

Expanding the prenatal and postnatal phenotype spectrum of HEATR5B-related disorders

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Brief Communication

Keywords: HEATR5B, pontocerebellar hypoplasia, hydrops fetalis, neurodevelopmental disorder, gene-disease validity, whole exome sequencing

Posted Date: September 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10836650/v1>

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Additional Declarations: There is no duality of interest

Abstract

Biallelic hypomorphic variants in *HEATR5B* were first reported in four individuals from two families with pontocerebellar hypoplasia, but no disease entity is listed for *HEATR5B* in OMIM, Mondo or similar databases; despite its ubiquitous expression across multiple organ systems, the gene-disease relationship remains limited to neurodevelopmental disorders. Genematching between 2021 and 2026, lead to the identification of 12 novel variants in *HEATR5B*, from 17 individuals in 11 unpublished families. Eight of these families showed autosomal recessive inheritance (seven homozygous, one compound heterozygous), while the remaining three carried *de novo* variants. Stillbirth and fetal hydrops were observed in 5/8 and 7/8 individuals respectively, and neurological phenotypes accounted for 10/14 of the postnatal cases; other organ systems, including genitourinary, cardiac, digestive and respiratory, were less frequently affected. This is the largest *HEATR5B* cohort to date, and highlights the perinatal lethality of *HEATR5B*-related disorders, and its multiorgan systemic involvement beyond neurodevelopmental disorders alone.

Introduction

HEAT Repeat Containing 5B (*HEATR5B*, HGNC:29273) is the third HEAT Repeat Containing gene to be implicated in human disease, following *HEATR2* (*DNAAF5*)-related primary ciliary dyskinesia, and *HEATR3*-related Diamond-Blackfan anemia (1–3). HEAT repeats are large helical motifs found in diverse eukaryotic proteins involved in intracellular signaling and transport; seven of the ten genes designated as HEATR remain uncharacterized (4,5).

Biallelic *HEATR5B* variants were first reported in four children from two unrelated families with pontocerebellar hypoplasia (PCH), neonatal seizures, severe intellectual disability, and motor delay (1). The same c.5051-1G > A splice variant (Family 2, published as PCH-2610) was later identified in one further individual with an overlapping PCH phenotype and shared Turkish ethnicity (6). Despite subsequent studies elucidating *HEATR5B*'s role in membrane trafficking, no additional genotypes have been reported, and phenotyping remains limited by the embryonic lethality of homozygous knockout mice (7, 8); gene-disease validity therefore remains limited. Here, we report unpublished *HEATR5B* variants in individuals with novel prenatal and postnatal phenotypes, strengthening the evidence for its role in human disease.

Materials and Methods

Individuals with unpublished *HEATR5B* variants were identified through GeneMatcher (9), public genomic and health data repositories, or collaborations with disease-focused cohorts. Sequencing, bioinformatic analyses, and (where applicable) segregation studies were performed independently at each institution. Clinical proformas were collected, and standardized across participating institutes using Human Phenotype Ontology (HPO) terminology. All variants were reannotated using GRCh38 HGVS nomenclature, gnomAD v4.1 allele frequencies, and REVEL and DynaMut2 scores for missense variants,

and reclassified by ACMG guidelines (10). Gene-disease validity for *HEATR5B* was reassessed using the guidelines outlined by the ClinGen Syndromic Disorders Gene Curation Expert Panel (11). Detailed clinical data and family history were collected using a standardized clinical proforma. Written informed consent for inclusion and data sharing was obtained for all participants in accordance with site-specific ethics approvals and local governance requirements.

During the preparation of this work, the authors used Claude.ai (Anthropic) to cross-check numerical consistency across the manuscript, tables and figures, and to improve readability. The authors reviewed and edited the resulting content as needed and take full responsibility for the content of the publication.

Results

Family 1: Index family

F1-1 is a deceased male fetus delivered at 23 + 4 weeks' gestation to consanguineous parents of Middle Eastern ancestry. The 31-year-old gravida four mother had no significant medical history. Early pregnancy was unremarkable, but ultrasound at 21 + 2 weeks demonstrated marked hydrops fetalis, with progression to anhydramnios, complex cardiac malformation, and cerebellar hypoplasia by 21 + 4 weeks. Following, intrauterine fetal demise at 23 + 3 weeks, postmortem examination (Table 1) confirmed severe hydrops, pulmonary hypoplasia, craniofacial dysmorphism, and cardiac anomalies. Neuroimaging showed severe global brain malformation, alongside smooth kidneys, gastrointestinal malrotation, and a hypomineralized skeletal phenotype.

Trio exome sequencing from the Australian Genomic Autopsy Study initially identified no candidate variants in known disease genes (12). Extended research analysis subsequently identified a homozygous frameshift variant (NM_019024.3(*HEATR5B*):c.4030dupG; p.Ala1344GlyfsTer2), which was absent from gnomAD v4.1, and in a gene with high genic intolerance (pREC = 1.0) to biallelic loss-of-function variants. At the time, *HEATR5B* was not a recognized OMIM/MONDO disease entity, and based on the lack of phenotypic overlap with the published cases, the findings were considered a gene of uncertain significance.

HEATR5B variants

In total, 14 unique *HEATR5B* (NM_019024.3) alleles were identified across 14 unrelated families (Fig. 1A, Table S1), including one recurring variant, c.5051-1G > A, in three individuals from two published Turkish families. Segregation analysis, where available, supported autosomal recessive inheritance in 11/14 families and *de novo* in three (Table 1). The prenatal cohort were comprised of five alleles: four homozygous loss-of-function, and one *de novo* missense (REVEL = 0.66); all absent from gnomAD v4.1. The postnatal cohort included: five homozygous, one compound heterozygous, and two *de novo* (Fig. 1A). The homozygous missense and splice donor variants were present at low frequency in gnomAD v4.1, while the rest were absent (Table S3). Only 1/4 postnatal missense variants had

supporting *in silico* evidence (REVEL = 0.66; Family 12), and of the two splice donor variants, only the 4th-base variant showed strong splice-altering evidence (SpliceAI donor loss = 0.68) versus a subthreshold effect at the 5th base (0.18). None of the nine postnatal alleles occurred in homozygotes in gnomAD v4.1 (Table S1).

Clinical characterization

In addition to the five published cases (3 families), 17 individuals (11 families) were identified through GeneMatcher and collaborative networks (Table 1). Two further individuals identified via ClinVar and DECIPHER were excluded from the cohort summary (n = 22) due to insufficient clinical information (Table 1, S1). From 22 individuals, there were eight prenatal and 14 postnatal cases (Table S2, S3). Consanguinity was reported in 8/14 families for seven prenatal and five postnatal cases.

Among the prenatal subcohort, the core phenotype was hydrops fetalis (7/8), and the most common outcome was fetal death *in utero* (5/8). Craniofacial and genitourinary abnormalities were each observed, affecting at least five fetuses in total (Fig. 1B-C). Nervous and musculoskeletal anomalies were less frequent, observed in three and four fetuses respectively; aside from cerebellar hypoplasia (2/8), most phenotypes occurred as isolated findings (Table S2). Forty-one phenotypes across six organ systems were documented among the eight fetal cases (Fig. 1D, Table S2).

Nine additional postnatal cases from six families were identified through GeneMatcher, bringing the total postnatal cohort to 14 individuals (Table 1, S1, S3). Neurological features were prominent in postnatal cases (12/14), primarily structural brain abnormalities on neuroimaging (10/14) and seizure disorders (6/14; Fig. 1D-F). Early death was the most common clinical course, in infancy (n = 2) and childhood (n = 4), with a median age at death of 3 years (Table S1, S3). Unlike the three published families, no further families showed nystagmus or visual impairment, though isolated craniofacial findings emerged (Fig. 1F). Immune involvement included meningitis and pneumonia in addition to recurrent infections reported in four published individuals. Altogether, 71 phenotypes spanning eight organ systems were observed among the 12 postnatal cases (Table S3).

Phenotypic concordance between prenatal and postnatal presentations was limited to two exact phenotype matches (Fig. 1G): cerebellar hypoplasia (6/22) and low-set ears (3/22). Related phenotypes based on overlapping structure or parent terms, included: agenesis or hypoplasia of the corpus callosum (7/22), brainstem dysplasia (6/22), craniofacial abnormalities (5/22). Other rare overlaps (2/22) include: cardiac septal defects, deep grey matter anomalies, lower limb abnormalities, and neuronal migration disorders (Fig. 1G, Table S2-3).

Discussion

In this study, we expand the molecular and phenotypic spectrum of 22 individuals with rare biallelic or monoallelic *HEATR5B* variants, establishing the gene as a novel multisystemic disorder with prominent

neurological involvement (Table 1, Fig. 1). The clinical spectrum ranged from perinatal lethality, with hydrops fetalis, pulmonary hypoplasia, and structural brain abnormalities, to childhood-onset neurodevelopmental disease with survival into adulthood. Biallelic loss-of-function variants caused the severe, early-onset phenotypes, including pontocerebellar hypoplasia, ventriculomegaly, corpus callosum abnormalities, seizures, hypotonia, developmental regression, and profound intellectual disability; whereas *de novo* missense variants caused milder, more variable phenotypes such as autism spectrum disorder, epilepsy, borderline intellectual disability, or isolated congenital anomalies. Intrafamilial variability (Table 1) among siblings in Families 2, 7, and 9, and interfamilial differences between Families 2 and 3 despite sharing the c.5051-1G > A variant, underscore the allelic and phenotypic heterogeneity of biallelic *HEATR5B*-related disorders, supporting a continuum from prenatal lethality to postnatal neurodevelopmental disorder.

The unpublished families substantially broadened this spectrum beyond previously reported *HEATR5B*-related pontocerebellar hypoplasia (1). Alongside the recurrent neurological phenotypes confirmed in the postnatal cohort, we observed prenatal and multisystemic features not previously described ((Fig. 1B-F). The early lethality and severity observed across the cohort point to a developmental role for *HEATR5B* during embryogenesis, consistent with its function as a conserved HEAT-repeat scaffold protein mediating dynein-dynactin trafficking and AP1-associated endosomal transport (7, 8), including retrograde vesicular trafficking to the Golgi and intracellular membrane organization essential for neuronal survival and organogenesis. Strong *HEATR5B* expression during human brain development, together with embryonic lethality in mouse knockouts, suggests complete loss of function is incompatible with life, consistent with Families 1, 5, 6, and 8 (1).

HEATR5B shares broad similarities with *HEATR2*- and *HEATR3*-related disorders, in that loss-of-function in any of the three causes severe early-onset multisystem disease, underscoring the importance of HEAT-repeat proteins in embryonic development (Table S4). Clinical manifestations otherwise differ considerably: *HEATR2* deficiency causes a motile ciliopathy with primary ciliary dyskinesia, chronic sinopulmonary disease, infertility, and laterality defects, while *HEATR3* deficiency causes a ribosomopathy resembling Diamond-Blackfan anemia, with erythroid failure, congenital anemia, and acromelic skeletal dysmorphism (2, 3). *HEATR5B*-related disorders are distinguished by severe prenatal manifestations and pontocerebellar and multisystem developmental defects, pointing to a distinct, critical role in embryogenesis and neuronal maintenance.

This study has several limitations, including variable phenotypic data from retrospective international ascertainment, and incomplete neuroimaging, segregation, and functional allelic series. Further evidence is needed to clarify genotype-phenotype correlations and the mechanisms underlying this heterogeneity. Nonetheless, this study provides strong genetic evidence for *HEATR5B* gene-disease validity (Table S5), supporting its inclusion in the differential diagnosis of severe prenatal and postnatal neurodevelopmental disorders, particularly pontocerebellar hypoplasia and nonimmune hydrops fetalis. Early molecular diagnosis will support genetic counseling, recurrence risk assessment, and reproductive planning.

Declarations

Data Availability Statement

The genomic data generated in this study are not publicly available due to participant consent restrictions; however, controlled access to de-identified data may be requested in accordance with the procedures outlined in (12).

Code Availability

No custom code was generated or used in this study; standard bioinformatic pipelines are cited in 'Materials and Methods'.

Ethical Approval

This study was performed in accordance with the Declaration of Helsinki, and was initiated as part of the NHMRC- and GHFM-MRFF-funded Australian Genomic Autopsy Study and was approved by the Human Ethics Committee of the Women's and Children's Health Network, South Australia, Australia (HREC/15/WCHN/35) and Melbourne Health as part of the Australian Genomics Health Alliance protocol (HREC/16/MH/251). Informed consent, including consent for publication, was obtained as part of the Genomic Autopsy Study. All other institutions who contributed human participant data received local REC/IRB approval, and obtained informed consent, including consent for publication, from their participants.

Competing Interests

The authors declare no competing interests.

Funding

This research was supported by the National Health and Medical Research Council (APP1123341), Genomics Health Futures Mission - Medical Research Futures Fund (GHFM76777), and Australian Genomic Health Alliance NHMRC Targeted Call for Research into Preparing Australia for the Genomics Revolution in Healthcare (GNT1113531) to H.S.S and C.P.B; by NHMRC Ideas Grant (APP2021396) to H.S.S, C.P.B, M.R.J, and T.T.H; by MRFF Infertility, Pregnancy Loss and Menopause grant (APP2044295) to M.R.J, T.T.H, C.P.B and H.S.S; by Australian Genomics and MRFF Early to Mid-Career Researchers grant (APP2023357) to M.R.J; and by the ACRF to H.S.S.

Author Contributions

Conceptualization: T.T.H, C.P.B., H.S.S; Data curation: T.T.H, A.G; Formal analysis: T.T.H, A.G; Funding acquisition: T.T.H, M.R.J, C.P.B., H.S.S; Investigation: T.T.H; Project administration: M.K.B, M.R.J; Resources: T.T.H., A.N, J.L, M.J, M.R, M.P, A.W, A.R, L.B, F.H, E.Z, L.J,H, M.K, F.R, T.M.J, M.B.L, L.K, H.D, M.R, C.W.T.C, C.P.B; Supervision: H.S.S; Writing - original draft: T.T.H; Writing - review and editing: M.K.B, M.R.J.

Acknowledgements

The authors would like to thank all the families who were involved in this study. We would also like to thank deCODE genetics for their contribution to this study.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

families from the novel cases in this cohort. G) Bar plot of phenotypes shared between prenatal and postnatal presentations (*italicised in blue*), including HPO terms describing the same anatomical finding at differing specificity (e.g. agenesis vs. hypoplasia of the corpus callosum; brainstem dysplasia vs. hypoplasia of the pons).

Supplementary Files

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- [260826HEATR5BSuppTablesTH.xlsx](#)
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