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Short Communication

The N370S/R496H genotype in type 1 Gaucher disease – Natural history and implications for pre symptomatic diagnosis and counseling

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ABSTRACT

Type 1 Gaucher disease (GD1) patients with the N370S/R496H (N409S/R535H) genotype are increasingly identified through carrier and newborn screening panels. However, limited information is available on the phenotype associated with this genotype. Here, we report our experience with 14 patients with this genotype. Our data suggests that most patients with N370S/R496H present with mild manifestations and often do not require treatment. This information is important for counseling newly diagnosed patients and GD1 carrier couples.

1. Introduction

Type 1 Gaucher disease (GD1) is an autosomal recessive lysosomal storage disease (LSD) characterized by reduced activity of acid β-glucosidase, due to deleterious mutations in the *GBA* gene, resulting in accumulation of glucosylceramide (GL1) predominantly in the cells of the monocyte-macrophage system [1]. Clinical manifestations include pancytopenia, hepatosplenomegaly, and skeletal disease. GD1 is clinically heterogeneous, and patients present across a wide phenotypic spectrum ranging from early onset severe disease requiring treatment to asymptomatic adults [2]. The carrier frequency of GD1 in the Ashkenazi Jewish (AJ) population is approximately 1 in 15, largely due to the common Type 1 founder mutation N370S (N409S) [3]. This mutation is known to be protective against the development of primary neurological manifestations seen in Type 2 and 3 GD. The less common R496H (R535H) mutation is reported almost exclusively in the AJ population [4,5], with frequencies of 0.0048 (1:207) in Israel [4] and 0.003 (1:335) in the US [3]. There is limited phenotype information for the N370S/R496H genotype, with only seven patients described in one publication [6].

As the use of prenatal and preconception carrier screening and direct-to-consumer (DTC) genetic testing grow in popularity, asymptomatic individuals are being diagnosed with GD1 more often in their second or third decade of life. Couples are also being identified as carriers before or during a pregnancy, and children are diagnosed prenatally or shortly after birth. As newborn screening for LSDs expands [7,8], more patients are expected to be identified and followed from birth. These growing trends have identified an increasing number of patients with this rare genotype [9]. Here, we characterize 14 patients with this genotype evaluated at our center.

2. Methods

GD1 patients with the N370S/R496H genotype evaluated at the LSD Program at the Icahn School of Medicine at Mount Sinai (ISMMS) until April 2018 were included. This case series was determined to be exempt by the ISMMS Program for the Protection of Human Subjects. Clinical data from initial and follow-up visits including physical exam, mode of diagnosis, laboratory values, biomarkers (chitotriosidase, plasma lyso-GL1), treatment status, ethnicity, and imaging studies (abdominal ultrasound or MRI, MRI of the femurs, and DXA scans) were reviewed. *GBA* genotypes were determined by targeted mutation analysis with the exception of two patients (patient #11 and #12) who had *GBA* sequencing. A validated GD severity score (GSS) [10] was assigned to pediatric patients retrospectively if applicable. For patients > 18 years, a validated disease severity scoring system (GD-DS3) was used to assess severity [11]. Hepatosplenomegaly was calculated using standardized weight-based formulas [12]. *Z*-scores for pediatric patients were corrected to height with the Zemel correction using the CHOP Pediatric Bone Density *Z*-Score Calculator (https://zscore.research.chop.edu/bmdCalculator.php) [13]. *CHIT1* genotyping was performed to evaluate for null mutations that would affect expression of chitotriosidase,

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Table 1
Demographic and clinical information for N370S/R496H patients.

Subject	Sex	Age[+] (yrs)	Treated?	Baseline						Follow up	
				Enzyme activity	ROS	Hepatosplenomegaly	Osteopenia	Adjusted Chito	Total GSS Score[+] Total GD1 DS3 Score*	Adjusted Chito	Plasma Lyso-GL1
1	F	8.0	N	1.4	−	L 0.99 S 0.79	.	5.6	2.3[a]	5.6	0.8
2	M	7.1	N	.	−	L 1.26 S 1.5	−	84	0	110.4	1.2
3	F	5.0	N	.	−	.	.	44	1[b]	104.8	1.1
4	F	8.1	N	.	−	L 0.8 S 0.5	−	17.2	0	22	.
5	F	6.2	N	.	−	.	+	.	1.5[c]	.	.
6	M	1.7	N	.	.	.	.	.	.	.	.
7	F	36.4	N	1.82	−	.	.	79.2	.	.	.
8	F	37.1	N	2.16**	−	L 1.06 S 2.49	−	41.2	0	264	.
9	F	25.1	N	1.8	−	.	.	572	.	.	.
10	F	41.4	N	1.0	Fx	L 0.98 S 2.6	++	.	2.0	.	.
11	M	67.1	Y	0.51	T, F	L 0.86 S 1.57	−	178	0.8	29.2	0.4
12	F	50.2	Y	0.96	T	L 1.64 S 9.02	++	3661	3.33	59.6	1.0
13	F	31.8	N	1.16	.	L 1.46 S 2.71	+	.	0.4	.	.
14	M	29.2	N	3.0**	−	L 0.98 S 2.69	−	12.8	0	.	.

. = data not available.
[+]At most recent evaluation.
*At baseline evaluation for treated patients and at last follow up for untreated patients.
Acid beta-Glucosidase enzyme activity: Normal range: 3.6–18.2 nmol/h/mg; Affected range = 0–2.0 nmol/h/mg; Inconclusive range (**) = 2.1–3.6 nmol/h/mg.
ROS: Review of systems; Abbreviations: F = fatigue, Fx = hx of fracture, T = thrombocytopenia.
Hepatosplenomegaly: L = Liver Multiples of Norm, S = spleen Multiples of Norm.
Osteopenia: - = Mild or none (≥ -1) at all sites measured, + = Moderate (≤ -1 to > -2.5) at one or more sites, ++ = Severe (≤ -2.5) at one or more sites.
Chitotriosidase enzyme activity: Normal range: 0–120 nmol/h/mL.
Gaucher Severity Score (GSS) – mild < 6, moderate 6–9, severe > 9.
a Height in the 20th %ile, 1SD below expected mid-parental height.
b Height in the 21st %ile.
c Mild osteopenia and height in the 12%ile, within 1 SD of expected mid-parental height.
Gaucher Disease Type 1 Disease Severity Scoring System (GD1 DS3).
Imaging studies were deferred for patients 7 & 9 due to gestational status therefore GD1 DS3 was not able to be calculated.
The maximum possible GD1 DS3 score is 19.
0–3 Borderline mild disease.
3–6 Moderate disease.
6–9 Marked disease.
9+ Severe disease.
Plasma Lyso-GL1: Normal range: 0–1.0 ng/ml; at follow up Plasma Lyso-GL1 was concurrent with Chitotriosidase level.

and baseline adjusted chitotriosidase activity was calculated [14].

3. Results

Fourteen patients (ages 4–66) were identified with the N370S/R496H genotype including 10 females (mean age 27.6 years, range 4–49) and 4 males (mean age 28.9 years, range 7–66). All patients reported AJ ancestry from both parents, with the exception of one patient who reported AJ and Dominican ancestry.

Five of six pediatric patients (4 females, 2 males; mean age 6.5 years, range 3–9 years) were tested for GD1 due to parents' known carrier status identified through prenatal screening. Two patients were diagnosed prenatally and three postnatally. Mean years of follow-up of the pediatric patients was 4 years.

All pediatric patients had a detailed physical examination including growth parameters based on previously described recommendations [15]. All children were asymptomatic, with a normal physical examination. None had cytopenia and all had normal chitotriosidase levels. Plasma lyso-GL1 measured at follow up was normal or minimally elevated (Table 1).

GSS scores were available for 5 of 6 pediatric patients, and ranged from 0 to 2.3, suggesting mild disease burden. Patient 1 had a GSS score of 2.3 due to height in the 20th percentile, approximately 1SD below expected mid-parental height with no other clinical manifestations. Patient 5 had a score of 1.5 due to mild osteopenia (Zemel-adjusted *Z*-scores of −2.32 at left femoral neck and −1.33 at right femoral neck) and height in the 12%ile (within 1 SD of expected mid-parental height). No pediatric patients were recommended treatment.

Five of eight adult patients (mean age 42.1 years) were diagnosed incidentally via prenatal or preconception carrier screening. One patient was diagnosed following his mother's carrier status determined by DTC testing through 23andMe. All 6 of these patients were asymptomatic with no significant medical history. The remaining 2 adult patients were diagnosed due to symptoms suggestive of GD. Mean years of follow-up in the adult population was 6.5 years.

All adult patients underwent a comprehensive baseline evaluation including laboratory studies and imaging based on published recommendations [16]. Cytopenia and hepatosplenomegaly were not seen in the 6 patients diagnosed incidentally and chitotriosidase levels (adjusted to *CHIT1* genotype) were mildly elevated or normal. Plasma

lyso GL-1 levels were normal in those with available data. GD-DS3 scores were in the borderline mild disease range in 5 of 6 patients [11]. Osteopenia was reported in 3 (50%) adult females (mean age at diagnosis 33 years) with baseline DXA T-scores of −2.48, −1.65, and −1.30. One patient developed a pathologic fracture during both her pregnancies; however, she had additional risk factors for osteopenia including low body mass index, history of previous fracture, vitamin D deficiency (19.1 ng/ml) and pregnancy and lactation related bone loss. For two of these three patients, osteopenia was an isolated finding and could be multifactorial in etiology, and unrelated to GD1. The third patient (#12) with osteopenia had normal vitamin D levels and no other identifiable risk factors, but had additional GD1 symptoms. Two adult patients were symptomatic and are receiving treatment with enzyme replacement therapy; patient 11 started treatment at age 45 for mild thrombocytopenia, vertebral marrow infiltration, fatigue and complaints of leg pain and patient 12 at age 33 for thrombocytopenia, hepatosplenomegaly, and osteopenia.

4. Discussion

These data suggest that patients with genotype N370S/R496H typically have mild GD1 manifestations and most often do not require treatment. The majority of patients were diagnosed either incidentally through prenatal or preconception carrier screening panels or because of known parental carrier status. All pediatric patients had minimal disease, with no evidence of systemic involvement or clinical indications for starting treatment.

In adult females, the most common finding was osteopenia including one patient with pathologic fracture during two pregnancies. This patient had an endocrine work up which was normal; however, she had other significant risk factors for osteopenia. In another patient, osteopenia was an isolated finding and could be unrelated. The two treated patients had improvement of clinical manifestations including normalization of thrombocytopenia and hepatosplenomegaly. Patient #12 was symptomatic in her 30's and presented with moderate disease symptoms unlike the other patients in our cohort. This case highlights the clinical variability often seen in GD1, which may be related to genetic modifiers.

Interestingly, two patients identified by screening had higher than expected acid beta-glucosidase levels, which were in the inconclusive range, consistent with the mild phenotype. Plasma levels of lyso-GL1, the most sensitive and specific marker of GD 1 were normal or low for those with available levels. This is in contrast to our experience with patients with other genotypes such as the N370S homozygotes where plasma lyso GL1 levels are elevated even in the absence of symptoms. Longer term follow up is needed to see if these levels increase over time.

As carrier and preconception screening becomes increasingly common, more couples are aware of their carrier status before or during a pregnancy and have additional reproductive options available to them. Many couples seek specialty counseling to assess interest in in-vitro fertilization (IVF) with preimplantation genetic diagnosis (PGD) or testing during a pregnancy. Phenotypic information regarding this mild genotype is important to distinguish from more severe presentations, with the understanding that there is significant variability in all genotypes for GD1. Due to the limited data available, counseling of couples at risk of having a child with this genotype is challenging and our site has received referrals for couples in this scenario considering termination of their pregnancy and others considering IVF with PGD.

Limitations of our study include the small sample size. However, this data significantly expands the number of previously reported patients with the N370S/R496H genotype [5]. A recent publication described 4 additional untreated patients with the R496H/other genotype with mild manifestations and low lyso-GL1 levels supporting our findings [17]. Our patients were predominantly AJ; therefore, the phenotype cannot be extrapolated to other ethnicities. Most patients had targeted *GBA* mutation analysis and not *GBA* sequencing which could miss additional mutations modifying the disease phenotype. Additionally, there is limited experience with pediatric bone density, particularly in very young patients and these results should be interpreted with caution. Another limitation is the young age of our cohort and the limited longitudinal follow up data. Ongoing monitoring of these patients will continue to expand the phenotype information.

5. Conclusions

In our experience, the N370S/R496H genotype is typically associated with mild GD1 manifestations and is usually diagnosed incidentally. Our data suggests that most patients with N370S/R496H present with mild manifestations and often do not require treatment. Patients should be regularly followed at an experienced center to avoid unnecessary interventions on the one hand while monitoring closely to prevent disease-related complications and allow for optimal treatment initiation, if needed. This information is important for counseling newly diagnosed patients and GD1 carrier couples in both prenatal and preconception settings.

Declaration of Competing Interest

Author NZ has received an honorarium for a lecture given at a meeting sponsored by Sanofi Genzyme.

Author CS has received honoraria for lectures given at meetings sponsored by Sanofi Genzyme and is a member of the Nurse Practitioner Advisory Board for Sanofi Genzyme.

Author AY has received honoraria for lectures given at meeting sponsored by Sanofi Genzyme.

Author HN declares no conflict.

Author LF declares no conflict.

Author JG declares no conflict.

Author MB is a member of the ICGG North American Scientific Advisory board and has received honoraria from Genzyme and Shire.

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Comments

This manuscript is submitted solely to this journal and was not published elsewhere. Several patients included in this analysis were included in a previous publication with composite data (Yang et al., 2017).

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