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Update of the Pompe variant database for the prediction of clinical phenotypes: Novel disease-associated variants, common sequence variants, and results from newborn screening

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Abstract

Pompe disease is an inherited disorder caused by disease-associated variants in the acid α-glucosidase gene (*GAA*). The Pompe disease *GAA* variant database (http://www.pompevariantdatabase.nl) is a curated, open-source, disease-specific database, and lists disease-associated *GAA* variants, in silico predictions, and clinical phenotypes reported until 2016. Here, we provide an update to include 226 disease-associated variants that were published until 2020. We also listed 148 common *GAA* sequence variants that do not cause Pompe disease. *GAA* variants with unknown severity that were identified only in newborn screening programs were listed as a new feature to indicate the reason why phenotypes were still unknown. Expression studies were performed for common missense variants to predict their severity. The updated Pompe disease *GAA* variant database now includes 648 disease-associated variants, 26 variants from newborn screening, and 237 variants with unknown severity. Regular updates of the Pompe disease *GAA* variant database will be required to improve genetic counseling and the study of genotype–phenotype relationships.

KEYWORDS

database, disease-associated variants, *GAA*, NBS, Pompe disease, SNP

1 | INTRODUCTION

Pompe disease (glycogen storage disease type II; MIM #232300) is an autosomal recessive disorder caused by disease-associated variants in the acid α-glucosidase (*GAA*) gene, resulting in a deficiency of the GAA enzyme, accumulation of lysosomal glycogen, and progressive muscle weakness. The clinical spectrum of Pompe disease is broad (Güngör & Reuser, 2013). The most severe classic infantile phenotype presents shortly after birth with hypertrophic cardiomyopathy and generalized muscle weakness. These patients die in the first year of life due to cardiorespiratory insufficiency if left untreated. The slower progressing phenotype is characterized by

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muscle weakness that can appear at any age from <1 year into adulthood. These patients are generally spared from cardiac symptoms (Kohler et al., 2018; van der Ploeg & Reuser, 2008). Enzyme replacement therapy (ERT) with intravenously applied recombinant human GAA is available since 2006. ERT normalizes hypertrophic cardiomyopathy, improves motor function, and extends survival.

The differences between phenotypes in Pompe disease can, in part, be attributed to the severity of the disease-associated variants present in the *GAA* gene. Classic infantile patients carry two disease-associated variants that completely disrupt the function of GAA (i.e., null alleles). This group of patients can be subdivided based on their cross-reactive immunological material (CRIM) status, which is defined by the disease-associated variants involved. When two *GAA* variants are present that do not result in GAA protein expression, the patient is classified as CRIM-negative. When at least one *GAA* variant gives rise to GAA protein expression (in which the GAA protein can be enzymatically inactive), the patient is classified as CRIM-positive. The clinical importance of CRIM status is highlighted by the fact that CRIM-negative classic infantile patients have a poorer prognosis compared with CRIM-positive classic infantile patients, possibly due to the formation of high sustained anti-GAA antibody titers upon treatment with ERT (Bali et al., 2012; van Gelder et al., 2015). Patients who do not have the classic infantile phenotype carry at least one disease-associated variant that allows some residual enzymatic activity. These patients are, by definition, CRIM-positive (Kroos et al., 2012b; Kulessa et al., 2020).

The "Pompe disease *GAA* variant database" (http://www.pompevariantdatabase.nl) is an open-access database that lists and classifies all reported variants in the *GAA* gene. We recently revised this database to include clinical data from patients collected from the literature, adapted the classification system for variant severity, and added (predicted) CRIM status for disease-associated variants. The database included literature up to May 2016, resulting in a total of 561 variants (Niño et al., 2019). In recent years, many new patients and *GAA* variants have been reported. These include findings from large patient populations, such as the French nationwide study (246 patients with late-onset Pompe disease) and the Pompe registry (1079 patients from 26 countries; Reuser et al., 2019; Semplicini et al., 2018).

In addition, various countries, including Taiwan, the United States, Italy, Brazil, and Japan, have implemented newborn screening (NBS) programs for Pompe disease, resulting in an increase of variants of unknown significance (VUS; Bravo et al., 2017; Burlina et al., 2018; Chien et al., 2019; Elliott et al., 2016; Momosaki et al., 2019; Yang et al., 2014). For variants associated with late onset, the associated phenotypes from NBS cases are still unknown as symptom onset could, in principle, be delayed until (late) adulthood. It will be important to monitor the onset and progress of symptoms in patients identified via NBS programs closely to determine the severity of the newly identified genetic variants.

Public databases, such as dbSNP (https://www.ncbi.nlm.nih.gov/snp) and gnomAD (https://gnomad.broadinstitute.org), provide a source of variants that have been detected in various genome-wide studies (Karczewski et al., 2020; Sherry et al., 2001). A large percentage of these variants represent common sequence variants that have a minor allele frequency (MAF) ≥ 1%. Several of these variants have already been reported for the *GAA* gene and have been ruled out to cause Pompe disease (Kroos et al., 2007; Labrousse et al., 2010; Turaça et al., 2015). However, most of the common sequence variants in these databases are listed as VUSs and may lead to misinterpretation during molecular diagnostics.

In this study, we provide an update of the Pompe disease *GAA* variant database with variants and patients described in the literature up to January 2020. We included information on novel *GAA* variants that were identified via NBS and for which no phenotype was yet known. Known common sequence variants in the *GAA* gene that do not cause Pompe disease have now also been added to prevent misdiagnosis. In addition, selected common missense variants were tested in expression studies and also this information was added to the updated database. The database provides a curated up-to-date reference source for the molecular diagnosis of Pompe disease.

2 | METHODS

The Pompe disease *GAA* variant database is publicly available at http://www.pompevariantdatabase.nl. The previous version of the database included literature until 2016; the update described here contains variants from publications up to January 2020. Additionally, NBS studies that screened for Pompe disease were now included if the authors provided the genotypes of the described cases. Novel variants were analyzed as described in Niño et al. (2019). Variants were annotated based on the reference sequences NM_000152.3 for *GAA* messenger RNA (mRNA), LRG_673 genomic sequence for describing variants in intronic sequences, and NP_000143.2 for GAA protein. Exon annotations were based on the human genomic build (GRCH37/hg19) for exons 2–20; however, changes were made to the annotation of exon 1 to reflect the findings of (GRCH38/hg38). Within this region, a new 195-bp intron was identified at positions c.−112 and c.−113. Therefore, the region that was previously annotated as exon 1 has been split between exons 1A and 1B, which are separated by intron 1A. Intron 1 has been renamed to intron 1B. This numbering was made to maintain the same numbering of subsequent exons compared with existing literature.

Common sequence variants in the *GAA* gene (hg38 Chr17:80,101,556-80,119,881) were extracted from gnomAD and were categorized as "not disease-associated." Combined Annotation-Dependent Depletion (CADD) in silico predictions were performed using the CADD (https://cadd.gs.washington.edu) platform, which compiles different tools for analysis of intronic insertion and deletion variants (Rentzsch et al., 2019). The MAF and CADD scores were obtained in April 2020. Predictions of effect on pre-mRNA splicing were performed using Alamut Visual v.2.15 (Interactive Biosoftware).

Functional studies were performed using site-directed mutagenesis (SDM) to generate complementary DNA (cDNA) expression

TABLE 1 Novel disease-associated variants added to the Pompe variant database

DNA nomenclature	Phenotype combined with a null allele	DNA nomenclature	Phenotype combined with a null allele
Ch37/hg19 chr17:78,059,821_78,076,592del	Unknown (disease-associated)	c.1057C>T	Unknown (disease-associated)
c.−113+2T>A	Unknown (disease-associated)	c.1057del	Unknown (disease-associated)
c.−32-17_−32-10delins(30)	Classic infantile	c.1099T>G	Unknown (disease-associated)
c.−32-1G>C	Unknown (disease-associated)	c.1106T>A	Unknown (disease-associated)
c.40_47del	Classic infantile	c.1109G>A	Unknown (disease-associated)
c.104T>C	Classic infantile	c.1114C>G	Unknown (disease-associated)
c.169C>T	Classic infantile	c.1114C>T	Unknown (disease-associated)
c.205C>T	Unknown (disease-associated)	c.1121G>A	Unknown (disease-associated)
c.258C>A	Unknown (disease-associated)	c.1127_1130del	Unknown (disease-associated)
c.265C>T	Unknown (disease-associated)	c.1129G>A	Unknown (disease-associated)
c.295_314del	Unknown (disease-associated)	c.1153del	Unknown (disease-associated)
c.323G>C	Unknown (disease-associated)	c.1192del	Unknown (disease-associated)
c.365del	Unknown (disease-associated)	c.1193del	Unknown (disease-associated)
c.380G>A	Unknown (disease-associated)	c.1201C>A	Unknown (disease-associated)
c.397T>G	Unknown (disease-associated)	c.1209C>A	Unknown (disease-associated)
c.437del	Classic infantile	c.1211A>C	Unknown (disease-associated)
c.445A>C	Unknown (disease-associated)	c.1211A>T	Classic infantile
c.484A>C	Classic infantile	c.1212C>G	Unknown (disease-associated)
c.502C>T	Unknown (disease-associated)	c.1216G>A	Childhood
c.505C>A	Unknown (disease-associated)	c.1219T>C	Unknown (disease-associated)
c.517_519del	Childhood	c.1221C>A	Classic infantile
c.541_545del	Classic infantile	c.1221del	Unknown (disease-associated)
c.547-1G>C	Unknown (disease-associated)	c.1226_1227insG	Classic infantile
c.568C>T	Unknown (disease-associated)	c.1231del	Unknown (disease-associated)
c.665T>G	Classic infantile	c.1240T>C	Unknown (disease-associated)
c.686G>C	Unknown (disease-associated)	c.1241del	Classic infantile
c.691C>T	Unknown (disease-associated)	c.1242C>A	Unknown (disease-associated)
c.692T>C	Unknown (disease-associated)	c.1249A>C	Unknown (disease-associated)
c.692+1G>T	Unknown (disease-associated)	c.1281G>T	Classic infantile
c.693-2A>C	Classic infantile	c.1292_1295dup	Classic infantile
c.693-1G>C	Unknown (disease-associated)	c.1293_1326+57del	Unknown (disease-associated)
c.715_716del	Unknown (disease-associated)	c.1298A>C	Classic infantile
c.730C>T	Classic infantile	c.1311_1312ins(26)	Classic infantile
c.736del	Unknown (disease-associated)	c.1320_1322del	Classic infantile
c.756_757insT	Unknown (disease-associated)	c.1327-54_1437+178del	Classic infantile
c.759del	Unknown (disease-associated)	c.1358_1361del	Classic infantile
c.766_784del	Unknown (disease-associated)	c.1378G>T	Unknown (disease-associated)
c.781G>A	Classic infantile	c.1388_1406del	Unknown (disease-associated)
c.784G>C	Unknown (disease-associated)	c.1396dup	Unknown (disease-associated)

(Continues)

TABLE 1 (Continued)

DNA nomenclature	Phenotype combined with a null allele	DNA nomenclature	Phenotype combined with a null allele
c.796C>A	Childhood	c.1402A>T	Unknown (disease-associated)
c.799_803delinsA	Unknown (disease-associated)	c.1409A>G	Unknown (disease-associated)
c.837G>C	Unknown (disease-associated)	c.1431del	Classic infantile
c.841C>T	Unknown (disease-associated)	c.1441del	Unknown (disease-associated)
c.876C>G	Classic infantile	c.1447G>T	Unknown (disease-associated)
c.878G>T	Unknown (disease-associated)	c.1456G>T	Unknown (disease-associated)
c.883C>A	Unknown (disease-associated)	c.1464dup	Classic infantile
c.930_932del	Classic infantile	c.1470C>A	Childhood
c.942C>A	Unknown (disease-associated)	c.1477C>T	Unknown (disease-associated)
c.947A>G	Classic infantile	c.1493G>A	Classic infantile
c.950C>T	Unknown (disease-associated)	c.1501_1515del	Unknown (disease-associated)
c.955+1G>A	Classic infantile	c.1507del	Classic infantile
c.971dup	Classic infantile	c.1526A>T	Unknown (disease-associated)
c.982_988del	Classic infantile	c.1531C>A	Unknown (disease-associated)
c.983T>C	Classic infantile	c.1537G>A	Unknown (disease-associated)
c.994_995insTT	Unknown (disease-associated)	c.1538A>G	Classic infantile
c.1000G>T	Classic infantile	c.1551+3A>T	Unknown (disease-associated)
c.1004_1005dup	Unknown (disease-associated)	c.1551+5G>A	Unknown (disease-associated)
c.1047del	Unknown (disease-associated)	c.1559A>G	Unknown (disease-associated)
c.1560C>G	Unknown (disease-associated)	c.2096T>C	Unknown (disease-associated)
c.1579_1580del	Classic infantile	c.2109del	Unknown (disease-associated)
c.1583G>C	Unknown (disease-associated)	c.2131A>C	Classic infantile
c.1594G>A	Adult	c.2146G>C	Unknown (disease-associated)
c.1597T>G	Classic infantile	c.2153_2156delinsACGCCG	Classic infantile
c.1602_1605delinsAGG	Classic infantile	c.2182_2183del	Unknown (disease-associated)
c.1610del	Unknown (disease-associated)	c.2190-345A>G	Unknown (disease-associated)
c.1627T>G	Unknown (disease-associated)	c.2205dup	Classic infantile
c.1629C>G	Unknown (disease-associated)	c.2213G>A	Classic infantile
c.1636G>C	Unknown (disease-associated)	c.2221G>A	Classic infantile
c.1636+5G>A	Classic infantile	c.2222A>T	Unknown (disease-associated)
c.1650del	Unknown (disease-associated)	c.2234T>C	Classic infantile
c.1657C>T	Classic infantile	c.2235dup	Classic infantile
c.1681_1699dup	Unknown (disease-associated)	c.2237G>T	Unknown (disease-associated)
c.1688A>T	Unknown (disease-associated)	c.2240G>A	Unknown (disease-associated)
c.1716C>A	Classic infantile	c.2261dup	Unknown (disease-associated)
c.1721T>C	Unknown (disease-associated)	c.2294G>A	Classic infantile
c.1753_2799del	Classic infantile	c.2296T>A	Classic infantile
c.1754+1dup	Unknown (disease-associated)	c.2297A>C	Classic infantile
c.1754+2T>C	Unknown (disease-associated)	c.2304del	Unknown (disease-associated)
c.1780C>T	Unknown (disease-associated)	c.2320G>A	Unknown (disease-associated)

TABLE 1 (Continued)

DNA nomenclature	Phenotype combined with a null allele	DNA nomenclature	Phenotype combined with a null allele
c.1784C>T	Unknown (disease-associated)	c.2331+5G>C	Classic infantile
c.1799G>C	Unknown (disease-associated)	c.2331+102del	Unknown (disease-associated)
c.1822del	Unknown (disease-associated)	c.2334_2335dup	Unknown (disease-associated)
c.1825T>G	Unknown (disease-associated)	c.2377_2378insAC	Classic infantile
c.1835A>C	Unknown (disease-associated)	c.2380dup	Unknown (disease-associated)
c.1835A>G	Unknown (disease-associated)	c.2395C>T	Unknown (disease-associated)
c.1837T>G	Unknown (disease-associated)	c.2407C>T	Unknown (disease-associated)
c.1839G>C	Unknown (disease-associated)	c.2411G>A	Classic infantile
c.1844_1846del	Unknown (disease-associated)	c.2459_2461del	Unknown (disease-associated)
c.1844G>T	Classic infantile	c.2460dup	Unknown (disease-associated)
c.1844G>A	Classic infantile	c.2474C>G	Unknown (disease-associated)
c.1847dup	Unknown (disease-associated)	c.2480A>G	Unknown (disease-associated)
c.1859C>A	Unknown (disease-associated)	c.2515C>T	Unknown (disease-associated)
c.1879_1881del	Classic infantile	c.2537C>A	Unknown (disease-associated)
c.1888+2_1888+15del	Classic infantile	c.2544del	Unknown (disease-associated)
c.1895T>C	Unknown (disease-associated)	c.2563G>C	Classic infantile
c.1895T>G	Classic infantile	c.2578G>A	Unknown (disease-associated)
c.1903A>G	Unknown (disease-associated)	c.2584G>A	Childhood
c.1913G>A	Classic infantile	c.2585del	Classic infantile
c.1944_1950del	Unknown (disease-associated)	c.2596del	Unknown (disease-associated)
c.1952dup	Unknown (disease-associated)	c.2619C>G	Unknown (disease-associated)
c.1961C>G	Unknown (disease-associated)	c.2636T>C	Classic infantile
c.2004C>A	Unknown (disease-associated)	c.2655_2656del	Unknown (disease-associated)
c.2015G>T	Unknown (disease-associated)	c.2716G>A	Unknown (disease-associated)
c.2020C>G	Unknown (disease-associated)	c.2720T>C	Unknown (disease-associated)
c.2020C>T	Unknown (disease-associated)	c.2725G>A	Unknown (disease-associated)
c.2024A>G	Classic infantile	c.2740dup	Unknown (disease-associated)
c.2040+2dup	Unknown (disease-associated)	c.2742dup	Classic infantile
c.2040+29_2190-270del	Classic infantile	c.2757del	Unknown (disease-associated)
c.2041-2A>G	Classic infantile	c.2799+5G>A	Unknown (disease-associated)
c.2051C>A	Unknown (disease-associated)	c.2800-1G>C	Classic infantile
c.2051C>G	Unknown (disease-associated)	c.2843dup	Classic infantile
c.2051C>T	Classic infantile	c.2845_2847del	Unknown (disease-associated)
c.2056_2057delinsCC	Unknown (disease-associated)		
c.2084dup	Unknown (disease-associated)		

constructs containing the missense variant of interest as described (in 't Groen et al., 2020). The activity of the GAA protein produced by the constructs was measured using 4-methylumbelliferyl-α-D-glucopyranoside (4-MU) as a substrate in transfected COS-7 cells, as described in Kroos et al. (2008). Statistical analysis was performed using one-way analysis of variance with Tukey honestly significant difference post hoc multiple testing corrections. $p < .05$ was considered significant.

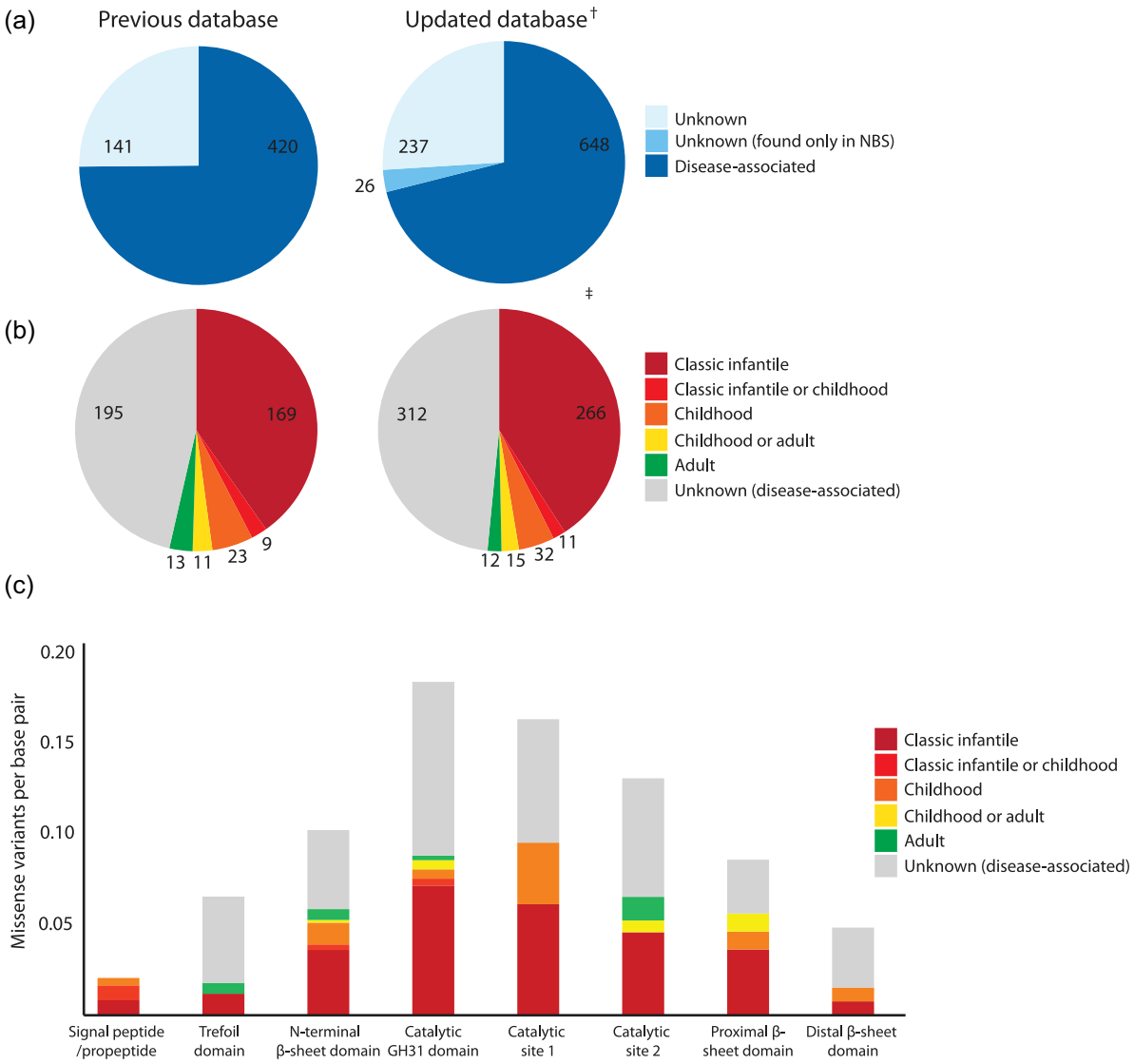

FIGURE 1 Overview of variants, comparing the previous (Niño et al., 2019) and updated version of the Pompe disease GAA variant database (http://www.pompevariantdatabase.nl). (a) Number of disease-associated and unknown variants in the previous database (left) and the updated version of the database (right). (b) Number of disease-associated variants classified based on the predicted clinical phenotype when combined with a null allele in the previous database (left) and in the updated version of the database (right). (c) Distribution of disease-associated missense variants listed in the updated database, based on the protein domains of GAA and the predicted clinical phenotype when combined with a null allele. Numbers are corrected for the length of each domain. †Two entries in the previous version of the database were removed as the variants were described twice using different nomenclatures. ‡For 36 variants listed in the previous version of the database, a reclassification of the phenotypic severity was performed due to the addition of novel patients included in this update

3 | RESULTS AND DISCUSSION

Table 1 provides an overview of the novel variants. We performed a literature search covering the past 4 years and identified 80 publications (listed in the updated database and Table S1) that described 350 novel variants, of which 226 were considered to be disease-associated (Table 1 and Figure 1a). Seventy-six novel variants (33%) were present in combination with a null allele, which allowed prediction of the clinical severity of these variants (Table 1 and Figure 1b). In addition, the inclusion of new patient information allowed us to classify the severity of 55 variants that were already present in the database. This resulted in a new total of 911 *GAA* variants, of which 648 were disease-associated (71%). In total, 336 out of 648 disease-associated variants (52%) could be associated with a clinical phenotype. The geographical or ethnical distribution of reported patients remained similar to what was described previously. The majority of patients had a Caucasian background or were of Caucasian descent (data not shown). This introduces a bias in the current version of the database and indicates the necessity of extending the database to patients of other descent. Mapping of missense variants to GAA protein domains revealed an even

TABLE 2 List of common sequence variants located within the boundaries of the *GAA* gene

Location	Variant	Variant ID	Global allele frequency (GnomAD)	Predictions of pre-mRNA splicing	CADD score PHRED
Exon 1A, 5′ UTR	c.−338C>G	rs144639114	2%	No effect on splicing	6.524
Exon 1A, 5′ UTR	c.−260G>C	rs2304849	16%	No effect on splicing	8.996
Exon 1A, 5′ UTR	c.−178G>A	rs77514632	2%	No effect on splicing	9.948
Exon 1B, 5′ UTR	c.−75C>G	rs80020206	0.9% (3% in African population)	No effect on splicing	9.989
Intron 1B	c.−33+219G>C	rs4889961	75%	No effect on splicing	0.866
Intron 1B	c.−33+316C>A	rs8077055	20%	No effect on splicing	9.079
Intron 1B	c.−33+317C>T	rs8077056	20%	No effect on splicing	8.579
Intron 1B	c.−33+671A>C	rs55751636	31%	No effect on splicing	1.456
Intron 1B	c.−33+757G>A	rs28413147	5%	No effect on splicing	4.974
Intron 1B	c.−33+903A>C	rs12450199	34%	No effect on splicing	8.196
Intron 1B	c.−33+1104A>G	rs11150841	75%	No effect on splicing	6.976
Intron 1B	c.−33+1172G>A	rs1442315	5%	No effect on splicing	0.064
Intron 1B	c.−33+1190G>T	rs12602593	10%	No effect on splicing	1.784
Intron 1B	c.−33+1309T>C	rs1442314	76%	No effect on splicing	1.752
Intron 1B	c.−32-1298G>C	rs12602610	33%	No effect on splicing	2.604
Intron 1B	c.−32-1124C>T	rs58959690	20%	No effect on splicing	5.825
Intron 1B	c.−32-884T>C	rs145362066	0.9% (3% in African population)	No effect on splicing	3.993
Intron 1B	c.−32-793C>G	rs55666739	2%	No effect on splicing	4.041
Intron 1B	c.−32-721G>C	rs75754966	2%	Generates a new cryptic splice accepter site	1.008
Intron 1B	c.−32-686A>G	rs147264695	0.3% (1% in Finnish population)	No effect on splicing	4.349
Intron 1B	c.−32-640C>T	rs12600845	51%	No effect on splicing	0.136
Intron 1B	c.−32-521G>T	rs115060925	1%	Generates a new cryptic splice donor site	0.639
Intron 1B	c.−32-494C>G	rs140325572	2%	No effect on splicing	0.036
Intron 1B	c.−32-462G>A	rs74003606	5%	No effect on splicing	0.226
Exon 2	c.271G>A	rs1800299	2%	No effect on splicing	0.256
Exon 2	c.324T>C	rs1800300	72%	No effect on splicing	8.391
Exon 2	c.447G>A	rs2289536	0.5% (3% in East Asian population)	No effect on splicing	1.252
Intron 2	c.546+293G>A	rs34746710	20%	No effect on splicing	1.899
Intron 2	c.547-243C>G	rs8065426	67%	No effect on splicing	2.529
Intron 2	c.547-238T>C	rs12452263	20%	No effect on splicing	5.667
Intron 2	c.547-67C>G	rs8069491	67%	No effect on splicing	1.337
Intron 2	c.547-39T>G	rs12452721	67%	Loss of cryptic splice donor site	2.78
Intron 2	c.547-4C>G	rs3816256	67%	No effect on splicing	4.721
Exon 3	c.596A>G	rs1042393	67%	No effect on splicing	0.548
Exon 3	c.642C>T	rs1800301	18%	No effect on splicing	1.805
Exon 3	c.668G>A	rs1042395	67%	No effect on splicing	1.46
Intron 3	c.692+38C>T	rs2304848	3%		5.574

(Continues)

TABLE 2 (Continued)

Location	Variant	Variant ID	Global allele frequency (GnomAD)	Predictions of pre-mRNA splicing	CADD score PHRED
				Generates a new cryptic splice donor site	
Intron 3	c.692+144A>G	rs2304847	67%	No effect on splicing	3.653
Intron 3	c.692+509T>C	rs8082405	66%	No effect on splicing	3.271
Intron 3	c.692+674G>C	rs8078350	67%	No effect on splicing	4.501
Intron 3	c.692+751T>C	rs8068051	67%	No effect on splicing	2.363
Intron 3	c.693-586G>A	rs112308142	3%	No effect on splicing	2.71
Intron 3	c.693-585T>C	rs8068555	67%	No effect on splicing	4.133
Intron 3	c.693-559C>T	rs12602422	67%	No effect on splicing	1.879
Intron 3	c.693-491G>A	rs12948631	67%	No effect on splicing	3.629
Intron 3	c.693-441C>G	rs12602440	67%	Loss of a cryptic splice acceptor site	7.559
Intron 3	c.693-434C>A	rs12941269	66%	No effect on splicing	4.416
Intron 3	c.693-414C>G	rs12941289	66%	Loss of a cryptic splice acceptor site	0.077
Intron 3	c.693-413A>G	rs12937590	67%	Loss of a cryptic splice acceptor site	1.544
Intron 3	c.693-216T>A	rs11150844	67%	No effect on splicing	4.13
Intron 3	c.693-94C>T	rs79849256	0.2% (3% in East Asian population)	No effect on splicing	9.666
Intron 3	c.693-78C>T	rs74003611	6%	No effect on splicing	0.06
Intron 3	c.693-49C>T	rs78855075	7%	No effect on splicing	2.374
Exon 4	c.852G>A	rs142626724	0.6% (1% in European population)	No effect on splicing	1.095
Intron 4	c.858+30T>C	rs2304845	66%	No effect on splicing	0.067
Exon 5	c.921A>T	rs1800303	8%	No effect on splicing	9.101
Intron 5	c.955+12G>A	rs2252455	69%	No effect on splicing	0.981
Intron 5	c.955+155C>A	rs9901190	5%	No effect on splicing	7.196
Intron 5	c.955+167C>T	rs77717164	0.7% (6% in East Asian population)	No effect on splicing	6.348
Intron 5	c.956-107G>A	rs2241888	73%	No effect on splicing	5.835
Intron 5	c.956-84C>T	rs2241887	67%	No effect on splicing	0.061
Intron 6	c.1075+13C>T	rs41292402	1%	No effect on splicing	7.496
Exon 8	c.1203G>A	rs1800304	67%	No effect on splicing	5.972
Exon 8	c.1286A>G	rs200294882	0.07% (1% in East Asian population)	Loss of cryptic splice acceptor site and generates a new cryptic splice donor site	0.068
Intron 8	c.1326+132G>A	rs894306	67%	No effect on splicing	1.999
Intron 8	c.1326+459C>T	rs74679377	0.7% (6% in East Asian population)	No effect on splicing	0.435
Intron 8	c.1326+460G>A	rs12150323	2%	No effect on splicing	0.322
Intron 8	c.1327-514G>A	rs72850826	5%	No effect on splicing	1.914
Intron 8	c.1327-356G>T	rs6565640	73%	No effect on splicing	0.258

TABLE 2 (Continued)

Location	Variant	Variant ID	Global allele frequency (GnomAD)	Predictions of pre-mRNA splicing	CADD score PHRED
Intron 8	c.1327-321del	rs140385114	7%	No effect on splicing	0.888
Intron 8	c.1327-269A>G	rs6565641	67%	No effect on splicing	4.207
Intron 8	c.1327-209C>T	rs76604157	0.3% (6% in East Asian population)	No effect on splicing	0.471
Intron 8	c.1327-179G>A	rs2278620	20%	No effect on splicing	0.643
Intron 8	c.1327-118A>G	rs74003628	7%	No effect on splicing	0.184
Intron 8	c.1327-18A>G	rs2278619	72%	No effect on splicing	0.124
Exon 9	c.1374C>T	rs1800305	7%	No effect on splicing	0.206
Intron 9	c.1438-220A>G	rs2278618	67%	No effect on splicing	6.607
Intron 9	c.1438-108G>A	rs12944802	67%	No effect on splicing	0.013
Intron 9	c.1438-19G>C	rs2304844	67%	No effect on splicing	3.529
Intron 10	c.1551+42G>A	rs115427918	0.9% (3% in African population)	No effect on splicing	5.792
Intron 10	c.1551+49C>A	rs2304843	67%	No effect on splicing	7.131
Exon 11	c.1581G>A	rs1042396	23%	No effect on splicing	6.758
Intron 11	c.1636+43G>T	rs2304842	5%	Generates a new cryptic splice accepter site	6.859
Intron 11	c.1636+117del	rs199788201	59%	No effect on splicing	0.045
Intron 11	c.1636+117C>T	rs12945868	11%	No effect on splicing	0.181
Intron 11	c.1636+118G>T	rs4889817	59%	No effect on splicing	3.161
Intron 11	c.1636+205C>T	rs79673008	3%	No effect on splicing	0.013
Intron 11	c.1636+210G>A	rs79487884	5%	No effect on splicing	1.463
Intron 11	c.1636+269C>T	rs111625854	2%	No effect on splicing	3.828
Intron 11	c.1636+284G>C	rs111551014	2%	No effect on splicing	1.81
Intron 11	c.1636+389C>G	rs7221675	63%	No effect on splicing	0.573
Intron 11	c.1636+390A>G	rs7209921	63%	No effect on splicing	1.829
Intron 11	c.1636+404A>G	rs4889818	74%	No effect on splicing	1.902
Intron 11	c.1637-185A>G	rs12951255	55%	No effect on splicing	0.576
Exon 12	c.1726G>A	rs1800307	2%	Generates a new cryptic splice acceptor	0.268
Intron 12	c.1754+12G>A	rs2304840	6%	No effect on splicing	4.325
Intron 12	c.1754+100C>T	rs113688685	0.9% (3% in African population)	No effect on splicing	8.142
Intron 12	c.1754+104C>G	rs2304839	5%	No effect on splicing	0.763
Intron 12	c.1754+144C>T	rs2304838	61%	No effect on splicing	1.787
Intron 12	c.1755-186A>G	rs62075593	2%	No effect on splicing	2.032
Intron 13	c.1888+21G>A	rs2304837	6%	No effect on splicing	3.378
Intron 14	c.2040+20A>G	rs2304836	72%	No effect on splicing	2.163
Intron 14	c.2040+66C>T	rs2304835	7%	No effect on splicing	3.54
Intron 14	c.2040+69A>G	rs2304834	6%	No effect on splicing	0.027
Intron 14	c.2041-64G>A	rs2304833	27%	No effect on splicing	0.371
Exon 15	c.2065G>A	rs1800309	6%	No effect on splicing	1.783

(Continues)

TABLE 2 (Continued)

Location	Variant	Variant ID	Global allele frequency (GnomAD)	Predictions of pre-mRNA splicing	CADD score PHRED
Exon 15	c.2133A>G	rs1800310	27%	No effect on splicing	1.134
Intron 15	c.2189+95C>T	rs72850840	5%	No effect on splicing	3,771
Intron 15	c.2189+263G>A	rs7221604	66%	Generates a new cryptic splice donor site	0.563
Intron 15	c.2189+510T>G	rs4889963	5%	No effect on splicing	1.444
Intron 15	c.2189+607G>A	rs112710614	7%	No effect on splicing	0.189
Intron 15	c.2189+616T>C	rs139307163	5%	No effect on splicing	1.94
Intron 15	c.2189+723G>A	rs4889819	20%	No effect on splicing	0.367
Intron 15	c.2189+729A>G	rs74737410	5%	No effect on splicing	0.498
Intron 15	c.2189+859A>G	rs4889964	5%	No effect on splicing	1.503
Intron 15	c.2189+884G>A	rs4889965	5%	No effect on splicing	0.355
Intron 15	c.2189+1153A>G	rs72850844	5%	No effect on splicing	3.687
Intron 15	c.2189+1201C>A	rs72850846	5%	No effect on splicing	2.352
Intron 15	c.2189+1208A>G	rs72850847	5%	No effect on splicing	0.367
Intron 15	c.2189+1263A>G	rs74700450	5%	No effect on splicing	2.97
Intron 15	c.2189+1290A>G	rs74003630	5%	No effect on splicing	6.015
Intron 15	c.2189+1600C>T	rs60668271	5%	No effect on splicing	0.481
Intron 15	c.2190-1531G>A	rs74702528	0.9% (3% in African population)	No effect on splicing	0.489
Intron 15	c.2190-1463G>A	rs116416508	0.9% (3% in African population)	No effect on splicing	0.328
Intron 15	c.2190-1139A>G	rs184803352	0.7% (2% in African population	No effect on splicing	0.095
Intron 15	c.2190-1005A>G	rs4889820	5%	No effect on splicing	2.452
Intron 15	c.2190-686G>A	rs12452616	19%	No effect on splicing	2.725
Intron 15	c.2190-647G>A	rs59362713	10%	No effect on splicing	0.227
Intron 15	c.2190-536G>A	rs60429724	10%	No effect on splicing	0.454
Intron 15	c.2190-490G>A	rs111477580	1%	No effect on splicing	3.101
Intron 15	c.2190-444A>G	rs4889967	73%	No effect on splicing	1.059
Intron 15	c.2190-336C>T	rs76178719	3%	No effect on splicing	1.566
Intron 16	c.2331+20G>A	rs2304832	75%	No effect on splicing	5.346
Intron 16	c.2331+24T>C	rs2304831	15%	No effect on splicing	0.204
Intron 16	c.2331+151C>T	rs111537160	2%	No effect on splicing	0.608
Intron 16	c.2332-198A>T	rs2304830	73%	No effect on splicing	3.363
Exon 17	c.2338G>A	rs1126690	72%	No effect on splicing	2.675
Exon 17	c.2446G>A	rs1800314	5%	No effect on splicing	5.793
Intron 17	c.2482-132C>T	rs113824706	0.9% (3% in African population)	No effect on splicing	0.066
Exon 18	c.2553G>A	rs1042397	57%	Weakens a cryptic splice donor site	1.241
Intron 18	c.2647-71G>C	rs4889821	5%	No effect on splicing	3.473
Exon 19	c.2780C>T	rs1800315	2%	No effect on splicing	0.222
Intron 19	c.2800-227C>G	rs9890469	66%	No effect on splicing	0.661
Intron 19	c.2800-60G>A	rs55662462	0.7% (11% in Latino population)	No effect on splicing	2.209
Exon 20, 3′ UTR	c.*3G>A	rs1800317	5%	No effect on splicing	0.03

TABLE 2 (Continued)

Location	Variant	Variant ID	Global allele frequency (GnomAD)	Predictions of pre-mRNA splicing	CADD score PHRED
Exon 20, 3′ UTR	c.*91G>A	rs2229221	12%	No effect on splicing	6.887
Exon 20, 3′ UTR	c.*223C>T	rs8132	22%	No effect on splicing	3.025
Exon 20, 3′ UTR	c.*419G>T	rs7567	19%	No effect on splicing	4.17

Abbreviations: CADD, Combined Annotation-Dependent Depletion; mRNA, messenger RNA; UTR, untranslated region.

stronger enrichment in the catalytic core compared with the mapping we performed previously (Niño et al., 2019; Figure 1c).

We included in the current version of the database common sequence variants that have a MAF ≥ 1% and do not cause Pompe disease. This resulted in a relative increase in the number of nondisease-associated variants (Table 2). We decided to include common sequence variants in response to the misreporting of these variants as the principal cause of disease in several patients. Examples of this are the c.547-67C>G (rs8069491) and 547-39T>G (rs12452721) variants, which were reported as the cause of disease while having an allele frequency of 67% in the global population (Bekircan-Kurt et al., 2017; Guevara-Campos et al., 2019). In total, the database now includes 148 variants with a MAF ≥ 1%. All variants had a low CADD score (<10; Table 2) and were classified as "unknown." We note that while these common sequence

(a)

Variant	Protein change	phenotype combined with a null allele	reported patients	Predictions on pre-mRNA splicing	CADD score PHRED
GAA + c.1597T>C	p.(Cys533Arg)	Classic infantile	1	no effect on splicing	25.5
GAA + c.307T>G	p.(Cys103Gly)	Classic infantile	11	loss of a cryptic splice donor site	25.1
GAA + c.309C>G	p.(Cys103Trp)	Unknown	1	no effect on splicing	5.6
GAA + c.655G>A	p.(Gly219Arg)	Classic infantile	14	no effect on splicing	28.2
GAA + c.670C>T	p.(Arg224Trp)	Classic infantile or Childhood	7	no effect on splicing	22.8
GAA + c.1655T>C	p.(Leu552Pro)	Classic infantile	41	no effect on splicing	29.9
GAA + c.1798C>T	p.(Arg600Cys)	Classic infantile	18	no effect on splicing	27.0

(b)

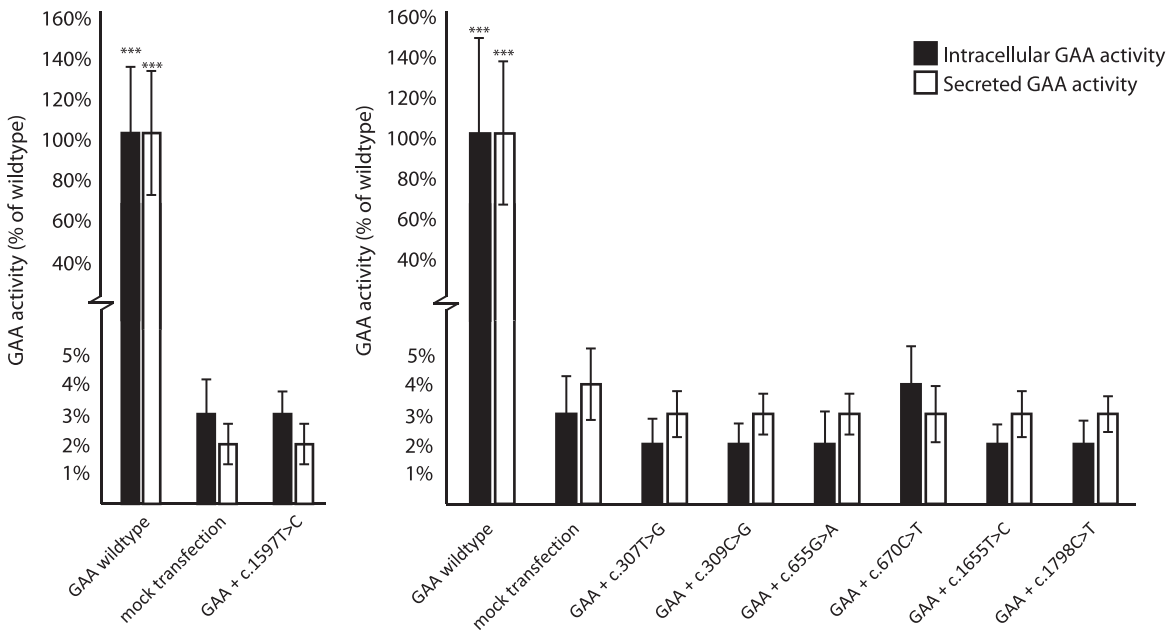

FIGURE 2 Expression study of seven disease-associated missense variants in the *GAA* gene. (a) Overview of basic information regarding the pathogenicity of selected variants. (b) Measured *GAA* activity in both cells and medium of COS-7 cultures after transfection with the generated constructs. Findings for the c.1597T>C variants are plotted separately as this was performed in a separate experiment. Data represent means, error bars represent *SD* ($n = 3$ biological replicates), ***$p < .001$. CADD, Combined Annotation-Dependent Depletion; mRNA, messenger RNA

TABLE 3 Variants of unknown significance that were found only through newborn screening programs

Variant	Protein change	Location	Type of variant (protein)	MAF	Predictions on splicing–Align GVGD–SIFT–Mutation taster–[CADD score]	Experimental data	Country and reference
c.317G>A*	p.(Arg106His)	Exon 2	Missense	MAF <1%	No effect on splicing–Class C0–Deleterious–Disease causing–[25.9]		Japan; Momosaki et al. (2019)
c.365T>A	p.(Met122Lys)	Exon 2	Missense	MAF not reported	No effect on splicing–Class C0–Tolerated–Polymorphism–[14.17]		USA; Scott et al. (2013)
c.424_440del	p.(Ser142Leufs*29)	Exon 2	Frameshift	MAF not reported	No effect on splicing–Results in an out of frame product–[32]		Taiwan; Chien et al. (2011)
c.533G>A*	p.(Arg178His)	Exon 2	Missense	MAF <1%	No effect on splicing–Class C0–Tolerated–Disease causing–[31]	No effect on splicing of exon 2 in minigene construct (Goina, et al., 2019)	Taiwan; Chien et al. (2011)
c.546+5G>T*	p.?	Intron 2	No category (splicing)	MAF <1%	Weakens exon 2 splice donor and generates a cryptic splice donor–[23.7]	Affects splicing of exon 2 in minigene construct (Goina, et al., 2019)	Taiwan; Labrousse et al. (2010)
c.705G>A	p.(=)	Exon 4	Silent	MAF <1%	No effect on splicing–[0.534]		Japan; Momosaki et al. (2019)
c.811A>G*	p.(Thr271Ala)	Exon 4	Missense	MAF not reported	No effect on splicing–Class C0–Tolerated–Polymorphism–[16.93]	71% residual activity of GAA in expression study (Kroos, et al., 2012a)	Taiwan; Labrousse et al. (2010)
c.1054C>T	p.(Gln352*)	Exon 6	Nonsense	MAF not reported	No effect on splicing–Introduces an early stop codon–[43]		Taiwan; Liao et al. (2014)
c.1080C>G	p.(Tyr360*)	Exon 7	Nonsense	MAF not reported	No effect on splicing–Introduces an early stop codon–[39]		Taiwan; Chien et al. (2011)
c.1082C>A	p.(Pro361Arg)	Exon 7	Missense	MAF <1%	No effect on splicing–Class C65–Deleterious–Disease causing–[25.5]		Japan; Momosaki et al. (2019)
c.1220A>G	p.(Tyr407Cys)	Exon 8	Missense	MAF <1%	No effect on splicing–Class C65–Deleterious–Disease causing–[25.9]		Mexico; Navarrete-Martínez et al. (2017)
c.1244C>T	p.(Thr415Met)	Exon 8	Missense	MAF <1%	No effect on splicing–Class C15–Deleterious–Disease causing–[24.6]		Japan; Momosaki et al. (2019)
c.1324G>A*	p.(Val442Met)	Exon 8	Missense	MAF <1%	No effect on splicing–Class C0–Deleterious–Disease causing–[23.8]		Taiwan; Chien et al. (2011)
c.1409A>C	p.(Asn470Thr)	Exon 9	Missense	MAF <1%	No effect on splicing–Class C25–Deleterious–Disease causing–[23.2]		Hungary; Witmann et al. (2012)

TABLE 3 (Continued)

Variant	Protein change	Location	Type of variant (protein)	MAF	Predictions on splicing–Align GVGD–SIFT–Mutation taster–[CADD score]	Experimental data	Country and reference
c.1574T>A	p.(Phe525Tyr)	Exon 11	Missense	MAF not reported	No effect on splicing–Class C15–Deleterious–Disease causing–[28.8]	10% residual activity of GAA in expression study (Kroos, et al., 2012a)	Taiwan; Chien et al. (2011)
c.1805C>T	p.(Thr602Ile)	Exon 13	Missense	MAF not reported	No effect on splicing–Class C0–Tolerated–Disease causing–[24.1]		USA; Elliott et al. (2016)
c.1840A>G	p.(Thr614Ala)	Exon 13	Missense	MAF not reported	No effect on splicing–Class C55–Deleterious–Disease causing–[24.3]		Taiwan; Liao et al. (2014)
c.1925T>A	p.(Val642Asp)	Exon 14	Missense	MAF not reported	No effect on splicing–Class C45–Deleterious–Disease causing–[29.2]		USA; Scott et al. (2013)
c.1958C>A	p.(Thr653Asn)	Exon 14	Missense	MAF <1%	No effect on splicing–Class C15–Tolerated–Disease causing–[25.4]		Taiwan; Chien et al. (2011)
c.2003A>G*	p.(Tyr668Cys)	Exon 14	Missense	MAF not reported	No effect on splicing–Class C65–Deleterious–Disease causing–[31]		Japan; Momosaki et al. (2019)
c.2055C>G	p.(Tyr685*)	Exon 15	Nonsense	MAF not reported	No effect on splicing–Introduces an early stop codon–[36]		Japan; Momosaki et al. (2019)
c.2174G>A	p.(Arg725Gln)	Exon 15	Missense	MAF <1%	No effect on splicing–Class C0–Tolerated–Disease causing–[32]		Hungary; Witmann et al. (2012)
c.2482-5T>C*	p.?	Intron 17	No category (splicing)	MAF not reported	No effect on splicing–[8.409]		Taiwan; Liao et al. (2014)
c.2482-2A>G	p.?	Intron 17	No category (splicing)	MAF <1%	Loss of exon 18 splice acceptor site–[35]		Hungary; Witmann et al. (2012)
c.2647-23del	p.?	Intron 18	No category (intron variant)	MAF <1%	No effect on splicing–[0.451]		Taiwan; Liao et al. (2014)
c.2843dup	p.(Val949Argfs*69)	Exon 20	Frameshift	MAF not reported	No effect on splicing–Results in an out of frame product–[23.1]		Taiwan; Liao et al. (2014)

Abbreviations: CADD, Combined Annotation-Dependent Depletion; MAF, minor allele frequency.

*Variants found in *cis* with the Asian pseudodeficiency allele c.[1726G>A; 2065G>A].

variants do not result in clinical manifestation of Pompe disease, it remains possible that they might modify disease progression when present in *cis* with a disease-associated variant. In Pompe disease, this is the case for the Asian pseudodeficiency allele (c.[1726G>A (p.Gly576Ser);2065G>A (p.Glu689Lys)]) and GAA2 (c.271G>A, (p.Asp91Asn)), which have a MAF of 14% for c.1726G>A, 23.5% for c.2065G>A (both East Asian), and 3.2% for GAA2 (European), and can be present in *cis* with known disease-associated variants (Kroos et al., 2006; Labrousse et al., 2010). Also, a variant with a low MAF in the general population, c.510C>T (p.=) (rs564758226), is known to be linked to the late-onset variant c.−32-13T>G (p.[=,0]) (IVS1). c.510C>T has a global MAF of 0.005%, but a MAF of 27.3% in compound heterozygous IVS1 patients with symptom onset at childhood. It worsens aberrant splicing caused by IVS1 and causes lower levels of leaky wild-type splicing and lower GAA enzyme activity, resulting in accelerated disease onset (Bergsma et al., 2019).

Figure 2a,b shows the results on the *GAA* variants we subjected to a more in-depth investigation. We selected the common missense variants c.307T>G (p.Cys103Gly), c.655G>A (p.Gly219Arg), c.670C>T (p.Arg224Trp), c.1655T>C (p.Leu552Pro), and c.1798C>T (p.Arg600Cys) and performed in vitro analysis of their severity using SDM of *GAA* cDNA expression constructs. In addition, c.1597T>C (p.Cys533Arg) and c.309C>G (p.Cys103Trp) were tested due to a request for diagnostic purposes. All of these variants fully abrogated GAA enzymatic activity following transfection in COS-7 cells (Figure 2, compare mutant GAA with mock transfections). The c.309C>G variant was included because the patient that harbored this variant in combination with c.525del p.(Glu176Argfs*45) showed an atypical Pompe disease phenotype (Mori et al., 2017). This case report described an adult patient with cardiomyopathy. Molecular analysis of primary skin fibroblasts identified a reduction in GAA activity, although not at pathogenic levels, and GAA activity was in the normal range for skeletal muscle tissue (Mori et al., 2017). We note that the c.309C>G variant was not detected in DNA from either parent and was described as a de novo variant (Mori et al., 2017). This variant might have been introduced during embryonic development, resulting in mosaicism similar to, as described previously in Labrijn-Marks et al. (2019) and in 't Groen et al. (2020). This might explain the “uneven pattern” of glycogen accumulation in histological sections derived from cardiac tissue (Mori et al., 2017). The in vitro analysis indicated that the c.309C>G variant is fully deleterious and has a predicted classic infantile phenotype in combination with a null allele. A comprehensive genetic analysis would be necessary to confirm this hypothesis.

Novel variants that have been reported only through NBS studies, but for which no clinical phenotype has been provided, were classified as “Unknown (found only in NBS)”. In the current version of the database, 26 variants have been classified as such (Table 3). Seven out of 26 variants were also present in *cis* with the Asian pseudodeficiency allele, indicating that additional testing is required because the Asian pseudodeficiency is known to result in false-positive outcomes in dried blood spot-based assays (Liao et al., 2014; Momosaki et al., 2019). It is currently unknown at what age symptoms will develop in neonates diagnosed with disease-associated variants that are potentially associated with a late-onset phenotype. Symptoms might be delayed until late adulthood or, for some genetic variants, might not even lead to disease. In these cases, further research on the effect of the genetic variants is essential to better inform patients, families, and doctors. As reported, in these cases, the uncertainty of the diagnosis, the possibility of an emerging disease, and the doubt on when to start treatment with ERT could lead to emotional stress (Bodamer et al., 2017). This underscores the importance of phenotype prediction for disease-associated variants, especially in the case of asymptomatic patients identified through NBS programs.

The sharp increase in reports on patients with Pompe disease and *GAA* disease-associated variants highlights the need for regular updates of the Pompe disease *GAA* variant database. Increased awareness and improved diagnostic technology with exome and genome sequencing and NBS programs are expected to further increase the number of entries in the database in the coming years. It will be important to link variants to clinical information and to test their deleterious effect in vitro using expression and splicing assays. Curated disease-specific databases such as the Pompe disease *GAA* variant database will be important to provide guidance to clinicians and clinical geneticists to establish an accurate molecular diagnosis.

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CONFLICT OF INTERESTS

Ans T. van der Ploeg has provided consulting services for various industries in the field of Pompe disease under an agreement between these industries and Erasmus MC, Rotterdam, the Netherlands. The remaining authors declare that there are no conflict of interests.

WEB RESOURCES

Pompe disease *GAA* variant database: http://www.pompevariantdatabase.nl/

LOVD: http://gaa.lovd.nl/

GnomAD: https://gnomad.broadinstitute.org/

dbSNP: https://www.ncbi.nlm.nih.gov/snp/

CADD score: https://cadd.gs.washington.edu/

DATA AVAILABILITY STATEMENT

The data described in this study is available upon request from the corresponding authors, and new variants have been added to the Pompe disease *GAA* variant database (http://www.pompevariantdatabase.nl/) and LOVD (http://gaa.lovd.nl/).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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