

Biallelic *ATOH1* Gene Variant in Siblings With Pontocerebellar Hypoplasia, Developmental Delay, and Hearing Loss

Tanja Višnjar, PhD, Aleš Maver, MD, PhD, Karin Writzl, MD, PhD, Ornela Maloku, PhD, Gaber Bergant, MD, Helena Jaklič, PhD, David Neubauer, MD, PhD, Federico Fogolari, PhD, Nuška Pečarič Meglič, MD, and Borut Peterlin, MD, PhD

Correspondence
Dr. Peterlin
borut.peterlin@kdj.si

Neurol Genet 2022;8:e677. doi:10.1212/NXG.0000000000000677

Abstract

Background and Objectives

To report on the novel association of biallelic variant in atonal basic helix-loop-helix transcription factor 1 (*ATOH1*) gene and pontocerebellar hypoplasia (PCH), severe global developmental delay, intellectual disability, and hearing loss in a family with 2 affected siblings.

Methods

A detailed clinical assessment and exome sequencing of peripheral blood sample were performed. Segregation analysis with Sanger sequencing and structural modeling of the variant was performed to support the pathogenicity of the variant.

Results

A homozygous missense variant (NM_005172.1:c.481C>G) in the *ATOH1* gene was identified in the proband and his affected sister. The segregation analysis subsequently confirmed its segregation with an apparently recessive PCH in this family. *ATOH1* encodes for the atonal basic helix-loop-helix (bHLH) transcription factor 1, a core transcription factor in the developing cerebellum, brainstem, and dorsal spinal cord, and in the ear. The identified variant results in the p.(Arg161Gly) amino acid substitution in the evolutionarily conserved DNA-binding bHLH domain of the *ATOH1* protein. Biallelic missense variants in this domain were previously reported to result in disordered cerebellar development and hearing loss in animal models. In silico homology modeling revealed that p.Arg161Gly in *ATOH1* protein probably disrupts a salt bridge with DNA backbone phosphate and increases the flexibility of the bHLH helix—both of which together affect the binding capability of the bHLH domain to the DNA.

Discussion

Based on the sequencing results and evidence from structural modeling of the identified variant, as well as with previous reports of *ATOH1* gene disruption, we conclude that *ATOH1* may represent a novel candidate gene associated with the phenotype of PCH, global developmental delay, and hearing loss in humans.

From the Clinical Institute of Genomic Medicine (T.V., A.M., K.W., G.B., H.J., B.P.), University Medical Centre Ljubljana, Slovenia; Medical Faculty (K.W., D.N.), University of Ljubljana, Slovenia; Division of Paediatrics (D.N.), Department of Child, Adolescent & Developmental Neurology, University Medical Centre Ljubljana, Slovenia; Clinical Institute of Radiology (N.P.M.), University Medical Centre Ljubljana, Slovenia; and Department of Mathematics (O.M., F.F.), Informatics and Physics, University of Udine, Italy.

Go to [Neurology.org/NG](https://www.neurology.org/NG) for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Glossary

ATOH1 = Atonal BHLH Transcription Factor 1; **bHLH** = basic helix-loop-helix; **ES** = exome sequencing; **LoF** = loss of function; **PCHs** = pontocerebellar hypoplasias; **NEUROD1** = neurogenic differentiation factor 1.

Pontocerebellar hypoplasias (PCHs) represent a group of disorders characterized by hypoplasia of the brainstem and cerebellum. Disorders in this group generally have a prenatal onset, and they are usually characterized by a progressive course and neurodegeneration. Apart from these features, there is a broad clinical and neuroradiologic variability within different types of PCH and among patients affected by the same PCH type. While some patients experience hypotonia, severe developmental delay, intellectual disability, seizures, and hearing loss, others may present with a milder phenotype and have the ability to walk or sit independently.^{1,2}

The current classification (as of March 01, 2021; OMIM database) encompasses 13 distinct types of PCHs with several subtypes based on clinical features and genetic causes. To date, 21 PCH-related genes have been reported in the literature, which play a role in a variety of biological processes, including messenger RNA degradation (*EXOSC3*, *EXOSC8*, and *EXOSC9*), tRNA synthesis and splicing (*AIMP1*, *RARS2*, *CLP1*, *TSEN15*, *TSEN2*, *TSEN34*, and *TSEN54*), RNA processing (*TOE1*), maintenance of mitochondrial function (*SLC25A46*), selenocysteine synthesis (*SEPSECS*), vesicular transport (*PCLO*, *VPS51*, *VPS53*, *TBC1D23*, and *CHMP1A*), neuronal migration (*VRK1*), and cellular metabolic pathways (*AMPD2* and *COASY*).² Although the diagnostic yield in patients with PCH is generally high, a notable proportion of patients remains undiagnosed, possibly due to noncoding pathogenic variants in established PCH-associated genes or due to presence of pathogenic variants in novel PCH genes. In the recent years, next-generation sequencing has enabled sequencing of the exomes and genomes of patients with undiagnosed diseases and thus also enabled identification of novel candidate genes, including those leading to PCH.

In this report, we present a family with 2 siblings affected by PCH, severe global developmental delay, and hearing loss. Using exome sequencing (ES), we identified the presence of an exceedingly rare homozygous missense variant in the association of biallelic variant in atonal basic helix-loop-helix transcription factor 1 (*ATOH1*) in both affected siblings. Based on the previous evidence of the *ATOH1* playing a central role in cerebellar development, properties of the identified variant and in *silico* protein simulation studies for the variant's effect on protein function, we hypothesize that *ATOH1* could represent a novel gene associated with a neurodevelopmental disorder with a striking cerebellar involvement.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Informed consent was obtained from the parents of the affected individuals in accordance with guidelines established by the institutional review boards at their primary site of care. CARE reporting guidelines were followed while preparing this article.³

Clinical Investigation

The index patient was referred to the genetic department because of possible genetic reason for PCH, severe global developmental delay, intellectual disability, and hearing loss. A genetic screening was performed within clinical routine; peripheral blood from the patient and family members was drawn for sequencing. MRI and CT of the brain were performed in the patient and his affected sister, and images were interpreted by an experienced neuroradiologist and further examined within the research group. A physical and neurologic examination was performed on the affected siblings.

ES and Bioinformatic Analyses

ES was performed in the affected proband (*II-1*, Figure 1) after in-solution capture with Illumina Nextera Coding Exome probes targeting 37 Mb of exonic coding sequences (214,405 coding sequences according to RefSeq gene models). Sequencing of the enriched shotgun library was performed on the HiSeq 2500 platform in the 2 × 100 reads paired-end sequencing mode. A median on-target coverage exceeding 90× was reached (more than 98% of regions were covered at 20×). Sequencing results were analyzed using an in-house analysis pipeline, as described in our previous publications.^{4,5}

First, an analysis of variants in a panel of 21 genes, known to be involved in PCH (*AIMP1*, *AMPD2*, *CHMP1A*, *CLP1*, *COASY*, *EXOSC3*, *EXOSC8*, *EXOSC9*, *PCLO*, *RARS2*, *SEPSECS*, *SLC25A46*, *TBC1D23*, *TOE1*, *TSEN15*, *TSEN2*, *TSEN34*, *TSEN54*, *VPS51*, *VPS53*, and *VRK1*), was performed. Afterward, we focused the analyses to the applicable PanelApp gene panels, including Cerebellar hypoplasia (version 1.39), Intellectual disability (version 3.33), and Hearing loss (version 2.8). Homozygosity mapping analyses were performed using an in-house algorithm, followed by the validation of the findings using Homozygosity mapper software.⁶

Sanger Sequencing

The candidate variant in the *ATOH1* gene was confirmed by Sanger sequencing. In brief, the region containing the missense mutation in the *ATOH1* gene was amplified using a set

of primers (forward primer: 5'-GCCGCCAGTATTTGC-TACA-3'; reverse primer: 5'-TCTTTTGCGCCATCATCGCT-3'). Sequencing data were analyzed by the Geneious software, v.2020.0.5.

Homology Modeling

The structure of ATOH1 has not been solved experimentally to date, so we performed homology modeling. The 2 most similar homologs found in the Protein Data Bank are complexes of 2 and 4 protein chains and DNA (pdb id: 2QL2 and 2YPA, respectively). We chose as a template the structure with pdb id. 2QL2, which showed the higher identity percentage (60% over the region 160–217, with no gaps) and had an overall resolution of 2.50 Å.⁷ The entry contains the crystal structure of the basic helix-loop-helix (bHLH) domains of the heterodimer of the neurogenic differentiation factor 1 (gene *NEUROD1*) and the bHLH domain of transcription factor E2-alpha (gene *TCF3*) bound to DNA.

The chain of the ATOH1 was modeled by homology on the *NEUROD1* chain, using the program DeepView 4.10.⁸ Of interest, transcription factor E2-alpha is reported to interact with ATOH1 in the STRING protein interaction database,⁹ and the DNA fragment bound by the 2 proteins matches the AtEAM motif. We thus retained the chains of the transcription factor E2-alpha and of the DNA double helix as they are found in the crystal structure to obtain a bona fide homology model of the heterodimer complex with DNA.

Free Energy Implicit Solvent Calculations

To estimate the change in free energy of binding upon the mutation R161 to G, we performed electrostatic calculations using the program Blueues.¹⁰ The structures of the complex and the isolated proteins (wild-type and mutant) and DNA were prepared using the program pdb2pqr.¹¹

Molecular surfaces were computed using the program MSMS¹² and read in the program Blueues. The dielectric constant of water was set to 78.5 and 75 at 298.0 and 310 K, respectively. The inner dielectric constant was set to 4, 2, and 1 to test the effect of this parameter on the computed free energy of binding.

Data Availability

Original data can be made available on request; genetic data or clinical data sharing may be subject to privacy restrictions.

Results

Clinical Presentation of a Family

We present a family with 2 affected siblings (the proband and his sister). The proband (Figure 1, *II-1*), a 14-year-old White boy, was born vaginally after an uneventful pregnancy as the first-born twin at 36 weeks of gestation. The second-born twin experienced intrauterine hypoxia; however, she showed no clinical and radiologic signs present in the proband and the affected sister. The proband's birth weight was

2,720 g (50 P), birth length 48 cm (50 P), and head circumference 35.5 cm (85 P); Apgar 8/9. At his birth, the ages of the mother and the father were 29 and 30 years, respectively. In the neonatal period, he was breastfed and had no feeding difficulties. Hypotonia was reported at age 3 months. Motor development was slow. He achieved head control at age 4 months, sat at age 3 years, and walked with a walker at age 8 years. He had severe speech delay, used one word (mum) only, and communicated nonverbally or by communication symbols. He had severe hearing loss but declined to use hearing aids. He learned to eat and dress mostly independently but was not toilet trained. His sleep pattern was normal. He never had seizures. Behavior disturbances included stereotypic hand movements, bursts of anger, and the episodes, when he gritted his teeth, looked up, and hugged his thighs with his hands. At age 14 years, his weight was 44.5 kg (25 P), height 160.5 cm (35 P), and head circumference 55.8 cm (44 P). A neurologic examination revealed horizontal nystagmus, low muscular tone, brisk reflexes, and intention tremor. He was unable to walk independently because of severe trunk ataxia. He had soft skin, hypermobility of the small joints, mild contractures of knees and elbows, and bilateral valgus feet.

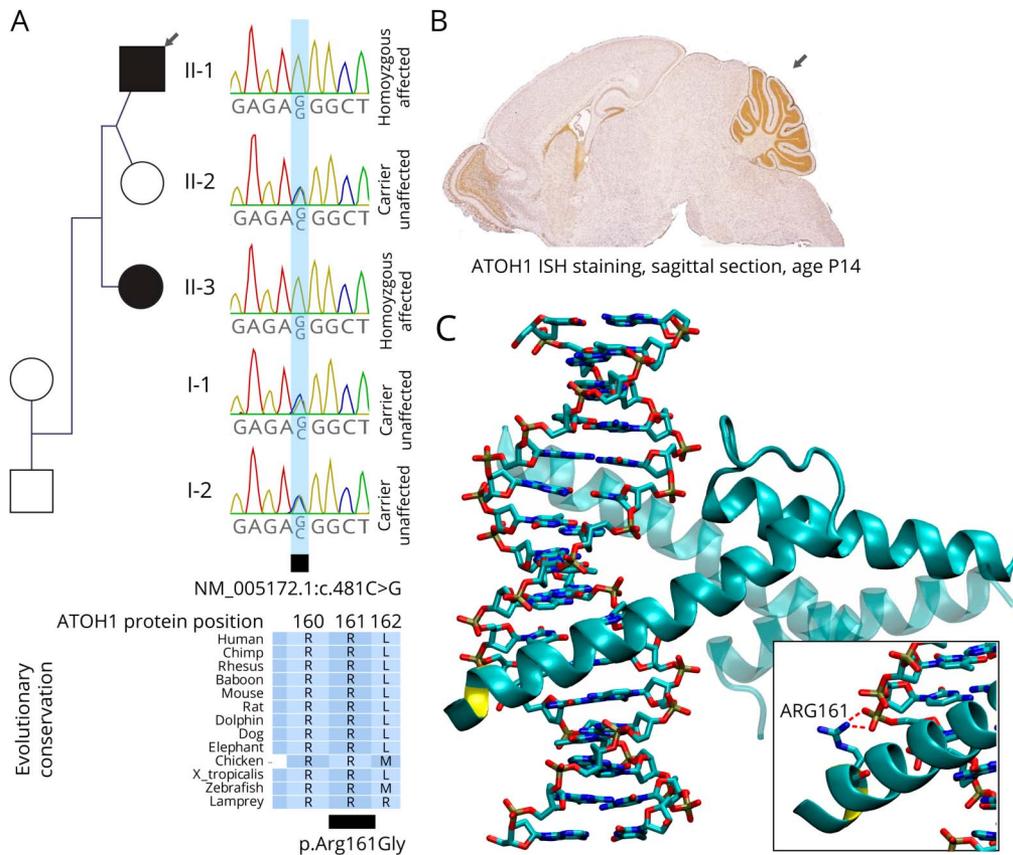
His older affected sister (Figure 1, *II-2*) was born at full term by normal vaginal delivery. The family moved to Slovenia when she was at the age of 10 years. Birth weight was 2,750 g (5 P). Other anthropometric data at birth were not recorded. The neonatal period was uneventful. She was breastfed. At the age of 6 months, hypertonus, hyperreflexia, and repeated episodes of opisthotonus were reported. Her development was extremely delayed. She achieved short-term head control but was not able to sit or walk. She had vision and hearing impairment and reacted to auditory and tactile stimuli by smiling and making sounds. She was fed orally and had problems with constipation. She had frequent apnea attacks after the age of 20 years. At the age of 10 years, her weight was 31 kg (25–50 P), and her development was estimated to be at the level of 4 to 6 months. She had no head control, nystagmus, opisthotonus, the lower limbs in an adduction position, and athetosis of the upper limbs.

General laboratory tests including blood cell counts, liver and kidney function tests, blood glucose levels, lactate and pyruvate blood levels, and amino acid and organic acid tests revealed normal results in both siblings.

An ophthalmologic examination of the siblings at the age of 10 years revealed horizontal nystagmus, convergent strabismus, and pale optic discs. In addition, the proband had mild peripapillary chorioretinal atrophy in the right eye. The proband showed severe visual impairment (6/50), and his sister presented with blindness (6/130).

Audiogram and brain-evoked response audiometry performed in the proband showed a severe bilateral sensorineural hearing loss. His sister was clinically assessed as experiencing

Figure 1 Segregation Analysis and Structural Modeling of c.481C>G Variant in *ATOH1* Gene



Panel (A) shows the pedigree of the reported family and the results of segregation analysis using Sanger sequencing. A homozygous missense variant in the *ATOH1* (NM_005172.1:c.481C>G) was detected in the proband II-1 and his affected sister II-3. The unaffected sister II-2, mother I-1, and father I-2 were heterozygous carriers of the variant. Panel (B) shows the staining pattern of the *ATOH1* in the developing brain of mice, according to the Allen developing mouse brain atlas, and demonstrates the widespread expression of *ATOH1* gene in the cerebellum in the postnatal day 14 (P14) (Image credit: Allen Institute, permission has been obtained from the resource creators for reproduction of the image). Panel (C) Homology model of *ATOH1* in complex with DNA. The partnering protein, transcription factor E2 alpha, is shown in transparency. The position of the mutation (R161) is shown in yellow. Panel (C) inlay shows the detail of the complex of the homology model of wild-type *ATOH1* with DNA.

profound hearing loss because she only responded to loud auditory stimuli.

Several brain imaging studies were performed in both siblings; however, only CT images from the proband and MRI from his affected sister were preserved (Figure 2 and eFigure 1, links.lww.com/NXG/A523). The consistent findings were posteriorly thin corpus callosum, small posterior fossa with the upward displacement of the tentorium, small cerebellum with more severely hypoplastic vermis than hemispheres, and small brainstem (eFigure 1 and eTable 1, links.lww.com/NXG/A523).

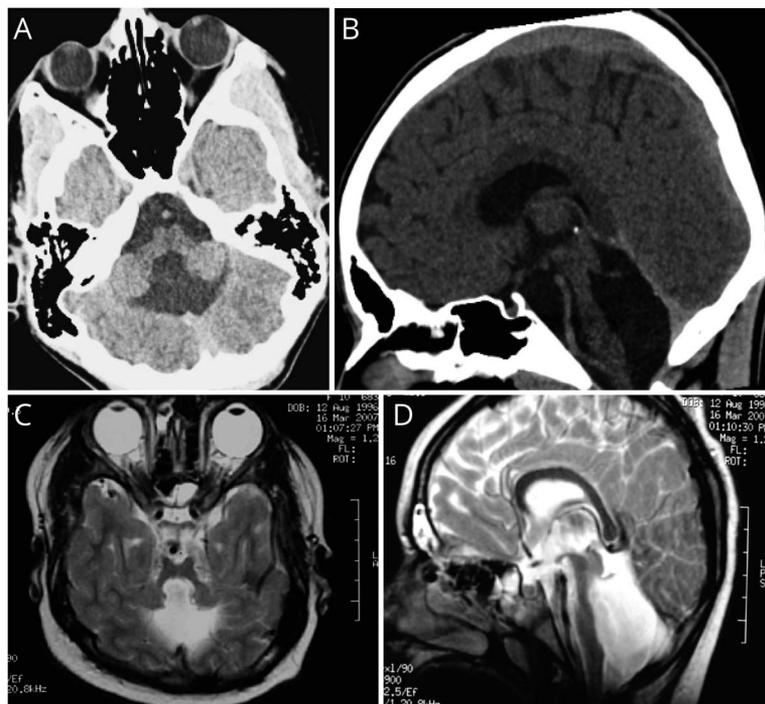
EEG performed in the proband at the age of 14 years showed slow and irregular background electrical activity with no epileptiform discharges. There was otherwise no family history of pontocerebellar dysplasia and hearing impairment, and the parents were healthy. Because the proband and his sister were likely affected by the same condition, an autosomal recessive inheritance was considered.

Genetic Investigation

Initially, we focused the analysis of ES data on the genes with established association with monogenic disorders. No known pathogenic or novel variants were identified in the set of genes associated with PCH or other neurodevelopmental disorders or hearing loss. In addition, the analysis of mitochondrial sequences, patterns of homozygosity, and copy number variants in the ES data revealed no convincing causative variants.

We therefore proceeded to the analysis of all variants identified by ES. We filtered and prioritized the ES variants as outlined in the Methods section. Performing the filtration under the recessive model, we identified a homozygous variant NM_005,172.1:c.481C>G (p.Arg161Gly) in the *ATOH1* that was predicted to result in amino acid substitution of arginine to glycine at position 161 in the sequence of the protein encoded by the *ATOH1*. The *ATOH1* gene encodes for the atonal bHLH transcription factor 1, a core transcription factor in the developing cerebellum, brainstem, and

Figure 2 CT and MR Images of the Proband II-1 and His Affected Sister II-3



(A) CT axial image of a proband II-1 demonstrating prominent hypoplasia of pons, middle cerebellar peduncles, and both cerebellar hemispheres. The vermis is absent. (B) CT sagittal image of a proband II-1 resulting in small posterior fossa and widened 4th ventricle, opened into the cerebellomedullary cistern and hypoplastic pons. Image courtesy of Allen Developing Mouse Brain Atlas. (C) MR axial image of an affected sister II-3 showing enlarged 4th ventricle with cerebellomedullary cistern, very hypoplastic pons, middle cerebellar peduncles, and cerebellar hemispheres. Please note also enlarged ocular bulbs. (D) MR sagittal image of an affected sister II-3 shows very hypoplastic pons, cystic 4th ventricle with absent vermis, and flattened posterior part of corpus callosum.

dorsal spinal cord, and in the ear. The identified missense variant results in the amino acid substitution within the evolutionarily conserved DNA-binding bHLH domain of the *ATOH1* protein.

Strikingly, the variant was found in a homozygous state despite being absent from all control populations (gnomAD, GoNL, UK10K, and the in-house population). No blocks of homozygosity or structural variants were identified in the region of the *ATOH1*. The gnomAD currently contains 332 variants of *ATOH1*, of which 215 are missense variants, 113 are synonymous mutations, and only 4 variants are predicted to result in the loss of function (LoF). Among the missense and LoF variants in gnomAD, only 4 are homozygous (p.Asp138Glu, p.His237Gln, p.Ala291Thr, and p.Glu326Val), and none of them lie in the evolutionary conserved bHLH domain (p.159-211) of the *ATOH1* protein. In addition, the *ATOH1* displays constraint against missense variants according to the gnomAD regional constraint data in DECIPHER genome browser (missense constraint: 0.637, p -value 6.03×10^{-7}).

The p.Arg161Gly variant is consistently predicted as damaging by most in silico predictors, and the affected amino acid position also shows a high degree of evolutionary conservation, with the arginine position being invariant across species. This affected amino acid position 161 lies at the beginning of the evolutionary conserved bHLH domain of the *ATOH1* protein called basic region of bHLH domain, which is involved in DNA binding. At the carboxy-terminal end of this domain is the HLH region, which facilitates interactions with

other transcription factors to form a heterodimer^{13,14} and activate target gene transcription. Because our identified variant is in a basic region of bHLH domain, it may affect the structural composition of this protein region or influence binding affinity to DNA.

Based on the evidence presented earlier, we analyzed the segregation of this variant in the reported family. Sanger sequencing confirmed the homozygous state of the NM_005172.1: c.481C>G *ATOH1* variant in the proband and affected sister. Both unaffected parents and the proband's twin were found to be heterozygous carriers of the variant, signifying that the variants segregated with the disease (Figure 1A). This variant was submitted to a ClinVar database (variation ID:873538).

We analyzed the images of the Allen mouse brain atlas¹⁵ and RNAseq data from the Allen developing human brain project¹⁶ and observed that the expression of the *ATOH1* and its mouse ortholog *Atoh1* is confined to developing cerebellum and primarily occurs in the prenatal stages of brain development (Figure 1B, eFigure 2, links.lww.com/NXG/A523).

Because the biallelic variants in *ATOH1* have not been conclusively associated with monogenic conditions in humans (apart from a single report without details on the clinical presentation¹⁷), we attempted to use the Matchmaker Exchange networks (GeneMatcher, Phenome Central, and MyGene2) to identify additional patients with biallelic *ATOH1* variants and PCH but failed to identify a suitable match.

In Silico Modeling of p.Arg161Gly in ATOH1

To determine the potential functional effect of the identified variant on the function of the ATOH1 protein, we performed in silico modeling of the p.Arg161Gly change. A bona fide homology model of the complex of the ATOH1/transcription factor E2 alpha with cognate DNA could be built using the structure of the NeuroD1/transcription factor E2 alpha/DNA complex (pdb id. 2QL2) (Figure 1C). A structural analysis of the homology model of the complex suggests that replacing R161 with G has 2 main effects:

1. Loss of a salt bridge with DNA backbone phosphate (Figure 1C), which should reduce the affinity of the protein for DNA and could also slightly change the orientation of the helix regarding the major groove of DNA, with possible implications on DNA recognition;
2. Glycine has a different conformational preference for arginine, and the helix of the bHLH motif could be more flexible or disrupted at the beginning, affecting the relative orientation of other parts of the protein.

We performed continuum electrostatic calculations to assess the effect of the variant on the free energy of binding DNA. Although the computed values depend on the assumed inner dielectric constant and the temperature, in all cases, the estimated change in the free energy of binding was unfavourable between 1.0 and 2.0 kcal/mol.

We also performed 60 ns molecular dynamics simulations in explicit water for the complex to check for structural and dynamical differences. The rather large fluctuations, possibly due to the small size of the DNA fragment, make, however, the differences observed in simulations not significant.

Discussion

We report on a family with 2 siblings similarly affected by PCH, severe global developmental delay, intellectual disability, and hearing impairment. Using ES, we identified a homozygous variant NM_005172.1:c.481C>G (p.Arg161Gly) in the *ATOH1* gene, which segregated with the apparently recessive PCH in our family with no other mutation explaining the disease. Although this gene has been shown to be a crucial factor in cerebellar and CNS development, biallelic *ATOH1* gene variants have been reported only once in a cohort of patients with intellectual disability and CNS abnormalities.¹⁷ In addition, a recent study reported cosegregation of a heterozygous *ATOH1* variant with progressive nonsyndromic hearing loss in a 5-generational pedigree.¹⁸

The *ATOH1* gene encodes a 354-amino acid protein that belongs to the bHLH family of transcription factors and is highly expressed during embryonic and early CNS development. The ATOH1 transcription factor is involved in the regulation of neurogenesis in the cerebellum, brainstem, and dorsal spinal cord, maintenance of the intestinal secretory cells, and differentiation of auditory hair cells.¹⁹⁻²¹

There are several lines of evidence supporting the possible role of *ATOH1* gene defects in cerebellar and neurodevelopmental disease. ATOH1 is one of the earliest markers of cerebellar granule neuron precursors that maintains cells in a proliferative state and is essential for their migration during development of cerebellum.²² It is important that mice models lacking *ATOH1* gene fail to form cerebellar granule cells and are born with a cerebellum that is devoid of an external germinal layer.²³ The knock-out mice models die shortly after birth because of respiratory failure.^{23,24}

To date, biallelic variants in *ATOH1* were reported only in 2 studies as a possible cause of the disease in humans. In a cohort of patients with intellectual disability, Anazi et al. reported a patient with a homozygous LoF variant in *ATOH1* (NM_005172.1:c.212del, p.(Gly71Alafs*36)) and a clinical presentation that largely overlaps with the presentation in the siblings of this study.¹⁷ The reported patient similarly presented with hypotonia, nystagmus, and global developmental delay. Furthermore, brain imaging studies revealed a strikingly similar pattern of severe hypoplasia of the cerebellum and brainstem with a mild frontal lobe atrophy, further supporting the role of biallelic *ATOH1* variants in PCH. Of interest a more recent report presented a novel association of a monoallelic *ATOH1* variant with hearing loss. The study reported a C-terminal extension variant (c.1030delC) segregating with hearing loss with onset at birth or early childhood and proposed the impaired degradation of abnormal ATOH1 protein.¹⁸ Although the siblings reported in our study also displayed hearing loss, it is not currently clear whether a similar mechanism of pathogenicity leading to hearing loss could be relevant in the case of biallelic variants.

The *ATOH1* variant we identified in our patients has not yet been reported in literature or databases of pathogenic or population variability. Considering it is an exceedingly rare variant and no consanguinity was reported for this family, we thought it was peculiar to find it in a homozygous state. Furthermore, there were no significant blocks of homozygosity identified in the proband's ES data to explain this observation. Analysis of segregation confirmed the variant to be present in homozygous state and that it segregated with PCH in our family (the affected sister had the same homozygous variant, while the unaffected parents and second sister were heterozygous carriers). The variant was also prioritized because of in silico predictions that consistently predicted the damaging effect of the p.(Arg161Gly) alteration, which affects an evolutionarily invariant arginine residue.

The variant we identified affects the DNA binding (bHLH) domain of the ATOH1 protein. Similarly, animal studies show that biallelic missense variants affecting this domain of the ATOH1 transcription factor result in defects of cerebellar development and function but do not cause lethality. Mouse models with a homozygous missense variant (p.Ser193Ala) exhibited cerebellar foliation defects, motor impairments, partial pontine nucleus migration defects, cochlear hair cell

degeneration, and profound hearing loss. The authors showed that this variant partially impairs ATOH1's ability to upregulate transcription of its target genes.²⁵ Similarly, another missense alteration in the bHLH region of ATOH1 (p.Met200Ile) caused progressive hearing loss because of inner ear hair cell loss and cerebellar atrophy, reduced cerebella in size, lacking of external granular cell layers, and improper localization and migration of Purkinje cells but with normal lifespan in mice.²⁶ It appears that milder defects of the ATOH1 protein function caused by biallelic missense variants with partial disruption of ATOH1 function may be associated with nonlethal disruption of cerebellar development and hearing loss. Of interest all studies in animal models consistently report disruption of ear development and hearing loss, which is also consistent with our observation of hearing loss in both affected siblings.

To further evaluate the pathogenicity of identified variant c.481C>G in *ATOH1* gene, we analyzed the characteristics of ATOH1 protein domain, where this mutation occurred. The p.Arg161Gly substitution lies at the beginning of evolutionary conservation bHLH domain (p.158-213) of the ATOH1 protein, which is involved in recognition and binding to a target DNA. Because the basic DNA binding region is crucial for proper recognition and binding to the target DNA sequence, the substitution of a positively charged amino acid Arg to Gly at the beginning of this region is probably disfavored. Arginine is strongly over-represented in protein sidechain/DNA backbone interaction,²⁷ providing overall stability of the complex, and possibly distortions of regular double helix structure, through salt bridges with DNA phosphates. Substitution of Arg161 with Gly implies loss of a salt bridge, resulting in an estimated change in free energy of ca. 1.5 kcal/mol unfavorable to complex formation. Moreover, glycine has unique conformational properties²⁸ that could introduce flexibility at the beginning of a helix crucial for proper contacts of the protein with DNA within the complex, with a possible slight repositioning of the complex partners, as suggested also by molecular dynamics simulations. Overall modeling shows that the mutation changes the stability of the protein-DNA complex and possibly also its geometry, implying dysregulation of transcription.

In conclusion, we confirm that *ATOH1* is a novel candidate gene for PCH, severe global developmental delay, intellectual disability, and hearing impairment. We hypothesize that biallelic missense variants causing partial loss of function of the DNA-binding domain of the transcription factor ATOH1 may lead to impaired cerebellar development and hearing loss. Although the evidence is currently too limited to conclusively prove causality, this report may provide a basis for further studies on the role of the *ATOH1* gene in human disease.

Acknowledgment

The authors are grateful to the family for its willingness to participate in this study.

Study Funding

This study was supported by the National Research Agency of the Republic of Slovenia, grant no. J3-9280.

Disclosure

The authors report no disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* November 9, 2021. Accepted in final form March 4, 2022. Submitted and externally peer reviewed. The handling editor was Alexandra Durr, MD, PhD.

Appendix Authors

Name	Location	Contribution
Tanja Višnjar, PhD	Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia	Major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Aleš Maver, MD, PhD	Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia	Major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Karin Writzl, MD, PhD	Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia; Medical Faculty, University of Ljubljana, Ljubljana, Slovenia	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Ornela Maloku, PhD	Department of Mathematics, Informatics and Physics, University of Udine, Udine, Italy	Analysis or interpretation of data
Gaber Bergant, MD	Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia	Drafting/revision of the article for content, including medical writing for content, and analysis or interpretation of data
Helena Jaklič, PhD	Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia	Drafting/revision of the article for content, including medical writing for content, and analysis or interpretation of data
David Neubauer, MD, PhD	Medical Faculty, University of Ljubljana, Ljubljana, Slovenia; Division of Paediatrics, Dept. of Child, Adolescent & Developmental Neurology, University Medical Centre Ljubljana, Ljubljana, Slovenia	Drafting/revision of the article for content, including medical writing for content, and analysis or interpretation of data
Federico Fogolari, PhD	Department of Mathematics, Informatics and Physics, University of Udine, Udine, Italy	Drafting/revision of the article for content, including medical writing for content, and analysis or interpretation of data
Nuška Pečarič Meglič, MD	Clinical Institute of Radiology, University Medical Centre Ljubljana, Ljubljana, Slovenia	Major role in the acquisition of data; analysis or interpretation of data
Borut Peterlin, MD, PhD	Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and study concept or design

References

- Rüsch CT, Bölsterli BK, Kottke R, Steinfeld R, Boltshauser E. Pontocerebellar hypoplasia: a pattern recognition approach. *Cerebellum*. 2020;19(4):569-582.
- van Dijk T, Baas F, Barth PG, Poll-The BT. What's new in pontocerebellar hypoplasia? An update on genes and subtypes. *Orphanet J Rare Dis*. 2018;13(1):92.

3. Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D. The CARE guidelines: consensus-based clinical case report guideline development. *J Clin Epidemiol*. 2014;67(1):46-51.
4. Bergant G, Maver A, Lovrecic L, Cuturilo G, Hodzic A, Peterlin B. Comprehensive use of extended exome analysis improves diagnostic yield in rare disease: a retrospective survey in 1,059 cases. *Genet Med*. 2018;20(3):303-312.
5. Maver A, Lovrecic L, Volk M, et al. Phenotype-driven gene target definition in clinical genome-wide sequencing data interpretation. *Genet Med*. 2016;18(11):1102-1110.
6. Seelow D, Schuelke M, Hildebrandt F, Nürnberg P. HomozygosityMapper: an interactive approach to homozygosity mapping. *Nucleic Acids Res*. 2009;37(Web Server issue):W593-W599.
7. Longo A, Guanga GP, Rose RB. Crystal structure of E47-NeuroD1/beta2 bHLH domain-DNA complex: heterodimer selectivity and DNA recognition. *Biochemistry*. 2008;47(1):218-229.
8. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis*. 1997;18(15):2714-2723.
9. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 2015;43(Database issue):D447-D452.
10. Fogolari F, Corazza A, Yarra V, Jalaru A, Viglino P, Esposito G. Blues: a program for the analysis of the electrostatic properties of proteins based on generalized Born radii. *BMC Bioinformatics*. 2012;13(suppl 4):S18.
11. Dolinsky TJ, Czodrowski P, Li H, et al. PDB2PQR: expanding and upgrading automated preparation of biomolecular structures for molecular simulations. *Nucleic Acids Res*. 2007;35(Web Server issue):W522-W525.
12. Sanner MF, Olson AJ, Spehner JC. Reduced surface: an efficient way to compute molecular surfaces. *Biopolymers*. 1996;38(3):305-320.
13. Gyoja F. Basic helix-loop-helix transcription factors in evolution: roles in development of mesoderm and neural tissues. *Genesis*. 2017;55(9). doi:10.1002/dvg.23051.
14. Jones S. An overview of the basic helix-loop-helix proteins. *Genome Biol*. 2004;5(6):226.
15. Lein ES, Hawrylycz MJ, Ao N, et al. Genome-wide atlas of gene expression in the adult mouse brain. *Nature*. 2007;445(7124):168-176.
16. Miller JA, Ding SL, Sunkin SM, et al. Transcriptional landscape of the prenatal human brain. *Nature*. 2014;508(7495):199-206.
17. Anazi S, Maddirevula S, Faqeih E, et al. Clinical genomics expands the morbid genome of intellectual disability and offers a high diagnostic yield. *Mol Psychiatry*. 2017;22(4):615-624.
18. Brownstein Z, Gulsuner S, Walsh T, et al. Spectrum of genes for inherited hearing loss in the Israeli Jewish population, including the novel human deafness gene ATOH1. *Clin Genet*. 2020;98(4):353-364.
19. Mulvaney J, Dabdoub A. Atoh1, an essential transcription factor in neurogenesis and intestinal and inner ear development: function, regulation, and context dependency. *J Assoc Res Otolaryngol*. 2012;13(3):281-293.
20. Le Dréau G, Escalona R, Fueyo R, et al. E proteins sharpen neurogenesis by modulating proneural bHLH transcription factors' activity in an E-box-dependent manner. *Elife*. 2018;7:e37267.
21. Zhong C, Fu Y, Pan W, Yu J, Wang J. Atoh1 and other related key regulators in the development of auditory sensory epithelium in the mammalian inner ear: function and interplay. *Dev Biol*. 2019;446(2):133-141.
22. Pan N, Jahan I, Lee JE, Fritsch B. Defects in the cerebella of conditional Neurod1 null mice correlate with effective Tg(Atoh1-cre) recombination and granule cell requirements for Neurod1 for differentiation. *Cell Tissue Res*. 2009;337(3):407-428.
23. Ben-Arie N, Bellen HJ, Armstrong DL, et al. Math1 is essential for genesis of cerebellar granule neurons. *Nature*. 1997;390(6656):169-172.
24. Chonko KT, Jahan I, Stone J, et al. Atoh1 directs hair cell differentiation and survival in the late embryonic mouse inner ear. *Dev Biol*. 2013;381(2):401-410.
25. Xie WR, Jen HI, Seymour ML, et al. An Atoh1-S193A phospho-mutant allele causes hearing deficits and motor impairment. *J Neurosci*. 2017;37(36):8583-8594.
26. Sheykholslami K, Thimmappa V, Nava C, et al. A new mutation of the Atoh1 gene in mice with normal life span allows analysis of inner ear and cerebellar phenotype in aging. *PLoS One*. 2013;8(11):e79791.
27. Mandel-Gutfreund Y, Schueler O, Margalit H. Comprehensive analysis of hydrogen bonds in regulatory protein DNA-complexes: in search of common principles. *J Mol Biol*. 1995;253(2):370-382.
28. Barnes MR. Bioinformatics for geneticists: a bioinformatics primer for the analysis of genetic data. In: Betts MJ, Russell RB, eds. *Amino-Acid Properties and Consequences of Substitutions*. John Wiley & Sons; 2007.

Neurology[®] Genetics

Biallelic *ATOH1* Gene Variant in Siblings With Pontocerebellar Hypoplasia, Developmental Delay, and Hearing Loss

Tanja Visnjar, Ales Maver, Karin Writzl, et al.

Neurol Genet 2022;8;

DOI 10.1212/NXG.0000000000000677

This information is current as of May 3, 2022

Updated Information & Services	including high resolution figures, can be found at: http://ng.neurology.org/content/8/3/e677.full.html
References	This article cites 27 articles, 1 of which you can access for free at: http://ng.neurology.org/content/8/3/e677.full.html##ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): All Genetics http://ng.neurology.org/cgi/collection/all_genetics Developmental disorders http://ng.neurology.org/cgi/collection/developmental_disorders Mental retardation http://ng.neurology.org/cgi/collection/mental_retardation Neonatal http://ng.neurology.org/cgi/collection/neonatal
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://ng.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://ng.neurology.org/misc/addir.xhtml#reprintsus

Neurol Genet is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.

