Case Report

# A Novel Hemizygous ARX Variant in an Infant with Global Developmental Delay

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## ABSTRACT

An ARX variant is a genetic change in the *ARX* (aristaless related homeobox) gene located on the X chromosome (Xp21.3). ARX encodes a transcription factor involved in early brain development, and pathogenic variants are associated with a broad spectrum of X-linked neurodevelopmental disorders. Interpreting a rare ARX variant can be challenging when acquired perinatal injury may also contribute to the phenotype. We describe a 12-month-old boy with profound global developmental delay, spastic quadriplegia, laryngomalacia, and neonatal seizures that were resolved during the neonatal period. His history suggested perinatal asphyxia, while routine brain magnetic resonance imaging (MRI) revealed no major structural abnormalities. Initial metabolic investigations, including a positive urinary glycosaminoglycan spot test, did not establish a metabolic diagnosis; mucopolysaccharidosis was excluded by lysosomal enzyme assays. Clinical exome sequencing identified a hemizygous ARX missense variant, NM_139058.3:c.634G>A (p.Gly212Ser), which was absent from the population databases. Conflicting computational predictions and the available ACMG/AMP evidence supported classification as a variant of uncertain significance (VUS), rather than a definitive molecular diagnosis. Independent review also identified an erroneous automated domain annotation. Parental segregation testing and Sanger confirmation remain pending. This case illustrates the importance of integrating phenotype, variant - level evidence, and careful annotation review while avoiding premature attribution of causality.

***Key words:*** Developmental Disabilities; Exome Sequencing; Genetic Variation

Global developmental delay is a common presentation in pediatric practice and may result from genetic, acquired, or multifactorial causes. Exome sequencing is increasingly utilized in the evaluation of unexplained neurodevelopmental disorders, especially when initial investigations fail to identify an etiology [1,2]. A significant challenge in genomic testing is the interpretation of variants of uncertain significance (VUS), which cannot be confidently classified as either pathogenic or benign and should not, on their own, guide clinical decision-making. The Aristaless-related homeobox (ARX) gene, located on chromosome Xp21.3, encodes a transcription factor that plays a crucial role in brain development, including the formation and function of GABAergic neurons [3]. Pathogenic variants of the ARX gene are associated with a broad phenotypic spectrum, encompassing developmental and epileptic encephalopathies, brain malformations, and intellectual disability. Both allelic and phenotypic heterogeneity have been documented [4]. When a child has a history suggestive of perinatal neurological injury, distinguishing the relative contributions of genetic and acquired factors can be challenging. We report a 12-month-old boy with profound global developmental delay in whom clinical exome sequencing identified a hemizygous ARX variant, initially classified by the testing laboratory as a VUS. This case underscores the importance of interpreting molecular findings in the context of the clinical phenotype, carefully reviewing automated annotations before applying classification criteria, and confirming nonspecific metabolic screening results with appropriate diagnostic tests.

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## CASE PRESENTATION

A 12-month-old boy was referred due to failure to achieve developmental milestones across gross motor, fine motor, language, and social domains. He was born at term via lower-segment cesarean section, with a birth weight of 2.7 kg.

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The history suggested perinatal asphyxia; however, Sarnat staging was not performed, and therapeutic hypothermia was not administered. Clinical seizures began on day 7 of life and resolved during the neonatal period. No neonatal electroencephalogram (EEG) was obtained. At the time of assessment, he was seizure-free and was not receiving antiepileptic medication. He is the first and only child of non-consanguineous parents, with no affected male relatives reported on the maternal side. No other relevant medical history or medication exposure.

**Clinical Examination**

At 12 months, the child's weight was 6.1 kg, and length was 64 cm, both below the third percentile. Head circumference measured 36 cm, approximately 4.5 standard deviations below the mean, consistent with severe microcephaly. Neurological examination revealed spastic quadriplegia, brisk deep tendon reflexes, and bilateral extensor plantar responses. Laryngomalacia was present, accompanied by stridor and suprasternal retractions. No dysmorphic features or organomegaly were observed. The child was receiving full oral feeds. Global developmental delay was documented across all four developmental domains.

**Investigations**

Routine urine examination revealed the presence of reducing substances; galactosemia was subsequently excluded. A urinary glycosaminoglycan (GAG) spot test was positive, initially raising suspicion of mucopolysaccharidosis (MPS). However, lysosomal enzyme assays ruled out MPS. Brain magnetic resonance imaging (MRI) at 12 months, using standard T1- and T2-weighted sequences, showed no major structural abnormalities. Given the profound developmental delay and unremarkable metabolic and neuroimaging workup, clinical exome sequencing was requested. The clinical and investigational findings are summarized in Table 1.

**Genetic Findings**

Clinical exome sequencing was performed by MedGenome Laboratories, Bengaluru, India, with an average depth of 226×, an on-target depth of 114×, over 99% of reads aligned, and 98.98% of bases covered at ≥20×. Testing identified a hemizygous missense variant in ARX: chrX:g.25013361C>T (GRCh38), NM_139058.3:c.634G>A, p.(Gly212Ser), located in exon 2. This variant was absent from gnomAD v2.1, gnomAD v4, and the 1000 Genomes Project and was not listed in ClinVar, HGMD, LOVD, DECIPHER, or SwissVar at the time of the original assessment. It has since been submitted to ClinVar (SCV007579660.1). The laboratory pipeline annotated residue 212 as part of a "polymerase alpha zinc finger domain. However, independent review against UniProt entry Q9UL36 and PFAM did not support this annotation; residue 212 was found to lie within the PA2–PA3 interdomain region, outside the canonical ARX homeodomain (residues 331–390). Based on the evidence provided, the PM1 criterion was not applied. The original ACMG/AMP assessment [5] considered PM2 (absence from population databases) and PP2 (gene-level evidence supporting a missense mechanism) as evidence supporting pathogenicity, while BP4 was applied because the reported computational predictions generally favored a benign effect (REVEL 0.183, CADD Phred 17.6, SIFT "tolerated," PolyPhen-2 "benign," MutationTaster2 "benign," and SpliceAI delta score 0.01). The variant was classified as a VUS. Parental segregation testing and Sanger confirmation of the exome finding remain pending. The genetic findings are summarized in Table 2.

**Table 1: Summary of clinical and investigational findings**

| Parameter | Finding |
|---|---|
| Age / Sex | 12 months / Male |
| Birth history | Term lower segment cesarean section; birth weight: 2.7 kg; history suggestive of perinatal asphyxia; Sarnat staging not performed; therapeutic hypothermia not given |
| Neonatal seizures | Day seven of life; no neonatal EEG; resolved; no antiepileptic drugs at assessment |
| Growth in 12 months | Weight: 6.1 kg, length: 64 cm (both below third centile); head circumference 36 cm (microcephaly, approximately -4.5 SD) |
| Developmental status | Global developmental delay — all four domains |
| Neurological findings | Spastic quadriplegia; brisk deep tendon reflexes; bilateral extensor plantars; laryngomalacia with stridor and suprasternal retractions |
| Urine GAG screening | Positive spot test — false positive; MPS excluded by lysosomal enzyme assay |
| MRI brain | Normal on standard T1/T2 sequences at 12 months; diffusion-weighted imaging and MR spectroscopy not performed; MRI report available as supporting document |
| EEG | Not performed |
| Consanguinity | Absent; no affected maternal male relatives |
| Reason for exome sequencing | Unexplained global developmental delay in hemizygous male; unrevealing metabolic and neuroimaging workup |

**Table 2: Genetic findings and ACMG/AMP classification summary**

| Parameter | Evidence | Interpretation |
|---|---|---|
| Gene / Transcript | ARX (OMIM *300382) / NM_139058.3 | X-linked; male |
| Variant | chrX:g.25013361C>T (GRCh38); c.634G>A; p.(Gly212Ser), exon two | Novel; not in any database |
| Domain location | PA2-PA3 interdomain region; outside homeodomain (residues 331-390) per UniProt Q9UL36/PFAM | PM1 not applicable |
| Population frequency | Absent from gnomAD v2.1, v4, and 1000 Genomes | PM2 — moderate pathogenic |
| Gene constraint | ARX missense Z-score 3.45 (threshold 3.09); missense is an established disease mechanism [4] | PP2 — supporting pathogenic |
| Computational tools | REVEL 0.183; CADD phred 17.6; SIFT tolerated; PolyPhen-2 benign; MutationTaster2 benign; SpliceAI delta 0.01 | BP4 — supporting benign; PP3 not applicable |
| De novo status | Pending parental segregation. If confirmed: PS2 (strong pathogenic) + PM2 + PP2 outweighs BP4 | PS2 pending — reclassification to Likely Pathogenic if confirmed |
| Net classification | PM2 + PP2 vs BP4 | Variant of Uncertain Significance |

**Management and Follow-Up**

Supportive care included physiotherapy, occupational therapy, and speech therapy. Nutritional rehabilitation was initiated due to significant growth faltering. The family received genetic counseling, with an explanation that a VUS is not a definitive diagnosis and that parental segregation testing may help clarify the result. Sanger confirmation of the exome finding is pending.

Further evaluation should include technical confirmation, parental testing, reassessment of the variant using current evidence, and ongoing developmental and neurological follow-up with genetic counseling. Any variant reclassification should be based on a formal assessment of the available evidence, as de novo occurrence alone does not establish pathogenicity.

## DISCUSSION

This case highlights the interpretive challenges posed by a rare ARX missense variant in a child whose phenotype may also be influenced by suspected perinatal injury. ARX is a well-established neurodevelopmental disease gene; however, the broad phenotypic spectrum associated with pathogenic variants does not confirm that this specific variant accounts for the child's presentation [3,4].

A normal routine MRI neither excludes a genetic contribution nor establishes perinatal injury as the sole cause. Current evidence supports maintaining the laboratory-reported VUS classification; therefore, the variant should not be presented as a confirmed molecular diagnosis.

Published studies and reviews describe significant clinical and molecular heterogeneity across ARX-related disorders, with phenotypes ranging from severe epileptic encephalopathy and structural brain abnormalities to intellectual disability without major malformations [4,6,7]. This spectrum supports considering ARX in the differential diagnosis of a male infant with severe neurodevelopmental impairment; however, it does not establish variant-specific pathogenicity. Unlike well-established disease-associated ARX variants reported in the literature, the p.(Gly212Ser) variant exhibits conflicting in silico predictions and lacks segregation or functional evidence in this case. The domain review is also clinically relevant: ACMG/AMP criteria should be applied only when the underlying evidence is valid, and an automated domain label alone is insufficient to invoke PM1 [5]. Similarly, a positive qualitative urinary GAG screen requires confirmation; in this patient, lysosomal enzyme testing excluded MPS, underscoring the importance of interpreting screening tests in the context of confirmatory studies [7, 8].

The pending Sanger confirmation and parental segregation testing, and the absence of a neonatal EEG, are the limitations of the present case [9]. Although a single-patient report cannot establish causality or confirm a new genotype–phenotype association, the false-positive result initially delayed the diagnostic process. Once excluded, it helped redirect the investigation toward exome sequencing [10]. Thus, this case report shows the significance of the risk of overinterpreting qualitative screening results in resource-limited settings [11].

## CONCLUSION

This case highlights the identification of a hemizygous ARX variant in an infant with profound in an infant with profound developmental impairment.

However, this variant remains classified as a VUS, and its contribution to the phenotype is unclear. Further clinical correlation, Sanger sequencing confirmation, parental segregation analysis, and careful evidence-based reinterpretation are necessary to establish causality.

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