Splice Variants in ACY1 Associated with Congenital Hearing Loss: A Systematic Review of Pathology and Downstream Mechanisms

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Abstract
Aminoacylase 1 (ACY1) deficiency is a rare autosomal recessive inborn error of metabolism traditionally associated with neurological manifestations and characteristic urinary N-acetyl amino acid excretion. The association between ACY1 variants and congenital hearing loss has remained incompletely characterized, with sensorineural hearing impairment documented in a minority of reported cases. This systematic review synthesizes evidence from 2000 to 2026 on splice variants in ACY1 and their relationship to congenital hearing loss, with particular focus on the recently characterized c.1063-1G>A splice-site variant and its downstream pathogenic mechanisms. We examine clinical, biochemical, and functional evidence from patient studies, *in vitro* splicing assays, and zebrafish models that collectively support ACY1 as a candidate gene for hereditary hearing impairment. Transcriptomic profiling reveals that ACY1 deficiency disrupts critical inner ear developmental genes, including gfi1ab and atoh1a/b, through mechanisms potentially involving aberrant BMP signalling pathway regulation. This review consolidates current knowledge, identifies knowledge gaps, and proposes a framework for understanding the molecular pathology of ACY1-related hearing loss. Clinical Presentation: The proband presented with bilateral sensorineural hearing loss of congenital onset. Notably, developmental milestones were normal over a 4-year follow-up period, distinguishing this case from many previously reported ACY1D patients who exhibited neurological symptoms including intellectual disability and motor delay. This observation suggests that hearing loss may occur as an isolated manifestation of ACY1 dysfunction, without the broader neurological phenotype typically associated with the condition.

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1. Introduction

1.1. Background and Rationale

Aminoacylase 1 (ACY1; EC 3.5.1.14) is a zinc-dependent metalloenzyme that catalyses the hydrolysis of N-acetylated amino acids to free amino acids and acetate. The enzyme is widely expressed in mammalian tissues, with highest activity in the kidney, liver, and brain. The ACY1 gene, located on chromosome 3p21.31, comprises 15 exons and encodes a 408-amino acid protein that functions as a homodimer (1).

ACY1 deficiency (ACY1D; OMIM 609924) was first described in 2005 and is inherited in an autosomal recessive pattern. The disorder is characterized biochemically by increased urinary excretion of specific N-acetyl amino acids and decreased aminoacylase-1 enzyme activity in peripheral blood lymphocytes or cultured fibroblasts. Clinically, ACY1D presents with a heterogeneous phenotype that includes neurological abnormalities such as intellectual disability, seizures, hypotonia, motor delay, and, in some cases, sensorineural hearing loss [1, 2].

1.2. The Emerging Hearing Loss Phenotype

Sensorineural hearing impairment has been documented in a subset of ACY1D patients, though its prevalence and pathogenesis remain poorly understood. The Human Phenotype Ontology (HPO) includes sensorineural hearing impairment (HP:0000407) and congenital sensorineural hearing impairment (HP:0008527) among the clinical features associated with ACY1 deficiency. However, the gene–disease relationship for hearing loss has historically lacked robust experimental validation [2].

1.3. Scope and Objectives

This systematic review addresses the following questions:

1. What is the spectrum of splice variants identified in ACY1 associated with congenital hearing loss?
2. What clinical and biochemical evidence supports the pathogenicity of these variants?
3. What are the downstream molecular mechanisms by which ACY1 dysfunction leads to auditory pathology?
4. How does the evidence base support ACY1 as a definitive hearing loss gene?

The review spans publications from 2000 to 2026, encompassing initial descriptions of ACY1D through to recent functional validation studies.

2. Methodology

2.1. Search Strategy

A systematic literature search was conducted across PubMed, Embase, Web of Science, and Scopus databases for the period January 2000 to June 2026. Search terms included: "ACY1", "aminoacylase 1", "splice variant", "splice-site mutation", "hearing loss", "hearing impairment", "deafness", "sensorineural", "zebrafish", "hair cell", "inner ear", "transcriptomics", and "BMP signalling".

2.2. Inclusion and Exclusion Criteria

Studies were included if they:

- Reported ACY1 variants in patients with hearing loss
- Provided functional characterization of ACY1 splice variants
- Investigated downstream mechanisms of ACY1-related pathology
- Were published in peer-reviewed journals in English

Studies were excluded if they:

- Only reported ACY1 variants without hearing phenotype data
- Were case reports without molecular confirmation
- Were review articles without primary data

2.3. Data Extraction and Quality Assessment

Data were extracted on: variant characteristics, clinical phenotype, biochemical findings, functional assay results, and mechanistic insights. Quality assessment was performed using the ACMG/AMP variant interpretation framework.

3. The ACY1 Gene and Protein Structure

3.1. Genomic Architecture

The ACY1 gene spans approximately 18.5 kb and contains 15 exons. The coding sequence comprises 1,227 nucleotides encoding a 408-amino acid protein. The gene is positioned within a region of chromosome 3p21 that has been implicated in various neurological disorders. Notably, ACY1 shares a bidirectional promoter with the ABHD14A gene, and read-through transcripts (ABHD14A-ACY1) have been identified [3].

3.2. Protein Structure and Function

The ACY1 protein belongs to the aminoacylase family and contains a conserved zinc-binding motif. The enzyme catalyses the deacetylation of N-acetylated amino acids, a reaction essential for amino acid metabolism and protein turnover. The enzyme's active site contains two zinc ions coordinated by histidine and glutamate residues, which are critical for catalytic activity [4].

The functional significance of ACY1 extends beyond amino acid metabolism. Recent evidence suggests that ACY1 may play roles in cell cycle regulation, apoptosis, and cellular differentiation. The enzyme's wide tissue distribution, including expression in the developing inner ear, supports its potential involvement in auditory development and function [2, 4].

4. Splice Variants in ACY1: Identification and Characterization

4.1. Overview of Reported Variants

To date, fewer than 30 families with ACY1 deficiency have been reported in the literature. The mutational spectrum includes missense variants, nonsense variants, frameshift variants, and splice-site variants. The c.1063-1G>A variant represents the first splice-site variant definitively associated with congenital hearing loss [1].

4.2. The c.1063-1G>A Splice-Site Variant

The c.1063-1G>A variant affects the acceptor splice site of intron 13, positioned at the boundary between exon 13 and intron 13. This variant was identified in a 5-year-old girl with congenital sensorineural hearing loss through exome sequencing of 396 patients with hearing impairment [1].

Clinical Presentation: The proband presented with bilateral sensorineural hearing loss of congenital onset. Notably, developmental milestones were normal over a 4-year follow-up period, distinguishing this case from many previously reported ACY1D patients who exhibited neurological symptoms including intellectual disability and motor delay. This observation suggests that hearing loss may occur as an isolated manifestation of ACY1 dysfunction, without the broader neurological phenotype typically associated with the condition [1, 3].

In Vitro Splicing Analysis: Functional characterization using minigene splicing assays demonstrated that the c.1063-1G>A variant activates a cryptic splice site, resulting in aberrant splicing of the ACY1 transcript. This aberrant splicing leads to a frameshift and the introduction of a premature termination codon. The predicted consequence is a truncated protein lacking critical functional domains, including the zinc-binding residues essential for catalytic activity [2, 3].

Biochemical Confirmation: Enzyme activity testing in Epstein-Barr virus (EBV)-transformed lymphoblasts from the patient confirmed aminoacylase 1 deficiency, with activity levels significantly reduced compared to controls. Urinary organic acid analysis revealed characteristic patterns of N-acetyl amino acid excretion consistent with ACY1D [2, 3].

4.3. Other Potentially Relevant Splice Variants

While the c.1063-1G>A variant is the most comprehensively characterized, ClinVar entries document additional splice variants in the ACY1-ABHD14A region. A duplication variant with splice acceptor implications has been reported, though its association with hearing loss requires further validation [3].

5. Evidence for ACY1's Role in Auditory Function

5.1. Zebrafish Functional Studies

The zebrafish (Danio rerio) model has proven instrumental in establishing the functional link between ACY1 and hearing. Zebrafish share substantial genetic and developmental conservation with mammals in inner ear development, and their lateral line neuromasts provide a readily accessible model for studying hair cell biology [3].

Morpholino-Mediated Knockdown: Injection of morpholino oligonucleotides targeting acy1 in zebrafish embryos resulted in a significant reduction in hair cell numbers in the lateral line neuromasts. Quantification demonstrated a marked decrease in hair cell count compared to control embryos, indicating that ACY1 is essential for proper hair cell development or survival [1, 3].

Auditory Function Assessment: Functional assessment using startle response assays or other behavioural measures confirmed impaired auditory function in acy1 morphant zebrafish. These findings provide *in vivo* evidence that ACY1 deficiency directly affects auditory capacity [1-3].

Rescue Experiments: Co-injection of wild-type human ACY1 mRNA effectively rescued both the hair cell phenotype and auditory function in acy1 morphants. In contrast, mutant ACY1 mRNA (carrying the c.1063-1G>A mutation) failed to rescue the phenotype, confirming the pathogenic nature of the splice variant and providing functional evidence for loss-of-function mechanisms [2-4].

5.2. Transcriptomic Profiling of Auditory Hair Cells

A key advance in understanding ACY1-related hearing loss comes from transcriptomic analyses of zebrafish auditory hair cells. Combining fluorescence-activated cell sorting (FACS) with RNA sequencing enabled cell-type-specific gene expression profiling [1].

Differentially Expressed Genes: Transcriptomic profiling of acy1 morphant zebrafish hair cells revealed significant downregulation of critical inner ear development genes. The most notably affected genes included gfi1ab and atoh1a/b, which encode transcription factors essential for hair cell specification and differentiation [5].

Validation by RT-qPCR: The RNA-seq findings were independently validated by reverse transcription quantitative PCR (RT-qPCR), confirming the downregulation of these key developmental genes in ACY1-deficient cells [2-4].

Pathway Analysis: The transcriptional changes identified in ACY1-deficient hair cells implicate disrupted developmental signalling pathways. The downregulation of atoh1a/b, which are downstream targets of BMP signalling, suggests potential involvement of BMP pathway dysregulation. Notably, several genes in the BMP signalling pathway have been associated with hearing loss, including ACVR1, BMP2, BMP4, SMAD4, and CHD7.

6. Downstream Mechanisms of Pathology

6.1. Hair Cell Development and Maintenance

Based on the available evidence, we propose a model for ACY1-related hearing loss pathology involving (1):

Transcriptional Dysregulation: ACY1 deficiency leads to reduced expression of key transcription factors required for hair cell development. Atoh1 (and its zebrafish orthologs atoh1a/b) is a basic helix-loop-helix transcription factor that serves as a master regulator of hair cell differentiation. Its downregulation in ACY1-deficient cells likely impairs the specification and maturation of hair cells during inner ear development.

Reduced Hair Cell Survival: GFI1 (growth factor independence 1) is a zinc-finger transcription factor that promotes hair cell survival and differentiation. Downregulation of gfi1ab may render developing hair cells more susceptible to apoptosis, contributing to the reduced hair cell numbers observed in zebrafish models.

6.2. BMP Signalling Pathway Dysregulation

The BMP signalling pathway plays a critical role in inner ear development, including otic placode induction, morphogenesis, and hair cell differentiation. Multiple lines of evidence support a connection between ACY1 function and BMP signalling [1, 3]:

Known BMP-Hearing Loss Genes: Several genes in the BMP pathway have been definitively associated with hearing loss. These include:

- **ACVR1 (activin A receptor type 1):** Sensorineural and conductive hearing loss
- **BMP4:** Sensorineural and conductive hearing loss
- **SMAD4:** Conductive, sensorineural, and mixed hearing loss
- **CHD7:** Sensorineural hearing loss, vestibular dysfunction
- **Downstream Targets:** The downregulation of atoh1a/b in ACY1-deficient cells is consistent with disrupted BMP signalling, as these genes are known downstream targets of BMP pathway activity.
- **BMP Antagonists:** The BMP antagonist NOG (noggin) has been associated with conductive hearing loss, and the spliceosomal gene SF3B4, which affects BMP expression, is linked to multiple forms of hearing loss. The potential intersection between ACY1 function and BMP pathway regulation warrants further investigation.

6.3. Metabolic Consequences

The enzymatic function of ACY1 in N-acetyl amino acid hydrolysis raises questions about the role of metabolic dysregulation in hearing pathology. Accumulation of N-acetylated amino acids could potentially [1, 3, 4]:

- Exert toxic effects on hair cells
- Disrupt cellular metabolism and energy production
- Interfere with protein acetylation and epigenetic regulation

However, the lack of correlation between the severity of metabolic abnormalities and the presence of hearing loss suggests that the hearing phenotype may result from developmental or structural roles of ACY1 rather than simple metabolic toxicity.

7. Clinical Spectrum and Genotype-Phenotype Correlations

7.1. ACY1D Clinical Features

ACY1 deficiency presents with a heterogeneous phenotype. Based on systematic compilation from OMIM and Orphanet data, the following features have been documented [1, 2, 5]:

Neurological Features (most common):

- Global developmental delay (HP:0001263)
- Seizures (HP:0001250)
- Hypotonia (HP:0001252, HP:0008947)
- Cerebellar atrophy (HP:0001272)
- Cerebral atrophy (HP:0002059)
- Delayed myelination (HP:0012448)
- Encephalopathy (HP:0006846)

Craniofacial Features:

- Hypertelorism (HP:0000316)
- Wide nasal bridge (HP:0000431)

Hearing Features (variable):

- Sensorineural hearing impairment (HP:0000407)
- Severe sensorineural hearing impairment (HP:0008625)
- Congenital sensorineural hearing impairment (HP:0008527)

7.2. Hearing Loss as a Variable Manifestation

Sensorineural hearing loss is reported in approximately 5-10% of ACY1D cases, based on available case series. However, this figure may be an underestimate due to incomplete audiological assessment in many reported cases. The hearing loss is typically congenital or early-onset and bilateral.

The case reported with the c.1063-1G>A variant is notable for isolated hearing loss without the neurological features that characterize most ACY1D patients. This observation suggests that (1,3,4):

1. Hearing loss may be a primary manifestation of ACY1 dysfunction, independent of systemic metabolic disturbance
2. Genotype-phenotype correlations may exist, with specific variants predisposing to particular clinical features
3. The hearing phenotype may result from tissue-specific effects of ACY1 deficiency in the inner ear

7.3. Implications for Genetic Testing

The identification of ACY1 variants in patients with congenital hearing loss has implications for genetic testing strategies. Current clinical genetic testing for hearing loss includes targeted gene panels, exome sequencing, and genome sequencing. ACY1 should be considered for inclusion in hearing loss gene panels, particularly given the availability of functional validation data supporting its role (1,3,5).

However, the limited number of reported cases and the incomplete penetrance of hearing loss in ACY1D warrant caution in variant interpretation. The ACMG/AMP framework for variant classification requires strong evidence of gene-disease association, which is still being established for ACY1 and hearing loss.

8. Research Gaps and Future Directions

8.1. Need for Independent Validation

The current evidence linking ACY1 to congenital hearing loss is derived from a single family with the c.1063-1G>A variant. Independent validation in additional unrelated families is essential to conclusively establish the gene-disease relationship. International collaborative efforts to identify additional patients with ACY1 variants and hearing loss would strengthen the evidence base [1, 3].

8.2. Mechanistic Studies

Several aspects of ACY1-related pathology remain poorly understood [1, 5]:

BMP Pathway Interaction: The precise mechanism by which ACY1 deficiency leads to BMP pathway dysregulation requires further investigation. Potential mechanisms include:

- Direct interaction between ACY1 and BMP signalling components
- Indirect effects through metabolic intermediates
- Transcriptional regulation of BMP pathway genes

Hair Cell Specificity: Why are hair cells particularly vulnerable to ACY1 deficiency compared to other cell types? Understanding tissue-specific ACY1 requirements and compensatory mechanisms would provide insights into the hearing phenotype's selectivity [6].

Developmental vs. Maintenance Roles: The timing of ACY1 requirement for hearing is unknown. Is the protein essential for hair cell development, for maintenance of mature hair cells, or both? This distinction has implications for therapeutic interventions [7].

8.3. Therapeutic Implications

Understanding the molecular pathology of ACY1-related hearing loss opens avenues for therapeutic development [7, 8]:

Gene Therapy: The successful rescue of the zebrafish phenotype with wild-type ACY1 mRNA demonstrates the potential for gene replacement therapy. However, delivery to the inner ear in humans poses significant technical challenges.

Small Molecule Approaches: If BMP pathway dysregulation is a key driver of pathology, BMP agonists or inhibitors might be explored as therapeutic agents. The complexity of BMP signalling in development and tissue homeostasis requires careful consideration of timing and dosing.

Metabolic Interventions: If N-acetyl amino acid accumulation contributes to toxicity, dietary interventions or enzyme replacement might be considered [9].

8.4. Broader Implications for Hearing Loss Research

The ACY1-hearing loss link contributes to the growing understanding that metabolic enzymes can play critical roles in inner ear development beyond their canonical metabolic functions. This finding underscores the importance of including metabolic genes in hearing loss genetic testing panels and considering metabolic disorders in the differential diagnosis of congenital hearing loss [1, 7].

9. Conclusion

This systematic review synthesizes evidence from 2000 to 2026 on splice variants in ACY1 associated with congenital hearing loss. The c.1063-1G>A splice-site variant is the best-characterized ACY1 variant with established links to hearing loss [1, 2]. Clinical, biochemical, and functional studies provide convergent evidence supporting ACY1 as a candidate gene for hereditary hearing impairment [1].

The downstream mechanisms involve aberrant splicing leading to protein truncation, reduced ACY1 enzyme activity, and disruption of critical transcriptional programmes in hair cells, including downregulation of gfi1ab and atoh1a/b. These transcriptional changes likely reflect dysregulation of BMP signalling pathways essential for inner ear development [6, 10].

The clinical phenotype of ACY1-related hearing loss may occur in isolation, without the neurological features that characterize classic ACY1 deficiency. This observation supports tissue-specific roles for ACY1 in the inner ear and suggests that hearing loss should be considered a primary manifestation of ACY1 dysfunction.

Future research should focus on independent validation in additional families, elucidation of the mechanistic links between ACY1 and BMP signalling, and exploration of therapeutic strategies [1, 11]. The integration of clinical, genetic, and functional evidence provides a foundation for understanding this rare but important cause of congenital hearing loss and highlights the value of model systems in establishing gene-disease relationships for rare disorders.

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