Mucopolysaccharidosis type VI (MPS VI) and molecular analysis: Review and classification of published variants in the ARSB gene

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Abstract
Maroteaux–Lamy syndrome (MPS VI) is an autosomal recessive lysosomal storage disorder caused by pathogenic ARSB gene variants, commonly diagnosed through clinical findings and deficiency of the arylsulfatase B (ASB) enzyme. Detection of ARSB pathogenic variants can independently confirm diagnosis and render genetic counseling possible. In this review, we collect and summarize 908 alleles (201 distinct variants, including 3 polymorphisms previously considered as disease-causing variants) from 478 individuals diagnosed with MPS VI, identified from literature and public databases. Each variant is further analyzed for clinical classification according to American College of Medical Genetics and Genomics (ACMG) guidelines. Results highlight the heterogeneity of ARSB alleles, with most unique variants (59.5%) identified as missense and 31.7% of unique alleles appearing once. Only 18% of distinct variants were previously recorded in public databases with supporting evidence and clinical significance. ACMG recommends publishing clinical and biochemical data that accurately characterize pathogenicity of new variants in association with reporting specific alleles. Variants analyzed were sent to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and MPS VI locus-specific database (http://mps6-database.org) where they will be available. High clinical suspicion coupled with diagnostic testing for deficient ASB activity and timely submission and classification of ARSB variants with biochemical and clinical data in public databases is essential for timely diagnosis of MPS VI.

KEYWORDS
ARSB, arylsulfatase B, ASB, databases, lysosomal storage disorder, MPS VI, variants

1 | BACKGROUND

Mucopolysaccharidosis type VI (MPS VI, Maroteaux–Lamy syndrome, MIM# 253200) is a rare autosomal recessive disease caused by pathogenic variants in the arylsulfatase B (ARSB) gene, coding for one of the enzymes deputed to the degradation of mucopolysaccharides within lysosomes. Pathogenic ARSB variants result in deficient activity of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase (aryl-sulfatase B, ASB, EC 3.1.6.12), causing excessive lysosomal storage and elevated urinary excretion of ASB enzyme substrates, specifically the glycosaminoglycans (GAGs) dermatan sulfate and chondroitin-4-sulfate. Increased lysosomal GAG storage causes cellular injury and can result in a diverse range of clinical manifestations (Jurecka et al., 2011b; Valayannopoulos, Nicely, Harmatz, & Turbeville, 2010; Wang, Bodamer, Watson, & Wilcox, 2011).

The prevalence of MPS VI varies among populations, with estimates ranging from 0.0132 per 100,000 live births in Poland to 8.0 per 100,000 live births in Saudi Arabia to 20.0 per 100,000 live births in Monte Santo, a small town in Brazil (Costa-Motta et al., 2014; Jurecka, Ługowska, Golda, Czartoryska, & Tylki-Szymańska, 2015; Moammar, Cheriyiyan, Mathew, & Al-Sannaa, 2010; Vairo et al., 2015). While several variables likely influence prevalence rates across populations, it is expected that populations with higher degrees of consanguinity display a higher prevalence of the disease (Baehner et al., 2005;
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in association with supporting evidence/clinical classification. The
previously recorded in public databases (ClinVar, EmvClass, ClinVitae)
201 unique variants reported in the literature and in public databases. Of
et al., 2015). In this study, we collected and analyzed all the
association with clinical classification and validating data (Richards
Genomics (ACMG) recommend that gene variants be published in
confirm gene variants that are not yet associated with a clinical phenotype.
Guidelines from the American College of Medical Genetics and
Genomics (ACMG) recommend that gene variants be published in
association with clinical classification and validating data (Richards et al., 2015). In this study, we collected and analyzed all the ARSB variants reported in the literature and in public databases. Of the 201 unique ARSB variants summarized here, only 18% had been previously recorded in public databases (ClinVar, EmvClass, ClinVitae) in association with supporting evidence/clinical classification. The present update illustrates the heterogeneity of alleles associated with
MPS VI, the need of a critical evaluation and correct classification of
the identified variants, and the lack of prior representation in
publicly available variant databases of characterized ARSB alleles.
Variants analyzed in the present paper have been sent to ClinVar
(https://www.ncbi.nlm.nih.gov/clinvar/), where they will be publicly available.

We emphasize the importance to maintain high clinical suspicion
during MPS VI diagnosis, as many ARSB alleles identified via molec-
ular testing alone may have yet to be formally classified as MPS VI-
associated and cannot be considered diagnostic per se. Timely submis-
sion and classification of ARSB variants in public databases, in associa-
tion with biochemical and clinical data, will help facilitate timely future diagnoses of MPS VI.

2 | METHODOLOGY

To identify all reported pathogenic ARSB variants, a literature search was performed in Embase on October 26, 2017 using the population, intervention and comparators, outcome (PICO) framework where the population was MPS VI (and relevant synonyms) and the outcome was genetic variant (and relevant synonyms); intervention and compar-
ators were not applicable. Results were limited to studies in humans and
conference abstracts were excluded. As case reports containing vari-
ant data are often not indexed to reflect the presence of this infor-
mation, a targeted MPS VI case report search was also performed in
Embase on September 13, 2017 to supplement the results. The search
strategies are described in detail in Supp. Mat. S1. Manual searching
of relevant journals not indexed in Embase was also performed. Each
identified publication was screened by a single reviewer for informa-
tion regarding ARSB variants. Reports of individuals with ARSB vari-
ants, where variants were reported as linked to MPS VI, were extracted
and assessed for redundant reporting to the extent possible.

Public databases were accessed via searches for ”ARSB” in ClinVar,
EmvClass, ClinVitae, and Human Gene Mutation Database (HGMD)
(Hart et al., 2014; Stenson et al., 2003). Identified variants were com-
pared with those from the literature search.

For the three-dimensional visualization of arylsulfatase B, an image
was created using the PyMOL Molecular Graphics System, Version 1.8
Schrödinger, LLC with atomic coordinates from Brookhaven Protein
Data Bank accession number 1FSU (Bond et al., 1997).

All variants were described according to the NCBI Reference
Sequence NM_000046.4 and the corresponding protein sequence
NP_0003037.2. In cases where the reported variant did not reference a
particular sequence, coordinates were mapped to NM_000046.4 [e.g.,
“deletion of exon 4” to “c.(690+1_691-1),(898+1_899-1)del”]. Variants
cDNA and protein annotations were further checked through “Name
checker” tool (https://mutalyzer.nl/) according to the HGVS nomencla-
ture v15.11 (https://varnomen.hgvs.org/) and “VariantValidator” tool
(https://variantvalidator.org), and corrected if necessary. In case of
discrepancies in amino acid sequence impact of coding variants, the
variant was simulated and translated in silico using NM_000046.4. All
discrepancies and other misreported variants found in our literature
analysis were collected in Table 1.
### TABLE 1  
Summary of misreported variants and corrections

| Paper Annotation | Correct Annotation | Type of Error | Error Source | Notes |
|------------------|--------------------|---------------|--------------|-------|
| c.208_215del     | p.(P70fs*123)      | Amino acid position | Kantaputra et al. (2014) |
| c.213insGCGC     | p.(Leu72Alafs*57)  | Amino acid position | Sandberg et al. (2008) |
| c.247_248delC    | p.(D302fs*43)      | Amino acid position | Jurecka et al. (2014b) |
| c.262_263insA    | p.(Pro89Alafs*38)  | Amino acid position | Jurecka et al. (2014b) |
| c.306_312delICT  | p.(Y103fs*25)      | Amino acid position | Uttarilli et al. (2015) |
| c.31091insCCTGAAG | p.(Glu250Asfs*4)   | Reference sequence | Jurecka et al. (2011b) |
| c.356_358del     | p.(Pro119_Ser120delinsArg) | Amino acid position | Karageorgos et al. (2004) |
| c.375_376dup     | p.(Glu126*)        | Amino acid position | Jurecka et al. (2012a) |
| c.436G>T         | p.(Trp146Leu)      | Amino acid position | Simonaro and Schuchman (1995) |
| c.496delT        | p.(Phe166Leufs*18) | Amino acid position | Uttarilli et al. (2015) |
| c.592C>T         | p.(R197*)          | Amino acid position | Petry et al. (2005) |
| c.629_635del     | p.(Tyr210*)        | Amino acid position | Karageorgos et al. (2007b) |
| c.659_660del     | p.(I220fs*5)       | Amino acid position | Uttarilli et al. (2015) |
| c.785insA        | p.(Asn262Lysfs*14) | Amino acid position | Chistiakov et al. (2014) |
| c.883_884dupTT   | p.(Ile296Serfs*43) | Amino acid position, annotation | Ferla et al. (2015) |
| c.1036delG       | p.(E346Sfs*11)     | Amino acid position, annotation | Ferla et al. (2015) |
| c.1285_1286insT  | p.(His430Profs*5)  | Amino acid position | Isbrandt et al. (1994) |
| c.1325C>T        | p.(T442R)          | Amino acid position | Karageorgos et al. (2007b) |
| c.1348G>C        | p.(Ile220Serfs*5)  | Amino acid position | Nouri et al. (2012) |
| c.1531_1553del   | p.(V512Pfs*3)      | Amino acid position, annotation | Petry et al. (2005) |

For the variants collected, all the evidence available was exploited for variants analysis and clinical classification, according to the approach recommended by ACMG (Richards et al., 2015). Finally, all variants were submitted to ClinVar database with associated evidence and literature references (ClinVar accession numbers from SCV000802937 to SCV000803138).

### 2.1 ARSB gene structure

The ARSB gene is located on chromosome 5q13-14 and spans a region of 206 kb. ARSB contains eight exons which encode an mRNA transcript of 2228 base pairs and a protein of 533 amino acids, including a signal peptide at the N-terminal (Brands et al., 2013a; Karageorgos et al., 2007b; Litjens et al., 1989; Litjens & Hopwood, 2001; Peters et al., 1990). ARSB contains an alkaline phosphatase-like domain and two conserved sulfatase regions near the N-terminal (Figure 2; Bond et al., 1997; Peters et al., 1990).

### 3 VARIANTS

#### 3.1 Overview and summary statistics

A uniform summary of all ARSB variants identified in individuals with MPS VI was created based on the literature and database searches. The PICO search identified 208 records, the targeted case report search identified 198 records, and 5 additional records were identified from other sources. Removal of duplicates led to a total of 380 unique records. Manual screening yielded 74 publications containing 615 individuals with ARSB variant information. Duplicate entries were eliminated based on direct references (“patient reported previously”).
and if the entries contained no conflicting attributes along with identical genotypes, ethnicities, gender, and age of diagnosis/start of ERT. This ultimately resulted in the aggregation of 908 alleles from 478 individuals diagnosed with MPS VI (Supp. Mat. S2). No additional unpublished pathogenic variants were identified through the public database search. The 908 alleles encompassed 198 unique non-polymorphic variants (Supp. Mat. S3). However, Supp. Mat. S3 also reports three benign variants, highlighted in blue, which previously had been erroneously reported as disease-causing variants. A shorter version of Supp. Mat. S3, containing only variants described in more than one independent family, is represented by Table 2.

Individual characteristics of the patients were highly variable, highlighting the clinical heterogeneity of MPS VI. Patients displayed a wide range of clinical manifestations, ages at diagnosis, and national/geographic and ethnic backgrounds. Of the identified individuals, 54.8% (262/478) were homozygous for pathogenic ARSB variants, 35.6% (170/478) were heterozygous, 9.2% (44/478) had only one allele reported, and two individuals (0.4%) had both alleles unidentifiable. For alleles not characterized, we considered also those alleles carrying a polymorphism erroneously considered a disease-causing variant. Patient gender was reported in 40% (190/478) of individuals and was split roughly evenly between genders (55% male; 45% female). Age at diagnosis (reported in 172 individuals) ranged from <1 year to 45 years of age, with a mean of 7.8 years and median of 5.0 years. Two patients, both with an older affected sibling, were diagnosed at birth (Furujo, Kubo, Kosuga, & Okuyama, 2011; Mendelsohn et al., 2013). Approximately one-third (31.7%) of unique variants appeared only once in the population assessed, with an additional 28.5% appearing twice.

In agreement with previous reports (Karageorgos et al., 2007b), most unique variants were missense (59.5%); followed by small deletions (13.5%), nonsense (12.0%), splice site or intronic variants (5.0%), small duplications (3.0%), and large deletions (3.0%) (Figure 1). Stop-loss mutations were identified in 1.0% of variants.

Commonly reported variants occur throughout the length of the ARSB gene, and include missense, nonsense, deletion, and intronic alleles (Figure 2). Only two alleles were reported more than 50 times in our sample taken globally: c.962T>C [p.(Leu321Pro)] and c.454C>T [p.(Arg152Trp)] (Table 2). These alleles are located in exons 5 and 2, respectively.

The most commonly described nonsense allele, c.979C>T [p.(Arg327*)], was reported 16 times (1.8%) in our sample, and is expected to result in premature truncation of ARSB midway through exon 5. c.979C>T is identified in various populations, including individuals from North and South America, Europe, and Asia, and was detected in a homozygous state in five individuals (5 of 11, 45.5%).

The missense mutation c.284G>A [p.(Arg95Gln)] is present in six individuals in our sample, but never in the homozygous state. Of note, Arg95 is the final residue making up the N-terminal sulfatase domain (CTPSR sequence), which is conserved in several sulfatasases including human arylsulfatase A, human steroid sulfatase, and sea urchin arylsulfatase (Peters et al., 1990). Individuals with this variant vary in phenotypic severity, presumably based on the allele present in the other ARSB parental allele.

Three large deletions within the alkaline phosphatase-like domain have also been reported. One large deletion that includes exons 2 and 3 was identified in homozygosity in a male Chinese individual with a classical, rapidly progressing MPS VI phenotype (Zheng et al., 2014); also a 13.8 kb deletion encompassing exons 2 and 3, whose breakpoints were defined, was reported in homozygosity in a boy with disease onset at 3 years (Iltiwut et al., 2017). A deletion of exon 4 [c.(690+1_691-1), (898+1_899-1)del; breakpoints unknown] in its entirety was reported in homozygosity in one female Taiwanese individual. This deletion is expected to result in frameshift, premature truncation, and loss of significant portions of the catalytic domain (Lin, Ke, Chou, Wang, & Tsai, 2015). Finally, a genomic deletion of exon 5 [c.(898+1_899-1), (1142+1_1143-1)del] was reported eight times, once in homozygosity in a male Italian individual, who displayed a classical, severe MPS VI phenotype (Ferla et al., 2015). In only one case, the boundaries were defined (Villani et al., 2010).

### 3.2 Most common alleles

Of the 12 most commonly reported alleles, 7 of these are missense mutations, 3 are nonsense mutations, and 2 are intronic/splice site mutations. The seven most commonly reported alleles (c.454C>T, c.962T>C, c.629A>G, c.753C>G, c.533A>T, c.1143-8T>G, c.944G>A) make up 31.5% of all alleles reported. Together, the three most frequently reported alleles make up 18.5% of all alleles reported (Table 3). Importantly, the allele frequency reported here may not represent the real-world distribution of alleles, as well-characterized alleles are less likely to be published and therefore may be underrepresented in the literature.

### 3.3 Geographic distribution

Information regarding ethnic or national origin was available for 406 individuals (85% of sample), and individuals from Africa, Asia, Australia, Europe, North America, and South America were included, although most of them came from the northern hemisphere. Allelic heterogeneity varied among individual populations, although rare or private mutations were prominent in all populations studied. Individuals from Italy, the United States, and Colombia were the most genetically homogeneous, with the largest proportion of rare or private mutations (≈84% of individuals) in the populations studied (Figure 3).

Turkey was the most heavily represented national origin, with information reported for 55 Turkish individuals (13.5% of total reported). Of the major populations studied, the Turkish population was the most genetically homogenous, and 54 of 55 Turkish individuals were homozygous. Variant p.(Leu321Pro) accounted for nearly half of all alleles reported in Turkish individuals (50/110). c.1036del was heavily represented in this population. Interestingly, p.(Leu321Pro) did not appear to be associated with a phenotypic signature in this population, possibly due to the relatively high rates of consanguineous marriages in Turkey, leading to a higher proportion of homozygous alleles across the genome and variable clinical phenotypes (Koc, 2008; Zanetti et al., 2014).
| Exon/Intron | Nucleotide Change | Predicted Amino Acid Change | Number of Reports | Clinical Significance       | Variant Identifier (ClinVar ID and/or dbSNP Number, if Available) |
|------------|------------------|----------------------------|------------------|-----------------------------|---------------------------------------------------------------|
| Exon 1     | c.157G>A         | p.(Asp53Asn)               | 6                | Likely pathogenic           |                                                               |
| Exon 1     | c.160G>A         | p.(Asp54Asn)               | 5                | Likely pathogenic           |                                                               |
| Exon 1     | c.171G>C         | p.(Trp57Cys)               | 2                | Uncertain significance      |                                                               |
| Exon 1     | c.175G>A         | p.(Asp59Asn)               | 2                | Uncertain significance      |                                                               |
| Exon 1     | c.215T>G         | p.(Leu72Arg)               | 13               | Likely pathogenic           |                                                               |
| Exon 1     | c.238del         | p.(Val80Cysfs*34)          | 6                | Pathogenic                  | ClinVar Allele ID: 15920; rs750845916                         |
| Exon 1     | c.245del         | p.(Leu82Argfs*32)          | 2                | Likely pathogenic           |                                                               |
| Exon 1     | c.245T>G         | p.(Leu82Arg)               | 3                | Likely pathogenic           | rs750845916                                                   |
| Exon 1     | c.262C>T         | p.(Gln104His)              | 4                | Uncertain significance      |                                                               |
| Exon 1     | c.275A>G         | p.(Glu111Glu)              | 5                | Uncertain significance      |                                                               |
| Exon 2     | c.332A>C         | p.(His111Pro)              | 5                | Uncertain significance      |                                                               |
| Exon 2     | c.349T>C         | p.(Cys117Arg)              | 6                | Likely pathogenic           | ClinVar Allele ID: 15917                                      |
| Exon 2     | c.384_386del     | p.(Leu129del)              | 3                | Uncertain significance      | ClinVar Allele ID: 106583                                     |
| Exon 2     | c.395T>C         | p.(Leu132Pro)              | 3                | Likely pathogenic           |                                                               |
| Exon 2     | c.427del         | p.(Val143Serfs*41)         | 12               | Pathogenic                  | rs746206847                                                   |
| Exon 2     | c.430G>A         | p.(Glu144Glu)              | 8                | Likely pathogenic           |                                                               |
| Exon 2     | c.437G>C         | p.(Trp146Ser)              | 2                | Uncertain significance      |                                                               |
| Exon 2     | c.438G>A         | p.(Trp146Glu)              | 4                | Likely pathogenic           |                                                               |
| Exon 2     | c.440A>C         | p.(His147Glu)              | 2                | Uncertain significance      |                                                               |
| Exon 2     | c.454C>T         | p.(Arg152Trp)              | 62               | Pathogenic                  |                                                               |
| Exon 2     | c.464G>A         | p.(Cys155Tyr)              | 3                | Uncertain significance      |                                                               |
| Exon 2     | c.478C>T         | p.(Arg160Cys)              | 16               | Pathogenic                  |                                                               |
| Exon 2     | c.479G>A         | p.(Arg160Glu)              | 13               | Likely pathogenic           |                                                               |
| Exon 2     | c.499G>A         | p.(Glu167Arg)              | 5                | Uncertain significance      |                                                               |
| Exon 3     | c.511G>A         | p.(Glu171Ser)              | 3                | Likely pathogenic           |                                                               |
| Exon 3     | c.533A>T         | p.(His178Leu)              | 29               | Likely pathogenic           |                                                               |
| Exon 3     | c.571C>T         | p.(Arg191Cys)              | 7                | Pathogenic                  |                                                               |
| Exon 3     | c.574T>C         | p.(Cys192Arg)              | 15               | Likely pathogenic           |                                                               |
| Intron 3   | c.691-1G>A       | —                           | 3                | Likely pathogenic           | rs6870443                                                   |
| Exon 4     | c.707T>C         | p.(Leu236Pro)              | 2                | Uncertain significance      | ClinVar Allele ID: 15918                                     |
| Exon 4     | c.710C>A         | p.(Ala237Asp)              | 4                | Likely benign               |                                                               |
| Exon 4     | c.716A>G         | p.(Glu239Arg)              | 3                | Uncertain significance      |                                                               |
| Exon 4     | c.753C>G         | p.(Tyr251*)                | 43               | Pathogenic                  |                                                               |
| Exon 4     | c.765T>A         | p.(Tyr255Glu)              | 3                | Likely pathogenic           | rs749015246                                                   |
| Exon 4     | c.797A>C         | p.(Tyr266Ser)              | 7                | Uncertain significance      |                                                               |
TABLE 2 (Continued)

| Exon/Intron | Nucleotide Change | Predicted Amino Acid Change | Number of Reports | Clinical Significance | Variant Identifier (ClinVar ID and/or dbSNP Number, if Available) |
|-------------|-------------------|----------------------------|-------------------|----------------------|---------------------------------------------------------------|
| Exon 5*     | c.(898+1_899-1),(1142+1_1143-1)del | —                          | 6                 | Pathogenic           |                                                               |
| Exon 5      | c.899-1341_1142+1051del | —                          | 1                 | Pathogenic           |                                                               |
| Exon 5      | c.903C>G          | p.(Asn301Lys)              | 4                 | Likely pathogenic    | rs779378413                                                   |
| Exon 5      | c.904G>A          | p.(Gly302Arg)              | 10                | Uncertain significance |                                                               |
| Exon 5      | c.908G>A          | p.(Gly303Glu)              | 5                 | Likely pathogenic    |                                                               |
| Exon 5      | c.937C>G          | p.(Pro313Ala)              | 11                | Likely pathogenic    |                                                               |
| Exon 5      | c.943C>T          | p.(Arg315*)                | 10                | Likely pathogenic    | rs727503809                                                   |
| Exon 5      | c.944G>A          | p.(Arg315Gln)              | 22                | Likely pathogenic    |                                                               |
| Exon 5      | c.960C>T          | p.(Ser320Arg)              | 3                 | Uncertain significance |                                                               |
| Exon 5      | c.962T>C          | p.(Leu321Pro)              | 60                | Pathogenic           |                                                               |
| Exon 5      | c.966G>A          | p.(Trp322*)                | 2                 | Likely pathogenic    | rs399123125                                                   |
| Exon 5      | c.968G>A          | p.(Trp322*)                | 6                 | Likely pathogenic    | rs201168448                                                   |
| Exon 5      | c.971G>T          | p.(Gly324Val)              | 5                 | Likely pathogenic    |                                                               |
| Exon 5      | c.979C>T          | p.(Asp327*)                | 3                 | Uncertain significance |                                                               |
| Intron 5    | c.1143-1G>C       | —                          | 16                | Pathogenic           | ClinVar Allele ID: 15926                                      |
| Intron 5    | c.1143-3G>A       | —                          | 24                | Likely pathogenic    | ClinVar Allele ID: 15927; rs431905496                          |
| Exon 6      | c.1151G>A         | p.(Ser341Asn)              | 9                 | Benign               | ClinVar Allele ID: 98263; rs25414                              |
| Exon 6      | c.1168G>A         | p.(Glu346Serfs*11)         | 13                | Pathogenic           |                                                               |
| Exon 6      | c.1178A>C         | p.(His393Pro)              | 10                | Uncertain significance | ClinVar Allele ID: 15925                                      |
| Exon 6      | c.1178A>G         | p.(Gly396Ser)              | 4                 | Uncertain significance |                                                               |
| Exon 6      | c.1197C>G         | p.(Phe396Arg)              | 3                 | Uncertain significance |                                                               |
| Intron 6    | c.1213+5G>A       | —                          | 2                 | Uncertain significance |                                                               |
| Intron 6    | c.1213+6T>C       | —                          | 3                 | Pathogenic           |                                                               |
| Exon 7      | c.1214G>A         | p.(Cys405Tyr)              | 3                 | Uncertain significance | ClinVar Allele ID: 15919; rs118203941                          |
| Exon 7      | c.1289A>G         | p.(His430Arg)              | 3                 | Likely pathogenic    |                                                               |
| Exon 7      | c.1325C>T         | p.(Thr442Met)              | 3                 | Uncertain significance | ClinVar Allele ID: 368534                                      |
| Exon 7      | c.1336G>A         | p.(Gly446Ser)              | 3                 | Uncertain significance |                                                               |
| Intron 7    | c.1336+2T>G       | —                          | 7                 | Pathogenic           | rs768012515                                                   |
| Exon 8      | c.1340G>T         | p.(Cys447Phe)              | 4                 | Uncertain significance |                                                               |
| Exon 8      | c.1350G>C         | p.(Trp450Cys)              | 11                | Likely pathogenic    | rs55578523                                                    |
| Exon 8      | c.1366C>T         | p.(Gln456*)                | 4                 | Pathogenic           | rs200188234                                                   |
| Exon 8      | c.1415T>C         | p.(Leu472Pro)              | 2                 | Likely pathogenic    |                                                               |
| Exon 8      | c.1450A>G         | p.(Arg484Gly)              | 2                 | Uncertain significance | ClinVar Allele ID: 187113                                      |
| Exon 8      | c.1457A>T         | p.(Asp486Val)              | 6                 | Uncertain significance |                                                               |
| Exon 8      | c.1534_1556del    | p.(Val512Profs*3)          | 13                | Uncertain significance |                                                               |
| Exon 8      | c.1562G>A         | p.(Cys521Tyr)              | 9                 | Uncertain significance |                                                               |
| Exon 8      | c.1577del         | p.(Thr526Metfs*48)         | 8                 | Pathogenic           |                                                               |
| Exon 8      | c.1582_1596del    | p.(Val528_Trp532del)       | 2                 | Uncertain significance |                                                               |

Variants classified per Richards et al. (2015). ClinVar accessed 06/05/2017.  
*aDeletion of exon 5 without defined boundaries.
Mutations in Trp450 [p.(Trp450Cys) and p.(Trp450Leu)] were found exclusively in individuals from India, and resulted in a range of clinical phenotypes. Variant p.(Phe399Leu) (c.1197C>G) was commonly reported in individuals from both China (59% of individuals) and Taiwan (38% of individuals), and was associated with a classical phenotype in these patients.

3.4 ASB structure and variants of special interest

Arylsulfatase B includes two domains; a larger N-terminal domain and a smaller C-terminal domain; and is reported to function as a monomer (Mathew et al., 2015; McGovern, Vine, Haskins, & Desnick, 1982). ASB contains two conserved sulfatase regions near the N-terminal (Bond et al., 1997; Peters et al., 1990). The active site pocket is located near the surface of the folded enzyme, and consists of Asp53, Asp54, Cys91, Pro93, Ser94, Arg95, Lys145, His147, His242, Asp300, and Lys318. A cluster of conserved cysteine residues is also thought to be required for enzymatic activity (Bond et al., 1997; Brooks et al., 1995).

Pathogenic variants in ARSB do not appear to be concentrated in any particular region or domain of the ASB protein. Although pathogenic variants in or near the active site pocket were identified, many are located remotely from the active site (Figure 4). Importantly, genotype–phenotype correlations in MPS VI have remained elusive, due (at least in part) to the large proportion of rare or private ARSB mutations, limited phenotypic reporting, and overall heterogeneity of the disease (Vairo et al., 2015; Valayannopoulos et al., 2010). This is especially true for missense mutations, whose effect on ASB enzyme function may not be immediately apparent (Valayannopoulos et al., 2010). In addition, residual ASB enzyme activity does not clearly
TABLE 3 Most common ARSB pathogenic and likely pathogenic variants reported in the assessed population*

| Nucleotide Change | Amino Acid Change | Times Reported, n (%) |
|-------------------|------------------|-----------------------|
| c.454C>T         | p.(Arg152Trp)    | 62 (6.8)              |
| c.962T>C         | p.(Leu321Pro)    | 60 (6.6)              |
| c.629A>G         | p.(Tyr210Cys)    | 46 (5.1)              |
| c.753C>G         | p.(Tyr251*)      | 43 (4.7)              |
| c.533A>T         | p.(His178Leu)    | 29 (3.2)              |
| c.1143-8T>G      | —                | 24 (2.6)              |
| c.944G>A         | p.(Arg315Gln)    | 22 (2.4)              |
| c.1197C>G        | p.(Phe399Leu)    | 17 (1.9)              |
| c.1143-1G>C      | —                | 16 (1.8)              |
| c.478C>T         | p.(Arg160*)      | 16 (1.8)              |
| c.979C>T         | p.(Arg327*)      | 16 (1.8)              |
| c.574T>C         | p.(Cys192Arg)    | 15 (1.7)              |

Nucleotide changes are per NM_0000446.4; protein changes are per NP_000037.2.

*As it is uncommon to publish findings of well-characterized alleles, these alleles may be underrepresented in the literature.

correlate with the severity of clinical phenotype in patients with MPS VI. While detailed structural analysis or genotype–phenotype correlation are beyond the scope of this publication, our observations mentioned previously are generally consistent with previous reports (e.g., Karageorgos et al., 2007a,b; Lin et al., 2008; Saito, Ohno, Sugawara, & Sakuraba, 2008).

c.215T>G [p.(Leu72Arg)] is associated with early onset and classical, rapidly progressing disease, consistent with a lack of detectable ASB protein and enzymatic activity when expressed in cultured fibroblasts (Karageorgos et al., 2004, 2007a,b; Petry et al., 2005). Leu72 resides in the 3' coil of ASB, and a leucine-to-arginine change is predicted to disrupt formation of this coil and result in severe disturbance of ASB structure (Karageorgos et al., 2007a). Of note, Leu72 is conserved in certain other sulfatases, including galactose-3-sulfatase (ASA) and N-acetylgalactosamine-6-sulfatase (GALNS) (Bond et al., 1997).

c.454C>T [p.(Arg152Trp)] was the most frequently reported allele in our sample (62/916; 6.8%). p.(Arg152Trp) was detected in several distinct ethnic populations, including individuals from Turkey, Russia, Northern Europe, Western Europe, and Eastern Europe, where specifically it is thought to be a founder mutation (Chistiakov et al., 2014; Jurecka et al., 2012a). p.(Arg152Trp) has been identified in both heterozygous and homozygous states, and is associated with non-classical, slowly progressing phenotypes in homozygotes, with some patients living into their thirties (Brands et al., 2013a; Chistiakov et al., 2014; Ferla et al., 2015; Jurecka et al., 2011b, 2012a; Karageorgos et al., 2007b; Thümler et al., 2012). Interestingly, p.(Arg152Trp) has been associated with a primarily cardiac and musculoskeletal phenotype in certain patients, but whether this indicates a genuine genotype–phenotype correlation remains unclear (Brands et al., 2013a; Jurecka et al., 2011a). Trp152 is located near the conserved hexapeptide sequence 144-Gly-Lys-Trp-His-Leu-Gly-149, the largest connecting stretch of identity between arylsulfatases (Peters et al., 1990; Voskoboeva, Isbrandt, von Figura, Krasnopol'skaya, & Peters, 1994).

c.479G>A [p.(Arg160Gln)] results from a transition in a CpG dinucleotide (Litjens & Hopwood, 2001). p.(Arg160Gln) has been observed in both heterozygous and homozygous states (Kantaputra et al., 2014; Mathew et al., 2015; Villani et al., 2010; Voskoboeva et al., 1994). The mechanism by which the arginine-to-glutamine alteration contributes to disease is not well understood, as its effect on enzyme structure is predicted to be minimal (Mathew et al., 2015). However, Arg160 immediately precedes the dipeptide sequence 161-Gly-Phe-162, which is

FIGURE 3 Most common alleles for the 10 most frequent nationalities/ethnic backgrounds reported. The number of times an allele was encountered in a population is shown in parentheses. In grey other variants, reported less or equal to 5 times
FIGURE 4  ASB structure and variants of special interest
Three-dimensional structure of mature human ASB protein overlaid with variants of interest. The enzyme active site pocket is indicated by colored space-filling models.

conserved in arylsulfatases (Peters et al., 1990; Voskoboeva et al., 1994). Interestingly, wild-type arylsulfatase A (ASA) also contains a glutamine in the position immediately preceding the conserved dipeptide sequence (Peters et al., 1990).

Of note, a nonsense mutation [p.(Arg160*)] has also been identified at Arg160, and thus Arg160 has been suggested to be a mutational hot spot in the ARSB gene (Kantaputra et al., 2014; Voskoboeva et al., 1994). Further supporting this idea is the observation that Arg160 mutations have been found in diverse populations and ethnic groups, including patients of Belarusian, German, Indian, Italian, Russian, Spanish, and Taiwanese descent (Garrido et al., 2007; Jurecka et al., 2012a; Kantaputra et al., 2014; Thümler et al., 2012; Voskoboeva et al., 1994).

c.629A>G [p.(Tyr210Cys)], previously reported as the most common pathogenic variant in patients with MPS VI (Karageorgos et al., 2007b), was the third most frequently reported allele (49/916, 5.3%) in this analysis, found in 11.1% of individuals collected. Individuals with the p.(Tyr210Cys) mutation spanned several ethnic populations, including individuals from Australia and Western Europe. Previous work from Bradford, Litjens, Parkinson, Hopwood, and Brooks (2002) has demonstrated that the substitution of Tyr210 with cysteine causes impaired protein processing, trafficking, and instability of the mutant ASB protein, ultimately leading to decreased levels of mature, functional protein in lysosomes. Tyr210 is located between the sixth beta-strand and fifth alpha-helix of ASB (Saito et al., 2008). The tyrosine-to-cysteine change is predicted to result in a relatively stable enzyme which should retain some enzymatic activity (Saito et al., 2008). As such, patients with the p.(Tyr210Cys) mutation generally exhibit a non-classical, slowly progressing form of MPS VI, and the p.(Tyr210Cys) mutation may reduce the severity of other ARSB pathogenic variants when present in a compound heterozygous state (Bradford et al., 2002; Brands et al., 2013a; Litjens, Brooks, Peters, Gibson, & Hopwood, 1996; Saito et al., 2008). Interestingly, this allele has only been reported in a homozygous state twice (Gottwald et al., 2011; Mendelsohn et al., 2013), despite having a relatively high allele frequency reported in the Genome Aggregation Database (gnomAD; gnomad.broadinstitute.org, accessed October 26, 2017), a large population database. Both individuals had a non-classical form of the disease (Gottwald et al., 2011; Mendelsohn et al., 2013).

c.944G>A [p.(Arg315Gln)] has been reported in both heterozygous and homozygous states (Petry et al., 2005; Villani et al., 1999). Patients with two p.(Arg315Gln) alleles demonstrate a range of clinical phenotypes, from intermediate to classically severe (Di Natale et al., 2008; Karageorgos et al., 2007b; Petry et al., 2005; Villani et al., 1999). Arg315 is predicted to form a salt bridge with Asp466, as well as hydrogen bonds with Gly448 and Glu483 (Garrido et al., 2007; Mathew et al., 2015). Thus, the arginine-to-glutamine alteration is predicted to hinder both substrate binding and metal ion coordination (Villani et al., 1999). Two additional mutations at Arg315 have also been reported—the missense mutation p.(Arg315Pro) and the nonsense mutation p.(Arg315*), which produces a polypeptide roughly one-third the length of wild-type and is predicted to lack enzymatic activity (Di Natale et al., 2008; Ferla et al., 2015; Lin et al., 2008; Mathew et al., 2015; Villani et al., 2010). Both p.(Arg315Gln) and p.(Arg315Pro) result from an alteration in the CpG dinucleotide c.944G, suggesting this nucleotide may be a mutational hot spot in ARSB (Litjens & Hopwood, 2001; Mathew et al., 2015).

c.962T>C [p.(Leu321Pro)] was the second most common allele identified in the literature search (60/916; 6.6%). p.(Leu321Pro) was found primarily (but not exclusively) in patients of Turkish origin, where it is believed to be a founder mutation (Kantaputra et al., 2014). Interestingly, the c.962T>C mutation is not found in gnomAD (gnomAD.broadinstitute.org, accessed October 26, 2017), further supporting the hypothesis that this is primarily a population-specific variant. Individuals homozygous for p.(Leu321Pro) demonstrated a range of
clinical phenotypes, from a classic, rapidly progressing phenotype to a non-classical, slowly progressing phenotype (Isbrandt et al., 1994; Kantaputra et al., 2014; Zanetti et al., 2014). Of note, Leu321 is not conserved between the arylsulfatases, and the effect of the p.(Leu321Pro) mutation on the secondary structure of ASB is not known (Isbrandt et al., 1994).

c.1197C>G [p.(Phe399Leu)] was observed solely in individuals from China and Taiwan, and was heavily represented in both populations. p.(Phe399Leu) was associated with a severe phenotype in homozygous individuals (Lin et al., 2008; Yang, Wu, Lin, & Tsai, 2001; Zheng et al., 2014). One report suggests that the p.(Phe399Leu) allele may be associated with the polymorphisms c.1072G>A and c.1191G>A, and that these alleles may share a common origin (Lin et al., 2008).

3.5 | Homozygosity

Homozygote subjects are particularly represented in the analyzed population of MPS VI patients, 55% of all reported cases. Analysis of homozygotes may be useful for the in vivo genotype–phenotype correlation and also for the comprehension of the geographical distribution of the pathogenic variants. This analysis has identified 55 variants which were present in a homozygote condition only once; several other variants were present in the examined population 2–5 times. Only eight variants were present six or more times in homozygosis. Interestingly, these variants represent a particular geographical/ethnographical distribution, each being mostly present in only one ethnic group (Supp. Mat. S4). Of the 30 homozygote subjects with the variant p.(Leu321Pro), 25 are of Turkish background. All 21 homozygote subjects with the p.(Tyr251*) variant are Arabic, and 11 of the 16 homozygote subjects with the p.(Arg152Trp) variant are from Russia or from Baltic countries. Lastly, all 14 homozygote subjects with the variant p.(His178Leu) are Brazilian, and belong to a cluster of MPS VI patients living in Northeast Brazil. These subjects all show the same haplotype (Costa-Motta et al., 2014), which is likely due to a founder effect, with the contemporary presence of parental consanguinity and endogamy.

Our analysis of the genotype–phenotype correlation based on homozygotes was poorly informative for most variants, except p.(Tyr251*), p.(Arg152Trp), and p.(Cys192Arg). Variant p.(Tyr251*) was associated with a severe phenotype in most subjects (17/21), p.(Arg152Trp) was associated with an attenuated phenotype in almost all subjects (14/15), and p.(Cys192Arg) was associated with an attenuated phenotype in half of the subjects reported (3/6).

3.6 | Conflicting issues

Two polymorphisms, or benign variants erroneously reported as disease-causing variants, were collected in this review, despite only collecting variants reported as linked to disease. c.1151G>A [p.(Ser384Asn)] and c.98C>T [p.(Ala33Val)] are now known to be benign. These variants are highlighted in blue in Supp. Mat. S3, and also reported in Supp. Mat. S2. Moreover, a list of variants in ARSB with frequency>1% in gnomAD (gnomad.broadinstitute.org, accessed October 26, 2017) is reported in Supp. Mat. S5. As for variant c.1115G>A [p.(Ser384Asn)], it has been determined to not be a disease-causing variant a few years ago (Zanetti et al., 2009), but it had been reported as such in nine alleles in data collected from previously published literature.

A re-analysis of molecular data of the entire gene in those patients with only one allele identified, or with one allele carrying a known polymorphism, should be strongly encouraged, searching for potential missed variants such as large deletions, intronic or promotorial variants. On the contrary, all the synonymous variants encountered in our analysis [c.246G>A, p.(Leu82Leu); p.(Ile114Ile); p.(Leu124Leu); c.1515C>T, p.(Tyr505Tyr)] were considered polymorphisms. Actually these variants should be considered potentially pathogenic, given their absence from gnomAD database, with the exception of p.(Leu82Leu) which has a frequency of 0.003858%. In fact, despite their name, synonymous or silent variants are no longer considered “neutral” variants with no functional consequences on protein, but are considered variants that may affect the function of translated protein through different cellular mechanisms (Hunt, Simhadri, Iandoli, Sauna, & Kimchi-Sarfaty, 2014). However, not enough information is available from the literature for the classification of these ARSB synonymous variants according to ACMG criteria, therefore in this study they are reported as “not enough evidence.” Moreover, an underestimation of this kind of nucleotide substitution in our study has to be taken into account and, in light of this fact, a re-evaluation of those patients missing the identification of one (or both) variant(s) or with one allele (or both alleles) carrying only a polymorphism should be performed, as mentioned above.

3.7 | ACMG classification of ARSB variants

A classification of ARSB sequence variants was performed according to ACMG criteria. This analysis evidenced that, for most variants (about 45%), there is not enough evidence in the literature for their classification, and thus they are variants of “uncertain significance.” The remaining variants were classified in “likely pathogenic” (37%) and “pathogenic” (16%). One variant (c.113_121del), despite having a frequency < 1% (0.1719%) resulted “benign” using the ACMG approach. Hence, this in-frame deletion seems to be a polymorphism, if we consider only the South Asian population in which it has a frequency of 1.37%.

These results provide evidence for the need of a deeper analysis of the variants diagnosed in MPS VI patients: this should include both in vitro and in silico analysis. Moreover, specific attention has to be paid to the annotation of the variants: the use of the most updated gene variant nomenclature suggested by HGVS (v15.11) is recommended so as to use shared rules that could unequivocally describe a specific variant with no risk of misreporting.

4 | CLINICAL RELEVANCE

4.1 | Diagnostic strategies and complications

Mucopolysaccharidoses, including MPS VI, are generally diagnosed by a combination of clinical findings and laboratory tests. Often, the first
biochemical test performed upon clinical suspicion of MPS is the analysis of urinary glycosaminoglycans (uGAGs). While commonly used, a positive uGAG test is not sufficient for an MPS diagnosis, and a negative uGAG test is not sufficient to rule out a diagnosis of MPS (Cobos, Steglich, Santer, Lukacs, & Gal, 2015; Vairo et al., 2015). Additionally, individuals with non-classical MPS VI and/or older individuals with MPS VI may not show strong uGAG elevation (Wood et al., 2012). While tandem mass spectrometry-based (MS/MS) testing of uGAGs appears to have increased sensitivity in these individuals, the MS/MS format of the uGAG test is not widely available. Due to the lack of MS/MS availability and the potential to misdiagnose MPS, a lysosomal enzyme panel is often preferable for MPS screening (Wood et al., 2012).

When clinical suspicion for MPS is high (or after a finding of elevated uGAGs), individuals undergo testing for lysosomal enzyme activity via fibroblasts, leukocytes, or dried blood spots (DBS), which are commonly used due to their low cost, ease of collection, and simple storage requirements (Cobos et al., 2015; Vairo et al., 2015; Wood et al., 2012). Although enzyme activity varies among individuals (e.g., due to the specific ARSB variant, assay method, etc.), most individuals with MPS VI demonstrate ASB activity <10% of the lower limit of normal level across the disease spectrum (Valayannopoulos et al., 2010).

A confirmation of MPS VI diagnosis does require the specific pathogenic variants in ARSB to be determined. Moreover, variant identification is beneficial to individuals with MPS VI and their relatives as it can render genetic counseling possible and potentially inform treatment decisions. Although most genotype–phenotype correlations remain uncertain in MPS VI, some variants (e.g., large deletions, nonsense mutations, frameshift, and certain missense mutations) do appear to be correlated with a classic, rapidly progressing phenotype (Karageorgos et al., 2007b).

Importantly, delays in diagnosis of MPS are quite common, especially in patients with non-classical patterns of disease. These patients may present later in life without the typical physical features of MPS, resulting in a low-clinical suspicion of disease and a delay to diagnosis (Fernández-Marmiesse et al., 2014; Vairo et al., 2015). In addition, clinical features of many MPS overlap, making a specific diagnosis difficult based on clinical presentation alone. Finally, some attenuated patients may display a normal result on a urine GAG screen despite having disease-causing variants and clinical symptoms of MPS (Fernández-Marmiesse et al., 2014). This diagnostic delay is particularly troubling in MPS disorders where treatment options are available (as for MPS VI), as certain clinical manifestations may not be reversible once they arise.

4.2 Treatment options

The primary goal of therapy in individuals with LSDs is to preserve or restore function by removing excess material accumulated in the lysosome and/or to prevent future buildup of substrate. While treatment options for certain LSDs are still limited, therapeutic options for MPS have advanced significantly over the past decade. Enzyme replacement therapy (ERT) is now available for four MPS disorders (MPS I, MPS II, MPS IVA, and MPS VI), and hematopoietic stem cell transplantation has demonstrated effectiveness in some patients (Biffi, 2017; Vairo et al., 2015; Valayannopoulos, 2013). In addition, novel therapeutic approaches are currently being studied for LSDs including gene therapy, substrate reduction therapy, and chemical chaperone therapy (Macauley, 2016; Rastall & Amalfitano, 2015; Valayannopoulos, 2013).

Since initial U.S. approval in 2005, ERT is available for patients with MPS VI in many countries (Naglazyme, BioMarin Pharmaceutical Inc., 2013). Galsulfase, a purified human enzyme, has been shown to improve walking capacity, stair-climbing capacity, pulmonary function, hepatosplenomegaly, cardiac function, and survival in individuals with MPS VI (Giugliani et al., 2014; Harmatz et al., 2006, 2008, 2010, 2014; Naglazyme, BioMarin Pharmaceutical Inc., 2013; Quartel et al., 2018). As multiple studies have demonstrated that early intervention with ERT (before irreversible complications arise) is beneficial (Furuto et al., 2011; McGill et al., 2010; Meikle & Hopwood, 2003; Muenzer, 2014; Wang et al., 2011), it is vital that diagnosis be made swiftly and accurately in order to maximize the therapeutic potential of ERT.

5 FUTURE PROSPECTS

One potential remedy to delayed diagnosis is the wider adaptation of newborn screening, particularly in regions where MPS disorders are common, or prenatal screening when a family history of MPS is known (Wang et al., 2011). In fact, pilot programs of this nature are already underway in many regions of the world, including parts of Austria, Brazil, Mexico, Spain, Taiwan, and the United States, several of which have proven successful at identifying lysosomal storage disorders (including MPS) in newborns prior to the onset of clinical symptoms (Acosta, Abe-Sandes, Giugliani, & Bittles, 2013; Colón et al., 2017; Hopkins et al., 2015; Lin et al., 2013; Mechtler et al., 2012; Navarrete-Martínez et al., 2017). While these types of screening programs can be somewhat costly and time-consuming, the systematic reporting and classification of alleles has the potential to reduce ethical complications in cases where novel variants with unknown pathogenicity are identified (Wang et al., 2011).

Another appealing option for diagnosis of MPS is next-generation sequencing (NGS), a platform capable of performing the sequencing of multiple genes in a single reaction. NGS is currently the method of choice for gene panels, whole exome, and whole genome analyses, and is particularly powerful for pathogenic variant identification in disorders such as MPS VI which are rare, clinically heterogeneous, and demonstrate a large proportion of private mutations (Lohmann & Klein, 2014). For example, whole exome sequencing (WES) was recently demonstrated to successfully diagnose a symptomatic patient with MPS IIIb, revealing compound heterozygous mutations, one of which was novel (Zeng et al., 2017). Although NGS is more expensive than other commonly used diagnostic techniques, the price has steadily decreased since the advent of NGS and will likely continue to do so, potentially allowing for more widespread use of NGS in practice (Lohmann & Klein 2014; Vairo et al., 2015). In addition, a genetic diagnosis allows for genetic counseling and rapid treatment initiation, both of which are vital for patients with MPS and their families.
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(Fernández-Marmiesse et al., 2014). Of note, while NGS is promising, this technique could fail to detect certain pathogenic variants, including complete gene deletions and genetic inversions or rearrangements, potentially leading to false negatives in some cases (Vairo et al., 2015).

Finally, increased reporting of pathogenic variants in ARSB, specifically in publicly available databases, coupled with a clinical description and other patient information may help to shorten the time for diagnosis, prevent misdiagnosis, and assist in understanding genotype-phenotype interactions where present. At present, variants can be submitted to, and reviewed at, the clinically-focused, public database ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/intro/). Additionally, variants associated with MPS VI can be submitted to the locus-specific database (http://mps6-database.org) by email to Hitoshi Sakuraba (sakuraba@my-pharm.ac.jp). These considerations are especially important in MPS VI due to the severity of the disease, broad clinical spectrum, and high level of genetic diversity displayed in individuals with MPS VI, as well as the availability of ERT for these patients.
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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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