Glycogen storage diseases: Twenty-seven new variants in a cohort of 125 patients

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1 | INTRODUCTION

Hepatic glycogen storage diseases (GSDs) are a group of inborn errors of metabolism that include 11 different diseases caused by defects in glycogenolytic pathway. These defects are caused by pathogenic variants that result in enzymatic deficiencies for glycogen breakdown or synthesis, or problems in proteins that regulate glycogen metabolism. The
consequence is accumulation of glycogen in tissues, especially in liver (Chen & Zhong, 2013).

The general GSD frequency is 1 in 2,000–43,000 and their distribution is pan-ethnic (Özen, 2007; Vega et al., 2016). Some forms of GSDs are underestimated due to mild symptoms, rare occurrence, or difficult diagnostic methods. Symptoms may range from neonatal to almost asymptomatic, and the age of onset, severity, morbidity, mortality, and prognosis are dependent of causal variants (Kishnani et al., 2014, 2010; Laforêt, Weinstein, & Smit, 2012; Özen, 2007; Wang et al., 2012). The main clinical symptoms are hypoglycemia and hepatomegaly, and long-term complications are frequent (Burda & Hochuli, 2015).

Different types of GSDs can be clinically indistinguishable and need liver biopsy, an invasive method. In this aim, the molecular diagnosis using blood samples generates an accurate diagnosis and allows prognosis and genetic counseling (Choi et al., 2017; Davit-Spraul et al., 2011). Similar diseases in clinical symptoms, metabolic routes, or genetic features are a challenge to diagnose. In this aim, next-generation sequencing (NGS) is an important tool to determine the cause of the disease with accuracy and efficacy, allowing a more suitable treatment.

Only two previous studies have characterized 13 patients with GSD Ia and Ib in Brazilian population (Carlin, Scherrer, Tommaso, Bertuzzo, & Steiner, 2013; Reis et al., 2001).

In the present study, we describe the results of variant analysis in a cohort of 125 patients with hepatic GSD suspected diagnosis by NGS.

2 | MATERIAL AND METHODS

This study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre (project no. 15-0556), and all patients and guardians provided written informed consent for participation.

Were analyzed 125 patients with clinical symptoms of hepatic GSD. Blood samples were collected in EDTA vacuum container. DNA was extracted with Easy-DNA Purification kit (Thermo Fisher). DNA samples were quantified in NanoDrop 1000 (Thermo Fisher) and through Qubit dsDNA HS Assay Kit (Thermo Fisher).

The gene panel amplicon was designed with Ion AmpliSeq Designer software (Thermo Fisher), and included the exons and flanking 40 bp into introns of 11 genes involved in hepatic GSD (Table 1). The sequencing was performed in Ion Torrent PGM platform (Applied Biosystems), based in PCR amplification with minimal coverage of 200X. Base calling and sequence read quality assessments were performed using Torrent Suite 5.0.5. Alignment of the sequence reads to a reference human genome (GRCh37.p13) was performed using IonStates alignment.

The softwares Enlis Genome Research (LLC), Variant Effect Predictor (Ensembl), Ion Reporter (Thermo Fisher) and Varstation® (Varstation) were used to detect and classify variants. To determine the variants causing disease, the following were considered: ACMG guideline (Richards et al., 2015); allele frequency under 1% in the 1,000 Genomes Project (Sabeti, 2015); location in exon or borderlines; impact in the protein (missense, nonsense or splicing sites); and pathogenicity by predictors SIFT and Polyphen 2. For score of pathogenicity predictions in missense novel variants were used the softwares Polyphen 2 (Adzhubei et al., 2010), SIFT (Vaser, Adusumalli, Leng, Sikic, & Ng, 2016), PROVEAN (Choi, Sims, Murphy, Miller, & Chan, 2012), Mutation Teaster (Schwarz, Cooper, Schuelke, & Seelow, 2014), Pmut 2017 (López-Ferrando, Gazzo, Cruz, Orozco, & Gelpí, 2017), SNP&Go (Profiti, Martelli, & Casadio, 2017), PhDsnp (Capriotti & Fariselli, 2017), Panther (Thomas et al., 2003), SNAP2 (Hecht, Bromberg, & Rost, 2015) and MutPred (Pejaver et al., 2017). For splice site variants, Genescan (Burge & Karlin, 1997) and MaxEntScan were used (Yeo & Burge, 2003).

Validations of NGS results were realized by Sanger sequencing in patients and in parents when the sample was available. The unbiased capture and deep coverage of each coding exon and adjacent intronic region of all genes in this panel ensure accuracy of variant detection.

3 | RESULTS

We analyzed 125 patients with clinical suspicion of hepatic GSD. All samples were successfully sequenced. We found 63 different variants in 110 families, and 27 of those were new variants (Tables 2 and Appendix S1).

Seventy-five patients are men. The patients included in the study are from all Brazilian regions: 63 from the southeast (SP n = 48, RJ n = 2, MG n = 10, ES n = 3), 50 from the south (RS n = 46, SC n = 4), eight from the northeast (BA n = 2, CE n = 3, PB n = 3), two from the Midwest (DF = 1, MT n = 1) and two from the north (PA n = 2) (Appendix S1).

Both pathogenic variants were identified in 118 patients confirming the molecular diagnosis of hepatic GSD. For two patients, only one variant was found (patients 84 and 85). In five patients, no variant was identified (Appendix S1). All identified variants were confirmed by Sanger sequencing and investigated in literature or databanks (Tables 2 and Appendix S1).

Eight families included in this study had multiple affected individuals. For patients 5, 11, and 12 (11 and 12 are sisters), their parents reported consanguinity (Appendix S1). These information were considered while counting alleles.

Sixty-three alleles were identified, in which 26 are missense variants (41.2%), 16 are nonsense variants (25.3%), six
are splice site variants (9.5%), 11 deletions (17.4%), three insertions (4.7%) and one duplication (1.5%) (Table 2).

Among the 125 patients analyzed, 53 were genetically diagnosed with GSD Ia (42%), 23 with GSD Ib (18%), 14 with GSD III (11%), two with GSD VI (1.6%), 16 GSD IXa (12%), six with GSD IXb (4.8%), six with GSD IXc (4.8%), and five were not diagnosed (4%) (Appendix S1).

The most frequent variants in patients were p.Arg83Cys, observed in 39 alleles (18.5%), and p.Gln347* present in 14 alleles (6.6%), both in G6PC gene, causing GSD Ia. The other frequent variant, p.Leu348Valfs in SLC37A4 gene, was observed in 10% of alleles causing GSD Ib.

Variants not described in the literature were evaluated for protein impact by nine in silico pathogenicity prediction algorithms. All new missense variants were predicted as pathogenic. In the bioinformatics analyses of new splice site variants, all were confirmed to modify the exon–intron structures in different forms importantly, showing sufficient entropy forces to perform the incorrect splicing.

4 | DISCUSSION

This is one of the largest screening of variants causing the different forms of GSDs in patients, including 125 patients and describing 63 different variants, of which 27 are novel.

The GSD Ia and GSD Ib represent 60% of the analyzed patients. In other analyzed cohorts, Vega et al. (2016) reported more than three-quarters of patients who had GSD III or GSD IXa (39% of each type), and Özen (2007) found GSD type IXa as the most common form of the disease. These data reflect the differences among populations, the existence of private pathogenic variants, and the differences in prevalence of variants in GSDs.

GSD type Ia is the most widely distributed. The most frequent pathogenic variant found in this work was p.Arg83Cys, present in 18.5% of all patients and 39% of alleles in GSD Ia patients. This is one of the most important variant found around the world in patients with GSD Ia (Chou & Mansfield, 2008; Matern, Seydewitz, Bali, Lang, & Chen, 2002). This variant in G6PC is in the active center of the enzyme G6Pase and presented no detectable activity in transient expression assays (Lei, Shelly, Pan, Sidbury, & Chou, 1993). p.Arg83Cys is present in 50% of alleles in French and Tunisian patients (Barkaoui et al., 2007; Triacho et al., 2000), 80% of Sicilian and 100% of alleles in Ashkenazi Jewish patients (Ekstein et al., 2004; Stroppiano et al., 1999). This variant is found in genomAD in a frequency of 0.0005 (Lek et al., 2016) and appears to be in a hotspot since two other variants are observed in the same position (p.Arg83His and p.Arg83=). There are another eight variants in amino acids 80, 81, and 82, six of them being pathogenic. In the same gene, the variant p.Gln347* was in 6.9% of all alleles or in
## Table 2
Variants found in 125 patients with hepatic GSDs, their references, frequencies in databanks, and ACMG classification

| Gene       | GenBank     | Allele       | Protein         | Location | Reference                                                                 | ExAC Frequency | ACMG                      |
|------------|-------------|--------------|-----------------|----------|---------------------------------------------------------------------------|----------------|--------------------------|
| *G6PC*     | NM_000151   | c.70C > T    | p.Gln24*        | E1       | Rocha, Cabral, and Vilarinho (2000)                                       | 0.00006        | PM2, PVS1Probably pathogenic |
|            |             | c.77delC     | p.Ser26fs       | E1       | Lei et al. (1995)                                                        | 0.00008        | PM1, PM2, PP2, PP3, PP5   |
|            |             | c.113A > T   | p.Asp38Val      | E1       | ChevalierPorst et al. (1996)                                             |                |                          |
|            |             | c.161A > C   | p.Gln54Pro      | E1       | Tria et al. (2000)                                                       |                |                          |
|            |             | c.189G > C   | p.Trp63Cys      | E1       | New                                                                      |                |                          |
|            |             | c.202G > A   | p.Gly68Arg      | E1       | Reis et al. (2001)                                                      |                |                          |
|            |             | c.231−1G > A| p.Arg83Cys      | E2       | Lei et al. (1993)                                                       | 0.0005         | BS1, PM1, PP2, PP3, PP5 |
|            |             | c.323C > T   | p.Thr108Ile     | E2       | Tria et al. (2000)                                                       |                |                          |
|            |             | c.401_402delCT| p.Thr134 = fs  | E3       | New                                                                      |                |                          |
|            |             | c.493A > T   | p.Arg147*       | E3       | New*                                                                    | 0.000008       | PM1, PM2, PVS1Pathogenic |
|            |             | c.509G > A   | p.Arg170Gln     | E4       | Barkaoui et al. (2007)                                                   | 0.00001        | PM1, PM2, PP2, PP3Pathogenic |
|            |             | c.563-3C > G| p.Gly270Val     | E5       | Lei et al. (1995)                                                        | 0.00001        | PM2, PP2, PP3Pathogenic  |
|            |             | c.969C > A   | p.Tyr323*       | E5       | Caldenaro et al. (2013)                                                  | 0.000008       | PM2, PVS1Pathogenic      |
|            |             | c.1012G > T  | p.Val338Phe     | E5       | Rake et al. (2000)                                                       | 0.00001        | PM2, PP2, PP3Uncertain   |
|            |             | c.1039C > T  | p.Gln347*       | E5       | Lei, Pan, Shelly, Liu, and Chou (1994)                                     | 0.0002         | BS1, PP5, PVS1Uncertain  |
| *SLC37A4*  | NM_001467   | c.59G > A    | p.Gly20Asp      | E3       | Veiga da Cunha et al. (1998)                                             | 0.00001        | PM1, PM2, PP2, PP3Pathogenic |
|            |             | c.92_94delTCT| p.Phe51_Ser32del| E3       | New                                                                      |                | PM2Pathogenic            |
|            |             | c.344_345dupGG| p.Leu116Glyfs   | E4       | Galli et al. (1999)                                                      | 0.00001        | PM2, PVS1Pathogenic      |
|            |             | c.446G > A   | p.Gly149Glu     | E5       | Galli et al. (1999)                                                      | 0.00002        | PM1, PM2, PP2, PP3Pathogenic |
|            |             | c.547T > C   | p.Cys183Arg     | E5       | Veiga da Cunha et al. (1998)                                             |                | PM1, PM2, PP2, PP3Pathogenic |
|            |             | c.557T > C   | p.Leu186Pro     | E5       | New                                                                      |                | PM1, PM2, PP2, PP3Pathogenic |
|            |             | c.703_705delGTG| p.Val236del    | E7       | Hou et al. (1999)                                                       |                | PM2, PP5Uncertain        |
|            |             | c.899G > A   | p.Arg300His     | E9       | Marcolongo et al. (1998)                                                 | 0.00003        | BS1, PP5, PVS1Uncertain  |
|            |             | c.1042_1043delCT| p.Leu348fs     | E9       | Veiga da Cunha et al. (1998)                                             | 0.00002        | PM2, PVS1Pathogenic      |
|            |             | c.1179G > A  | p.Tryp393*      | E10      | New*                                                                     |                |                          |

(Continues)
| Gene GenBank | Allele | Protein | Location | Reference | ExAC Frequency | ACMG |
|-------------|--------|---------|----------|-----------|----------------|------|
| AGL NM_000642 | c.293 + 2T>A | p.Val109Leu | I3 | Hadjigeorgiou et al. (1999) | 0.00009 | PM2, Uncertain |
|             | c.325G > T | p.Trp248* | E4 | New* | PM1, PM2, PP3, Uncertain |
|             | c.344G > A | p.Trp461* | E11 | New* | PM1, PM2, PP3, Uncertain |
|             | c.1418G > A | p.Arg494His | E12 | Goldstein et al. (2010) | 0.008 | BP1, BS1, PM1, PP3, Probably Benign |
|             | c.1571G > A | p.Arg524His | E12 | Lucchiarì et al. (2006) | 0.000008 | BP1, PM1, PM2, PP3, Uncertain |
|             | c.1734A > T | p.Arg578Ser | E13 | New | BP1, PM1, PM2, PP3, Uncertain |
|             | c.1858_1859delCT | p.Leu620Valfs | E14 | New | PM2, PV51, Probably pathogenic |
|             | c.2455_2458delAAAC | p.Lys819 = fs | E19 | New | PM2, PV51, Probably pathogenic |
|             | c.2728C > T | p.Arg910* | E21 | Lucchiarì et al. (2006) | 0.000008 | PM1, PM2, PV51, Pathogenic |
|             | c.2904_2905insT | p.Tyr969Leufs | E22 | New | PM2, PV51, Probably pathogenic |
|             | c.3124_3125delGA | p.Glu1072Aspfs | E24 | Goldstein et al. (2010) | 0.000008 | PM2, PV51, Pathogenic |
|             | c.3475_3476insA | p.Gln1159fs | E26 | New | PM2, PV51, Probably pathogenic |
|             | c.3484C > T | p.Gln1162* | E26 | New | PM1, PM2, PV51, Pathogenic |
|             | c.3625C > T | p.Gln1209* | E27 | New | PM1, PM2, PV51, Pathogenic |
|             | c.3814_3815delG | p.Arg1272 = fs | E28 | New | PM2, PV51, Probably pathogenic |
|             | c.3904delA | p.Lys1302fs | E29 | New | PM2, PV51, Probably pathogenic |
|             | c.3989G > A | p.Trp1327* | E30 | Lucchiarì et al. (2002) | 0.00002 | PM1, PM2, PV51, Pathogenic |
|             | c.4528_4529insA | p.Tyr1510* | E34 | Shen and Chen (2002) | 0.00001 | PM2, PV51, Probably pathogenic |
| PYGL NM_001163940 | c.131G > A | p.Arg44His | E1 | Hoogeveen et al. (2015) | | PM2, PP2, PP3, Uncertain |
|             | c.697G > A | p.Gly233Ser | E6 | New | PM2, PP2, PP3, Uncertain |
| PHKA2 NM_000292 | c.133C > T | p.Arg45Trp | E2 | Beauchamp et al. (2007) | | PM1, PM2, PP2, PP3, Probably pathogenic |
|             | c.557G > A | p.Arg186His | E6 | Burwinkel et al. (1996) | | PM1, PM2, PP2, PP3, PP5, Probably pathogenic |
|             | c.883C > T | p.Arg295Cys | E9 | Ban, Sugiyama, Goto, Mizutani, and Togari (2003) | | PM1, PM2, PP2, PP3, PP5, Probably pathogenic |
|             | c.1965 + 1G>A | p.Gln818* | I18 | Rodriguez-Jimenez et al. (2017) | 0.00001 | PM2, Uncertain |
|             | c.2452C > T | p.Gln818* | E22 | New | PM2, PV51, Probably pathogenic |
|             | c.3614C > T | p.Pro1205Ser | E33 | van den Berg et al. (1995) | | PM2, PP2, PP3, PP5, Uncertain |
|             | c.3629G > A | p.Gly1210Glu | E33 | Rudolfová, Slováčková, Trbušek, Pešková, and Kozák (2001) | | PM2, PP2, PP3, Uncertain |

(Continues)
15% of alleles in GSD I. They both represent approximately 54% of variants found in GSD Ia patients. The second most frequently found variant among all patients was p.Leu348Valfs in SLC37A4 gene, present in 10% of patients, and 47.7% of alleles (21/44 alleles) in GSD Ib. This variant was present in 39% of Serbians patients (Skakic et al., 2018) and 31% of White patients reviewed in Chou, Jun, and Mansfield (2010).

Twenty-seven novel variants were identified among the 125 patients, observed mainly among patients with GSD type III and type IX. AGL, that causes GSD III, is one of the largest genes, and has the highest number of variants reported in HGMD – The Human Gene Mutation Database (Stenson et al., 2003), which proves its heterogeneity. The increased number of variants in type IX patients can be justified by their lower characterization.

Some of the novel variants have already been detected in database projects involving the search for variants in a large number of individuals but never related to patients. We investigate the variants in "The Exome Aggregation Consortium" – ExAC – composed of 60,706 unrelated individuals, and the Online Archive of Brazilian Mutations – AbraOM – composed of 609 elderly individuals, as in other databases (Lek et al., 2016; Naslavsky et al., 2017; Sabeti, 2015). Seven of 27 novel variants were in ExAC, all in very low frequencies (Table 2).

In five patients with no identified variants, the clinical suspicions are mild or inconclusive, once they did not have clear clinical indications or laboratory findings supporting 15% of alleles in GSD Ia. They both represent approximately 54% of alleles found in GSD Ib patients. The second most frequently found variant among all patients was p.Leu348Valfs in SLC37A4 gene, present in 10% of patients, and 47.7% of alleles (21/44 alleles) in GSD Ib. This variant was present in 39% of Serbians patients (Skakic et al., 2018) and 31% of White patients reviewed in Chou, Jun, and Mansfield (2010).

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the diagnosis of GSD, besides hypoglycemia and/or hepatomegaly. The NGS was a diagnostic exclusion test; therefore, it was an expected result. These patients probably do not have GSD, once hepatomegaly and hypoglycemia are difficult to distinguish from other metabolic storage disorders without more clinical findings. Another possibility is the presence of variants in nontargeted deep intronic and regulatory regions (Wang et al., 2013).

Relationships of synergistic heterogeneity should be considered for GSDs, since the disease-causing deficient enzymes share metabolic pathways, however it was not observed in the present study (Vockley, Rinaldo, Bennett, Matern, & Vladutiu, 2000).

The patient 120 is possibly a GSD patient because he presented clinical symptoms such as hypoglycemia and ketonuria but no other clinical signs, however only synonymous variants were found in GYS2 (that causes GSD type 0), which does not justify the disease.

This variety of results reflects the profile of an extremely large country with an interesting and important mix of people from all over the world. The presence of immigrants from the most diverse origins, such as Africans, Asians, and Amerindians justifies the variability of alleles found in a highly mixed population. Genetic analyses indicate that Latin Americans trace their ancestry mainly in the intermixing of Native Americans, Europeans, and Sub-Saharan Africans. Historically, Latin America has a continuous, differential, and diverse intra- and intercontinental migration events, and presents higher prevalence of metabolic diseases (Adhikari, Chacón-Duque, Mendoza-Revilla, Fuentes-Guajardo, & Ruiz-Linares, 2017; Chacón-Duque et al., 2018; Giolo et al., 2012; Quinto-Sánchez et al., 2017; Resque et al., 2016).

Among the advantages of NGS diagnosis, patients undiagnosed by traditional means were investigated and correctly diagnosed in the present study. This method is especially promising for mixed populations with high level of heterogeneity. This method also allows the identification of unexpected diagnoses in the supposed typical phenotypes. Rare genetic diseases can be a diagnostic challenge, sometimes an odyssey. The NGS technologies can provide a fast diagnosis, advantages for treatment management, in reproductive choices, genetic counseling, and fertility services (Schofield et al., 2017). The GSD traditional diagnosis methods involve liver biopsy, an invasive and risked method, that can be avoided with a well-established molecular method (Bali, Chen, Austin, & Goldstein, 2016; Lévesque et al., 2016).

This diagnosis is an important advancement for patients with nontypical forms of disease, especially for those who need agile actions, since it evaluates 11 genes at the same time.

This was an important step to increase the knowledge about the genetics of the different types of hepatic GSDs in Brazilian patients, since they have a genetically heterogeneous origin, and it is reflected in the variability of types and variants (Vega et al., 2016; Wang et al., 2013). The evaluation by NGS also allows to detect cases of synergistic heterogeneity that cannot be perceived by Sanger sequencing.

Differentiated therapeutic management among GSD justifies the population characterization of patients. If NGS analyses are not available or expensive, the molecular diagnosis should be conducted first through the search for the pathogenic variants p.Arg83Cys and p.Gln347* in G6PC in case of GSD Ia or p.Leu348Valfs in SLC37A4 for GSD Ib. Sanger sequencing approach is the most cost-effective to solve up to 40% of the cases. However, for the cases without prevalent mutations or without suspected type of GSD, NGS is the most effective solution.

This study emphasizes that molecular genetic analysis is a reliable and convenient alternative to the assay of enzymatic activity in a fresh liver biopsy specimen for the diagnosis of GSDs. This type of study is an important tool for the estimation of disease progression, since different types of GSDs present variations in their clinical course and treatment, besides serving as a basis for genetic counseling and prenatal diagnosis.

The discovery of a significant number of new mutations reinforces the allelic variability of different GSDs and proves that the diagnosis of GSDs in Brazil can be challenging, showing the validity of NGS gene panel use for diagnosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Adhikari, K., Chacón-Duque, J. C., Mendoza-Revilla, J., Fuentes-Guajardo, M., & Ruiz-Linares, A. (2017). The genetic diversity of the Americas. Annual Review of Genomics and
Capriotti, E., & Fariselli, P. (2017). PhD-SNPg: A webserver and lightweight tool for scoring single nucleotide variants. *Nucleic Acids Research*, 45(W1), W247–W252. https://doi.org/10.1093/nar/gkx369

Carlin, M. P., Scherrer, D. Z., Tommaso, A. M. A. D., Bertuzzo, C. S., & Steiner, C. E. (2013). Determining mutations in G6PC and SLC37A4 genes in a sample of Brazilian patients with glycogen storage disease types Ia and Ib. *Genetics and Molecular Biology*, 36(4), 502–506. https://doi.org/10.1590/S1415-4757201300010400007

Chacón-Duque, J.-C., Adhikari, K., Fuentes-Guajardo, M., Mendoza-Revilla, J., Acuña-Alonzo, V., Barquera, R., … Ruiz-Linares, A. (2018). Latin Americans show wide-spread Condroplasty ancestry and imprint of local Native ancestry on physical appearance. *Nature Communications*, 9. https://doi.org/10.1038/s41467-018-07748-z

Chen, Z., & Zhong, C. (2013). Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: Implications for diagnostic and therapeutic strategies. *Progress in Neurobiology*, 108, 21–43. https://doi.org/10.1016/j.pneurobio.2013.06.004

Chevalier-Porst, F., Bozon, D., Bonardot, A. M., Bruni, N., Michieux, G., Mathieu, M., & Maire, I. (1996). Mutation analysis in 24 French patients with glycogen storage disease type Ia. *Journal of Medical Genetics*, 33, 358–360. https://doi.org/10.1136/jmg.33.5.358

Choi, R., Park, H. D., Ko, J. M., Lee, J., Lee, D. H., Hong, S. J., … Choe, Y. H. (2017). Novel SLC37A4 mutations in Korean patients with glycogen storage disease Ib. *Annals of Laboratory Medicine*, 37(3), 261–266. https://doi.org/10.3343/alm.2017.37.3.261

Choi, Y., Sims, G. E., Murphy, S., Miller, J. R., & Chan, A. P. (2012). Predicting the functional effect of amino acid substitutions and indels. *PLoS ONE*, 7(10), e46688. https://doi.org/10.1371/journal.pone.0046688

Chou, J. Y., Jun, H. S., & Mansfield, B. C. (2010). Glycogen storage disease type I and G6Pase-β deficiency: Etiology and therapy. *Nature Reviews Endocrinology*, 6(12), 676. https://doi.org/10.1038/nrendo.2010.189

Chou, J. Y., & Mansfield, B. C. (2008). Mutations in the glucose-6-phosphatase-α (G6PC) gene that cause type Ia glycogen storage disease. *Human Mutation*, 29(7), 921–930. https://doi.org/10.1002/humu.20772

Davit-Spraul, A., Piraud, M., Dobbeltrae, D., Valayanopoulos, V., Labrune, P., Habes, D., … Baussan, C. (2011). Liver glycogen storage diseases due to phosphorylase system deficiencies: Diagnosis thanks to non-invasive blood enzymatic and molecular studies. *Molecular Genetics and Metabolism*, 104(1), 137–143. https://doi.org/10.1016/j.ymgme.2011.05.010

Ekstein, J., Rubin, B. Y., Anderson, S. L., Weinstein, D. A., Bach, G., Abeliovich, D., … Risch, N. (2004). Mutation frequencies for glycogen storage disease Ia in the Ashkenazi Jewish population. *American Journal of Medical Genetics. Part A*, 129(2), 162–164. https://doi.org/10.1002/ajmg.a.30232

Galli, L., Orrico, A., Marcolongo, P., Fulceri, R., Burchell, A., Melis, D., … Sorrentino, V. (1999). Mutations in the glucose-6-phosphate transporter (G6PT) gene in patients with glycogen storage diseases type Ib and 1c. *FERS Letters*, 459, 255–258. https://doi.org/10.1016/S0014-5793(99)01248-X

Giolo, S. R., Soler, J. M., Greenway, S. C., Almeida, M. A., De Andrade, M., Seidman, J. G., … Pereira, A. C. (2012). Brazilian urban population genetic structure reveals a high degree of admixture. *European Journal of Human Genetics*, 20(1), 111. https://doi.org/10.1038/ejhg.2011.144

Goldstein, J. L., Austin, S. L., Boyette, K., Kanaly, A., Veerapandiyan, A., Rehder, C., … Bali, D. S. (2010). Molecular analysis of the AGI gene: Identification of 25 novel mutations and evidence of genetic heterogeneity in patients with glycogen storage disease type III. *Genetics in Medicine*, 12(7), 424. https://doi.org/10.1097/PGM.0b013e3181d94aea

Hadjigeorgiou, G. M., Comi, G. P., Bordoni, A., Shen, J., Chen, Y. T., Salani, S., … Rodolico, C. (1999). Novel donor splice site mutation in the human G6PC gene in a patient with glycogen storage disease type Ia. *Human Genetics*, 105(5), 303–305. https://doi.org/10.1007/s001140050672
mutations of AGL gene in glycogen storage disease type IIIa. *Journal of Inherited Metabolic Disease, 22*(6), 762–763. https://doi.org/10.1023/a:1005572906807

Hecht, M., Bromberg, Y., & Rost, B. (2015). Better prediction of functional effects for sequence variant. *BMC Genomics, 16*(S8), S1. https://doi.org/10.1186/1471-2164-16-s8-s1

Hedell, R., Dufva, C., Ansell, R., Mostad, P., & Hedman, J. (2015). Enhanced low-template DNA analysis conditions and investigation of allele dropout patterns. *Forensic Science International: Genetics, 14*, 61–75. https://doi.org/10.1016/j.fsigen.2014.09.008

Hoogeveen, I. J., van der Ende, R. M., van Spronsen, F. J., de Boer, F., Heiner-Fokkema, M. R., & Derks, T. G. (2015). Normoglycemic ketonemia as biochemical presentation in ketotic glycogen storage disease. *Journal of Inherited Metabolic Disease Report, 28*, 41–47. https://doi.org/10.1007/8904_2014_511

Hou, D.-C., Kure, S., Suzuki, Y., Hasegawa, Y., Haru, Y., Inoue, T., ... Narisawa, K. (1999). Glycogen storage disease type Ib: Structural and mutational analysis of the microsomal glucose-6-phosphate transporter gene. *American Journal of Medical Genetics, 86*(3), 253–257. https://doi.org/10.1002/(SICI)1096-8628(19990917)86:3<253::AID-AJMG11>3.0.CO;2-7

Inokuchi, S., Kitayama, T., Fujii, K., Nakahara, H., Nakanishi, H., Saito, K., ... Sekiguchi, K. (2016). Estimating allele dropout probabilities by logistic regression: Assessments using Applied Biosystems 3500XL and 3130xl Genetic Analyzers with various commercially available human identification kits. *Legal Medicine, 19*, 77–82. https://doi.org/10.1016/j.legalmed.2015.07.006

Kishnani, P. S., Austin, S. L., Abdunur, J. E., Arn, P., Bali, D. S., Boney, A., ... Watson, M. S. (2014). Diagnosis and management of glycogen storage disease type I: A practice guideline of the American College of Medical Genetics and Genomics. *Genetics in Medicine, 16*, e1. https://doi.org/10.1038/gim.2014.128

Kishnani, P. S., Austin, S. L., Arn, P., Bali, D. S., Boney, A., ... Case, L. E., ... Watson, M. S. (2010). Glycogen storage disease type III diagnosis and management guidelines. *Genetics in Medicine, 12*(7), 446–463. https://doi.org/10.1097/GIM.0b013e3181e655b6

Kishnani, P. S., Chuang, T. P., Bali, D., Koeberl, D., Austin, S., Weinstein, D. A., & Chen, Y. T. (2009). Chromosomal and genetic alterations in human hepatocellular adenomas associated with type Ia glycogen storage disease. *Human molecular genetics, 18*(24), 4781–4790.

Laforêt, P., Weinstein, D. A., & Smit, G. P. A. (2012). The glycogen storage diseases and related disorders. In *Inborn metabolic diseases* (pp. 115–139). Berlin: Heidelberg, Springer.

Lei, K.-J., Chen, Y.-T., Chen, H., Wang, L.-J.-C., Liu, J.-L., McConkie-Rosell, A., ... Chou, J. Y. (1995). Genetic basis of glycogen storage disease type 1a: Prevalent mutations at the glucose-6-phosphatase locus. *American Journal of Human Genetics, 57*, 766–771.

Lei, K.-J., Pan, C.-J., Shelly, L. L., Liu, J.-L., & Chou, J. Y. (1994). Identification of mutations in the gene for glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1a. *Journal of Clinical Investigation, 93*, 1994–1999. https://doi.org/10.1172/JCI117192

Lei, K.-J., Shelly, L. L., Pan, C.-J., Sidbury, J. B., & Chou, J. Y. (1993). Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. *Science, 262*, 580–583. https://doi.org/10.1126/science.8211187

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., & Tukiainen, T. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature, 536*, 285–291. https://doi.org/10.1038/nature19057

Lévesque, S., Auray-Blais, C., Gravel, E., Boutin, M., Dempsey-Nunez, L., Jacques, P.-E., ... Kishnani, P. (2016). Diagnosis of late-onset Pompe disease and other muscle disorders by next-generation sequencing. *Orphanet Journal of Rare Diseases, 11*(1), 8. https://doi.org/10.1186/s13023-016-0390-6

López-Ferrando, V., Gazzo, A., de la Cruz, X., Orozco, M., & Gelpí, J. L. (2017). PMut: A web-based tool for the annotation of pathologic variants on proteins 2017 update. *Nucleic Acids Research, 45*(W1), W222–W228. https://doi.org/10.1093/nar/gkx313

Lucchieri, S., Donati, M. A., Parini, K., Melis, D., Gatti, R., Bresolin, N., & Comi, G. P. (2002). Molecular characterisation of GSD III subjects and identification of six novel mutations in AGL. *Human mutation, 20*(6), 480–480.

Lucchieri, S., Pagliarani, S., Salani, S., Filocamo, M., Di Rocco, M., Melis, D., ... Comi, G. P. (2006). Hepatic and neuromuscular forms of glycogenosis type III: Nine mutations in AGL. *Human Mutation, 27*(6), 600–601. https://doi.org/10.1002/humu.9426

Marcolongo, P., Barone, V., Priori, G., Pirola, B., Giglio, S., Biasucci, G., ... Sorrentino, V. (1998). Structure and mutation analysis of the glycogen storage disease type 1b gene. *FEBS Letters, 436*, 247–250. https://doi.org/10.1016/S0014-5793(98)01129-6

Matern, D., Seydewitz, H., Bali, D., Lang, C., & Chen, Y. T. (2002). Glycogen storage disease type I: Diagnosis and phenotype/genotype correlation. *European Journal of Pediatrics, 161*(1), S10–S19. https://doi.org/10.1007/BF02679989

Naslavsky, M. S., Yamamoto, G. L., de Almeida, T. F., Ezquina, S. A., Sunaga, D. Y., Pho, N., … Zatz, M. (2017). Exomic variants of an elderly cohort of Brazilians in the ABrAOM database. *Human Mutation, 38*(7), 751–763. https://doi.org/10.1002/humu.23220

Özen, H. (2007). Glycogen storage diseases: New perspectives. *World Journal of Gastroenterology, 13*(18), 2541. https://doi.org/10.3748/wjg.v13.i18.2541

Pejaver, V., Urestii, J., Lugo-Martinez, J., Pagel, K. A., Lin, G. N., Nam, H., … Radivojac, P. (2017). MutPred2: inferring the molecular and phenotypic impact of amino acid variants. *bioRxiv*. https://doi.org/10.1101/134981

Profitti, G., Martelli, P. L., & Casadio, R. (2017). The Bologna Annotation Resource (BAR 3.0): Improving protein functional annotation. *Nucleic Acids Research, 45*(W1), W285–W290. https://doi.org/10.1093/nar/gkx330

Quinto-Sánchez, M., Cintas, C., Silva de Cerqueira, C. C., Ramallo, V., Acuña-Alonzo, V., Adhikari, K., … González-José, R. (2017). Socioeconomic status is not related with facial fluctuating asymmetry: Evidence from Latin-American populations. *PLoS ONE, 12*(1), e0169287. https://doi.org/10.1371/journal.pone.0169287

Rake, J. P., ten Berge, A. M., Verlind, E., Visser, G., Verlind, E., Nienkoning, K. E., … Scheffer, H. (2000). Glycogen storage disease type Ia: Recent experience with mutation analysis, a summary of mutations reported in the literature and a newly developed diagnostic flowchart. *European Journal of Pediatrics, 159*, 322–330. https://doi.org/10.1007/s004310051281

Reis, F. C., Caldas, H. C., Norato, D. Y., Schwartz, I. V. D., Giugliani, R., Burin, M. G., & Sartorato, E. L. (2001). Glycogen storage disease type Ia: Molecular study in Brazilian patients. *Journal of Human Genetics, 46*(3), 146. https://doi.org/10.1007/s100380170102

Resque, R., Gusmão, L., Geppert, M., Roewer, L., Palha, T., Alvarez, L., … Santos, S. (2016). Male lineages in Brazil: Intercontinental
admixture and stratification of the European background. *PLOS ONE*, 11(4), e0152573. https://doi.org/10.1371/journ al.pone.0152573

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., … Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17(5), 405. https://doi.org/10.1038/gim.2015.30

Rocha, H., Cabral, A., & Vilariño, L. (2000). Identification of a novel mutation (Q24X) in the glucose-6-phosphatase gene of a Portuguese patient with GSD-Ia. *Human Mutation*, 16, 449. https://doi.org/10.1002/1098-1004(20000116)15:6<449::aid-humu25>3.0.co;2-l

Rodríguez-Jiménez, C., Santos-Simarro, F., Campos-Barros, Á., Camarena, C., Lledín, D., Vallespin, E., … Rodríguez-Nóvoa, S. (2017). A new variant in PHKA2 is associated with glycogen storage disease type IXa. *Molecular Genetics and Metabolism Reports*, 3(10), 52–55. https://doi.org/10.1016/j.jmgmr.2017.01.003

Rudolfová, J., Slováčková, R., Trbušek, M., Pešková, Št'astná, S., & Rudolfová, J., Slováčková, R., Trbušek, M., Pešková, Št'astná, S., & Kozák, L. (2001). Identification of three novel mutations in the PHKA2 gene in Czech patients with X-linked liver glycogenosis. *Journal of Inherited Metabolic Disease*, 24(1), 85–87. https://doi.org/10.1023/a:1005635629149

Sabeti, P. C. (2015). A global reference for human genetic variation. *Nature*, 526(7571), 68–74.

Schofield, D., Khurshid Alam, K., Douglas, L., Shrestha, R., MacArthur, D. G., Davis, M., … O’Grady, G. L. (2017). Cost-effectiveness of massively parallel sequencing for diagnosis of paediatric muscle diseases. *Genomic Medicine*, 2(1), 4. https://doi.org/10.1038/s41525-017-0006-7

Schwarz, J. M., Cooper, D. N., Schuelke, M., & Seelow, D. (2014). MutationTaster2: Mutation prediction for the deep-sequencing age. *Nature Methods*, 11(4), 361–362. https://doi.org/10.1038/nmeth.2890

Shen, J., & Chen, Y. (2002). Molecular characterization of glycogen storage disease type III. *Current Molecular Medicine*, 2(2), 167–175. https://doi.org/10.2174/15655240234605752

Skakic, A., Djordjevic, M., Sarajlija, A., Klaassen, K., Tosic, N., Keccman, B., & Stojiljkovic, M. (2018). Genetic characterization of GSD I in Serbian population revealed unexpectedly high incidence of GSD Ib and 3 novel SLC37A4 variants. *Clinical Genetics*, 93(2), 350–355. https://doi.org/10.1111/cge.13093

Stenson, P. D., Ball, E. V., Mort, M., Phillips, A. D., Shiel, J. A., Thomas, N. S., & Cooper, D. N. (2003). The Human Gene Mutation Database (HGMD®): 2003 update. *Human Mutation*, 21, 577–581. https://doi.org/10.1002/humu.10212

Stroppiano, M., Regis, S., DiRocco, M., Caroli, F., Gandullia, P., & Gatti, R. (1999). Mutations in the glucose-6-phosphatase gene of 53 Italian patients with glycogen storage disease type Ia. *Journal of Inherited Metabolic Disease*, 22(1), 43–49. https://doi.org/10.1016/s1015-4596(99)515118

Thomas, P. D., Campbell, M. J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R., & Narechania, A. (2003). PANTHER: A library of protein families and subfamilies indexed by function. *Genome Research*, 13(9), 2129–2141. https://doi.org/10.1101/gr.772403

Trioche, P., Francois, J., Chalas, J., Capel, L., Lindenbaum, A., Odiévre, M., & Labrune, P. (2000). Genetic heterogeneity of glycogen storage disease type Ia in France: A study of 48 patients. *Human Mutation*, 16(5), 444. https://doi.org/10.1002/1098-1004(20001116)16:5<444::aid-HUMU10>3.0.CO;2-F

van den Berg, I. E., van Beurden, E. A., Malingre, H. E., van Amstel, H. K., Poll-The, B. T., Smetinik, J. A., & Berger, R. (1995). X-linked liver phosphorylase kinase deficiency is associated with mutations in the human liver phosphorylase kinase alpha subunit. *American Journal of Medical Genetics*, 56(2), 381.

Vaser, R., Adsumalli, S., Leng, S. K., Sikic, M., & Ng, P. (2016). SIFT missense predictions for genomes. *Nature Protocols*, 11, 1–9. https://doi.org/10.1038/nprot.2015.123

Vega, A. I., Medrano, C., Navarrete, R., Desviat, L. R., Merinero, B., Rodríguez-Pombo, P., … Pérez, B. (2016). Molecular diagnosis of glycogen storage disease and disorders with overlapping clinical symptoms by massive parallel sequencing. *Genetics in Medicine*, 18(10), 1037–1043. https://doi.org/10.1038/gim.2015.217

Veiga-da-Cunha, M., Gerin, I., Chen, Y.-T., de Barys, T., de Lonlay, P., Dionisi-Vici, C., … Van Schaftingen, E. (1998). A gene on chromosome 11q23 coding for a putative glucose-6-phosphate translocase is mutated in glycogen-storage disease types Ib and Ic. *American Journal of Human Genetics*, 63, 976–983. https://doi.org/10.1086/302068

Vockley, J., Rinaldo, P., Bennett, M. J., Matern, D., & Vladutiu, G. D. (2000). Synergistic heterozygosity: Disease resulting from multiple partial defects in one or more metabolic pathways. *Molecular Genetics and Metabolism*, 71, 10–18. https://doi.org/10.1006/mgme.2000.3066

Wang, D. Q., Carreras, C. T., Fiske, L. M., Austin, S., Boree, D., Kishnani, P. S., & Weinstein, D. A. (2012). Characterization and pathogenesis of anemia in glycogen storage disease type Ia and Ib. *Genetics in Medicine*, 14(9), 795. https://doi.org/10.1038/gim.2012.41

Wang, J., Cui, H., Lee, N.-C., Hwu, W.-L., Chien, Y.-H., Craigew, W. J., … Zhang, V. W. (2013). Clinical application of massively parallel sequencing in the molecular diagnosis of glycogen storage diseases of genetically heterogeneous origin. *Genetics in Medicine*, 15(2), 106–114. https://doi.org/10.1038/gim.2012.104

Yeo, G., & Burge, C. B. (2003). Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. *Journal of Computational Biology*, 11(2–3), 377–394. https://doi.org/10.1089/106652041401418

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