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ORIGINAL ARTICLE

Mutational spectrum and genotype–phenotype correlation in Mexican patients with infantile-onset and late-onset Pompe disease

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Abstract

Background: Pompe Disease (PD) is a metabolic myopathy caused by variants in the GAA gene, resulting in deficient enzymatic activity. We aimed to characterize the clinical features and related genetic variants in a series of Mexican patients.

Methods: We performed a retrospective study of clinical records of patients diagnosed with LOPD, IOPD or pseudodeficiency.

Results: Twenty-nine patients were included in the study, comprising these three forms. Overall, age of symptom onset was 0.1 to 43 years old. The most frequent variant identified was c.-32-13T>G, which was detected in 14 alleles. Among the 23 different variants identified in the GAA gene, 14 were classified as pathogenic, 5 were likely pathogenic, and 1 was a variant of uncertain significance. Two variants were inherited in cis arrangement and 2 were pseudodeficiency-related benign alleles. We identified two novel variants (c.1615 G>A and c.1076-20_1076-4delAAGTCGGCGTTGGCCTG).

Conclusion: To the best of our knowledge, this series represent the largest phenotypic and genotypic characterization of patients with PD in Mexico. Patients within our series exhibited a combination of LOPD and IOPD associated variants,

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which may be related to genetic diversity within Mexican population. Further population-wide studies are required to better characterize the incidence of this disease in Mexican population.

KEYWORDS

acid alpha-glucosidase, *GAA* gene, metabolic myopathy, phenotype–genotype correlation, Pompe disease, pseudodeficiency allele, rare disease

1 | INTRODUCTION

Pompe disease (PD) is a low-frequency metabolic myopathy stemming from variants in the *GAA* gene, resulting in deficient or absent acidic alpha-glucosidase (GAA) activity, which leads to the accumulation of glycogen within muscular lysosomes (Kishnani, Hwu, et al., 2006). Overall incidence has been estimated at approximately 1 case per 40,000 individuals. However, this may vary depending on factors such as ethnicity and geographical location (Kishnani, Hwu, et al., 2006). To date, there are no reliable population-wide reports on the incidence of PD in the Mexican population. The clinical spectrum of PD is heterogeneous, as it varies depending on the age of onset, organ involvement, and rate of disease progression. In an attempt to classify the disease, two clinical forms have been proposed: (1) infantile-onset PD (IOPD) and (2) late-onset PD (LOPD). Both can manifest with either classic or non-classic symptoms (Dubrovsky et al., 2014).

Patients with classic IOPD typically exhibit a characteristic phenotype of hypotonia and cardiomyopathy, often manifesting within the early months of life. Without prompt initiation of enzyme therapy, mortality occurs before the first year of life (Dubrovsky et al., 2014). In contrast, LOPD typically begins after 12 months of age, and 95% of patients do not present with cardiac involvement. The clinical presentation is more heterogeneous, characterized by progressive muscle weakness that spans from childhood into adulthood (Barba-Romero et al., 2012). However, it is important to emphasize that clinical classification and phenotype are not always clear.

The diagnosis of PD is established through GAA enzyme measurement and molecular studies of the *GAA* gene (Musumeci & Toscano, 2019). Phenotype/genotype correlation is crucial for predicting the clinical behavior of the disease (Kroos et al., 2012). Therefore, we aimed to characterize the clinical features associated with genetic variants of the *GAA* gene. Previously, we reported genetic variants in a series of 19 cases of LOPD in Mexico (Grijalva-Pérez et al., 2018; Sánchez-Sánchez, Ávila-Rejón, et al., 2022). In this series, we have expanded the phenotypic characterization of these patients and included patients with the IOPD form and pseudodeficiencies within the same population.

2 | METHODS

2.1 | Study design

This was a retrospective study of clinical records of patients diagnosed with PD. The study was conducted in two phases. The first phase involved the records of patients diagnosed with either LOPD or IOPD. In the second phase, clinical records of patients with GAA pseudodeficiency were reviewed. Clinical geneticists in several institutions were contacted and invited to share the clinical records of their patients. Informed consent from the patients or their guardians, as guided by the institution where genetic care was received, was a prerequisite for inclusion in the study. Each clinician filled out a questionnaire and patients' personal data were masked to preserve confidentiality. The specifics of these phases are illustrated in Figure 1.

The clinical records of patients were investigated to assess the following parameters: state of birth, sex, age of symptom onset, age of diagnosis, presence of neuromuscular symptoms such as muscle weakness, gait disorders, and hyperlordosis, as well as EMG results (when available). Respiratory symptoms such as sleep apnea and history of pneumonia, along with ventilatory test values were also evaluated. Additionally, laboratory values including creatine phosphokinase (CPK) serum concentration and GAA activity were investigated. The use of enzyme replacement therapy (ERT) and familial cases were also noted.

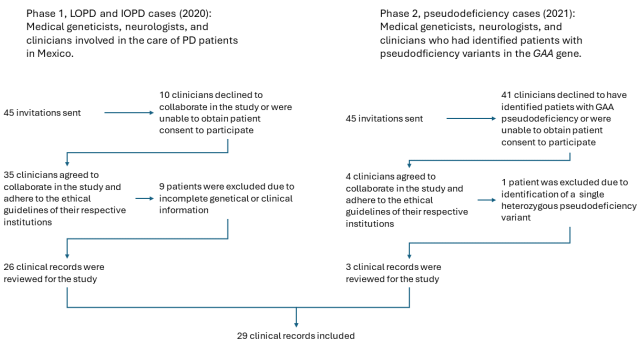

FIGURE 1 Study design.

Patients were classified as having an IOPD or LOPD form, according to both Güngör and Kishnani, Hwu, et al. (2006) proposals. Those with onset before 12 months of age, null enzyme activity, and cardiomyopathy were classified as IOPD, while those with onset after 12 months old (during childhood, adolescence, or adulthood) and residual enzymatic activity were classified as LOPD Patients with non-classical symptoms were classified based on clinicians' assessment, *GAA* gene variants, and CRIM status prediction.

As institutions employ different genotyping methodologies, only patients with full *GAA* gene sequencing by next-generation sequencing (NGS) with Sanger sequencing confirmation, conducted either by in-house teams or external providers, were included in the study.

2.2 | Data classification and analysis

Low enzymatic activity was determined using the dried blood spot (DBS) method and molecular studies of the *GAA* gene. Variant classification was performed based on the guidelines of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015). In addition, reputable genomic databases were consulted to obtain more information on variant classification, such as Franklin by Genoox (https://franklin.genoox.com/clinical-db/home), Clin-Var (https://www.ncbi.nlm.nih.gov/clinvar/), Leiden Open Variation Database or LOVD (https://www.lovd.nl/), and Pompe Variant Database (https://www.pompevariantdatabase.nl/). The latter was consulted to determine the cross-reactive immunological material (CRIM) status.

2.3 | Statistical analysis

No comparative statistical tests were required due to the nature of this study.

2.4 | Ethical compliance

Clinicians involved in this study were required to adhere to the ethical guidelines of their respective institutions and obtain informed consent from patients or their guardians before submitting their medical records for this study.

3 | RESULTS

Twenty-nine patients diagnosed with PD or pseudodeficiency across Mexico were included in this study. Figure 2 illustrates the geographical distribution of these patients alongside the corresponding genetic variants.

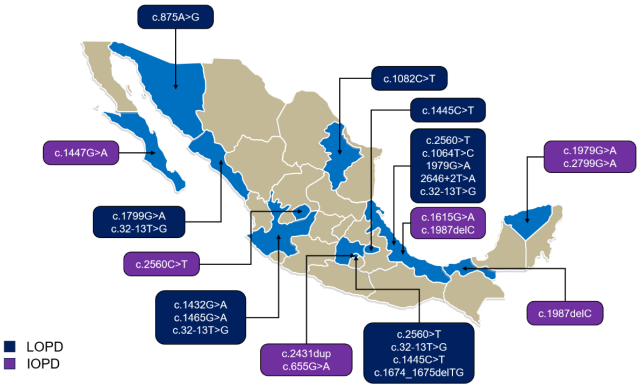

FIGURE 2 Geographical distribution of the patients with LOPD and IOPD diagnosis, along with the corresponding variants.

The female population predominated in our series, accounting for 22 out of 29 patients (75.8%). Symptom onset ranged from 0.1 to 43 years, encompassing both IOPD and LOPD forms. Among the LOPD subgroup (P1–P19), the median age of symptom onset was 19 years (range: 2–43 years), with a median age of diagnosis of 36 years (range: 9–52 years). Notably, two asymptomatic women (P18, P19) were included in the study, as they were relatives of a patient previously diagnosed with PD. Further evaluation classified both of them as having LOPD. For LOPD, the median time from symptom onset to diagnosis was 16 years, ranging from 2 to 30 years. Clinical and demographic characteristics of the LOPD subgroup are delineated in Table 1.

Patients 20 to 26 were infants diagnosed with IOPD, the median age at symptom onset was 3 months (range: 1–9 months) and the median age at diagnosis was 8 months (range: 4–18 months). Among these seven IOPD patients, six (85.7%) had passed away at the time of study due to PD-related complications, the sole survivor of this cohort is currently undergoing enzyme replacement therapy (ERT). Clinical and demographic details of this subgroup can be found in Table 2.

Patients 27 to 29 exhibited typical symptoms and low GAA enzyme activity, suggesting PD. However, the lack of identification of two pathogenic *GAA* gene variants suggests a probable pseudodeficiency. The clinical and demographic characteristics of these unconfirmed cases are outlined in Table 3.

In summary, 26 patients (P1 to P26) received a diagnosis of PD due to low serum GAA enzyme levels and the presence of two pathogenic variants in the *GAA* gene. The remaining patients (P27 to P29) carried *cis* variants recognized as pseudodeficiency alleles. Notably, several patients belonged to the same family. Thus, we analyzed 21 families: P4–P6 from family 4 (F4) were siblings, P9–13 from

TABLE 1 Clinical and demographic characteristics of the LOPD subgroup.

Patient ID	State of birth	Sex	Age at symptom onset (years)	Age at diagnosis (years)	Axial/ proximal muscle weakness	Gait disorders	Hyper-lordosis	Sleep apnea	Forced vital capacity/ polysomnography
P1	Veracruz	F	33	47	Yes	Yes	Yes	No	None available
P2	Tlaxcala	M	13	21	Yes	Yes	No	Yes	Yes (variation higher than 10%—sitting to supine position)/NA
P3	Mexico City	F	25	52	Yes	Yes	Yes	Yes	Yes (variation higher than 10%—sitting to supine position)/NA
P4	Nuevo León	F	13	29	Yes	Yes	Yes	Yes	None available[c]
P5	Nuevo León	F	15	40	Yes	Yes	Yes	Yes	None available[d]
P6	Nuevo León	F	14	30	Yes	Yes	Yes	Yes	None available
P7	Jalisco	F	13	29	Yes	Yes	Yes	Yes	None available
P8[e]	Mexico City	F	2	9	Yes	Yes	No	No	None available
P9	Sinaloa	M	39	51	Yes	Yes	Yes	No	Yes (restrictive spirometric pattern)/No
P10	Sinaloa	F	19	49	Yes	Yes	No	No	Yes (restrictive spirometric pattern)/No
P11	Sinaloa	M	36	48	Yes	Yes	No	No	Yes (normal)/No
P12	Sinaloa	F	31	36	Yes	Yes	No	No	Yes (normal)/No
P13	Sinaloa	F	25	33	Yes	Yes	No	No	Yes (normal)/No
P14	Veracruz	F	10	13	Yes	Yes	No	Yes	None available
P15	Sonora	F	40	49	Yes	Yes	No	NA	None available
P16	Veracruz	F	38	40	Yes	Yes	No	Yes	Yes (variation higher than 20%—sitting to supine position)/No
P17	Jalisco	F	14	37	Yes	Yes	No	Yes	Yes (restrictive spirometric pattern)/Yes (confirmed OSAHS)
P18	Jalisco	F	Asymptomatic	40	No	No	No	No	Yes (normal)/Yes
P19	Jalisco	F	Asymptomatic	31	No	No	No	No	Yes (normal)/Yes

Note: Abnormal laboratory values are displayed in bold.

Abbreviations: CPK, creatine phosphokinase; EMG, electromyography; ERT, enzyme replacement therapy; F, female; GAA, acid alpha-glucosidase enzyme; M, male; NA, not available; OSAHS, obstructive sleep apnea/hypopnea syndrome.

[a]Enzymatic activity of GAA was reported in different units based on third-party diagnostic lab: nmol/ml/h (normal standard values: 1.29–25.7 nmol/mL/h), μmol/L/h (normal standard value: >4.46 μmol/L/h), nmol/mg of protein/h (normal standard value: >6.7 nmol/mg of protein/h).

[b]Electrode position: normal. Rest: positive waves and fibrillation in deltoid, trapezius, rectus femoris, psoas, paraspinal, thorax., and lumbar (bilateral). Contraction: Increased polyphasic motor unit action potential (MUAP) in selected muscles.

[c]Patient with tracheostomy and mechanical ventilation.

[d]Oxygen-dependent patient in a wheelchair.

[e]Echocardiogram: septal asymmetrical hypertrophic cardiomyopathy, septal thickness up to 24 mm, no obstructive. MRI [Nov 5, 2015]: Non-obstructive asymmetrical septal hypertrophic. Echocardiogram [Mar 14, 2016]: LVEF 50%, Tei index 0.9 (left ventricular diastolic dysfunction), No left ventricular outflow tract obstruction, hypertrophic cardiomyopathy. Echocardiogram [Mar 18, 2016]: sinus bradycardia, isolated ventricular extrasystole.
A St. Judes's DDD pacemaker had to be implanted.

History of pneumonia	Baseline CPK (IU/L)	EMG/were paraspinal muscles tested?	Enzymatic GAA activity[a]	ERT
No	**1011**	Yes (bilateral radiculopathy: L5-S1)/No	**0.24 nmol/mL**	Yes
No	**3000**	Yes (myoclonic myopathy with paraspinal muscle discharging)/Yes	**1.6 nmol/mL**	Yes
No	**340**	Yes (myopathic pattern and muscle membrane irritability)/No	NA	Yes
1 event	**358**	Yes (myopathic pattern)/No	**<0.01 µmol/L/h**	Yes
2 annually events	**402**	Yes (myopathic pattern)/No	**<0.43 µmol/L/h**	Yes
2 annually events	**432**	Yes (myopathic pattern)/No	**<0.29 µmol/L/h**	Yes
No	**439**	Yes[b] (probably limb-girdle muscular dystrophy)/Yes	**0.54 nmol/mL**	No
No	**926**	Yes (myopathic pattern in right tibialis anterior and deltoid muscles, normal nerve conduction velocity)/No	**0.25 nmol/mL/h**	Yes
No	216	Yes (chronic proximal myopathy)/Yes	**0.40 nmol/mL/h**	Yes
No	**496**	Yes (chronic proximal myopathy)/Yes	**0.73 nmol/mL/h**	Yes
No	**454**	Yes (chronic proximal myopathy)/Yes	**0.51 nmol/mL**	Yes
No	**495**	Yes (chronic proximal myopathy)/Yes	**0.8 nmol/mL/h**	Yes
No	**659**	Yes (chronic proximal myopathy)/Yes	**0.48 nmol/mL/h**	Yes
No	**926**	Yes (neuropathic pattern with evidence of active denervation in spinal nerve and left myotome L3, remarkable evidence of acute and chronic degeneration in proximal muscles with moderate loss of motor units, mild dysfunction of the proprioception in inferior limb, mainly right side)/Yes	**0.08 µmol/L/h**	Yes
NA	221	Yes (myopathic/neuropathic pattern with evidence of membrane irritability and some myotonic discharges in paraspinal muscles)/Yes	**0.20 µmol/L/h**	Yes
No	**498**	Yes (myopathic pattern, bilateral neurapraxia of the median nerve at the wrist with right predominance)/No	**2.10 µmol/L/h**	Yes
No	307	Yes (myopathic pattern)/No	**0.0638 nmol/mg of protein/h**	Yes
No	**471**	Yes (myopathic pattern)/No	**0.0901 nmol/mg of protein/h**	No
No	Not reported	Yes (normal myopathic pattern)/No	**0.0994 nmol/mg of protein/h**	No

TABLE 2 Clinical and demographic characteristics of the IOPD subgroup.

Patient ID	State of birth	Sex	Age at symptom onset (months)	Age at diagnosis (months)	Family health history	Current state (death age)	Enzymatic GAA activity[b]	Baseline CPK (IU/L)	Cardiomyopathy	Mechanical ventilation	Age of death (months)
P20	Mexico City	F	1	4	No	Deceased	**0.13 nmol/mL**	**1830**	Yes	Yes	4
P21	Aguascalientes	M	3	4	No	Deceased	**0.97 nmol/mL**	Not reported	Yes	Yes	7
P22	Yucatán	F	2	12	No	Alive[a] (60 months)	**0.06 nmol/mL**	**786**	Yes	No	-
P23	Mexico City	M	3	4	Yes	Deceased	**0.6 nmol/mL**	246	Yes	No	57
P24	Veracruz	F	4	10	No	Deceased	NA	**1924**	Yes	Yes	10
P25	BCS	M	12	16	No	Deceased	**0.14 nmol/mL**	**666**	Yes	Yes	18
P26	Tabasco	F	6	6	No	Deceased	**0.06 nmol/mL/h**	Not reported	Yes	Yes	7

Note: Abnormal laboratory values are displayed in bold.

Abbreviations: BCS, Baja California Sur (Mexican state); CPK, creatine phosphokinase; F, female; GAA, acid alpha-glucosidase enzyme; M, male; NA, not available.

[a]Current age during the study.

[b]Enzymatic activity of GAA was reported in different units based on third-party diagnostic lab: nmol/ml/h (normal standard values: 1.29–25.7 nmol/mL/h), μmol/L/h (normal standard value: >4.46 μmol/L/h), nmol/mg of protein/h (normal standard value: >6.7 nmol/mg of protein/h).

TABLE 3 Clinical and demographic characteristics of the unconfirmed (pseudodeficient) subgroup.

Patient ID	Origin	Sex	Age at symptom onset (years)	Age at diagnosis (years)	Axial/ proximal muscle weakness	Gait disorders	Hyper-lordosis	Sleep apnea	Forced vital capacity/ Polysomnography	History of pneumonia	Baseline CPK (IU/L)	EMG/were paraspinal muscles tested?	Enzymatic GAA activity[a]	ERT
P27	Mexico City	F	1 month	NA	Yes	No	No	No	None available	No	152	NA	**0.29 nmol/mL/h**	Not yet
P28	Tabasco	F	3	NA	Yes	Yes	Yes	No	None available	No	289	NA	**1.06 nmol/mL/h**	Not yet
P29	Mexico City	F	4	NA	Yes	No	Yes	No	None available	Yes	250	NA	**0.48 nmol/mL/h**	Not yet

Note: Abnormal laboratory values are displayed in bold.

Abbreviations: CPK, creatine phosphokinase; EMG, electromyography; ERT, enzyme replacement therapy; F, female; GAA, acid alpha-glucosidase enzyme; M, male; NA, Not available.

[a]Enzymatic activity of GAA was reported in different units based on third-party diagnostic lab: nmol/ml/h (normal standard values: 1.29–25.7 nmol/mL/h), μmol/L/h (normal standard value: >4.46 μmol/L/h), nmol/mg of protein/h (normal standard value: >6.7 nmol/mg of protein/h).

family 7 (F7), and P17–19 from family 11 (F11). Seven of the 29 patients displayed homozygous pathogenic variants and 22 were compound heterozygotes. Details of the variants found in each patient are displayed in Table 4.

Regarding the genotype, variants (cDNA level) identified in this case series were as follows: 36 alleles were identified as substitutions (36/58: 62.1%), five were deletions (5/58: 8.6%), two were duplications (2/58: 3.4%), and 15 intronic variants (15/58: 25.9%). Classification of variants according to impact on protein structure identified 29 alleles as missense variants (29/58: 50%), 14 as intronic (14/58: 24.2%), six as nonsense (6/58: 6.9%), seven as frameshift (7/58:12.07%), one as canonical splicing site (1/58: 1.7%), and one as synonymous (1/58:1.7%) variants. The most prevalent variant was c.-32-13T>G (*rs386834236*), which was identified in 14 alleles of our cohort with all instances occurring in compound heterozygous form. The variant c.2560C>T (p.Arg854Ter, rs121907943) was identified in four alleles, comprising one homozygote (P21) and two compound heterozygous patients (P1 and P3). Additionally, the variant c.1445C>T (p.Pro482Leu, rs2039212985) was detected in three alleles, one homozygote (P2), and one compound heterozygote (P8). Variant c.1987delC (p.Gln663Serfs*33) was detected in three alleles, including one homozygote (P26) and one compound heterozygote (P24). Among the 23 variants identified in the *GAA* gene within this cohort, 16 were classified as pathogenic. Regarding the three classified as likely pathogenic, two of them were inherited in *cis*, along with the c.2560C>T and c.1726G>A pseudodeficiency allele (P27), whereas the other one was found in a compound heterozygous state, along with variant classified as pathogenic (P24). One variant was classified as a variant of uncertain significance (VUS, P28), and 2 were classified as benign variants associated with pseudodeficiency (P27, P28, P29), as per ACMG criteria (Richards et al., 2015). The frequency of pseudodeficiency alleles found in this cohort was 3/29 (10.3%). Details of these variants and their predicted impact are displayed in Table 5.

4 | DISCUSSION

PD is a rare metabolic myopathy with a heterogeneous clinical spectrum. In an effort to classify the disease according to clinical presentation, it has been categorized as infantile-onset (classic and non-classical) and late-onset (infantile, juvenile, and adult-onset) forms (Kroos et al., 2012). Incidence rates of PD exhibit variability across ethnicities and geographical regions. The overall incidence is estimated to be approximately 1:40,000 (Kishnani, Hwu, et al., 2006).

The most severe form of the disease presents typically in early infancy. Previous reports indicate a median age of onset at 2 months (range: 0–12 months), with diagnosis typically confirmed by 4.7 months, initiation of mechanical ventilation by 5.9 months, and death occurring at 8.7 months (range: 0.3–73.4 months) (Kishnani, Steiner, et al., 2006). Our findings align with reported data in the IOPD patient subgroup.

Patients presenting with LOPD form may exhibit symptom onset ranging from late childhood to early adulthood. Typical symptoms for the diagnosis include proximal and axial muscle weakness, which may involve the diaphragm, leading to respiratory distress. Disease progression and appearance of symptoms are typically slow, insidious, and confusing, often overlapping with other muscle pathologies. Patients suffering from LOPD often experience a significant delay in diagnosis, which usually spans several years (Barba-Romero et al., 2012). A survey-based study conducted in several European countries reported a median time from the onset of the symptoms to diagnosis of 12 years. This delay in diagnosis was attributed to the fact that they were not sent directly to specialized or reference centers for patients with PD due to a lack of knowledge about to whom and where these patients should be referred once PD is suspected (Lagler et al., 2019). Among the 19 patients diagnosed with LOPD in this series, the average time between symptom onset and diagnosis was 16 years, surpassing previous reports. We suspect this delay in diagnosis may be related to insufficient awareness of the disease.

We identified 23 variants in this series, comprising the largest PD series in the Mexican population, which included 29 patients across 21 families, with two novel variants.

4.1 | c.-32-13T>G (rs386834236) was the most common variant in this series

The c.-32-13T>G variant, which corresponds to rs386834236, was the most common in our series, accounting for 14 alleles (26%). In our series, all occurrences were in a heterozygous state and were associated with the LOPD form. Previous reports have indicated a frequency of this variant ranging from 40% to 70%, with the exception of East Asia, where this variant has been found in <1% of cases. (Park, 2021; Park et al., 2013) In this series, the median age of symptom onset was 25 years (range: 4–40), with a median age of diagnosis of 40 years (range: 29–52). Regarding neuromuscular manifestations, 11/13 patients (85%) portrayed axial/proximal weakness, 10/13 (77%) displayed gait disorders, and 4/13 (30%) had hyperlordosis, EMG results revealed an array of findings, including bilateral radiculopathy, muscle membrane irritability, muscular dystrophy, chronic proximal myopathy, myotonic

TABLE 4 Characteristics of gene variants identified and classified in 29 Mexican patients with confirmed or suspected PD.

Family	Patient ID	Gene	NM ID	Genotype	Exon/ intron	cDNA	Protein	ACMG	Status CRIM prediction	Reference
LOPD										
F1	P1	*GAA*	NM_000152.5	Heterozygous	18	c.2560C>T	p.Arg 854Ter	Pathogenic	Positive	Cheng et al. (2019)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic	Positive	Musumeci et al. (2015), ClinVar: RCV000055770.60
F2	P2	*GAA*	NM_000152.5	Homozygous	10	c.1445C>T	p.Pro 482Leu	Pathogenic	Positive	Kishnani et al. (2019)
F3	P3	*GAA*	NM_000152.5	Heterozygous	18	c.2560C>T	p.Arg 854Ter	Pathogenic	Positive	Cheng et al. (2019)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic	Positive	Musumeci et al. (2015)
F4	P4, P5, P6	*GAA*	NM_000152.5	Homozygous	8	c.1082C>T	p.Pro 361Leu	Pathogenic	Positive	Huang et al. (2021)
F5	P7	*GAA*	NM_000152.5	Heterozygous	9	c.1432G>A	p.Gly 478Arg	Pathogenic	Positive	Luo et al. (2017)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic	Positive	Musumeci et al. (2015)
F6	P8	*GAA*	NM_000152.5	Heterozygous	10	1445C>T	p.Pro 482Leu	Pathogenic	Positive	Kishnani et al. (2019)
			NM_000152.5	Heterozygous	12	c.1674_1675delTG	p.Ala559LeufsTer76	Pathogenic	Positive	Bali et al. (2012)
F7	P9, P10, P11, P12, P13	*GAA*	NM_000152.5	Heterozygous	13	c.1799G>A	p.Arg 600His	Pathogenic	Positive	Sánchez-Sánchez, Ávila-Rejón, et al. (2022)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic	Positive	Musumeci et al. (2015)
F8	P14	*GAA*	NM_000152.5	Heterozygous	6	c.1064T>C	p.Leu 355Pro	Pathogenic	Positive	Reuser et al. (2019)
			NM_000152.5	Heterozygous	14	c.1979G>A	p.Arg 660His	Pathogenic	Positive	Reuser et al. (2019); Sánchez-Sánchez, Martínez-Montoya, et al. (2022)
F9	P15	*GAA*	NM_000152.5	Heterozygous	5	c.875A>G	p.Tyr 292Cys	Pathogenic	Positive	Park et al. (2013)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic	Positive	Musumeci et al. (2015)
F10	P16	*GAA*	NM_000152.5	Heterozygous	Int 18	c.2646+2T>A		Pathogenic	Positive	Viamonte et al. (2021)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic	Positive	Musumeci et al. (2015)
F11	P17, P18, P19		NM_000152.5	Heterozygous	10	c.1465G>A	p.Asp 489Asn	Pathogenic	Positive	Pittis et al. (2008)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic	Positive	Musumeci et al. (2015)

TABLE 4 (Continued)

Family	Patient ID	Gene	NM ID	Genotype	Exon/ intron	cDNA	Protein	ACMG	Status CRIM prediction	Reference
IOPD										
F12	P20	*GAA*	NM_000152.5	Homozygous	17	c.2431dup	p.Leu 811ProfsTer73	Pathogenic	Negative	Sánchez-Sánchez, Martínez-Montoya, et al. (2022); Vorgerd et al. (1998)
F13	P21	*GAA*	NM_000152.5	Homozygous	18	c.2560C>T	p.Arg 854Ter	Pathogenic	Negative	Cheng et al. (2019)
F14	P22	*GAA*	NM_000152.5	Heterozygous	14	c.1979G>A	p.Arg 660His	Pathogenic	Positive	Reuser et al. (2019) Sánchez-Sánchez, Martínez-Montoya, et al. (2022)
				Heterozygous	19	c.2799G>A	p.Lys933Lys	VUS	Positive	Sánchez-Sánchez, Martínez-Montoya, et al. (2022)
F15	P23	*GAA*	NM_000152.5	Homozygous	3	c.655G>A	p.Gly 219Arg	Pathogenic	Positive	Sánchez-Sánchez, Martínez-Montoya, et al. (2022)
F16	P24	*GAA*	NM_000152.5	Heterozygous	14	c.1615G>A[a]	p.Glu 539Lys	Likely Pathogenic	Negative	Not reported (novel)
				Heterozygous	14	c.1987delC	p.Gln 663SerfsTer33	Pathogenic	Negative	Sánchez-Sánchez, Martínez-Montoya, et al. (2022); Esmer et al. (2013); ClinVar Variation ID: 1454730
F17	P25	*GAA*	NM_000152.5	Homozygous	10	c.1447G>A	p.Arg 331	Pathogenic	Positive	Sánchez-Sánchez, Martínez-Montoya, et al. (2022); Kishnani et al. (2019)
F18	P26	*GAA*	NM_000152.5	Homozygous	14	c.1987delC	p.Gln 663SerfsTer33	Pathogenic	Negative	Sánchez-Sánchez, Martínez-Montoya, et al. (2022); Esmer et al. (2013); ClinVar Variation ID: 1454730

(Continues)

TABLE 4 (Continued)

Family	Patient ID	Gene	NM ID	Genotype	Exon/ intron	cDNA	Protein	ACMG	Status CRIM prediction	Reference
No PD										
F19	P27	*GAA*	NM_000152.5	Heterozygous *cis*	5	c.2065G>A/c.1726G>A	p.Glu 689Lys p.Gly 576Ser	Benign: Pseudodeficiency allele Benign: Pseudodeficiency allele	NA	Huang et al. (2021)
			NM_000152.5	Heterozygous *cis*	4 4	c.752C>T/c.761C>T	p.Ser 251Leu p.Ser 254Leu	Likely Pathogenic Likely Pathogenic		Fukuhara et al. (2017) Musumeci and Toscano (2019)
F20	P28	*GAA*	NM_000152.5	Heterozygous *cis*	5 12	c.2065G>A/c.1726G>A	p.Glu 689Lys p.Gly 576Ser	Benign: Pseudodeficiency allele Benign: Pseudodeficiency allele	NA	Huang et al. (2021)
				Heterozygous	7	c.1076-20_1076-4delAA GTCGGCGTTGGCCTG[b]		VUS		Not reported (novel)
F21	P29	*GAA*	NM_000152.5	Heterozygous *cis*	5 12	c.2065G>A/c.1726G>A	Glu689Lys p.Gly 576Ser	Benign: Pseudodeficiency allele Benign: Pseudodeficiency allele		Huang et al. (2021) Dubrovsky et al. (2014)
			NM_017950.4	Heterozygous		c.-32-13T>G		Pathogenic		Musumeci et al. (2015)

Abbreviation: *GAA*, alpha-glucosidase acid gene.

[a]Synonymous variant (novel).

[b]Intronic variant (novel).

TABLE 5 Summary of pathogenic variants by location, severity score, phenotype, and CRIM status in LOPD and IOPD cases[a].

Location	Gene variant	Protein	Severity score[b]	Phenotype prediction (with null allele)	CRIM prediction	Number of cases with the variant
Intron 1	c.-32-13T>G	NA	Potentially mild	Childhood/adult	Positive	13
Exon 3	c.655G>A	p. Gly219Arg	Potentially less severe	Infantile-onset (classic)	Positive	1
Exon 5	c.875A>G	p.Tyr292Cys	Potentially mild	Infantile-onset (classic)	Positive	1
Exon 6	c.1064T>C	p. Leu355Pro	Potentially less severe	Infantile-onset (classic)/ childhood	Positive	1
Exon 7	c.1082C>T	p. Pro361Leu	Potentially less severe	Infantile-onset (classic)	Positive	3
Exon 9	c.1432G>A	p. Gly478Arg	Potentially less severe	Infantile-onset (classic)	Positive	1
Exon 10	c.1445C>T	p. Pro482Leu	Unknown	Unknown (associated with the disease)	Positive	2
Exon 10	c.1465G>A	p. Asp489Asn	Potentially less severe	Infantile-onset (classic)	Positive	3
Exon 10	c.1447G>A	p. Gly483Arg	Less severe	Infantile-onset (classic)	Positive	1
Exon 12	c.1674_1675delTG	NA	Unknown	Unknown	Unknown	1
Exon 13	c.1799G>A	p. Arg600His	Potentially less severe	Infantile-onset (classic)	Positive	5
Exon 14	1979G>A	p. Arg660His	Potentially less severe	Childhood	Positive	1
Exon 14	c.1615G>A	p. Glu539Lys	Unknown	Unknown	Unknown	1
Exon 14	c.1987delC	p. Gln663Serfs*33	Too severe	Infantile-onset (classic)	Negative	2
Exon 17	c.2431dup	p. Leu811Profs*73	Too severe	Infantile-onset (classic)	Negative	1
Intron 18	c.2646+2T>A	NA	Too severe	Infantile-onset (classic)	Negative	1
Exon 18	c.2560C>T	p. Arg854Ter	Too severe	Infantile-onset (classic)	Negative	3
Exon 19	c.2799G>A	p. Lys933Lys	Unknown	Unknown	Unknown	1

Abbreviation: NA, not available.

[a]This table listed all pathogenic variants found in 26 patients (19 non-related families) as heterozygote or homozygote (only one individual could have two or more variants as heterozygote or homozygote).

[b]The severity prediction was reported based on the effect on gene expression and the type of variant as described by Kroos et al. (2012). The severity score is based on the percentage of residual enzymatic activity, as well as the quantity and quality of the molecular sample. The results are expressed as A: too severe, B: potentially less severe, B: less severe, D: potentially mild, E: presumably no pathogenic, F: no pathogenic.

discharges, and bilateral neurapraxia of the median nerve. As for respiratory manifestations, 4/13 patients (30%) had a history of sleep apnea and 1/13 (8%) had a history of pneumonia. Additionally, 4/13 patients (30%) had evidence of spirometric alterations. Among these 13 patients, 12 of them (92%) had low GAA enzymatic activity, with nine of them (69%) undergoing ERT at the time of the study.

4.2 | c.2560C>T (rs121907943) was present in patients with LOPD and IOPD forms

The c.2560C>T (p.Arg854Ter) variant, which corresponds to rs121907943, was found in four alleles (8%), spanning three patients (10%). Two unrelated patients (66%) were compound heterozygotes, with the other variant being c.-32-13T>G (P1, P3) and displayed a LOPD form, while one of them (33%) was a homozygote (P21) and presented with an IOPD form.

The c.2560C>T variant has been described as highly prevalent in many countries, for instance, its frequency among IOPD patients has been reported as high as 16.7% in Brazil (Oba-Shinjo et al., 2009).

Clinical findings in patients with the c.2560C>T variant who exhibited the LOPD form (P1, P3) were similar. The median age of symptom onset was 29 years (range: 25–33) and the median age of diagnosis was 49.5 years (range: 47–52). Both patients displayed axial/proximal muscle weakness, gait disorders, and hyperlordosis. EMG study revealed alterations in both cases, with P1 exhibiting bilateral radiculopathy and P3 showing a myopathic pattern with muscle membrane irritability. One of the LOPD patients (P3) had a history of sleep apnea and slight alterations in ventilatory tests. Only P1 had documented low GAA enzymatic activity; however, both were undergoing ERT at the time of the study. In contrast, P21 displayed classical IOPD manifestations, with symptom onset at 3 months of age, diagnosis confirmed at 4 months of age, and death occurring at 7 months of age. This patient had

no familial history of PD, exhibited cardiomyopathy, and required mechanical ventilation. The c.2560C>T (p.Arg854Ter) variant has been associated with a classical IOPD form and CRIM-negative status prediction. The mechanisms underlying the difference in PD form in relation to the accompanying variant are beyond the scope of this work.

4.3 | c.1445C>T (rs2039212985) was associated with cardiomyopathy in LOPD

The c.1445C>T (p.Pro482Leu, rs2039212985) variant was found in two (6.8%) unrelated patients within this series, comprising three alleles (6%). Patient 2 was homozygous for this variant without a history of consanguinity or endogamy documented. Initial symptoms appeared at 13 years of age, being diagnosed at age 21. The patient exhibited axial/proximal muscle weakness and gait disorders; EMG tests revealed myoclonic myopathy with paraspinal muscle discharging. Sleep apnea and slight spirometric alterations were also noted. Additionally, the patient also displayed dilated right ventricular cardiomyopathy. This variant was previously reported in a patient with cardiomyopathy and sudden cardiac death, no mobility or respiratory support was documented for this patient (Puri et al., 2021).

Conversely, P8 presented the c.1445C>T variant in a compound heterozygous state, along with c.1674_1675delTG (p.Ala559LeufsTer76). The latter is a loss-of-function variant with unknown CRIM status. Symptoms first appeared at 2 years of age with axial/proximal muscle weakness. Cardiomyopathy and dyspnea with non-obstructive asymmetric septal hypertrophy were observed at 5 years of age. Family history revealed an unspecified cardiomyopathy-related death in an older brother. PD diagnosis was confirmed at 9 years of age, leading to the initiation of ERT. In contrast, a Spanish study reported on one patient with the c.1445C>T variant along with a nonsense variant in the second allele, predicting a severe phenotype and childhood onset. This case had a LOPD juvenile phenotype that appeared at 2 years old with cardiomyopathy and hypotonia. Similarly to our patient, this case presented symptoms in childhood along with cardiomyopathy. There is ongoing debate regarding the functional consequence of the c.1445C>T variant, leading us to suggest that the phenotype and early onset in childhood may be attributed to the effect of the accompanying variant. The c.1674_1675delTG variant has been classified as pathogenic based on the ACMG criteria. Since this variant results in a frameshift and a truncated protein, a severe impact on GAA enzymatic activity is predicted, which may explain the early onset of our patient. Although the c.1445C>T variant has been extensively reported in the literature, few cases of LOPD with cardiomyopathy during childhood have been described. (Bali et al., 2012; Barba-Romero et al., 2012; Cheng et al., 2019; Huang et al., 2021; Kishnani, Hwu, et al., 2006; Kishnani, Steiner, et al., 2006; Kishnani et al., 2019; Lagler et al., 2019; Luo et al., 2017; Musumeci et al., 2015).

4.4 | c.1979G>A (rs374143224) was associated with an atypical clinical presentation

The c.1979G>A (p.Arg660His) variant, which corresponds to rs374143224, was found in two patients (6.8%) in a heterozygous state, comprising two alleles in total (4%), with one (P14) presenting a LOPD form and the other one (P22) with an IOPD form.

P14 displayed early symptoms at 10 years of age, characterized by proximal muscle weakness with a predominance in the pelvic girdle, myopathic facies, slight macroglossia, severe scoliosis, and disordered swallowing mechanics. Electromyography revealed active denervation of the spinal cord and left L3 myotome, as well as acute and chronic denervation of proximal muscles with moderate loss of motor units. Nerve conduction tests showed pure motor axonal involvement for median nerves with F-waves persistence in median ulnar and fibula nerves on both sides. Clinically, the patient displayed a tendency to flex the thorax, rigid spine, winged scapula, and absence of hyperlordosis and pelvic tilt, which are typical for a patient with LOPD. Initially, spinal muscular atrophy (SMA) type IV was suspected. However, due to proximal weakness and elevated creatine kinase levels, a comprehensive diagnostic approach including PD was conducted. The patient was found to be compound heterozygous for the c.1979G>A (p.Arg660His) along with c.1064T>C (p. Leu355Pro), both of them have been documented in several patients worldwide.

The c.1979G>A variant has been classified in the Pompe Variant Database as potentially less severe and associated with a childhood phenotype. In a cohort of Iranian patients, this homozygous sequence variant was identified in two Iranian brothers with a history of consanguinity among parents. The boys presented with symptoms of proximal muscle weakness between 12 and 13 years old, winged scapula, rigid spine, and severe scoliosis, similar to our patient (Nazari et al., 2017). The c.1064T>C (p.Leu355Pro) has been associated with the IOPD form and is predicted to be moderately severe (Moravej et al., 2018). Montalvo et al. (2004) described a case with this variant in a homozygous state, causing an infantile-onset phenotype in a 2-year-old patient with severe hypotonia. The patient developed recurrent respiratory infections, respiratory

failure, signs of heart failure, hepatomegaly, and generalized dystrophy, which required full ventilatory support by 4 years of age. The Pompe Registry classified it as a type B variant, which manifests between 12 months and 12 years of age-old, without associated cardiomyopathy, and therefore cannot be included in group A (Bali et al., 2012; Barba-Romero et al., 2012; Cheng et al., 2019; Huang et al., 2021; Kishnani et al., 2019; Lagler et al., 2019; Luo et al., 2017; Musumeci et al., 2015; Reuser et al., 2019; Sánchez-Sánchez, Martínez-Montoya, et al., 2022).

Our patient presented with an atypical clinical phenotype for the LOPD form with childhood-onset, potentially attributed to the convergence of the previously described variants, both have been associated with childhood phenotypes of PD. This case highlights that atypical clinical data, such as rigid spine and neuropathy without a clear diagnosis, PD could be a differential diagnosis to consider.

4.5 | c.1987delC was found in families not previously identified as carriers

The c.1987delC (p.Gln663SerfsTer33) variant was identified in two patients (7.1%) of our series, appearing in three alleles (6%). This variant has been previously reported in Mexican patients with PD (Grijalva-Pérez et al., 2018; Sánchez-Sánchez, Ávila-Rejón, et al., 2022). Cases in the Huasteca region of the country are particularly interesting, as the admixture suggests a founder effect (Grijalva-Pérez et al., 2018). Interestingly, the patients of our series with this variant are not from this geographical area. P24 was a compound heterozygote for this variant, with c.1615G>A variant co-occurring in this patient, while P26 was a homozygote. Both exhibited an IOPD form, with symptom onset at 4 and 6 months of age, and diagnosis at 10 and 6 months of age, respectively. Both patients had cardiomyopathy and required mechanical ventilation, eventually leading to death. Notably, none of these patients had a family history of PD. This sequence variant has recently been reported as having a good response to ERT. We suggest that based on the geographical area in which the patients were diagnosed, this variant could have a high prevalence in other regions of Mexico (Esmer et al., 2013; Sánchez-Sánchez, Ávila-Rejón, et al., 2022; Sánchez-Sánchez, Martínez-Montoya, et al., 2022).

4.6 | c.1726G>A (rs1800307) and c.2065G>A (rs1800309) was the most common pseudodeficiency alelle

We found three cases of c.1726G>A (p.Gly576Ser) and c.2065G>A(p.Glu689Lys), corresponding to rs1800307 and rs1800309, respectively. In the three patients (10%) with these variants, they were found in *cis* and are known to cause low GAA enzymatic activity in normal individuals, for which this allele is recognized as a pseudodeficiency allele. This pseudodeficiency allele is relatively common in Asian populations, with a frequency of 4% of individuals presenting in a homozygous state. For instance, Momosaki et al. documented 71 cases of low GAA activity among 103,204 newborns. Further analysis revealed a prevalence of 45% for homozygotes and 52% for heterozygotes of this allele. (Esmer et al., 2013; Fukuhara et al., 2017; Huang et al., 2021; Kishnani et al., 2019; Málaga et al., 2017; Momosaki et al., 2019; Pittis et al., 2008; Richards et al., 2015; Viamonte et al., 2021; Vorgerd et al., 1998) It remains unknown if the individuals in our series are of Asian descent. In this series, all three patients (100%) with this variant presented axial/proximal muscle weakness, two (66%) had hyperlordosis and 1 (33%) had gait disorders. One of the patients (33%) had a history of pneumonia, and none had a history of sleep apnea. Unfortunately, no records of EMG or ventilatory function tests were available for any of these patients. Notably, all of them had low enzymatic activity. Several studies have reported on the frequency of pseudodeficiency alleles in different populations. For instance, Bravo et al. found only one carrier among 10,000 individuals in a newborn screening program in Brazil (Bravo et al., 2017). In an Italian screening study involving 44,414 children, only two carriers were identified (Burlina et al., 2018). In Mexico, Navarrete et al. reported eight patients with pseudodeficiency variants, two of whom were compound heterozygotes with *trans* variants associated with PD, whereas six were homozygotes without *trans* variants, identified through a screening program involving 20,018 newborns (Navarrete-Martínez et al., 2017). The frequency of pseudodeficiency alleles in Mexico has also been reported to be higher compared to that of Hungary or Australia (Sawada et al., 2020; Vorgerd et al., 1998).

Interestingly, in our study, these three patients were compound heterozygotes for the pseudodeficiency allele, along with accompanying variants in *trans*. Specifically, c.-32-13T>G (P29), c.752C>T and c.761C>T (P27), and c.1076-20_1076-4delAAGTCGGCGTTGGCCTG (P28), which have been classified as pathogenic, likely pathogenic, and VUS, respectively. PD has been reported in patients with pseudodeficiency alleles in *trans* with pathogenic or likely pathogenic variants. It is well known that the c.1726G>A variant is a modifier of pathogenic variants because it produces a greater reduction in GAA enzymatic activity than the pathogenic variant alone (Sawada et al., 2020). Strict surveillance for patients with evident PD manifestations

and complex genotypes involving pseudodeficiency alleles is mandatory. Extensive molecular testing, including whole genome sequencing (WGS) and RNA-Seq, has been proposed to increase the diagnostic rate of PD and improve timely ERT therapy initiation in patients with inconclusive molecular screening (In't Groen et al., 2020).

4.7 | Two novel variants were identified in this series

We identified two novel variants in two patients, (P24, P28) which expand the genotypic spectrum associated with this disease. For P24, the heterozygous variant c.1615G>A (p.Glu539Lys) was classified as likely pathogenic based on the ACMG guidelines (PM1, PP2, PM2, and PP3). In silico models (Revel and MetaLR) predicted a deleterious effect on the protein. Furthermore, these alleles have not been identified in other populations reported in the gnomAd database. Interestingly, this variant predicts a negative CRIM status. The patient showed respiratory failure which required ventilator support and died at 10 months after a single dose of the ERT. Interestingly, the accompanying variant in *trans* was *c.1987delC*, which also displays a negative CRIM status prediction.

We found an intronic variant c.1076-20_1076-4delAAGTCGGCGTTGGCCTG in P28, which was classified as VUS based on the ACMG criteria (PM2). This variant was identified together with a pseudodeficiency allele 2065G>A/c.1726G>A (p.Glu689Lys/p.Gly576Ser) in *trans*. The patient presented symptoms of LOPD at 3 years of age, which consisted of proximal and axial muscle weakness and progressive hepatomegaly. A liver biopsy showed intracytoplasmic storage of periodic acid–Schiff (PAS)-positive material associated with portal fibrosis without cirrhosis. The pathology report indicated glycogenosis type II. Also, this patient had low enzymatic activity in leukocytes. Unfortunately, it was not possible to perform enzymatic activity in fibroblasts to confirm the low GAA enzymatic activity. The enzymatic activity in leukocytes measured by DBS confirmed a value of 1.06 nmol/mL/h (low value). It was not possible to perform and extend the initial molecular screening of NGS with further techniques, such as multiplex-ligation-dependent probe amplification (MLPA) or WGS to rule out variants not identified by NGS. Future molecular studies could be used to determine whether the intronic sequence variant identified in the current study has a causal relation with PD, RNA-Seq may help characterize the functional involvement of noncanonical splicing site variants. However, due to the low allelic frequency shown in different populations and the patient's phenotype, we suggest this variant may cause a significant function impact on GAA enzyme in both muscular and hepatic locations. Further analysis and studies should be performed to confirm this hypothesis.

It has not escaped our notice that patients in our series with a LOPD form present a combination of infantile and adult-onset PD-associated variants, such as c.-32-13T>G. We hypothesize that due to the heterogeneous genetic profile of the Mexican population, the LOPD form occurs much more frequently, especially in the 8–19 years old age group. Timely evaluation of this age group by pediatricians and geneticists in cases where there is clear data suggestive of PD is very important because the diagnosis could be confirmed earlier. The molecular mechanisms associated with the phenotypes arising from different combinations of variants are beyond the scope of this work.

In summary, we present the largest series of PD cases in Mexico, comprising 23 variants, including two novel variants. The phenotype in most LOPD cases was associated with atypical PD and onset in childhood and youth, contrasting with other populations, who have symptoms until adulthood. We believe that prompt clinical and molecular assessment for PD should be conducted in a timely manner, enabling earlier diagnosis and the opportunity for treatment in earlier stages of the disease. Future studies will better characterize the genotype–phenotype association of PD in the Mexican population. Implementation of newborn screening programs at a national level, including PD in the evaluation, will help us better comprehend the epidemiological impact of PD and associated variants, as well as allowing for timely treatment initiation, especially in children with severe IOPD.

AUTHOR CONTRIBUTIONS

Valentina Martínez-Montoya and Luz María Sánchez-Sánchez performed the molecular analyses, interpreted the data, prepared the figures and tables, and wrote and submitted the manuscript. Ekaterina Kasakova and Dr. Pablo Radillo only participated in the organization and logistics management of this study. All further authors were involved in the investigation, resources, and formal analysis of each case reported in this study.

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CONFLICT OF INTEREST STATEMENT

Dr. Valentina Martinez-Montoya is a former employee of Sanofi-Genzyme Mexico, this study was conducted independently of this affiliation. Dr. Ekaterina Kasakova and Dr. Pablo Radillo currently are working in the Medical Department for Rare Diseases of Sanofi-Genzyme Mexico. They only helped in coordination and logistics management for this research project at the beginning and they did not participate in data analysis, results discussion, and project conceptualization.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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