

# Prominent Respiratory Involvement Featured in High-Risk Screening for Late-Onset Pompe Disease in China

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## Abstract

**Background:** Pompe disease is a rare metabolic disorder with available enzymatic replacement therapy. Contrasting with the classic infantile form, the others subtypes have a heterogeneous presentation that makes an early and accurate diagnosis difficult. A multicenter observational study was aimed to assess the prevalence of late-onset Pompe disease (LOPD) in high-risk population, using dried blood spot (DBS) as a main screening tool.

**Methods:** 20 Chinese neuromuscular centers were involved in the early LOPD screening study. Inclusion criteria were: (1) age  $\geq 1$  years, (2) either one of a) persistent hyperCKemia; or b) muscle weakness of axial and/or limb-girdle muscles; or c) unexplained restrictive respiratory insufficiency. Enzymatic activity of acid  $\alpha$ -glucosidase (GAA) was measured on DBS by tandem mass spectrometry (MS/MS) assay. For the final diagnosis genotype was assessed by next-generation sequencing.

**Result:** In a 9-month period, we studied 492 cases: 26 positive samples (5.3%) were detected by DBS screening. Molecular studies finally confirmed LOPD diagnosis in 8 cases (1.6%). The GAA activities in individuals bearing pseudodeficiency alleles were well separated from those in LOPD patients by MS/MS assay. The median interval from the onset of symptoms to diagnosis was 5 years. Besides axial/proximal muscle weakness, all patients showed respiratory insufficiency with a mean forced vital capacity of predicted of 48%. The level of creatine kinase ranged from normal to no more than 5-fold of upper normal limit. LOPD with isolated hyperCKemia was not identified.

**Conclusions:** This study confirms that DBS test is a reliable method for screening for LOPD. Respiratory insufficiency is earlier and more severe among Chinese LOPD patients. LOPD presented with paucisymptomatic hyperCKemia. Therefore, a prompt diagnosis is critical to prognosis.

## Background

Pompe disease, also known as glycogen storage disease type 2 or acid maltase deficiency, is a multi-systemic metabolic disease caused by deficiency of the lysosomal enzyme acid  $\alpha$ -1,4-glucosidase (GAA) [1]. Late-onset Pompe disease (LOPD) has a variable age at onset and phenotype. In most patients, the presenting symptom is axial and proximal skeletal muscle weakness; while morbidity and mortality are in particular related to respiratory insufficiency[2]. There is growing evidence to suggest that early treatment of LOPD with enzyme replacement therapy, before there is extensive, possibly irreversible muscle damage, is more efficacious than later treatment, emphasizing the importance of early diagnosis[3]. However, LOPD patients presents with a wide spectrum of symptoms including pauci-neuromuscular symptoms at onset, and the diagnosis can be easily overlooked[4, 5]. Patients with LOPD are generally diagnosed 144 (12-480) months after the onset of first symptoms[6]. In mainland China the mean delay in diagnosis of LOPD was 7.2 years[7].

The development of more rapid diagnostic tools, such as the dried blood spot (DBS), were widely used to detect GAA activity in large-scale screening studies in order to facilitate earlier diagnosis. A number of studies have proven the value of DBS in screening for Pompe disease in patients with unclassified limb-girdle weakness or asymptomatic hyperCKemia, with prevalence between 1.1%-4.6%[8–15]. In Chinese patients with LOPD, GAA mutational hotspots are different from European populations[16]. Compared with patients in other parts of the world, the Chinese patients with LOPD seems to have a more aggravated phenotype, especially regarding the respiratory involvement and the requirement of mechanical ventilation[7, 17]. Unfortunately, high risk screening for LOPD in Chinese population has not yet been reported.

In this study, we conducted a prospective, multicenter, observational study to identify undiagnosed LOPD in a large high-risk population, based on measuring GAA activity by DBS, in order to explore the appropriate screening criteria for Chinese LOPD patients.

## Patients And Methods

The participants were prospectively identified through medical examination at 20 in- and outpatient neuromuscular clinics in China between 2020 August and 2021 April. Inclusion criteria were patients with age over 1 year, and at least one of the following: (1) unexplained persistent hyperCKemia; (2) axial/limb-girdle muscular weakness, (3) unexplained respiratory insufficiency. Persistent hyperCKemia was defined as serum creatine kinase (CK) levels above 1.5-fold the upper normal limit (UNL) evidenced at least twice with a minimum interval of 1 month. Axial/limb-girdle muscular weakness was defined as weakness of the trunk muscles and proximal muscles in the arms and legs, including paravertebral muscles, especially the neck flexors, the muscles of the shoulders, upper arms, pelvic and thighs. Respiratory insufficiency was defined as development of respiratory symptoms (dyspnea at rest, exertional dyspnea, orthopnea, hypersomnolence, headache on awakening), hypercapnia, or abnormal pulmonary tests suggestive of neuromuscular weakness. Exclusion criteria were relatives of patients with diagnosed Pompe disease.

## Sampling And Tandem Mass Spectrometry Assay

A blood sample was collected from a peripheral vein and was immediately spotted onto a filter paper. All samples were anonymized and analyzed at Suzhou PerkinElmer Medical Laboratory, China. GAA activity was analyzed using tandem mass spectrometry (MS/MS) assay as previously reported[18, 19].

## Gene Sequencing

Next-generation sequencing was performed if the DBS activity of GAA was below 1.46  $\mu\text{mol/L/h}$ . Genomic DNA was extracted from the peripheral blood leukocytes using a genomic DNA extraction kit (Tiangen, China). Targeted next-generation sequencing (NGS) covering 603 muscular disorder related genes and subsequent Sanger confirmation was performed in 26 patients (Shanghai Amplicon-gene Bioscience Co., Ltd.). Human Gene Mutation Database was used to address the novelty of variants. Predicted severity for each known mutation was based on information from the Pompe mutation database, which is

maintained by the Erasmus Medical Center ([www.pompecenter.nl](http://www.pompecenter.nl)). The novel variants were interpreted and classified according to the American College of Medical Genetics and Genomics (ACMG) recommendation[20].

## Statistical analysis

For descriptive statistical analyses, the statistics program SPSS for Windows version 23.0 (IBM Corp., Armonk, NY) was used.

## Standard Protocol Approvals, Registrations, And Patient Consents

Oral and written informed consent was obtained from all participants before blood sampling. The study was conducted in accordance with ethical principles deriving from the Declaration of Helsinki. It was approved by the Ethics Committees of Huashan Hospital and participant centers.

## Results

The study included 492 patients aged between 1 and 86 years with a median of 42 years of participation (male: female≈1.2:1). The demographic and clinical presentation of the cohort is summarized in Table 1.

Table 1  
Summary of demographic and clinical characteristics of participants

	Patients (n=492)	
Age (years, mean±SD, range)	42±19.1, 1-86	
Sex (M:F)	264:228	
Inclusion criteria		
HyperCKemia	251 (51.0%)	isolated 66 (13.4%), 164 (33.3%) with LGMW, 3 (0.6%) with RI, 18 (3.7%) with LGMW and RI
LGMW/Axial weakness	413 (83.9%)	isolated 214 (43.5%), 167 (33.9%) with hyperCKemia, 13 (2.6%) with RI, 19 (3.9%) with LGMW and RI
Respiratory insufficiency	40 (8.1%)	isolated 5 (1.0%), 13 (2.6%) with LGMW, 3 (0.6%) with hyperCKemia, 19 (3.9%) with LGMW and hyperCKemia
LGMW, limb-girdle muscle weakness; RI, respiratory insufficiency.		

GAA activity results were within normal range in 466 patients (94.7%). DBS activity of GAA was below the cut off of 1.46  $\mu\text{mol/L/h}$  in 26 patients with a median age of 38.5 years. A total of 8 patients (1.6%) were finally diagnosed by mutational analysis as having late-onset Pompe disease. Next-generation sequencing revealed 2 heterozygous or 1 homozygous known pathogenic mutation in 7 patients, confirming the diagnosis. A novel variant, c.568C>G (p.R190G), was detected *in trans* with a known pathogenic variant in one patient (Pt1), which was classified as "pathogenic" according to ACMG guideline (PS3, PM1, PM2, PM3, PM5, PP1, PP3). An alternative diagnosis was finally established in 8 of the other 18 patients (facioscapulohumeral dystrophy [n=2], statin-induced myopathy [n=1], myofibrillar myopathy [n=1], spinal muscular atrophy type 3 [n=1], amyotrophic lateral sclerosis [n=1], peripheral neuropathy [n=1] and hyperhomocysteinemia [n=1]). (Figure 1)

The 8 patients with finally confirmed LOPD showed an average GAA activity of 0.33  $\mu\text{mol/L/h}$ . Among the rest with decreased DBS activity of GAA, 11 patients (42.3%) carried at least one pseudodeficiency allele. Individual GAA activities, genotypes, and creatine kinase levels are provided in Supplementary Table 1. The set of pseudodeficiency DBS were well separated from the LOPD samples when measured with MS/MS (Figure 2). Seven patients didn't have any GAA mutation. Since other enzymes (including acid  $\beta$ -glucocerebrosidase for Gaucher, acid  $\alpha$ -galactosidase A for Fabry, and acid  $\alpha$ -L-iduronidase for MPS-I) tested in the same multiplex assay were generally moderately low in these patients, we presumed that poor sampling or shipment might contribute to the false positivity. A second DBS test was not performed.

Among the 8 newly diagnosed patients with LOPD, 5/8 were women (Table 2). The median age at onset was 33.5±10.8 years, while the median age at diagnosis was 41.0±7.4 years. The median time from disease onset to the final diagnosis was 5 years. Five patients presented with hyperCKemia, while only 2 patient (25%) with CK above 2-fold the UNL. No individuals with isolated hyperCKemia were identified. All of the 8 patients revealed the axial muscle and/or lower limb weakness. According to percentage of predicted FVC in upright position (FVC-U) as well as blood gas test, a respiratory involvement was demonstrated in all patients with a mean FVC of 48±17% of that predicted (range 21-70%). All of them experienced exertional dyspnea, dyspnea at rest, or hypercapnia except for one patient. Six of the 8 patients required nocturnal non-invasive ventilation at the time of diagnosis. Notably, three patients had experienced acute physiological decompensation, resulting in admission to intensive care for at least once before.

Table 2  
Molecular analysis of the 8 newly diagnosed patients with LOPD

Pt	Sex	Age at onset/ Dx (y)	Dx Delay (y)	Clinical presentation			FVC (%Pred)	GAA activity (μmol/L/h)	Allele 1	Transcript	Effect (Erasmus database)	Allele 2
				CK/UNL	Muscle weakness	Respiratory insufficiency						
1	M	31/51	20	<1.5	A, LL	No complaint	70	0.18	c.568C>G	p.R160G	Pathogenic (ACMG)*	c.1082C>T
2	F	36/43	7	<1.5	A, LL	RF#, NNV	59	0.19	c.2238G>C	p.W746C	Potentially mild	c.2238G>C
3	F	43/44	1	<1.5	A	Dyspnea, NNV	39	0.19	c.2238G>C	p.W746C	Potentially mild	c.2238G>C
4	M	14/39	25	1.6	A, UL, LL	RF, NNV	44	0.5	c.953T>A	p.M318K	Potentially less severe	c.953T>A
5	F	20/33	13	3.9	A, UL, LL	RF, NNV	21	0.19	c.2238G>C	p.W746C	Potentially mild	c.2024_2026del
6	F	37/37.5	0.5	2.7	A, LL	RF#, NNV	56	0.49	c.2238G>C	p.W746C	Potentially mild	c.1799G>A
7	M	45/48	3	1.5	UL, LL	RF	ND	0.41	c.953T>A	p.M318K	Potentially less severe	c.953T>A
8	F	29/29.5	0.5	1.7	A, UL, LL	RF#, NNV	NA <sup>‡</sup>	0.46	c.2238G>C	p.W746C	Potentially mild	c.2662G>T

A, axial muscle weakness; ACMG, American College of Medical Genetics and Genomics; CK, creatine kinase; Dx, diagnosis; F, female; FVC, forced vital capacity; weakness; M, male; NA, not applicable; ND, not done; NNV, nocturnal noninvasive ventilation; RF, respiratory failure; UL, upper limb muscle weakness; UNL, upper limb muscle weakness

\* A novel GAA variant c.568C>G p.R160G is classified as a pathogenic variant based on the ACMG standard (PS3, PM1, PM2, PM3, PM5, PP1, PP3).

# With acute physiological decompensation, resulting in admission to intensive care for at least once.

<sup>‡</sup> Not applicable due to tracheotomy.

## Discussion

This is the first prospective cohort screening for late-onset Pompe disease in a high-risk population in China. In our study, the prevalence of adult Pompe disease is 1.6% in patients with unclassified axial or limb-girdle muscle weakness/ hyperCKemia/ respiratory insufficiency. This finding is slightly lower compared with previous published studies in other populations, with a combined prevalence of 2.4% (Table 3) [8–15]. Although it was reported that there is a higher prevalence of Pompe disease in Taiwan, the patients with LOPD in Taiwan often showed onset of symptoms in their second decade of life with rapid disease progression[17, 21]. In this study, since most centers involved are adult neurology departments, the prevalence of younger LOPD patients might largely be overlooked.

Table 3  
High risk screening studies in late-onset Pompe disease

Year of publication	Country	Inclusion criteria	No. of investigated patients	Patients DBS positive	Patients with confirmed Pompe	LOPD prevalence (%)	HyperCKemia (%)	LGMW/AW (%)	Respiratory insufficiency (%)
Present study	China	LGMW/AW, hyperCKemia, RI	492	26	8	1.6	5 (62.5)	8 (100)	8 (100)
2019	Poland	LGMW, hyperCKemia, RSS, dyspnea, myalgia	337	18	10	3.0	10 (100)	7 (70)	1 (10)
2018	Turkey	>18 years, undiagnosed myopathic syndrome	350	21	4	1.1	0	4 (100)	0
2017	Korea	Proximal muscle weakness, axial muscle weakness, lingual weakness, RI, hyperCKemia	90	16	2	2.2	2 (100)	2 (100)	1 (50)
2017	Portugal	LGMW, hyperCKemia±hypotonia	99	4	4	4.0	4 (100)	4 (100)	2 (50)
2016	Germany and UK	LGMW, unexplained hyperCKemia	3076	232	74	2.4	74 (100)	63 (85.1)	47 (63.7)
2015	Spain	>18 years, LGMW, asymptomatic or pauci-symptomatic hyperCKemia	348	20	16	4.6	12 (75)	14 (87.5)	10 (62.5)
2015	Italy	>5 years, hyperCKemia, LGMW	1051	30	17	1.6	15 (94)	11 (65.05)	14 (82)
2014	Denmark	Unclassified LGMD, hyperCKaemia, unexplained myopathy on muscle biopsy, unexplained RI, unspecified myopathy	103	3	3	2.9	3 (100)	3 (100)	0

AW, axial weakness; LGMW, limb-girdle muscle weakness; RI, respiratory insufficiency; RSS, rigid spine syndrome; UNL, upper normal limit

The efficacy of DBS has been described in newborn screening and other studies in Pompe disease[8–15]. Deficient DBS GAA activity can be caused by incorrect DBS spotting and sampling, and environmental circumstances such a high temperature during transport. In the Asian population, the high prevalence of the pseudodeficiency allele, which lowers GAA activity to near the Pompe disease range in normal individuals, complicated the screening for Pompe disease[10, 21–23]. The pseudodeficiency variant p.[G576S; E689K] (c.[1726G>A; 2065G>A]) has been identified at high frequency in the Japanese and Chinese populations[24]. Since newborn screening study in Taiwan indicated that mass spectrometry distinguished affected and pseudodeficiency patients well[21], we chose GAA sequencing as the second test for confirmation of the diagnosis. In this study 11 of the 18 false positive samples carried at least one pseudodeficiency allele. In previous Asian studies, pseudodeficiency heterozygote/homozygote was reported to complicate Pompe disease screening[10, 21, 22]. However, mass spectrometry could help distinguish between patients with LOPD and carriers of pseudodeficiency.

In our cohort, axial/proximal limb muscle weakness remained as the core feature of LOPD. However, the serum CK levels in the newly diagnosed LOPD patients were generally low. Five out of 8 patients presented with a CK level above 1.5-fold the UNL, and only 2 patients above 2-fold the UNL. None of the patients with “asymptomatic hyperCKemia” proved to have LOPD. In contrast, in the Spanish cohort, CK was elevated, in a variable manner, in 13 of the 16 patients, with values between 2 and 8 times the upper limit of normal (UNL) [25]. In the Italian study, 5/17 (29.4%) were identified as presymptomatic LOPD patients with isolated hyperCKemia[14]. Another Spanish study revealed 2.2% prevalence of LOPD in isolated hyperCKemia[26]. Caucasian patients with LOPD have a higher prevalence of c.-32–13 T > G (around 40%). Moreover, all those presymptomatic patient were heterozygous for the common c.-32–13T>G mutation[14, 26], which is at a very low frequency in Chinese LOPD cohort[16]. This difference of mutational spectrum may explain the lower CK level observed in Chinese patients.

In LOPD, as respiratory dysfunction progresses patients develop sleep disordered breathing which progresses to nocturnal hypoventilation, and eventually development of diurnal respiratory failure. The addition of ventilation use is a significant event in disease progression, negatively affecting quality of life. The respiratory involvement in the 8 newly diagnosed patients is earlier and more severe compared to previous high risk screening studies[8, 12, 14]. One patient (Pt.3) initially manifested with exertional dyspnea with preserved limb-girdle muscle strength and biceps muscle biopsy showed no vacuolar pathology. This was in accordance with a recent report that, compared with patients in other parts of the world, the lung function of Chinese patients with LOPD were worse, manifested by lower forced vital capacity and maximum expiratory and inspiratory pressure[7]. Generally, the predictive threshold of FVC-U for nighttime ventilation was 39% of predicted[27]. However, hypercapnia could happen in patients with larger ventilatory reserve, as Pt 2 and 6 in our study. It may involve both the presence of sleep-disordered breathing and blunted ventilatory responsiveness[27, 28]. Monitoring of respiration and/or gas exchange during sleep is especially of importance for the onset of sleep disordered breathing can be easily missed.

In conclusion, we recommend that DBS and tandem mass spectrometry test be undertaken early in the diagnosis workup of high-risk patients to exclude LOPD. Extension to patients with sleep disordered breathing rather than isolated hyperCKemia is recommended in Chinese population. Such approach will

facilitate early diagnosis and allows to start the treatment at less advanced stages of the disease.

## Abbreviations

LOPD, Late-onset Pompe Disease; UNL, upper limit of normal; DBS: dried blood spot; GAA $\alpha$ -1,4-glucosidase $\mu$ MS/MS $\mu$ tandem mass spectrometry

## Declarations

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### Author Contributions

All authors contributed to the development of the project and data collection and management. WZ and QF devised the study. WZ, JD and KJ analyzed the data, designed graphic illustrations and wrote the first draft of the manuscript. WZ and JD revised the manuscript. WZ oversaw the general direction of the article and critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

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### Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

### Ethical publication statement and consent to participate

This study was approved by Medical Ethics Committee of Huashan Hospital, Shanghai Medical College, Fudan University (approval no. KY2021-537).

### Consent for publication

Not applicable.

### Competing interest:

The authors declare that they have no competing interests.

### Disclosure

Dr. Zhu has received honoraria and travel funding from Genzyme, a Sanofi company, during the past 5 years.

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## Figures

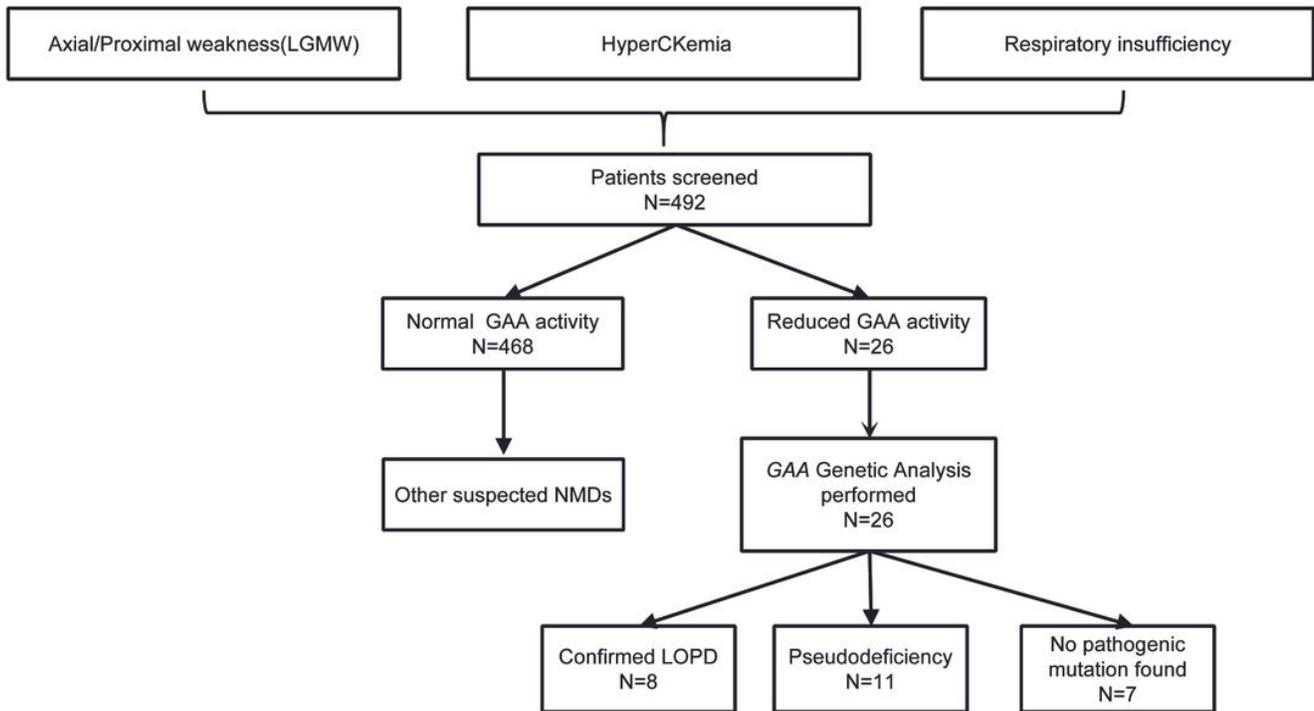
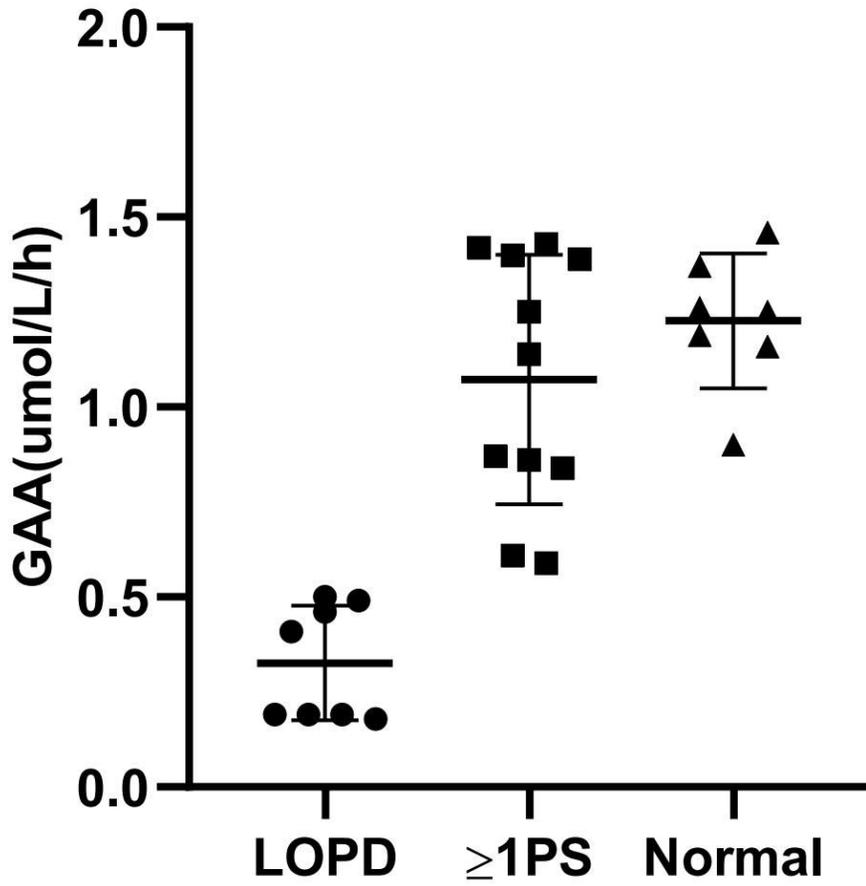


Figure 1

A flow diagram of the study design and analysis process.



**Figure 2**  
 Correlation between GAA activity and genotype. LOPD: Late-onset Pompe Disease; PS: pseudodeficiency with or without GAA mutations; Normal: normal controls.

**Supplementary Files**

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- [SupplementaryTable1zwh210929.xlsx](#)