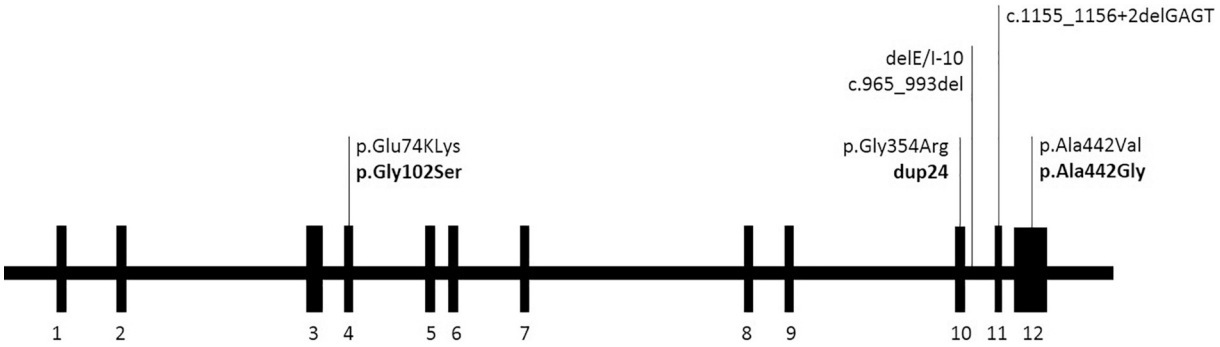

Fig. 1. *CHIT1* gene structure and location of variants. Variants found in this study are in bold.

osteoclastogenesis [7,10,11]. Furthermore, ChT activity has shown to be related to ERT dosage [13]. However, monitoring the efficacy of therapy by measuring ChT activity has some limitations. To date, eight mutations have been described in *CHIT1* associated with reduced ChT activity (HGMD 2018) with the most common being a 24-bp duplication in exon 10 (c.1049_1072dup24, dup 24, rs3831317) (Fig. 1). This variant, present in homozygosis in 6% of Caucasians, causes reduced activity of ChT even in heterozygosis, and homozygous for dup24 can present null or deficient activity, limiting its use as a biomarker [8,14,15]. GD patients who are dup24/dup24 can present null or deficient activity, below the reference value [14]. Other important variants for *CHIT1* gene, either by the frequency with which they occur or by the effect on ChT catalytic activity are: c.304G > A (p.Gly102Ser, rs2297950), c.1060G > A (p.Gly354Arg, rs9943208), c.1025C > T/C (p.Ala442Val/Gly, rs1065761) [16–18]; c.1155_1156 + 2delGAGT [19] and the rearrangement E/I-10 junction (c.1060G4A; 1155G4A; 1156 + 5_1156 + 8delGTAA [20,21] (Fig. 1).

The present study sought to ascertain the frequency of most common variants in *CHIT1*: dup24, p.Gly102Ser, p.Gly354Arg, p.Ala442Val/Gly and delE/I-10 variants in patients with GD seen at the Reference Center for GD from Rio Grande do Sul, Brazil (RCGD-RS), and evaluate which factors are associated with plasma activity of ChT before therapeutic intervention.

2. Methodology

This study was approved by the local Ethics Committee. All patients, or guardians, signed the informed consent form before inclusion. For being included, patients should have a deficient activity of GCase compatible with the diagnosis of GD.

2.1. CHIT1 genotyping

Peripheral blood samples were collected into tubes containing EDTA and DNA extraction was performed with a commercially available kit (EasyDNA™-gDNA Purification Kit, Invitrogen). Genotyping for dup24 allele was performed by PCR, using primers as described by Boot et al. [8] (Supplementary Tables S1 and S2). Genotypes were determined by the presence or absence of 75-bp (wild-type) or 99-bp (mutated allele) fragments in a 2% agarose gel. Patients were also genotyped for the variants p.Gly102Ser; p.Gly354Arg; p.Ala442Val/Gly and delE/I-10 by PCR-sequencing. The primers used for this purpose were those described by Grace et al. [20] (Supplementary Table S1). The PCR reaction and cycling conditions are described in Supplementary Table S2. The amplification products were purified with PEG 8000 (50% PEG, 2.5 mM NaCl). Samples were sequenced in an ABI 3500 system (Applied Biosystems), analyzed in *Chromas lite* v.2.1.1 software, and aligned with the NG_012867.1 reference sequence in NCBI *BLAST* (Basic Local Alignment Search Tool). When the results of sequencing were inconclusive, a second round of amplification with another primer was performed. Since DNA parental samples were not available for analysis, it was not possible to determine the *cis-trans* status of the variations.

2.2. Clinical and biochemical data

Clinical and biochemical data were obtained by means of a chart review, using a standard data collection form. This form included demographic and clinical information such as sex, age, type of GD, date of treatment initiation, type of treatment (ERT or SRT), ERT dosage, hematologic parameters (Hb levels and platelet counts), presence of visceral manifestations (hepatomegaly, splenomegaly), ChT activity, bone marrow burden score (BMB) and Gaucher severity scores (SSI and DS3). Data were collected at the initiation of therapy and 12 months thereafter.

Patients followed at the RCGD-RS are routinely evaluated in relation to the levels of Hb and platelets, as well as to the size of liver and spleen. Since 1998, ChT activity in plasma has been measured yearly. The enzyme analysis is performed according to Hollak et al. [9], using the substrate 4-methylumbelliferyl β-D-*N*,*N*′,*N*″-triacetylchitotrioside (4MU-chitotrioside, Sigma-Aldrich). The reference range value used is 8.8–132 nmol/h/mL. BMB and Gaucher severity scores are available since 2010.

2.3. Statistical analysis

Only variables with data available for > 50% of the sample were considered for analysis. Data were analyzed in PASW Statistics v.18.0 (SPSS Inc.). Categorical variables were expressed as absolute and relative frequencies for descriptive analyses; the McNemar or Pearson chi-square test were used for inferential analyses. For quantitative variables, descriptive analyses were expressed as mean and standard deviation or median and interquartile range, while inferential analyses were performed using nonparametric (e.g., Mann–Whitney *U*, Wilcoxon, Kruskal–Wallis, Spearman correlation) or parametric methods (e.g., *t*-test, Pearson correlation) as appropriate. The significance level was set at $p < .005$ for all tests. All tests were two-tailed.

3. Results

Forty-two patients with GD (type 1 = 37; type 2 = 2; type 3 = 3) were included in the study (male = 21; mean age = 34.2 ± 15.6 years, range, 4–67 years). Out of these, 37 were on ERT and 2 on SRT, with a mean age at treatment initiation of 24.5 ± 19.4 years (range, 0.25–61 years). Seven of the 39 treated patients had undergone splenectomy.

DNA samples were available for all patients. ChT activity in plasma was available for 32/42 naïve to treatment patients, being reduced in only one patient (8.2 nmol/h/mL; NR = 8.8–132 nmol/h/mL) who was eventually confirmed as being homozygous for dup24 and excluded from the correlations between ChT activity and clinical parameters. BMB and Gaucher severity scores were available for < 50% of the

Table 1
Clinical and biochemical characteristics of patients included in the study.

Patient	DG Type	Sex	Splenectomy	Age	Age at first symptoms	Age at diagnosis	Age at the start of treatment	Type of treatment	Hepatomegaly at the start of treatment	Hepatomegaly after 12 months of treatment	Hemoglobin at the start of treatment	Hemoglobin after 12 months of treatment	Platelets at the start of treatment	Platelets after 12 months of treatment	ChT at diagnosis	ChT after 12 months of treatment
1	2	M	No	N.I.	0.08	0,17	0.25	IMI	Yes	0	8,2	8,74	85,000	240,000	3553	3010
2	1	M	No	24	5	10	11	IMI	No	0	11,8	12,81	74,000	158,000	12,645	6578
3	1	F	Yes	64	48	59	60	IMI	No	0	11,5	13,3	207,000	326,000	9806	1685
4	1	F	No	20	7	7	7	IMI	Yes	0	9,85	10,46	101,000	148,000	9196	3202
5	1	F	No	58	30	42	42	CER	Yes	0	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
6	1	F	No	23	9	10	10	IMI	No	N.I.	11,9	N.I.	109,000	N.I.	32,221	N.I.
7	2	M	No	N.I.	0	0,041	N.I.	N.I.	Yes	N.I.	14,9	N.I.	16,000	N.I.	2718	N.I.
8	3	F	Yes	25	2.5	3	4	CER	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
9	1	F	No	32	22	27	27	IMI	Yes	1	10,1	10,5	81,000	115,000	24,676	19,595
10	1	M	No	53	N.I.	50	51	IMI	No	0	14,5	15,8	106,000	152,000	14,76	5212
11	1	F	No	19	N.I.	16	16	IMI	Yes	1	10,2	11,5	79,000	82,000	6876	3005
12	3	M	No	17	0.83	1,17	0.83	IMI	Yes	1	9,4	11,5	66,000	277,000	8627	N.I.
13	1	F	No	59	N.I.	57	57	IMI	No	0	14,2	15,1	86,000	125,000	6605	589
14	1	M	No	41	18	22	29	IMI	No	0	12,05	14,5	61,000	54,000	N.I.	9430
15	1	F	No	4	1.67	0,92	N.I.	N.I.	No	N.I.	11,6	N.I.	150,000	N.I.	3584	N.I.
16	1	M	No	24	5	7	7	CER	Yes	0	11,4	11,7	153,000	123,000	N.I.	N.I.
17	1	M	No	27	3	14	14	IMI	Yes	1	12,2	12,8	150,000	235,000	19,792	1531
18	1	F	No	22	2	15	16	IMI	Yes	1	10,1	11,9	115,000	115,000	8484	2880
19	1	M	Yes	38	16	26	27	IMI	Yes	1	12	12,5	355,000	294,000	N.I.	7212
20	1	F	No	16	9	13	13	IMI	No	N.I.	11,8	12	84,000	112,000	13,702	N.I.
21	1	M	Yes	58	42	49	52	IMI	Yes	1	13,7	16,1	237,000	312,000	7271	3080
22	1	M	No	41	N.I.	38	38	IMI	Yes	1	13	14,1	134,000	161,000	6544	2860
23	1	M	No	49	N.I.	44	46	IMI	Yes	0	13,7	14,3	60,000	135,000	20,581	9220
24	1	M	No	48	N.I.	43	43	IMI	Yes	0	15,3	15,9	76,000	92,000	7934	3328
25	1	F	No	29	8	11	11	CER	Yes	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
26	1	F	No	25	2	4	5	CER	Yes	N.I.	8,3	9,8	460,000	470,000	N.I.	N.I.
27	1	F	No	32	10	12	14	CER	Yes	0	11,4	N.I.	103,000	N.I.	N.I.	N.I.
28	1	M	No	18	6	8	8	IMI	Yes	1	11,4	12,5	136,000	196,000	28,072	4167
29	1	M	No	20	2	2	3	IMI	Yes	1	8	N.I.	160,000	N.I.	N.I.	N.I.
30	1	M	No	23	3	10	11	IMI	Yes	0	11,8	13,9	315,000	101,000	6751	2643
31	1	M	No	34	27	27	30	IMI	Yes	0	13,9	15	53,000	77,000	11,273	7980
32	1	F	No	36	9	29	29	IMI	Yes	1	12,1	14,2	79,000	117,000	15,375	6817
33	1	F	No	48	11	45	48	MIG	Yes	0	11,6	11,7	192,000	207,000	2,97	1667
34	1	F	No	45	33	42	43	MIG	No	0	14,6	13,7	113,000	141,000	9609	6050
35	1	M	No	60	N.I.	54	56	IMI	No	0	13,2	12,9	45,000	83,000	12,899	5731
36	1	F	No	21	4	8	8	IMI	Yes	1	8,35	9,5	195,000	224,000	17,53	10,970
37	1	F	Yes	67	25	34	35	IMI	Yes	0	11,94	13,24	460,000	367,000	19,853	4615
38	1	F	Yes	33	3	11	14	CER	Yes	N.I.	11,4	N.I.	360,000	N.I.	N.I.	N.I.
39	1	M	No	24	3	5	4	CER	Yes	N.I.	9,1	N.I.	55,000	N.I.	N.I.	N.I.
40	3	M	Yes	23	1	3	2	CER	Yes	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
41	1	M	No	N.I.	16	61	61	IMI	No	0	10,1	11,7	30,000	31,000	4,5	3,5
42	1	F	No	35	10	35	N.I.	N.I.	No	N.I.	11,6	N.I.	83,000	N.I.	14,295	N.I.

M: Male; F: Female; IMI: Imiglucerase; CER: Cerezyme; Hemoglobin unit: g/dL; Platelet count unit: $x10^3/mm^3$; Plasma ChT activity unit: nmol/h/mL; N.I: no information.

Table 2
Summary of features of Gaucher disease patients at baseline (no therapy), who had chitotriosidase activity data available, and at 12 months on therapy.

Features	Baseline (n = 32)	At 12 months on therapy (n = 26)	P-value
GD type			
1	29	25	NA
2	2	1	
3	1	0	
Splenectomy	4	4	NA
Hemoglobin (g/dL)	12.0 ± 1.8 (n = 32)	13.0 ± 1.9 (n = 26)	*< 0.001*
Plateletcount ($\times 10^3$/mm^3)	93.5 (IQR = 75.5) (n = 32)	144.5 (IQR = 128) (n = 26)	*0.010*[†]
Plasma ChTactivity (nmol/h/mL)	11,273 (IQR = 10,103) (n = 31)	4167 (IQR = 4144) (n = 25)	*< 0.001*[†]
Hepatomegaly	20/32	10/26	*0.008*[§]
Splenomegaly	25/28	16/22	0.125[§]
ERT	NA	24	NA
Imiglucerase	NA	22	NA
Taliglucerase alfa	NA	2	NA
Dosage (IU/kg/2 weeks)	NA	30 (IQR = 31)	NA
Number of infusions/patient/year	NA	20 (IQR = 5)	NA
SRT (Miglustat)	NA	2	NA

ERT: enzyme replacement therapy; SRT: substrate reduction therapy; NA: not applicable.
Normally distributed data expressed as mean ± standard deviation.
Asymmetrically distributed data expressed as median (IQR).
[§] McNemar's test.
[†] Wilcoxon test.

sample, being excluded from the analysis. Table 1 presents the main clinical and biochemical findings, and Table 2 summarizes the statistical analysis of findings in patients presenting ChT activity pre and at 12 months on treatment.

3.1. Treatment-naïve patients

No correlation was found between median ChT activity and patient's age (ρ = 0.076; n = 31; p = .684), Hb levels (ρ = −0.016; n = 31; p = .934), or platelet counts (ρ = −0.023; n = 31; p = .901). Median ChT activity before therapy was similar between patients who had hepatomegaly (11,252, IQR = 9605 nmol/h/mL; n = 20/31) and those who did not have (12,899, IQR = 11,118 nmol/h/mL; n = 11/31) (p = .804; Mann–Whitney *U*), nor between female (13,502 ± 7472 nmol/h/mL; n = 15/31) and male (12,764 ± 7280 nmol/h/mL) (p = .783; independent *t*-test). Patients with GD type II (n = 2) and III (n = 1) presented the following values of Cht activity, respectively: 3553; 2718; and 8627 nmol/h/mL. Median ChT activity declined significantly, by approximately 63%, at 12 months on therapy (Table 2).

3.2. CHIT1 genotyping

Table 3 presents the allelic and genotypic frequencies found for dup24, p.Gly102Ser and p.Ala442Gly. No patients presented p.Gly354Arg, p.Ala442Val or delE/I-10 variants.

Out of the 42 included patients, 11 did not present any variant. Twenty presented one variant in heterozygosis, one presented dup24 variant in homozygosis (c.[1048_1072dup24];[1048_1072dup24]), 3 presented other variant in homozygosis and a second variant in heterozygosis (p.[Gly102Ser];[p.Gly102Ser;Ala442Gly]), ChT activity = 24,676/11,231 nmol/h/mL; (p.[Ala442Gly];[Ala442Gly;Gly102Ser]), ChT activity = 3432 nmol/h/mL). One presented two variants in homozygosis (p.[Gly102Ser; Ala442Gly];[Gly102Ser;Ala442Gly]), ChT activity = 32,221 nmol/h/mL. Four patients presented two variants in heterozygosis (c.1048_1072dup24 and p.Gly102Ser = 3; ChT activity = 7334/8627/7749 nmol/h/mL; p.Gly102Ser and p.Ala442Gly = 1; ChT activity not available) and two patients presented three variants in heterozygosis (dup24, p.Gly102Ser and p.Ala442Gly); ChT activity = 3553 and 6876 nmol/h/mL.

Pretreatment ChT activity was also compared between wild-type homozygotes for dup24 (n = 23), for p.Gly102Ser (n = 12), and for p.Ala442Val (n = 24), and heterozygotes for those variations (n = 8, 16 and 5, respectively), being significantly higher in the wild-type homozygotes than in the heterozygotes for dup24 (Table 4). After 12 months on therapy, however, there was no difference in the ChT activity between wild-type and heterozygotes for dup24 (Table 4).

Table 3
Genotype and allele frequencies for *CHIT1*.

	dup24		p.Gly102Ser		p.Ala442Gly	
	c.1049_1072dup24		c.304G > A		c.1325C > G	
	(n = 42)		(n = 42)		(n = 42)	
Genotype Frequencies	N/N	31 (73.8%)	G/G	18 (42.86%)	C/C	34 (80.5%)
	N/D	10 (23.8%)	G/A	21 (50%)	C/G	6 (14.6%)
	D/D	1 (2.4%)	A/A	3 (7.14%)	G/G	2 (4.9%)
Allelic Frequencies	N	0.86	G	0.68	C	0.88
	D	0.14	A	0.32	G	0.12

dup24: N, normal allele; D, mutated allele.

4. Discussion

Few studies have evaluated the association of ChT activity with genotype and clinical parameters in Brazilian patients with GD. The greatest analysis so far had been made by Adelino et al. [19], who evaluated 33 Brazilian GD type 1 patients from the state of Minas Gerais, that found 4 patients with low ChT. The *CHIT1* gene was genotyped in those 4 patients, and their genotype revealed one patient homozygous for dup24 allele; one patient heterozygous for dup24 and bearing two variants in heterozygosity in exon 10, p.Gly354Arg and a 4 bp deletion at the exon-intron 10 boundary (first time described); one patient homozygous for p.Gly102Ser; and one patient heterozygous for p.Gly102Ser Similar to our study, the authors found no correlation between ChT activity and age or gender, but did observe higher ChT activity in wild-type homozygotes for dup24 than in heterozygotes.

As expected, our study reports high ChT levels in 31/32 patients with GD from Southern Brazil. Plasma ChT levels are up to 1000-fold higher in patients with GD, and can be used to monitor treatment response and disease progression [9,22]. Activity of ChT in plasma has been correlated with several clinical parameters of interest in GD. In the present study, we did not find significant correlations between pretreatment ChT activity and hemoglobin levels or platelet counts, and presence of hepatomegaly or splenomegaly. Some other studies,

Table 4
Comparison between ChT activity and *CHIT1* genotypes (wild homozygotes vs heterozygotes). #

	dup24			p.Gly102Ser			p.Ala442Gly		
ChT activity (nmol/h/mL)	−/− (n = 23)	−/+ (n = 8)	P-value	−/− (n = 12)	−/+ (n = 16)	P-value	−/− (n = 24)	−/+ (n = 5)	P-value
Baseline	15,230 (IQR = 9164; n = 23)	6936 (IQR = 3269; n = 8)	< 0.001†	13,300 (IQR = 8458; n = 12)	9492 (IQR = 9553; n = 16)	0.710†	12,086 (IQR = 9247; n = 24)	11,231 (IQR = 13,974; n = 5)	0.564†
On therapy	5212 (IQR = 5120; n = 19)	3045 (IQR = 1803; n = 6)	0.227†	4615 (IQR = 4435; n = 9)	3265 (IQR = 4503; n = 14)	0.801†	4913 (IQR = 4198; n = 20)	3007 (IQR = 12,537; n = 4)	0.699†
Delta	8558 (IQR = 7289; n = 19)	4001 (IQR = 4788; n = 6)	0.009†	9531 (IQR = 10,465; n = 9)	5116 (IQR = 5913; n = 14)	0.231†	7863 (IQR = 7408; n = 20)	4476 (IQR = 6158; n = 4)	0.121†

Delta: difference between median ChT activity at baseline and at 12 months after therapy.
Asymmetrically distributed data expressed as median (IQR).
Pearson's chi-square test.
† Mann–Whitney *U* test.

however, have reported a strong negative correlation of pretreatment ChT activity with platelet counts and hemoglobin levels, and a strong positive correlation between pretreatment ChT activity and organ volume [11,23,24]. One limitation of the present study is the lack of data of other clinical parameters of GD often used to monitor disease severity and progression, such as severity scores (SSI and DS3) [25,26], and degree of bone-marrow infiltration (BMB score) [27] which could not be correlated with ChT activity, as other parameters [7,25–27].

It is well known that ChT activity declines with therapeutic intervention and rises again when therapy is discontinued [9,10,12]. In our patients, we observed an average reduction of approximately 63% in ChT activity after 12 months of therapy. It is interesting to point out that after 12 months on therapy, wild homozygotes and heterozygotes for dup24 did not differ in relation to their ChT activity. The same was observed by Giraldo et al. [10] who found that, after 12 months on ERT the plasma activity of ChT declines by the same percentage in both, heterozygous carriers of the 24 bp duplication and homozygous for the wild type allele, but thereafter CT activity decline more slowly in carriers than non-carriers.

Regarding *CHIT1* genotype, we found that only the dup24 allele affected ChT activity before treatment, with a significant difference between wild-type patients and those heterozygous for this allele, as the found by Tylki-Szymańska et al. [15]. Only one patient had deficient ChT activity due to dup24 recessive status. The frequency of homozygosity for the dup24 allele varies widely across populations. Some studies have reported higher rates among Asians and Indigenous Mexicans (56% and 44.5% respectively) than in European (2–7%), Iranians (6,1%) and South Africans (0%) samples [8,17,21,28,29]. In the present study, 2.4% and 23.8% of patients were homozygous and heterozygous for dup24 respectively.

There was no significant difference in pretreatment ChT activity between wild-type homozygotes and heterozygotes for the p.Gly102Ser and p.Ala442Gly alleles. ChT levels were high in essentially all patients, even those heterozygous for dup24 and homozygous for p.Gly102Ser and p.Ala442Gly. Frequencies of both polymorphisms vary across populations (Supplementary Table S3) and the effect of p.Gly102Ser on ChT activity seems to be controversial. Lee et al. [17], in a study of subjects with European, African, and Asian ancestry, found no association of p.Gly102Ser or p.Ala442Gly status and reduced plasma ChT activity. The same was reported by Irún et al. [30] in relation to p.Gly102Ser variant. However, other studies have reported opposite results for p.Gly102Ser, i.e. significant reductions in ChT activity even in wild-type heterozygotes [18,19].This reduction occurs due to a glycine-to-serine substitution at the 102 position of ChT that alters its transglycosidase activity, leading to a misinterpretation when measured with the common fluorogenic substrate 4MU-chitotrioside under saturating conditions [18]. Use of the superior 4MU–deoxy-chitobiose substrate avoids such complications, because activity towards this substrate under saturating conditions is not affected by the p.Gly102Ser substitution [18,31].

5. Conclusions

In our sample, there is no evidence that higher activity of ChT is associated with a more severe symptomatology in untreated GD patients. The pretreatment ChT activity appears to be mainly dependent of the presence/absence of the dup24 allele. So, our data does not support the routinely genotyping of other variants in *CHIT1* gene in patients with GD.

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Conflicts of interest

The authors report no conflicts of interest.

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Ethical considerations

This study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre (project no. 14-0257), and all patients provided written informed consent for participation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.01.018.

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