Case Report

Rare GBA1 genotype associated with severe bone disease in Gaucher disease type 1

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Aims: To report on the case of two sisters with GD type 1 who bear a genotype never reported in the literature.

Introduction: Gaucher disease (GD) type 1 is a lysosomal disease characterised by hepatosplenomegaly, anemia, thrombocytopenia, bone changes, and bone marrow infiltration. The disease is caused by biallelic pathogenic variants in GBA1 which codes for glucocerebrosidase, an enzyme involved in the catabolic pathway of complex lipids.

Methods: To report on the case of two sisters with GD type 1 who bear a genotype never reported in the literature. Case report: Patient 1 is a 47-year-old female diagnosed at 42 years of age with chronic lumbar pain, mild splenomegaly, slightly reduced platelets and normal hemoglobin values, severe Bone Marrow Burden (BMB) score, high chitotriosidase activity, and low glucocerebrosidase. Patient 2 is a 50-year-old female, sister of patient 1, who was diagnosed after familial screening. At 45 years of age, she had osteonecrosis of the left femur and a total hysterectomy because of uncontrollable bleeding. At first evaluation, she had bone pain with a high BMB score, mild splenomegaly, normal hemoglobin, normal platelets count, elevated chitotriosidase activity, and low glucocerebrosidase activity. Both patients were found to be compound heterozygotes for the p.Glu388Lys and the p.Ser405Asn variants in GBA1.

Conclusions: This is the first family with GD and this combination of variants which causes a phenotype remarkable for severe bone disease with no or mild hematological manifestations.
used as a first line therapy for GD [4,5]. GD presents a broad range of phenotypes that are partially explained by the different GBA1 genotypes; therefore, we herein report on two sisters with a novel genotype associated with severe bone disease and mild or no hematological phenotype.

2. Case reports

Patient 1 is a 47-year-old female diagnosed with GD type 1 at the age of 42 years. She was born to a non-consanguineous couple and has 4 siblings of whom 3 were healthy and screened negative for GD, and one sister was symptomatic, described below as patient 2, see Fig. 1. There is no history of Parkinsonism or other neurologic symptoms in the family.

She was referred to the GD Reference Center at the Hospital de Clínicas de Porto Alegre (HCPA), Brazil, because of hyperferritinaemia (ferritin = 588 ng/mL) resistant to phlebotomy treatment; chronic lumbar pain (Visual Analogue Scale = 8); and recurrent epistaxis. Laboratory exams at admission showed hemoglobin of 13 g/dL, leucocyte count at 2320 cells/mm³, platelets at 143,000/mm³, and chitotriosidase activity at 9609 nmol/h/mL (NRV = 8.8–132). Abdominal ultrasonography revealed normal liver and spleen volumes. She had normal bone metabolism markers (parathyroid hormone, alkaline phosphatase, calcium, phosphate, and vitamin D), bone mineral density (BMD) with normal Z scores, however the bone marrow burden score (BMB) with normal T scores and BMB of 14/16 [Fig. 3]. Glucocerebrosidase activity of 5 nmol/h/mg prot in leukocytes (NRV = 10–45 nmol/h/mg prot) and 132 nmol/h/mg prot in fibroblasts (NRV = 257–688 nmol/h/mg prot) confirming the diagnosis of GD type 1. At diagnosis, the Disease Severity Scoring System [7] (DS3) was 3.6/19 (scoring only in bone subscore) and SSI = 1/49. The patient started treatment with miglustat 300 mg/day and followed a low-carbohydrate diet. Soon after, due to diarrhea and unintended 6 kg weight loss (10% of total body weight), the patient was found to also have lactose intolerance (lactase non-persistence CC-genotype) and streptococcal pharyngitis, and received treatment with lactose-free diet and albenazol. Due to persistence of gastrointestinal symptoms and slight clinical improvement (see Table 1), miglustat was switched after one year to taliglucerase alfa 30UI/kg/biweekly; since the patient presented an allergic reaction after 2 months of infusions to taliglucerase, it was switched to imiglucerase 30UI/kg/biweekly (see Table 1) - which regimen has been kept uneventfully, with improvement of the symptoms (Table 1).

Patient 2 is a 50-year-old female diagnosed with GD type 1 when she was 45 years old. Four years before the first appointment with Medical Genetics, the patient underwent a prosthetic replacement of the left femoral-acetabular joint for osteonecrosis, and, one year after, underwent total hysterectomy for uncontrollable bleeding during uterine polyp removal surgery. Laboratory tests at admission to our Center showed hemoglobin at 11.5 g/dL, leukocyte count at 8710 cells/mm³, platelets at 195,000/mm³, ferritin of 880 ng/mL, and chitotriosidase activity of 2970 nmol/h/mL (NRV = 8.8–132). Further investigation revealed mild hepatosplenomegaly and hepatic steatosis by abdominal ultrasonography. She had normal bone metabolism markers, BMD with normal T scores and BMB of 14/16 [Fig. 3]. Glucocerebrosidase activity of 2.8 nmol/h/mg protein in leukocytes (NRV = 10–45 nmol/h/mg protein) and 60 nmol/h/mg protein in fibroblasts (NRV = 257–688 nmol/h/mg protein) confirmed the diagnosis of GD. The severity scores were DS3 = 2/19 (scoring only in bone subscore) and SSI = 1/49. Because of needle phobia, she started on treatment with miglustat 300 mg/day together with a low-carbohydrate diet.

After 3 years of treatment with miglustat with unsatisfactory response (Table 1), treatment was switched to taliglucerase alfa 15UI/kg/biweekly. After 2 years of treatment with ERT, the hematological parameters and chitotriosidase activity improved, however ferritin remained high and bone marrow infiltration remained severe.

Upon genotyping with next-generation sequencing (NGS) both patients 1 and 2 were discovered to be compound heterozygotes for c.1162G > A (p.Glu388Lys) (E349K) and c.1214G > A (p.Ser405Asn) (S366N) GBA1 pathogenic variants. Both patients tested negative for the chitotriosidase gene (CHIT1) null variant. Patients are also heterozygote for the HFE1 pathogenic variant c.187C > G (p.His63Asp).

3. Discussion

3.1. Genotype

The patients described herein were compound heterozygotes for two uncommon GBA1 pathogenic variants, E349K and S366N. The former has been previously described by Grabowski and colleagues in 2006 [9]; however, no clinical phenotype description nor if it was in homozygosis or compound heterozygosis with a different variant was provided. The latter, was described in compound heterozygosis with R48W (p.Arg87Trp) by Demina and Beutler in 1998 [10] in an African-American female GD type II patient whose sister had anemia, mild thrombocytopenia, mild neutropenia, and moderate hepatosplenomegaly – however, no more details on the patient’s phenotype are provided. The E349K residue is on a coil motif at the eighth exon, in a region of neutral hydropathy. This variant is predicted to cause a reduction of 88% of the normal enzyme activity [11]. The S366N variant lays on an alpha-helix at the 3’ end of the eighth exon, in a region of neutral hydropathy, and impairs a phosphorylation site. Of note, the combination of these variants in our patients caused enzyme activity higher than expected for classical GD patients.

Both variants are considered pathogenic when applying the ACMG [12] classification criteria: they are absent from gnomAD (PM2), were...
previously detected in trans with a pathogenic variant (PM3), multiple in silico algorithms (such as DANN, FATHMM-MKL, SIFT, LRT, MutationTaster) predict both variants to be deleterious (PP3), patient’s phenotype and family history are highly specific for GD (PP4), UniProt classifies this variant as ‘disease’ (PP5), and the variant segregates with the phenotype in a gene definitively known to cause the disease (PP1).

4. Phenotype

Much is being studied about secondary modifier genes in Mendelian disorders, including GD [13–15]; however, still little is known about how strong is the genotype-phenotype association in GD. In the presented case, both patients harboured the same variants in GBA1, and although quite similar overall, there were some differences between the two sisters’ phenotypes: while patient 2’s bone phenotype may be considered somewhat more severe, patient 1’s chitotriosidase – a biomarker for GD activity – was more than three times higher at admission than patient 2’s. Liver and metabolic profiles, on the other side, were quite similar. This perhaps may be explained by the action of an unidentified modifier gene harboured by only one of the patients, or it may be due to environmental factors.

Osteonecrosis is a common manifestation of GD, with up to one third of GD patients experiencing it [16,17]. The most common site affected is the femoral head [18], as was the case of patient 2. In a study published by the International Collaborative Gaucher Group (ICGG) searching for risk factors for osteonecrosis [16], the only identified ones were anemia and splenectomy. Being their genotype for GBA1 the same and neither having been submitted to splenectomy, we cannot but wonder whether patient 2 being anemic at admission was related to her having had osteonecrosis, and her sister, which was not anemic at admission, having it not.

Another common hallmark of GD is bone marrow infiltration, which can be best assessed through the method of Dixon quantitative chemical shift (Dixon’s QCSI) [19], but unfortunately this method is not available worldwide. Because of that, different other semiquantitative methods are worldwide used to measure the bone marrow infiltration [6,20]. The method that correlates the best with the Dixon’s QCSI method, and evaluates both the axial and the peripheral skeleton is the MRI-based BMB score published by Mass et al., which relies on signal intensity as a measure of fat substitution for Gaucher cells in the bone marrow of femurs and lumbar spine [6]. Bone manifestations of GD are secondary to Gaucher cells infiltration in the bone marrow, together with possible phenotype modifiers genes [21]. What constitutes severe bone disease in GD is open to debate. Although both sisters presented with a normal BMD and no fractures, and only one had hip necrosis, both had a severe BMB score, which may imply a more severe bone phenotype caused by

Fig. 2. MRI images of patient 1 at baseline. A.1) Coronal T1-weighted image of femurs. A.2) Coronal T2-weighted image of femurs. B.1) Sagittal T1-weighted image of lumbar spine. B.2) Sagittal T2-weighted image of lumbar spine. Femur total score = 6 (T1 = 2, T2 = 2, Sites = 2); Lumbar spine total score = 8 (T1 = 3, T2 = 2, Pattern = 3).
the unusual combination of the E349K/S366N GBA1 variants. Besides that, as the DS3 subscores show, for both patients the compromise of bone is more severe than the compromise of visceral and hematological systems. Also, the pattern of decrease of the BMB score during treatment shows that patient 1, who was being treated with ERT for 24 months, presented a fast response with a significantly drop in the total score when compared to patient 2, who was being treated with SRT for 24 months and only 1 month with ERT. This is in accordance with previous studies that have shown that BMB tend to decrease during the first years of ERT, but this cannot be observed with SRT, and, also, the response is not known to reflect disease severity [22,23].

At admission, neither patient was profoundly thrombocytopenic nor anemic. Nor did the patients present overt hepatosplenomegaly, although patient 2 had mild hepatosplenomegaly and mild hepatic steatosis. Overall, the patients could be described as having predominantly severe bone disease and few, mild visceral and hematological manifestations. Whether this is due to environmental factors or indeed to the patients' rare genotype is still unclear, and more reports of patients with the same GBA1 genotype are needed before a conclusion may be confidently drawn. Response to substrate reduction therapy with miglustat was not satisfactory for both sisters, whereas response to enzyme replacement therapy was satisfactory regarding hematological and visceral parameters; both patients reached their goals following the Brazilian Guideline [24] and the European Working Group on Gaucher Disease in 2018 [25].

Mehta et al published in 2019 [26] the presenting signs and patient co-variables in Gaucher disease, and highlighted that physicians can fail to recognise the early stages of GD, which can lead to significant diagnostic delays and sometimes irreversible but avoidable morbidities. When it comes to a classic GD phenotype with massive splenomegaly, bone pain and cytopenias, diagnosis is more intuitive. On the other hand, if the patient has mild symptoms, or, as in our patients' cases only bone disease, the diagnosis becomes trickier and less intuitive, requiring greater expertise to be defined.

5. Conclusions

This is the first GD family with the E349K/S366N GBA1 genotype which is associated with severe bone disease and mild visceral and hematological manifestations. More genotype-phenotype studies are needed to fully establish a causal relationship between this rare genotype and the patients' unique phenotype.

Declaration of competing interest

The authors declare no conflict of interest.

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### Table 1

| Patient 1 | Patient 2 |
|-----------|-----------|
| **GBA1 genotype** | E349K/S366N | E349K/S366N |
| **Glucocerebrosidase activity** | | |
| Leucocyte (NRV: 10-45 nmol/h/mg protein) | 5 | 2.8 |
| Fibroblast (NRV: 257-668 nmol/h/mg protein) | 132 | 60 |
| **Baseline** | | |
| **Age at diagnosis (years)** | 42 | 45 |
| Bone Marrow Burden score | 13 | 14 |
| Hb (g/dL) | 14.6 | 11.6 |
| Platelets (μL): | 143,000 | 192,000 |
| Ferritin (ng/mL) | 588 | 880 |
| Chitotriosidase activity (nmol/h/mL) | 15,581 | 3432 |
| DS3 subscores | | |
| Bone | 3.6 | 2 |
| Visceral | 0 | 0 |
| Hematological | 0 | 0 |
| **After 1 year of treatment** | | |
| **Drug** | Miglustat 300 mg/day | Miglustat 300 mg/day |
| **Time in months of the current treatment** | 12 | 12 |
| Bone Marrow Burden score | 13 | 14 |
| Hemoglobin (g/dL) | 14 | 11.7 |
| Platelets (μL): | 172,000 | 207,000 |
| Ferritin (ng/mL) | 536 | 1160 |
| Chitotriosidase activity (nmol/h/mL) | 8591 | 1667 |
| DS3 subscores | | |
| Bone | 3.6 | 2 |
| Visceral | 0 | 0 |
| Hematological | 0 | 0 |
| **After 2 years of treatment** | | |
| **Drug** | Imiglucerase 30 Ui/kg/inf | Miglustat 300 mg/day |
| **Time in months of the current treatment** | 2 | 24 |
| Bone Marrow Burden score | NA | 14 |
| Hemoglobin (g/dL) | 14.5 | 11.8 |
| Platelets (μL): | 224,000 | 184,000 |
| Ferritin (ng/mL) | 937 | 1117 |
| Chitotriosidase activity (nmol/h/mL) | 5821 | 1984 |
| DS3 subscores | | |
| Bone | 3.6 | 2 |
| Visceral | 0 | 0 |
| Hematological | 0 | 0 |
| **After 3 years of treatment** | | |
| **Drug** | Imiglucerase 30 Ui/kg/inf | Taliglucerase 15Ui/kg/inf |
| **Time in months of the current treatment** | 14 | 1 |
| Bone Marrow Burden score | 3 | 14 |
| Hemoglobin (g/dL) | 14.5 | 12.4 |
| Platelets (μL): | 259,000 | 202,000 |
| Ferritin (ng/mL) | 608 | 1025 |
| Chitotriosidase activity (nmol/h/mL) | 1472 | 1689 |
| DS3 subscores | | |
| Bone | 1.6 | 2 |
| Visceral | 0 | 0 |
| Hematological | 0 | 0 |
| **After 4 years of treatment** | | |
| **Drug** | Imiglucerase 30 Ui/kg/inf | Taliglucerase 15Ui/kg/inf |

### Table 1 (continued)

| Patient 1 | Patient 2 |
|-----------|-----------|
| **Time in months of the current treatment** | 36 | 13 |
| Bone Marrow Burden score | NA | NA |
| Hemoglobin (g/dL) | 15.3 | 13.2 |
| Platelets (μL): | 267,000 | 234,000 |
| Ferritin (ng/mL) | 689 | 1053 |
| Chitotriosidase activity (nmol/h/mL) | 881 | 1133 |
| DS3 subscores | | |
| Bone | 1.25 | 0 |
| Visceral | 0 | 0 |
| Hematological | 0 | 0 |

NA = Not Available.
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Fig. 3. MRI images of patient 2 at baseline. A.1) Coronal T1-weighted image of femurs. A.2) Coronal T2-weighted image of femurs. B.1) Sagittal T1-weighted image of lumbar spine. B.2) Sagittal T2-weighted image of lumbar spine. Femur total score = 7 (T1 = 2, T2 = 2, Sites = 3); Lumbar spine total score = 7 (T1 = 2, T2 = 2, Pattern = 3).
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