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Investigating Late-Onset Pompe Prevalence in Neuromuscular Medicine Academic Practices

The IPaNeMA Study

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Abstract

Background and Objectives

We investigated the prevalence of late-onset Pompe disease (LOPD) in patients presenting to 13 academic, tertiary neuromuscular practices in the United States and Canada.

Methods

All successive patients presenting with proximal muscle weakness or isolated hyperCKemia and/or neck muscle weakness to these 13 centers were invited to participate in the study. Whole blood was tested for acid alpha-glucosidase (GAA) assay through the fluorometric method, and all cases with enzyme levels of ≤10 pmoL/punch/h were reflexed to molecular testing for mutations in the GAA gene. Clinical and demographic information was abstracted from their clinical visit and, along with study data, entered into a purpose-built REDCap database, and analyzed at the University of California, Irvine.

Results

GAA enzyme assay results were available on 906 of the 921 participants who consented for the study. LOPD was confirmed in 9 participants (1% prevalence). Another 9 (1%) were determined to have pseudodeficiency of GAA, whereas 19 (1.9%) were found to be heterozygous for a pathogenic GAA mutation (carriers). Of the definite LOPD participants, 8 (89%) were Caucasian and were heterozygous for the common leaky (IVS1) splice site mutation in the GAA gene (c -32-13T>G), with a second mutation that was previously confirmed to be pathogenic.

Discussion

The prevalence of LOPD in undiagnosed patients meeting the criteria of proximal muscle weakness, high creatine kinase, and/or neck weakness in academic, tertiary neuromuscular practices in the United States and Canada is estimated to be 1%, with an equal prevalence rate of pseudodeficiency alleles.

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

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The IPaNeMA Study Group coinvestigators are listed in Appendix 2 at the end of the article.

Glossary

CK = creatine kinase; **DBS** = dried blood spotted; **GAA** = acid alpha-glucosidase; **LGMD** = limb girdle muscular dystrophy; **LOPD** = late-onset Pompe disease; **MDA** = muscular dystrophy association; **NBS** = newborn screening program.

Trial Registration Information

Clinical trial registration number: NCT02838368.

Pompe disease is an inherited autosomal recessive lysosomal storage disease caused by the deficiency of acid alpha-glucosidase (GAA) resulting in the accumulation of glycogen within lysosomes in skeletal and cardiac myofibers and progressive muscle dysfunction, and significant motor disability and respiratory failure.[1-3] Enzyme replacement therapy with recombinant alpha-glucosidase, approved since 2006, has been shown to be effective in improving cardiac and respiratory functions in infant and juvenile patients and stabilizing pulmonary functions in adults.[4]

Earlier studies of the incidence of Pompe disease estimated the prevalence of infantile-onset Pompe to be 1 in 138,000 and that of late-onset Pompe disease (LOPD) to be 1 in 57,000.[5] The prevalence of late-onset Pompe in France was recently estimated to be 1 in 69,927.[6] On the other hand, Pompe is exceedingly rare in Finland, and a recent study of 108 subjects found no new cases of Pompe disease.[7] True prevalence of the disease is unknown. Most large centers specializing in neuromuscular disorders have fewer than 10 LOPD patients. On the one hand, this raises the question of whether the incidence of LOPD is overestimated, and on the other hand, whether LOPD is being underdiagnosed, including in large academic neuromuscular practices. Certain ethnic groups are particularly at a higher risk, such as Dutch, African American, and Southeast Asians, especially Chinese and Taiwanese.[8]

Taiwan was the first country in the world to institute a national Pompe newborn screening program (NBS), and Taiwanese authorities estimated the incidence of Pompe disease in Taiwan to be 1 in 16,919 cases.[9] Pompe disease has recently become a part of the national newborn screening panel in the United States, and Missouri was the first state in the United States to start a statewide screening program. By December 2018, with approximate 467,000 births screened in Missouri, 274 cases screened positive for low GAA enzyme levels.[10] Of these, 46 cases were confirmed to be Pompe (10 infantile and 36 late-onset). The incidence of Pompe is now estimated to be 1:9,625 live births in Missouri,[11] much higher than what was previously estimated.[5]

A number of groups around the world have recently investigated the prevalence of Pompe disease in their region. Starting with the effort in Canada, similar work was performed in a number of European countries, including Denmark, Italy, Finland, Spain, combined the United Kingdom and Germany group and Turkey.[6,7,12-24] Each group used different criteria, and the number of cases in each cohort differed. Overall, the prevalence of LOPD ranged from 1.6% to 5.7%.[6,7,13-24]

We do not have similar data in North America. There is tremendous ethnic and racial diversity within the different regions, and the academic neuromuscular practices by and large reflect this diversity. Thus, the prevalence of Pompe is likely to be determined by the population mix in the clinic, and it is probable that clinics with larger number of African American or Asian patients are more likely to see Pompe disease. We undertook a multicenter (13 sites) investigation to determine the prevalence of LOPD in Tertiary Academic Neuromuscular Practices across the United States and in Montreal, Canada.

Methods

Participants

Our primary objective was to determine the prevalence of LOPD in North American tertiary neuromuscular practices. Patients undergoing an evaluation for muscle disease were enrolled at 13 academic centers from July 2015 to July 2018. Patients aged ≥8 years were eligible for study participation if either had isolated hyperCKemia (above 350 IU/L in men and >250 IU/L in women) or there was proximal upper or lower extremity weakness, or neck weakness, on examination. Patients with previously diagnosed LOPD or a known muscle disease (including muscular dystrophies or inflammatory myopathies) were excluded from the study, as were those who refused to provide consent or follow study procedures. At most centers, the research visit was conducted during routine clinic visits, and patients were carefully examined to document muscle weakness. Six patients were included as an exception: 4 had isolated neuromuscular pattern of respiratory insufficiency and 2 had a family history of Pompe disease.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the UC Irvine Institutional Review Board (HS# 2014-1320) and then subsequently approved by each participating institution's local IRB or a relying commercial IRB. The study was listed on the public trials registry ClinicalTrials.gov (identifier NCT02838368). Written informed consent was obtained from all subjects by their treating physicians at the time of routine clinic visits.

Data Collection

Clinical details were abstracted from the subject's medical records, and whole blood was collected in EDTA tubes and sent to Duke Biochemical Genetics Laboratory (Durham, NC) for GAA enzyme assay and reflex molecular testing. If the patient consented for the optional additional DNA study, a separate tube for DNA was collected and sent to the Center for Neurobehavioral Genetics at the University of California, Los Angeles, where DNA was extracted and stored for future use.

Sample Analysis

Whole blood samples received at the Duke Biochemical Genetics Laboratory were dried blood spotted (DBS), and the samples were analyzed for the GAA enzyme activity, measured by the optimized GAA assay, using their published method.[25] GAA enzyme levels of ≤ 10.0 pmoL/punch/h were classified as abnormal in this study; Duke Laboratory's upper limit for deficiency is GAA enzyme level of < 3.88 pmoL/punch/h. The more conservative upper limit was selected to allow for the identification of carriers or patients with pseudodeficiency haplotypes. All abnormal samples were automatically reflexed to the biochemical genetics laboratory for GAA single-gene sequencing for the confirmation of diagnosis, as described previously.[26] As part of their clinical care at UCI, some participants also underwent free-of-cost limb girdle muscular dystrophy (LGMD) 36-gene panel through next-generation high-throughput sequencing testing provided through the Muscular Dystrophy Association and Emory Genetics Laboratory collaboration. This testing included GAA gene sequencing (20× coverage), and this information was available to us particularly for study participants whose GAA enzyme assay was greater than 10.0 pmoL/punch/h and therefore did not trigger reflex gene sequencing as part of our study. Gene sequence variations for such patients were also included in our study data.[27] We included the results of these gene sequence variations when available.

If the GAA assay returned normal, the participants were either informed by mail or in person during their next follow-up visit. If the GAA assay returned abnormal, disclosure of the results was delayed until the genetic testing results came back. The results were given to the participants in person during a follow-up visit. If the enzyme assay and molecular testing showed a definite diagnosis of LOPD, standard of care procedures such as pulmonary function tests and serum creatine kinase (CK) were recommended. Genetic counseling was also either offered or provided at this visit. As part of the research study, we requested that previously performed EMG, nerve conduction studies, and/or muscle biopsy results be included in the data collection.

Data from the clinical visit, the laboratory data, and the GAA results were entered in a study-specific purpose-built REDCap database, housed at the University of California, Irvine. Data were exported and analyzed by the study team (M.W. and T.M.) using Graphpad Prism version 8.0 (San Diego, CA). Statistical analyses consisted of descriptive statistics. Data are shown as mean (SD).

This was an investigator-initiated study that was funded through an investigator-initiated study mechanism by Sanofi-Genzyme (Cambridge, MA). Sanofi-Genzyme had no role in study design, data collection, or data analysis and had no contribution to the final manuscript.

Data Availability

Anonymized data will be shared by request from any qualified investigator.

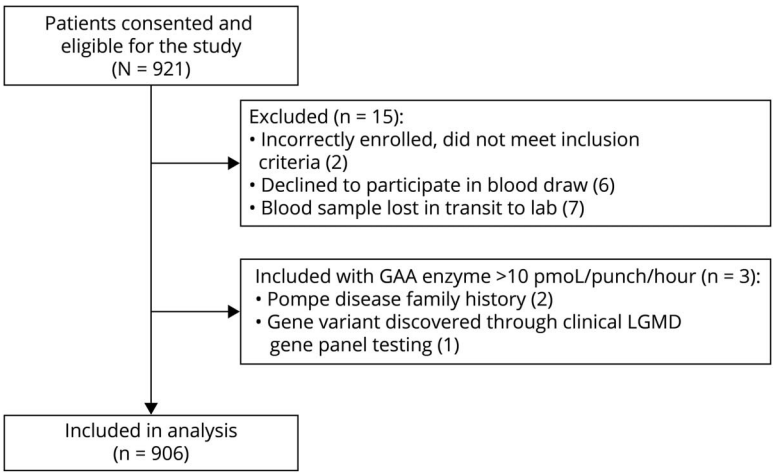
Results

The study enrolled participants from July 2015 through July 2018. Study flow is shown in the CONSORT diagram in Figure 1. A total of 921 participants were enrolled in the study. Two participants were enrolled but did not meet the inclusion/exclusion criteria; their data were excluded from the analysis. An additional 13 participants enrolled in the study but could not have blood tested. Of these 13 participants, 6 left clinic before a blood draw could take place and did not return for a subsequent blood draw. Seven participants had blood drawn, but the samples were lost in transit to the laboratory and were not tested. In all, 906 participants completed the study.

Study demographics are shown in Table 1. The mean age of participants was 52 (15.8) years. Of these, 408 (45%) were women and 496 (55%) were men. Sex information was missing on 2 subjects. Ethnic distribution is shown in Table 1. Whites, African Americans, Hispanic, and Asians made up 76%, 16%, 9%, and 4% of the cohort, respectively. Ethnicity was not identified for 4 patients.

Clinical details are shown in Tables 2 and 3. Thirty-one percent (280) of the participants had isolated elevation of CK, 25.8% (234) had proximal muscle weakness only, and 2.2% (20) had neck weakness only. Twenty percent (180) had both proximal muscle weakness and elevated CK, and 10% (99) had neck and proximal weakness, whereas 1.2% (11) had neck weakness and elevated CK. Eight percent (76) had neck and proximal muscle weakness, and elevated CK. Figure 2 shows the distribution of CK levels (inset) and the Pearson correlation between the CK and GAA values for the overall population. CK was elevated in 474 of the 751 participants for whom CK values were available (180 women and 294 men). The mean CK for all participants was 1,472 (3,649) IU/L. The mean CK value for male participants was 1,332 (3,328) IU/L, whereas in women, the mean CK value was 801.9 (2,437) IU/L. The difference between male and female CK values was significant ($p = 0.01$). The range for GAA enzyme assay was 0.4–402 pmoL/punch/h with a mean value of 17.42 (14.4) pmoL/punch/h. No difference was seen between men and women. There was no correlation between CK and GAA

Figure 1 CONSORT Diagram for the Study



values (r = 0.06; p = 0.09). One male subject had a GAA enzyme level of 402 pmoL/punch/h. This was much higher than the remainder of the cohort (max 70 pmoL/punch/h). He had been evaluated for the symptoms of myalgia and proximal weakness. He did not have a diagnosis of Pompe disease and was not on enzyme replacement therapy.

Of the 906 participants, 820 had normal GAA results, and their participation in this study was completed at this point. Eighty-six (9.5%) participants had an abnormal GAA enzyme level (≤10 pmoL/punch/h) and were automatically reflexed for molecular diagnostic testing. Of these 86 participants, 38 had at least 1 GAA gene variant detected by gene sequencing. Nine participants (1.0% of total study population) had 2 pathogenic variants in the GAA gene and were confirmed to have LOPD. Seventeen (1.9%) were found to have at least 1 pathogenic GAA gene sequence variation and, coupled with their low enzyme activity levels, were diagnosed as carriers of Pompe disease. Two additional participants (0.2%) were classified as likely carriers because of a likely pathogenic variant in the GAA gene, whereas 6 (0.7%) participants were classified as "undetermined" status for Pompe disease because an exact determination on the GAA gene abnormality could not be made, but their GAA levels were abnormal (below our cut off of 10 pmoL/punch/h). Nine (1.0%) had heterozygous pseudodeficiency alleles; although most of these participants had abnormal or low normal GAA enzyme levels, 1 had completely normal level (34.6 pmoL/punch/h). This participant's gene variant was detected by the LGMD gene panel testing performed as clinical standard of care.

Table 1 Demographics (n = 906)

Age (y)	52.5	(15.8)
Sex	**N**	**%**
Female/Male	408/496	45.0/54.7
Unreported	2	0.2
Ethnicity		
Hispanic/Not Hispanic	78/820	8.6/90.5
Unreported	8	0.9
Race		
American Indian	4	0.4
Asian	40	4.4
Black or African American	142	15.8
Native Hawaiian/Pacific Islander	2	0.2
White	690	76.2
Other	34	3.8
Unreported	9	1.0

Details the demographic characteristics of the 906 participants who completed the study.

Table 2 Symptoms and Signs at the Time of Inclusion Into the Study

Inclusion criteria clinical features	N	%
High creatine kinase (CK) only	280	30.9
Proximal weakness only	234	25.8
Proximal weakness and high CK	180	20.0
Proximal weakness and neck weakness	99	10.9
Proximal weakness, neck weakness, and high CK	76	8.4
Neck weakness only	20	2.2
Neck weakness and high CK	11	1.2
Respiratory insufficiency (inclusion exception)	4	0.4
Family history (inclusion exception)	2	0.2

Table 3 Participant Characteristics (All Participants vs Pompe-Positive)

Clinical features	Feature	All participants (n)	All participants (%)	Pompe-positive (n)	Pompe-positive (%)
—	Proximal weakness in upper extremities	485/887	54.7	6	66.7
—	Proximal weakness in lower extremities	586/887	66.1	8	88.9
—	Distal weakness in upper extremities	316/886	35.7	4	44.4
—	Distal weakness in lower extremities	333/886	37.6	5	55
—	Neck weakness	246/885	27.8	3	33.3
—	Muscle weakness in trunk	194/881	22.0	4	44.4
—	Elevated creatine kinase	571/898	63.6	7	77.8
—	Scapular winging	85/877	9.7	0	0
—	Scoliosis	64/881	7.3	2	22.2
—	Lordosis	55/875	6.3	2	22.2
—	Currently ambulatory	849/889	95.5	9	100
—	Ambulatory with difficulty	398/836	47.6	6	66.7
—	Using ambulatory devices	209/889	23.5	4	44.4
—	Muscle pain or cramps	479/887	54	5	55.6
—	Ptosis	65/881	7.4	0	0.0
—	Shortness of breath	263/884	29.8	4	44.4
—	Currently receiving respiratory support	124/879	14.1	5	55.6
—	Sleep apnea	154/877	17.6	2	22.2
—	Dysphagia	139/883	15.7	0	0.0
—	Medical history of aneurysm	6/885	0.7	0	0.0
—	Medical history of cardiovascular involvement	267/886	30.1	2	22.2

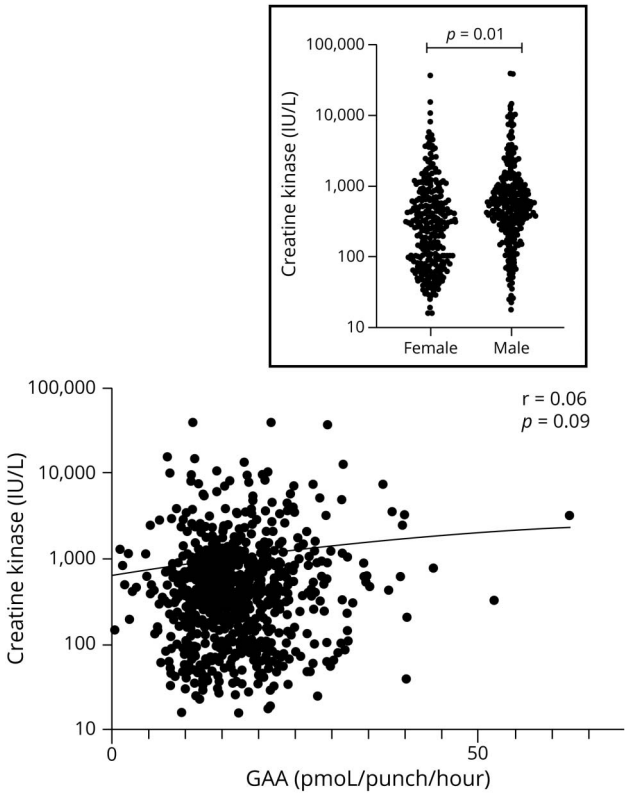
Table 4 shows the demographic and clinical characteristics of the participants diagnosed with definite LOPD, whereas Table 5 shows their mutation and enzyme details. Of the 9 participants who tested positive for definite LOPD, there were 6 women and 3 men. Eight were Caucasian, and 1 was African American. One identified as Hispanic. All 9 had proximal weakness on history and examination, 5 had additional neck weakness, and 7 had elevated serum CK. Mean age in this cohort was 53 (20.5) years. Eight (89%) were heterozygote for the common leaky IVS1 splice site mutation in the GAA gene (c.-32-13T>G); almost all of these subjects had a second mutation that were known (or previously confirmed) to be pathogenic. One subject had a novel pathogenic mutation (c.840_842dup). The ninth subject, an African American, had 2 pathogenic point mutations. All had GAA enzyme levels below 3.8 pmoL/punch/h with a range of 0.4–3.3 pmoL/punch/h.

In the carriers, the GAA enzyme levels ranged from 5.3 to 16.5 pmoL/punch/h (2 participants with greater than 10 pmoL/punch/h were included because of their family history of Pompe disease). Nine of the 17 participants classified as carriers had carried the common IVS1 splice site mutation. A variety of other point mutations or deletions were seen in the carriers. Nine participants were compound heterozygous for the pseudodeficiency haplotype (c.1726G>A and c.2065G>A) and had GAA enzyme levels ranging from 5.3 to 34.6 pmoL/punch/h. Six of these 9 were Asian, and 3 were Caucasian (2 Hispanic and 1 non-Hispanic).

Discussion

We found the prevalence of LOPD to be 1% in a large cohort of patients from 13 US and Canadian tertiary neuromuscular practices. There have been 2 previous studies of this size or larger. The Italian study enrolled 1,051 participants from 17 sites with very similar clinical criteria found 17 new cases of LOPD with a prevalence rate of 1.6%.[18] Lukacs et al.,[24] in a prospective study of 3,076 consecutive adult patients from 7 German and British neuromuscular centers found GAA gene mutations in 74 patients (2.4%) of a total of 3,076 patients who provided a DBS sample. Of these, 70 (94%) patients were heterozygous for the common GAA gene splice-site mutation c.-32-13T>G.[24] Smaller studies from South Korea

Figure 2 CK Values



There is no correlation between serum creatine kinase (CK) and acid alpha-glucosidase (GAA) levels. On Pearson correlation testing, there was no correlation between the CK levels and the GAA levels (r = 0.06; p = 0.09) in the overall population. Inset: Serum CK distribution in all 766 subjects (340 women and 424 men, 2 sex unspecified). Mean CK for all subjects was 1,472 (3,649) IU/L. Mean CK value for male subjects was 1332.3 (3,328) IU/L, whereas in women, the mean CK value was 801.9 (2,437) IU/L. The difference between CK values in men and women was significant (p = 0.01).

(90 subjects) and Denmark (103) found prevalence of 2.2% and 2.9%, respectively.[15,16] The highest prevalence rates were seen in Spain in a study of 348 participants, where a prevalence of 5.7% was found.[28] A comparison of these studies to ours is limited by differences in study design, inclusion criteria, and populations studied.

There has been a paradigm shift in the United States and in Europe, where more and more physicians are using next-generation sequencing-based disease-specific gene panels to diagnose new cases of Pompe disease.[6,27,29,30] A number of laboratories are offering free or inexpensive testing to diagnose these cases. In the United States, free testing for GAA enzyme levels had also been available for a number of years before our study was launched and a number of patients with LOPD had already been discovered through this mechanism. These may be reasons why the prevalence in our study is low compared with some of the other studies. Our prevalence does match a recent large study where Pompe prevalence was investigated through molecular methods rather than through biochemical assay.[27] In this study of 4,656 participants in the United States, the investigators identified 28 cases with 2 pathogenic variants in GAA among patients suspected to have LGMD. An additional 10 cases had 1 pathogenic variant along with 1 VUS in the GAA gene, with no other mutations identified in the remainder genes included in the panel.[27] Thus, a total of 38 cases of late-onset Pompe were identified in this cohort of 4,656 cases, equaling a prevalence of 0.8%. Eighty-one percent of the cases were heterozygous for the common GAA gene splice-site mutation c.-32-13T>G. LOPD thus ranks within the top 10 diagnoses in patients who present with limb-girdle muscle weakness.[27]

The patients captured in this study had clinical disease manifestations, yet most had not received next-generation sequencing. Despite the increasing availability of next-generation sequencing, patients with rare diseases still go through a diagnostic odyssey to achieve a definitive diagnosis. A part of this odyssey is seeing many doctors of various specialties and backgrounds. Different clinics have different criteria and thresholds on when to order this sequencing. This study shows that we are not yet at the point when the availability of next-generation sequencing is the single tool needed to diagnosis patients with Pompe. Increasingly, in the United States, the GAA enzyme assay has become a second-line option rather than the primary modality for diagnosis of Pompe disease. For cases, where the pathogenicity of sequence variations cannot be resolved, low levels of GAA on the enzyme assay would add value for secondary confirmation of Pompe. However, in developing countries and countries where the health system cannot afford the economic burden of large-scale genetic testing, GAA enzyme assay as a primary screening modality is still very important. There are large scale prevalence studies using GAA enzyme assay currently under way in Asia, Africa, and South America; such studies reduced their costs by doing the more expensive molecular testing in only a fraction of the patients.

One surprising result of the study is the frequency of pseudodeficiency in our cohort. We found as many cases of

Table 4 Diagnosed Pompe Patient Characteristics (n = 9)

Age (y)	52.2	(20.5)
	N	**%**
Sex		
Female/Male	6/3	66.7/33.3
Ethnicity		
Hispanic/Not Hispanic	1/8	11.1/88.9
White/Caucasian	8	88.9
Black/African American	1	11.1
Inclusion criteria		
Proximal weakness	9	100
Neck weakness	5	55.6
High creatine kinase	7	77.8

Table 5 Positive Participants

Subject ID	Disease status	GAA enzyme level[a]	Gene sequence 1	Gene sequence 2
001	Positive	2.8	c.172C > T	c.841C > T[a]
002	Positive	1.5	c.-32-13T > G	c.2481 + 102_2,646 + 31del
003	Positive	1.1	c.-32-13T > G	c.1912G > T
004	Positive	0.4	c.-32-13T > G	c.1912G > T
005	Positive	1.7	c.-32-13T > G	c.1933G > T
006	Positive	1.9	c.-32-13T > G	c.1655T > G
007	Positive	2.2	c.-32-13T > G	c.1548 G > A
008	Positive	2.4	c.-32-13T > G	c.840_842dup[a]
009	Positive	3.3	c.-32-13T > G	c.2560C > T

[a] Novel mutations.

pseudodeficiency as actual deficiency (9 for pseudodeficiency and 9 for LOPD). Because the pseudodeficiency alleles are more common in the Asian population (estimated to be present in 4% of Southeast Asian population),[31,32] it was not surprising that 6 of the 9 participants who had pseudodeficiency alleles were Asians. However, these alleles were also seen in 2 Hispanic and 1 Caucasian participants. Of interest, the findings parallel the experience from the NBS in the United States. By December 2018 with approximate 467,000 births screened in Missouri, 274 cases screened positive for low GAA enzyme levels.[10] Of these, 46 cases were confirmed to be Pompe (10 infantile and 36 late-onset), whereas 53 cases were determined to have pseudodeficiency and 65 cases were deemed to be carriers. Similar data are emerging from NBS in Illinois and California.[33,34] It is thus important to make sure that diagnosis of Pompe is never based on GAA enzyme testing alone but confirmed with a second line of evidence (such as mutation analysis, tissue based enzyme levels, etc).

Based on the NBS, the incidence of Pompe is much higher than what was previously established, and the incidence of Pompe is now estimated to be 1:9,625 live births in Missouri and 1:22,000 in Illinois.[11] The numbers emerging from California are similar, with an incidence of 1:25,200 (combined for infantile and late-onset),[34] up considerably from the previous figure of 1:40,000.[5] This poses a major public health issue because 75% of babies found to have GAA deficiency have the late-onset variant and will need surveillance to pick up early signs of disease development. Optimal time to start enzyme-replacement therapy is not known.

A limitation of the study is that the participants were patients actively seeking medical care from neuromuscular clinics. People with early/mild symptoms may not yet be treated by specialists and therefore would not have been captured in this cohort, thus not reflecting the true prevalence of the disease.

In summary, our large-scale prevalence study found a prevalence of 1% for LOPD in undiagnosed patients meeting the criteria of proximal muscle weakness, high CK, and/or neck weakness seen in large tertiary care neuromuscular practices, with an equal prevalence rate for GAA pseudodeficiency and a high rate of carrier status for Pompe disease. Given that Pompe disease is a treatable condition, it is imperative that we consider it in the differential diagnosis of limb-girdle muscle weakness, especially in the presence of respiratory insufficiency and/or neck weakness, and consider it for patients presenting with symptomatic or asymptomatic hyperCKemia.

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Disclosure

M. Wencel, Z. Rasheed, M. Hays, S. Hopkins, A. Shaibani, S. Bandyopadhyay, N.A. Goyal, J. Florence, and J.W. Ralph report no conflicts. T. Mozaffar has served on advisory boards for AbbVie, Alexion, Amicus, argenx, Audentes, Momenta, Sanofi-Genzyme, Sarepta, Spark Therapeutics, and UCB. In relation to these activities, he has received travel reimbursement and honoraria. He has also served on the

speaker's bureau for Alexion, CSL, Grifols, and Sanofi-Genzyme. Dr. Mozaffar serves on the medical advisory board for the Myositis Association, Neuromuscular Disease Foundation, Myasthenia Gravis Foundation of California, and Myasthenia Gravis Foundation of America and has received travel funding from the Myositis Association and the Neuromuscular Disease Foundation. Dr. Mozaffar receives research funding from the Myositis Association, the Muscular Dystrophy Association, the National Institutes for Health, and from the following sponsors: Alexion, Amicus, argenx, Audentes, Bristol Myers Squib, Cartesian Therapeutics, Grifols, Momenta, Ra Pharmaceuticals, Sanofi-Genzyme, Spark Therapeutics, UCB, and Valerion. He serves on the data safety monitoring board for Acceleron, Avexis, and NIH. N. Goyal has received research support from Brainstorm Cell Therapeutics, Cytokinetics, Fulcrum, Kezar, Octapharma, Orion, and Orphazyme. Dr. Goyal has served on the Advisory Boards for Acceleron, Alexion, argenx, CSL Behring, MT Pharma, Sanofi-Genzyme, Sarepta, and UCB. In relation to these activities, she has received travel reimbursement and honoraria. She has also served on the speaker's bureau for CSL. M. Dimachkie serves or recently served as a consultant for argenx, Catalyst, Cello, CSL Behring, EcoR1, Kezar, Momenta, Nufactor, Octapharma, RMS Medical, Sanofi-Genzyme, Shire Takeda, Spark Therapeutics, and UCB Biopharma. Dr. Dimachkie received research grants or contracts from Alexion, Alnylam Pharmaceuticals, Amicus, BioMarin, Bristol Myers Squibb, Catalyst, Corbus, CSL Behring, FDA/OOPD, GlaxoSmithKline, Genentech, Grifols, Kezar, Mitsubishi Tanabe Pharma, MDA, NIH, Novartis, Octapharma, Orphazyme, Ra Pharma/UCB, Sanofi-Genzyme, Sarepta Therapeutics, Shire Takeda, Spark, UCB Biopharma, ViroMed/ Helixmith, & TMA. J. Trivedi has received research support from Sanofi-Genzyme as a site in a multicenter clinical trial. She is also the recipient of an NIH-funded Wellstone grant as the site PI of training core. N. Johnson has received grant funding from NINDS (4K23NS091511; R01NS104010), CDC (DD19-002), and the FDA (7R01FD006071-02). He receives royalties from the CCMDHI and the CMTHI. He receives research funds from Dyne, AveXis, CSL Behring, Vertex Pharmaceuticals, Fulcrum Therapeutics, ML Bio, Sarepta, and Acceleron Pharma. He has provided consultation for AveXis, AMO Pharma, Strongbridge BioPharma, Acceleron Pharma, Fulcrum Therapeutics, Dyne, Avidity, Arthrex, and Vertex Pharmaceuticals. He receives licensing fees from the University of Rochester for the CCMDHI and CMTHI. He has received stock options from ML Bio. Dr. Laurie Gutmann has received research support from Alexion Pharmaceuticals and NIH. M.P. Wicklund has received honoraria from Sanofi-Genzyme and MDA and research support from ML Bio Solutions and Sarepta Therapeutics. He is on the scientific advisory boards for Affinia Therapeutics, Amicus Therapeutics, ML Bio Solutions, Sanofi-Genzyme, and Sarepta Therapeutics. A.L. Genge consults for AL-S Pharma, Biogen, Canopy Health, QurAlis, MTPA, Cytokinetics, Wave Life Sciences, Calico, Alexion, and Orion. Dr. Miriam Freimer has served on the scientific advisory board for Alexion, Immunovant, and argenx and receives research support from Alnylam, UCB, Amicus, NIH, Orphazyme, and Catalyst. A. Pestronk has research support and grants from Acceleron, Cytokinetics, Biogen, Ionis, Fulcrum, Genzyme, Idera, Ra, and Sanofi. He is on the advisory committee and has licensing/product development agreements or royalties for inventions/IP with Athena Diagnostics. C. Karam has served as an advisor to Akcea, Alnylam, argenx, Sanofi Genzyme, and CSL Behring and received research support from Sanofi Genzyme and Akcea. Go to Neurology.org/NG for full disclosure.

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Appendix 1 Authors

Name	Location	Contribution
Marie Wencel, BS	University of California, Irvine	Designed and conceptualized the study, supervision of the overall study, analyzed the data, performed statistical analysis, and drafted the manuscript for intellectual content
Aziz Shaibani, MD	Nerve and Muscle Center of Texas, Houston	Major role in the acquisition of data
Namita A. Goyal, MD	University of California, Irvine	Designed and conceptualized the study, analyzed the data, and drafted the manuscript for intellectual content
Mazen M. Dimachkie, MD	University of Kansas Medical Center	Major role in the acquisition of data
Jaya Trivedi, MD	University of Texas Southwestern, Dallas	Major role in the acquisition of data
Nicholas Johnson, MD	University of Utah, Salt Lake City	Major role in the acquisition of data
Laurie Gutmann, MD	University of Iowa	Major role in the acquisition of data
Matthew P. Wicklund, MD	Pennsylvania State University, Hershey	Major role in the acquisition of data
Sankar Bandyopadhyay, MD	Pennsylvania State University, Hershey	Major role in the acquisition of data
Angela L. Genge, MD	McGill University, Montreal, Quebec, Canada	Major role in the acquisition of data
Miriam L. Freimer, MD	Ohio State University, Columbus	Major role in the acquisition of data
Neelam Goyal, MD	Stanford University, Palo Alto, CA	Major role in the acquisition of data
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Name	Location	Contribution
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Chafic Karam, MD	Oregon Health & Science University, Portland	Major role in the acquisition of data
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Zinah Rasheed, MD	Nerve and Muscle Center of Texas, Houston	Major role in the acquisition of data
Melissa Hays, BA	University of Kansas Medical Center	Major role in the acquisition of data
Steve Hopkins, BS	University of Texas Southwestern, Dallas	Major role in the acquisition of data
Tahseen Mozaffar, MD	University of California, Irvine	Designed and conceptualized the study, supervision of the overall study, analyzed the data, performed statistical analysis, and drafted the manuscript for intellectual content

Appendix 2 Coinvestigators

Name	Location	Role	Contribution
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Name	Location	Role	Contribution
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Name	Location	Role	Contribution
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Appendix 2 *(continued)*

Name	Location	Role	Contribution
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