Molecular Basis of Mucopolysaccharidosis IVA (Morquio A Syndrome): A Review and Classification of *GALNS* Gene Variants and Reporting of New Variants

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Background

- Mucopolysaccharidosis IVA (Morquio A syndrome, MPS IVA) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the N-acetylgalactosamine-6-sulfatase (*GALNS*) gene
- Reduced/absent activity of the GALNS enzyme causes impaired degradation of the glycosaminoglycans chondroitin-6-sulfate and keratan sulfate and their accumulation in tissues
- The clinical presentation of MPS IVA is heterogeneous and varies from a classical rapidly progressing form to a nonclassical form[1,2]
- Diagnosis usually starts from a clinical suspicion, followed by biochemical and genetic analyses
  - With advances in next-generation sequencing and disease-associated gene panels, molecular diagnosis may now precede enzyme testing in the diagnostic pathway
- Delayed diagnosis and consequent late introduction of appropriate management are common[3]
- All patients with MPS IVA may benefit from early intervention with enzyme replacement therapy[4,5]
- Timely submission and classification of *GALNS* variants with biochemical and clinical data in public databases is essential for early diagnosis of MPS IVA

Objectives

- To collect, analyze, and uniformly summarize all published *GALNS* gene variants together with new/unpublished variants provided by 7 laboratories worldwide
- To classify variants according to their pathogenicity following the most recent American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines,[6] thus updating the previous mutation review issued in 2014[7]

Methods

- A literature search was performed in PubMed and Google using the search terms "*GALNS* variants", "*GALNS* mutations", and similar terms
  - Publications were filtered from 2014 (last GALNS Mutation Update[7]) to October 2020
  - Additional variants were retrieved from the Human Gene Mutation Database Professional 2020.1
- Unpublished data of patients with MPS IVA, as well as of newborns who tested positive for MPS IVA in pilot newborn screening programs, were collected from 7 reference laboratories in Europe, the Middle East, Latin America, Asia, and the US
- The majority of variants were identified by Sanger sequencing, a few used next-generation sequencing (targeted gene sequencing, whole exome sequencing), and comparative genomic hybridization array was used as second-level analysis in some cases
- All collected data were then analyzed to define allele frequency, geographic distribution, level of homozygosity, and genotype-phenotype correlation. Variants were classified according to their pathogenicity as suggested by the ACMG and the AMP[6]
- *In silico* analyses of novel variants' pathogenicity were performed by ANNOVAR (http://annovar.openbioinformatics.org/)[8]
- All variants will be submitted to the ClinVar database, with associated evidence and literature references, to make them publicly available[9]

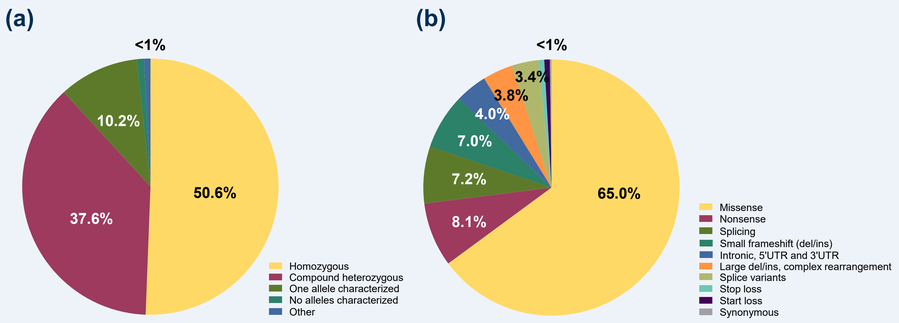
Results

Summary of Genotypes

	Number of Genotypes		
	2014 Update[7]	2020 Update	Total
Patients' genotypes from literature	536	448	984
Patients' genotypes from laboratory communications	0	206	206
Patients' genotypes from literature and laboratory communications	536	654	1190

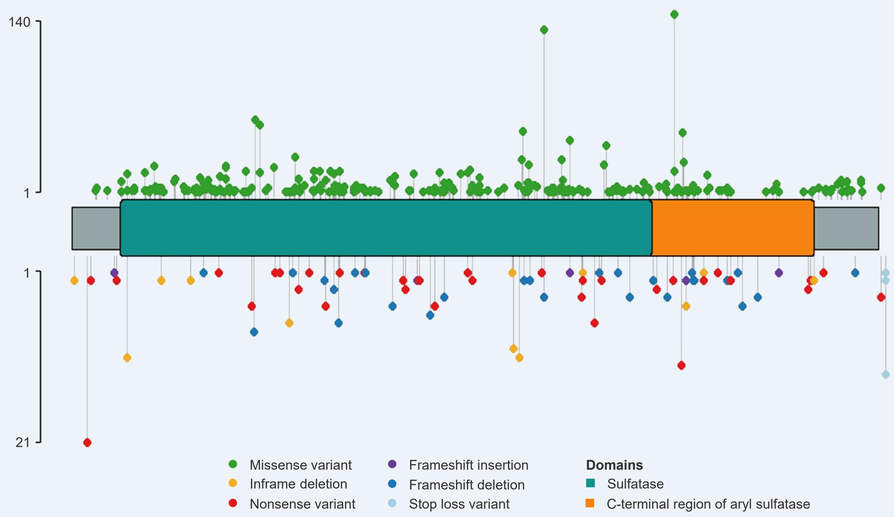
- Overall, including those previously published, we have assembled 446 unique variants (among which 68 were novel genetic alterations) from 1190 patients with MPS IVA
- The current update provides an additional 169 unique variants to the 277 previously reported
- Of the 654 genotypes identified in the current update, 448 (68.5%) were collected from the literature and 206 (31.5%) were reported by laboratory communication
- This ultimately resulted in the aggregation of 2323 characterized alleles from 1190 individuals diagnosed with MPS IVA

Summary of (a) Zygosity and (b) Unique Variants by Type



- Of the 1190 identified individuals, 602 (50.6%) were homozygous for *GALNS* variants, 448 (37.6%) were compound heterozygotes, 121 (10.2%) had only 1 allele characterized, and 11 (0.9%) individuals had no alleles characterized. Finally, in 9 (0.8%) individuals, more than 2 variants were identified (reported as "Other")
- Most unique variants were missense (65.0%), followed by nonsense (8.1%), splice site variants (7.2%), and small frameshift deletions or insertions (7.0%)
- All other variant types accounted for fewer than 4% of unique variants

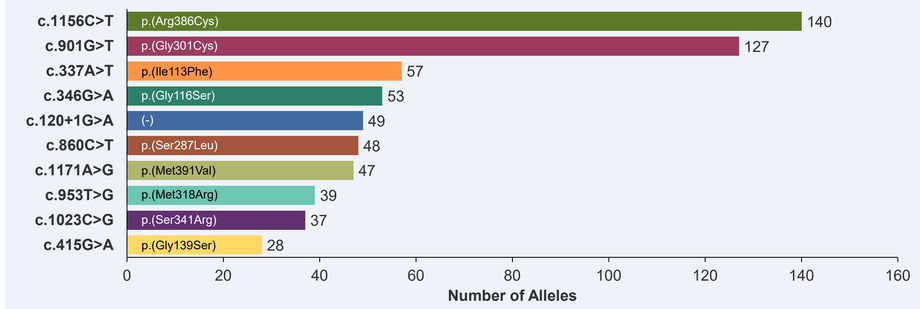
Distribution of Variants Along the GALNS Protein



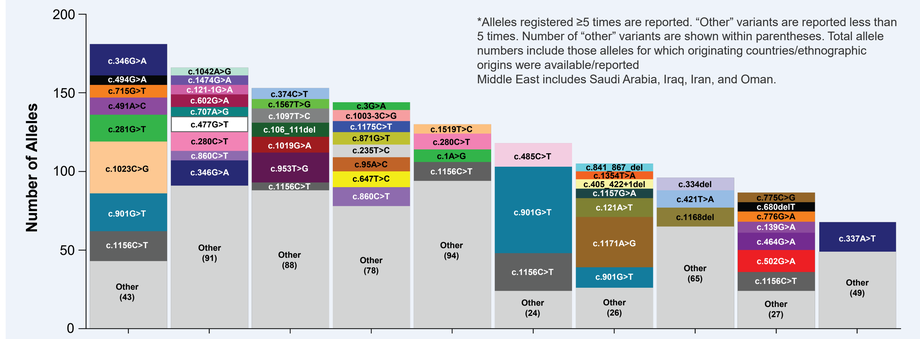
Exonic variants, excluding large deletions, insertions and complex rearrangements, are represented in a schematic of the GALNS protein (522 amino acids). Missense variants are reported at the top of the figure and the remaining exonic variants are reported at the bottom. "Lollipop" lengths represent variant frequency.

- Commonly reported variants occurred throughout the length of the *GALNS* gene, with no particular hotspot regions for variation

10 Most Frequent *GALNS* Alleles



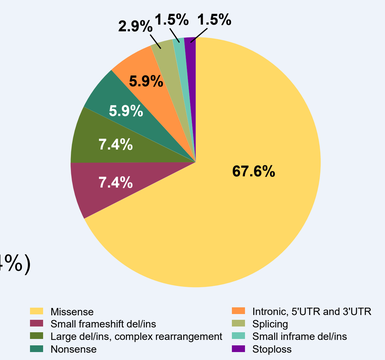
Most Common *GALNS* Alleles* for the 10 Most Frequent Nationalities/Ethnic Backgrounds Reported



- Allelic heterogeneity was observed within and between countries/ethnic backgrounds, with China and Italy presenting the highest heterogeneity and Brazil and Colombia presenting the lowest
- The variant c.1156C>T (p.[Arg386Cys]) was the most frequent, with 140 alleles identified overall, and was among the most common alleles, specifically in Colombia (n=24), Brazil (n=19), Italy (n=12), and Poland (n=12)

Novel Variants

- A total of 206 genotypes were collected from 7 laboratories, among which 68 novel (unpublished) genetic alterations carried by 119 alleles were identified
- Among the 206 subjects, 91 (44.2%) were homozygotes
- The majority of novel variants were missense (67.6%), followed by small frameshift deletions/insertions (7.4%), and large deletions/insertions, complex rearrangements (7.4%)
- As expected, most novel variants were identified in a single reported case (76.5%)
- For 23.5% of the novel variants, the allele was reported in 2 to 4 patients



Conclusions

- Overall, 446 unique variants were identified from 1190 patients with MPS IVA
  - The current update identified 169 additional variants since the 2014 update[7]
  - Among these, 68 novel variants were identified in the 206 new genotypes provided through laboratories' communication
- The majority (334, 74.8%) of variants were reported in fewer than 5 alleles
  - Results highlight the heterogeneity of *GALNS* alleles
- Mutation update is instrumental for the correct molecular diagnosis, genetic counseling, and disease management of patients with MPS IVA
- Using the information provided in mutation updates, a timely and precise diagnosis may improve clinical outcomes and quality of life for patients with MPS IVA

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