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Research Article

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The correlation between bone biomarkers, glucosylsphingosine levels, and molecular findings in Gaucher type 1 patients under enzyme therapy

Enzim tedavisi alan Gaucher tip 1 hastalarında kemik biyobelirteçleri, Glukozilsfingosin düzeyleri ve moleküler bulgular arasındaki korelasyon

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Abstract

Objectives: We aimed to determine the relationship of Lyso-Gb1 levels, bone biomarkers, and mutation findings with bone marrow burden (BMB) scores.
Methods: Lyso-Gb1 and bone biomarkers, and BMB scores of 10 Gaucher type 1 (GD1) patients under enzyme therapy were prospectively evaluated.
Results: Ten GD1 patients, aged between 4.5 and 40 (mean 23 ± 11 years), were included in the study. Four patients were homozygous for L444P/L444P, and six patients were compound heterozygous for N370S/R415H. We found positive correlations between pain and BMB scores with Lyso-Gb1 levels (r=0.889, p=0.001 and r=0.701, p=0.035, respectively). There were negative correlations between bone mineral density (BMD) of both the lumbar spine and femoral neck between Lyso-Gb1 levels (r=−0.929, p=0.001 and r=−0.893, p=0.007, respectively). Patients with L444P/L444P mutation had higher Lyso-Gb1 levels and BMB, pain scores and lower BMD measurements than patients with N370S/R415H (p=0.01, p=0.02, p=0.03, p=0.04, p=0.04, respectively).
Conclusions: There was an apparent correlation between, Lyso-Gb1 levels, BMB scores and genotype in evaluating bone involvement in Gaucher patients.

Keywords: bone biomarkers; enzyme therapy; Gaucher disease; Lyso-Gb1; mutation.

Öz

Amaç: Lyso-Gb1 düzeyleri, kemik biyobelirteçleri ve mutasyon bulgularının kemik iliği yükü (BMB) skorları ile ilişkisini belirlemeyi amaçladık.
Gereç ve yöntem: Enzim tedavisi altındaki on Gaucher tip1 hastasının Lyso-Gb1 düzeyleri, kemik biyobelirteçleri ve BMB skorları prospektif olarak değerlendirildi.
Bulgular: Yaşları 4.5 ile 40 arasında (ortalama 23 ± 11 yıl) 10 Gaucher tip 1 hastası (GD1) çalışmaya dahil edildi. Dört hasta L444P/L444P homozigot ve altı hasta N370S/R415H bileşik heterozigottu. Ağrı ve BMB skorları ile LysoGb1 seviyeleri arasında pozitif korelasyon bulduk (sırasıyla r=0.889, p=0.001 ve r=0.701, p=0.035). Lyso-Gb1 seviyeleriyle hem lomber omurga hem de femur boynunun kemik mineral yoğunluğu (KMY) arasında negatif korelasyon

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vardı (sırasıyla r=−0.929, p=0.001 ve r=−0.893, p=0.007). L444P/L444P mutasyonu olan hastalarda N370S/R415H olan hastalara göre daha yüksek Lyso-Gb1 düzeyleri ve BMB, ağrı skorları ve daha düşük BMD ölçümleri vardı (sırasıyla p=0.01, p=0.02, p=0.03, p=0.04, p=0.04).

Sonuç: Gaucher hastalarında kemik tutulumunun değerlendirilmesinde Lyso-Gb1 seviyeleri, BMB skorları ve genotip arasında belirgin bir ilişki vardı.

Anahtar kelimeler: enzim tedavisi; Gaucher hastalığı; kemik biyobelirteçleri; Lyso-Gb1; mutasyon.

Introduction

Gaucher disease type 1 (GD1, OMIM#230800) is an autosomal recessive lysosomal storage disease of glycosphingolipid metabolism caused by a deficiency of β-glucocerebrosidase, resulting in the progressive accumulation of the substrates related to glycosphingolipids in macrophages, transforming them into Gaucher cells [1]. Hepatosplenomegaly, pancytopenia, and bone involvement are the main manifestations with a broad spectrum of disease severity from infancy to adulthood [2]. GD1 differs from type-2 and type-3 GD by the absence of accompanying neuronopathic findings [3].

The clinical findings of GD have improved rapidly and effectively with the advent of enzyme replacement therapy (ERT) since the '90s' [4]. While visceral and hematological findings improve faster, recovery of bone manifestations take much longer [5, 6]. In fact, bone pain is still one of the leading complaints in some patients under ERT. It leads to the most debilitating complications of the disease, which reduce the quality of life [7]. GD effects the bone marrow and the mineralized components of bone [8, 9]. Bone remodeling is impaired as a result of the deterioration of the balance between osteoblasts and osteoclasts. Thus, osteopenia, lytic lesions, pathologic fractures, avascular osteonecrosis, and cortical and medullary infarcts result, which cause the main skeletal manifestations of GD [10, 11].

Glucosylsphingosine (Lyso-Gb1) is a highly sensitive and specific biomarker for diagnosing and monitoring patients in routine follow-up [12]. It is the lyso-derivate of the common glycolipid glucocerebroside. Pre-treatment values and the rate of decrease of these values with ERT also provide an indication of the disease's prognosis [13]. In the event of interruption, inefficiency, or absence of the treatment, Lyso-Gb1 rises before clinical signs worsen, providing a reliable time-saving biomarker for clinicians [14]. While many studies show the correlation between visceral, hematological findings and Lyso-Gb1, there is no study evaluating its relationship with bone clinical and imaging findings to date. According to the International Collaborative Gaucher Group Registry (ICGGR), there is an unmet need in the literature and consensus studies to determine the correlation between the treatment response of bone involvement and the biomarkers [15].

Magnetic resonance imaging (MRI) is a valuable tool in assessing bone and bone marrow and is the gold standard for monitoring bone involvement in GD patients [16]. The most sensitive and quantitative actual MRI technique is Dixon quantitative chemical shift imaging (QCSI). This technique quantifies the fat content of bone marrow by using the difference in resonant frequencies between fat and water. It can detect the reduction in the fat fraction that occurs when Gaucher cells displace the normal fat-rich cells in bone marrow [17, 18]. High bone marrow fat fractions as detected by QCSI have been shown to correspond with decreased clinical disease and bone complications. In addition, after ERT initiation, QCSI can monitor the response to therapy [19]. However, it is a technic that requires special software and is not easily accessible, limiting its use in practice. For these reasons, several semiquantitative scoring systems (Rosenthal staging system, Dusseldorf score, Terk Classification, vertebra disc ratio, and bone marrow burden [BMB] score) [20–23] with conventional MRI technology, which is widely available, are preferred. The semiquantitative method, the BMB scoring system, that we use in our study is less validated, reliable, and sensitive than Dixon QCSI, but it shows enough sensitivity to examine the detection of bone marrow response to enzyme supplementation therapy [24].

We aimed to determine the relationship of Lyso-Gb1 levels, biochemical bone biomarkers, and clinical symptoms with BMB scores, and evaluate the utility of these tools in monitoring the severity of bone involvement.

Materials and methods

Nineteen patients with GD were followed in our center between January 2015 and June 2021. One patient with type-2 GD and two patients with type-3 GD died during the follow-up. Ten patients with GD1 from five different families under the ERT treatment regime consented to participate in the study. This study was designed as a cross-sectional case-control, prospective methodology. Demographic and genetic data were extracted from medical records while collecting current clinical, laboratory, and imaging findings. Studies conducted to date have indicated the adequacy of the relationship between dose and target organ responses for 48 months of treatment [25, 26]. Therefore, patients whose treatment duration was over 48 months were included in the study.

Biochemical bone biomarkers including calcium, phosphorus, magnesium, bone alkaline phosphate, parathormone, 25 hydroxy-vitamin D, and C-terminal telopeptide of type-I collagen (CTX) were

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documented. The corrected calcium level was calculated according to the albumin level using the formula: (corrected calcium = serum calcium + 0.8 × [4 – patient's albumin]). Lyso-Gb1 levels were measured using liquid chromatography-mass spectrometry (LC-MS/MS) of DBS samples. Lyso-Gb1 (GluSph) (Matreya Cat. No. 2086), and N-glycinated glucosylspingosine (Matreya Cat. No. 2089) were used as internal standards, respectively.

Bone mass density (BMD) assessment was performed, and postmenopausal females or males aged >50 years with a T score ≤−2.5 SD at the lumbar spine, femoral neck, or distal radius were diagnosed with osteoporosis based on the World Health Organization (WHO) criteria. In premenopausal females or males aged ≤50 years, a Z score ≤−2.0 SD was also described as osteoporosis.

Bone marrow was evaluated by means of the MR Imaging BMB semiquantitative scoring system. The BMB score system considers femur and lumbar spine findings. In our study, the femur was scored according to signal intensity (T1, 0–2 and T2, 0–3) and site of involvement (proximal/distal epiphysis and/or diaphysis; 0–3).

Similarly, the sum of lumbar spine scores according to signal intensity (T1, 0–2 and T2, 0–3) and infiltration pattern (patchy or diffuse, and absence of fat in the basivertebral vein region; 0–2) was calculated as a maximum of eight points. Total BMB is obtained by adding the scores for the femur (up to eight points) and lumbar spine (up to eight points), with a maximum score of 16 points (range 0–16). Higher scores reflect more significant marrow infiltration [24]. BMB score examinations were performed by the same radiology, thus avoiding interobserver discrepancy.

We applied the "universal subjective pain intensity scale" to determine patients' pain levels for both adult and pediatric patients over three-year-old [27]. No pain: 0; Minor: able to adapt to pain (1–2–3); Moderate: interferes with many activities (4–5–6); Severe: disabled or unable to function independently (7–8–9–10).

Statics

Statistical analyses were performed using SPSS version 22.0. The categorical variables were defined as frequency and percentage rate, and the numerical variables were determined as mean ± standard deviation (SD). The Kolmogorov–Smirnov test assessed the normality of the distribution of the quantitative variables. The student's t-test was performed for normally distributed numerical variables, and the Mann–Whitney U test was carried out for non-normally distributed data for independent group comparison. Categorical variables were compared using the Chi-square test. Bivariate correlations were expressed by Pearson's correlation analysis or Spearman's correlation analysis. Statistically significant results were defined as those with a p-value of <0.05.

Ethical approval

This study was conducted under the ethical principles of the World Medical Association Declaration of Helsinki (2000). It was approved by the local Ethics Committee (Approval number: 2021/394, Istanbul [15/11/2021]).

Results

The current ages of the patients range from 4.5 to 40 years (median 22.5; mean 23 ± 11 years). Six in 10 (60%) patients were female. Three of the patients (P7, 9, 10) were in the pediatric age group. Four patients were diagnosed with typical visceral findings, and six cases were diagnosed through family screening. Their genetic diagnosis was based on identifying biallelic pathogenic or likely pathogenic variants in the GBA gene. Ten patients were homozygous for c.1448T>C (p.L444P), and six patients were compound heterozygous for c.1226A>G (p.N370S) and c.1505G>A (p.R415H). The mean duration of ERT (imiglucerase; in the dose of 30 U/kg) was 7.2 ± 4.7 (range = 2–18) years (Table 1).

Eight patients with GD (80%) had bone pain complaints, and one patient (P8) had undergone a splenectomy before ERT initiation. Patients with bone complaints did not report to have bone crisis and fracture in their medical history.

Table 1: Characteristics of patients with Gaucher disease type 1.

ID	Sex	Age at diagnosis, years	Duration of ERT, years	ERT dosage İmigluceras, U/kg	Pathogenic variation Allele 1	Pathogenic variation Allele 2
P1	M	20	8	30	c.1448T>C (p.Leu483Pro)	c.1448T>C (p.Leu483Pro)
P2	F	16	7	30	c.1448T>C (p.Leu483Pro)	c.1448T>C (p.Leu483Pro)
P3	F	30	6	30	c.1448T>C (p.Leu483Pro)	c.1448T>C (p.Leu483Pro)
P4	M	17	11	30	c.1226A>G (p.Asn409Ser)	c.1505G>A (p.Arg502His)
P5	M	16	6	30	c.1226A>G (p.Asn409Ser)	c.1505G>A (p.Arg502His)
P6	F	17	4	30	c.1226A>G (p.Asn409Ser)	c.1505G>A (p.Arg502His)
P7	M	15	2	30	c.1226A>G (p.Asn409Ser)	c.1505G>A (p.Arg502His)
P8	F	22	18	30	c.1226A>G (p.Asn409Ser)	c.1505G>A (p.Arg502His)
P9	F	0.9	4	30	c.1226A>G (p.Asn409Ser)	c.1505G>A (p.Arg502His)
P10	F	0.2	7.5	30	c.1448T>C (p.Leu483Pro)	c.1448T>C (p.Leu483Pro)

L444P: c.1448T>C (p. Leu483Pro); **N370S**: c.1226A>G (p. Asn409Ser); **R415H**: c.1505G>A (p. Arg502His). F, female; M, male; ERT, enzyme replacement therapy.

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In four patients (40%), moderate to severe bone pain was detected. Eight patients (80%) had MRI findings of varying severity. Distal epiphyseal involvement was seen in one patient (P1), and diaphyseal involvement was detected in seven patients. T1AG heterogeneous hypo-isointense and T2AG heterogeneous hyperintense lesions were detected in the medullary plane, consistent with Gaucher's nodule, in the medial part of the proximal diaphysis of the tibia in one patient (P1). Osteoporosis was found in two patients (P2, P3). Since vitamin D deficiency is expected to pose an additional risk for osteopenia, osteoporosis, and fractures in patients with GD, at least 1,500–2,000 IU/day of supplemental vitamin D were recommended to patients according to their 25-OH-D vitamin levels. However, only three of our patients (P1, P2, P10) used supplemental vitamin D regularly in this process and they have optimal 25-OH-D vitamin levels (above 30 ng/mL) (Our study was carried out in the winter months and the vitamin D level was measured in a period when the sun exposure was low).

There were positive correlations between pain and BMB scores with Lyso-Gb1 levels (r=0.889, p=0.001; r=0.701, p=0.035, respectively). There were negative correlations between BMD of both the lumbar spine and femoral neck with Lyso-Gb1 levels (r=–0.929, p=0.001; r=–0.893, p=0.007, respectively). There was also a negative correlation between Z scores of lumbar spine DXA with Lyso-Gb1 levels (r=–0.862, p=0.006). There was a positive correlation between BMB scores with pain scores and magnesium levels (r=0.725, p=0.027; r=0.708, p=0.033, respectively). There was a negative correlation between BMB with the Z score of femoral neck DXA and phosphorus levels (r=–0.863, p=0.012; r=–0.864, p=0.003, respectively) (Table 2).

Patients with L444P/L444P mutation had significantly higher Lyso-Gb1 levels, BMB and pain scores than patients with N370S/R415H mutation (p=0.01, p=0.02, and p=0.03, respectively). In addition, lumbar and femoral neck bone mineral density (BMD) measurements were lower in patients with L444P/L444P mutation than those with N370S/R415H (p=0.04 and p=0.04, respectively) (Table 3).

Table 2: Correlation between Lyso-Gb1 levels and BMB scores with patients' parameters.

N=10	Lyso-Gb1 level		BMB score	
	r	p-Value	r	p-Value
Lys-Gb1, ng/mL	_	_	**0.701**	**0.035**
Bone score on MR	**0.701**	**0.035**	_	_
Current age, years	–0.104	0.774	0.528	0.144
Female (n)/male (n)	0.018	0.960	–0.133	0.732
Pain score	**0.889**	**0.001**	**0.725**	**0.027**
Duration of ERT, years	–0.214	0.553	0.345	0.363
ALP, U/L	0.033	0.932	–0.497	0.210
Corrected calcium, mg/dL	–0.104	0.776	–0.293	0.444
Phosphorus, mg/dL	–0.413	0.235	**–0.864**	**0.003**
Magnesium, mg/dL	0.616	0.058	**0.708**	**0.033**
Parathormone, pg/mL	0.359	0.309	0.342	0.368
25 hydroxy-vitamin D, ng/mL	–0.050	0.898	0.424	0.295
Bone alkaline phosphatase, µg/L	0.949	0.051	0.500	0.500
CTX, ng/mL	0.200	0.800	–0.866	0.333
Lumbar spine DXA BMD, g/cm^2	**–0.929**	**0.001**	–0.514	0.238
T score	–0.750	0.052	–0.765	0.076
Z score	**–0.862**	**0.006**	–0.514	0.238
Femoral neck DXA BMD, g/cm^2	**–0.893**	**0.007**	–0.716	0.070
T score	–0.771	0.072	–0.794	0.059
Z score	–0.714	0.071	**–0.863**	**0.012**

p<0.05 statistically significant. Significant p-values are shown in bold. Data's were given as mean ± standard deviation. ALP, alkaline phosphatase; BMB, bone marrow burden; BMD, bone mineral density; CTX, collagen type I C-telopeptide; DXA, dual-energy X-ray absorptiometry; Lyso-Gb1, glucosylsphingosine.

Discussion

This study enabled the evaluation of bone involvement of GD1 patients under long-term enzyme therapy with clinical, biochemical, and MRI findings. Skeletal involvement was found to persist even under long-term effective ERT therapy in some patients, and it was determined that there was a significant correlation between bone pain, Lyso-Gb1 levels, BMB scores, and the genotype of the study group.

Skeletal complications in GD have a significant impact on patients' quality of life [28]. Bone pain is the most common and debilitating of these complications. According to the international registry of Gaucher patients worldwide, two-thirds of Gaucher patients had one of radiologic bone disease, and about half of them had bone pain before the initiation of ERT [29]. Several prospective studies have been performed to evaluate the effectiveness of ERT in treating skeletal involvement [28–31]. Some patients showed improvement in this aspect, but 40% of patients continue to suffer from this symptom after 3–4 years of treatment [28]. It is clear that there are Gaucher patients with early response, late response, or no response to ERT, but the underlying reasons have not been clearly revealed. According to the universal subjective pain intensity scale applied to our patients, 80% had pain complaints before ERT initiation. Moderate to severe pain complaints continued in 40% of the patients after a mean of seven years of ERT initiation, and the fact that 75% (n=3) of the patients with ongoing pain complaints were L444P-homozygous indicates that the genotype has a clear impact on the severity of pain and treatment response.

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Table 3: Comparison of the features of the patients according to the type of genetic mutation.

N=10 (all patients)	Patients with N370S/R415H (n=6)	Patients with L444P/L444P (n=4)	p-Value
Lyso-Gb1 levels, ng/mL	86.9 ± 52.9	444.4 ± 245.5	**0.01**
BMB score	1.2 ± 0.8	3.7 ± 2.2	**0.02**
Pain score	2.3 ± 1.0	4.7 ± 1.7	**0.03**
Corrected calcium, mg/dL	8.8 ± 0.4	8.8 ± 0.2	NS
Phosphorus, mg/dL	4.2 ± 0.8	3.4 ± 0.7	NS
Magnesium, mg/dL	1.8 ± 0.05	2.0 ± 0.06	**0.01**
Parathormone, pg/mL	50.0 ± 21.6	45.9 ± 6.9	NS
25 hydroxy-vitamin D, ng/mL	15.1 ± 11.5	21.4 ± 10.2	NS
N=7 (adult patients)	**(n=4)**	**(n=3)**	
ALP, U/L	63.0 ± 15.5	70.6 ± 17.0	NS
Lumbar spine DXA BMD, g/cm^2	1.085 ± 0.104	0.848 ± 0.096	**0.03**
T score	−0.4 ± 0.9	−1.4 ± 1.1	NS
Z score	−0.2 ± 0.8	−2.0 ± 0.9	**0.04**
Femoral neck DXA BMD, g/cm^2	1.076 ± 0.083	0.792 ± 0.124	**0.03**
T score	0.4 ± 0.6	−1.3 ± 0.6	**0.02**
Z score	0.4 ± 0.7	−1.1 ± 0.6	**0.04**

p<0.05 statistically significant. Significant p-values are shown in bold. ALP, alkaline phosphatase; BMD, bone mineral density; BMB, bone marrow burden; CTX, collagen type I C-telopeptide; DXA, dual-energy X-ray absorptiometry; Lyso-Gb1, glucosylsphingosine.

None of the bone biomarkers appear to reflect the presence of bone disease or the response to ERT [32, 33]. There was no correlation between biochemical bone biomarkers (Bone specific ALP and CTX) with Lyso-Gb1, BMB, and pain scores. Although the relationship of phosphorus and magnesium levels with BMB score and genotype was shown statistically, this does not constitute a general opinion associated with a small sample. Remarkably, both of the two patients who developed osteoporosis were L444P homozygous.

In our study, the mean 25-OH-D vitamin level of the patients was 17.2 ± 10.8 ng/mL (5.1–37.1), and no correlation was found between 25-OH-D vitamin levels and Lyso-Gb1, pain, and BMB scores. However, it is known that vitamin D has anti-inflammatory and immunomodulatory effects on the bone turnover by regulating calcium-phosphorus homeostasis. Since there is chronic basal inflammation in GD, monitoring vitamin D level and keeping it as high as possible are recommended. Several immune cells are impaired in GD, including monocytes, macrophages, and T- and B-cells, leading to systemic and local activation of the immune system that increases the inflammation [34–36]. This mechanism may be critical in terms of the effects on aging, autoimmune, malignant, and Parkinson's diseases rather than the early findings of the Gaucher disease. However, it is necessary to establish studies on this subject and evaluate long-term follow-up results for evidence.

Lyso-Gb1 is of special importance among chitotriosidase, CCL18 and other biomarkers for GD. Lyso-Gb1 is a highly sensitive, specific, and easily accessible biomarker for GD in terms of diagnosing and monitoring patients. Pre-treatment and reduction values of Lyso-Gb1 by ERT provide insight into prognostic status. Numerous publications have reported the correlation between Lyso-Gb1 and visceral organs and hematological findings. However, extensive studies on skeletal involvement and treatment response have not been conducted [37]. Lyso-Gb1 levels revealed a significant correlation with patients' pain scores, BMD measurements, and BMB scores in our study. In this case, Lyso-Gb1 can also be used as a reliable bone biochemical marker. Patients with N370S mutation exhibited lower Lyso-Gb1 concentrations than patients with L444P mutation [37–39]. Interestingly, Cullufi et al. presented the clinical and biochemical manifestations of four different mutations in a single Gaucher family, and similarly, the presence of the N370S mutation indicated both the absence of bone involvement and lower Lyso-Gb1 levels [40]. In our study, while the Lyso-Gb1 levels, pain, and BMB scores of patients with N370S mutation were lower, their BMDs were found to be higher, at a statistically significant level. This supports the theory that genotype alone affects bone status in GD independently. At this point, it is necessary to explain why bone biomarkers do not correlate with bone findings, but Lyso-Gb1 levels do. ERT reduces the burden of glucocerebroside in macrophages. Thus, this helps to prevent remodeling deformities, sclerotic lesions due to infarcts and decreases Lyso-Gb1 levels. Since different factors can play role in the development of osteoporosis and osteopenia, a

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significant relationship may not be found between bone biomarkers and bone findings. However, new studies are needed to explain this issue.

The BMB semiquantitative scoring system developed by Maas et al. [24] has been used successfully and widely to evaluate bone and bone marrow in GD patients. Compared with the Dixon QCSI scoring system, which best evaluates peripheral and axial bone marrow, it gave the most accurate pre-and post-treatment results [24]. Our study showed for the first time that the BMB scoring system was also correlated with clinical findings (pain scores) and biochemical biomarkers (Lyso-Gb1) in the monitoring of GD patients.

As part of this study, we had the chance to evaluate the patients according to their genotypes. Genotype is one of the critical parameters that determine the severity of the disease. Those of our patients who have the N370S/R415H compound heterozygous genotype seemed to have a better prognosis than those with L444P/L444P.

Conclusions

Our study revealed an apparent correlation between pain scores, Lyso-Gb1 levels, genotype and BMB scores in Gaucher patients. Therefore, consideration of all these non-invasive and easily accessible parameters is recommended in the follow-up of bone involvement in patients with GD.

Limitation of the study

Since GD1 is in the "Rare Diseases" group and is a single-center study, a small sample size was the limitation of the study.

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Author contributions: M.E, D.Y, and H.P planned, designed, and conducted the study. **M.E and H.P** were involved in the long-term care of patients and collected patient data. **M.E** was responsible for the genetic characterization of the patients. **M.E and H.P** were responsible for collecting the clinical and laboratory data of the patients. **D.Y** was responsible for evaluating the MRI BMB scores. All authors read and agreed with the manuscript before submission.
Conflicts of interest: The authors state that there were no potential conflicts of interest.

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