Congenital immobility and stiffness related to biallelic *ATAD1* variants

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Abstract

Objective
To delineate the phenotype associated with biallelic *ATAD1* variants.

Methods
We describe 2 new patients with *ATAD1*-related disorder diagnosed by whole-exome sequencing and compare their phenotype to 6 previous patients.

Results
Patients 1 and 2 had a similar distinctive phenotype comprising congenital stiffness of limbs, absent spontaneous movements, weak sucking, and hypoventilation. Both had absent brainstem evoked auditory responses (BEARs). Patient 1 carried the homozygous p.(His357Argfs*15) variant in *ATAD1*. In the light of the finding in patient 1, a second reading of exome data for patient 2 revealed the novel homozygous p.(Gly128Val) variant.

Conclusions
Analysis of the phenotypes of these 2 patients and of the 6 previous cases showed that biallelic *ATAD1* mutations are responsible for a unique congenital encephalopathy likely comprising absent BEAR, different from hyperekplexia and other conditions with neonatal hypertonia.

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Glossary

AMPA = α-amino-3-hydroxy-5-methylisoxazole-4-propionate; **BEAR** = brainstem evoked auditory response; **gnomAD** = Genome Aggregation Database; **NIV** = noninvasive ventilation; **SNEL** = severe neonatal episodic laryngospasm; **WES** = whole-exome sequencing.

Neonatal unexplained muscular stiffness is a relatively rare condition. Various genetic syndromes/etiologies such as hyperekplexia,[1,2] encephalopathies due to *BRAT1*[3,4] or *GRIA4*[5] genes, and Crisponi syndrome[6] are causes of persistent neonatal stiffness. Paroxysmal stiffness is observed in newborns with *SCN4A*-related severe neonatal episodic laryngospasm (SNEL),[7] and facial paroxysms are a hallmark of Crisponi syndrome. Features other than hypertonia may be part of these phenotypes: developmental delay with or without epilepsy (*BRAT1* and *GRIA4*), laryngeal spasms (*SCN4A* and Crisponi), permanent (*BRAT1*) or paroxysmal (*SCN4A* and Crisponi) sucking difficulties, and exaggerated startles (hyperekplexia, *GRIA4*, and Crisponi).

Biallelic variants in the *ATAD1* gene are the most recently described cause of persistent neonatal stiffness.[8–10] The phenotype ascribed to these genotypes is referred to as hyperekplexia 4 (MIM 618011). Six patients belonging to 3 different families have been reported to date in the literature. We describe here the *ATAD1*-related disorder in 2 additional patients of unrelated families, diagnosed by whole-exome sequencing (WES).

Methods

DNA was extracted from parent and proband samples. Trio WES was performed for each patient on a NextSeq 500 Sequencing System (Illumina, San Diego, CA), with a 2× 150 bp high-output sequencing kit after a 12-plex enrichment with the SeqCap EZ MedExome kit (Roche, Basel, Switzerland), according to the manufacturer's specifications. Sequence quality was assessed with FastQC 0.11.5; then, the reads were mapped using BWA-MEM (version 0.7.13), sorted, and indexed in a bam file (samtools 1.4.1), duplicates were flagged (sambamba 0.6.6), and coverage was calculated (picard-tools 2.10.10). Variant calling was performed with GATK 3.7 Haplotype Caller. Variants were then annotated with SnpEff 4.3, dbNSFP 2.9.3, Genome Aggregation Database (gnomAD), ClinVar, Human Gene Mutation Database, Variome Great Middle East, and an internal database. Coverage for these patients was at least 93% at a 20× depth threshold. This work was approved by the local ethics committee (Comité de Protection des Personnes).

Standard protocol approvals, registrations, and patient consents

The parents of both patients gave their consent for genetic studies and for the publication of the results and videos and have been given the opportunity to review the manuscript.

Data availability

Anonymized data not published within this article will be made available by request from qualified investigators.

Patient description and results

A full description is available in supplemental data 1 (links. lww.com/NXG/A322).

Patient 1 (video 1, links.lww.com/NXG/A323, figure, A) was the second child of healthy parents. He was born at 36 weeks of gestation with normal parameters. Respiratory distress and hypotonia occurred at 20 minutes of life requiring oxygen therapy and noninvasive ventilation (NIV). At 3 hours of life, the patient's weak sucking required parenteral nutrition. The day after, physical examination revealed marked generalized stiffness, brisk tendon reflexes, truncal hypotonia, tremor of limbs, absent spontaneous movements and absent eye contact, weak respiratory movements, and a constant moaning. These clinical findings remained the same from that point on. During the following weeks, patient 1 needed a nasogastric tube. After NIV removal, he required oxygen supply and experienced many episodes of apnea and desaturation. Two brain MRIs were normal. Electroencephalographic (EEG) recordings were initially normal (at days 2, 4, 11, and 21), then showed a slow, monotone and nonreactive activity without spatial organization, amplitude and frequency were asymmetric with scarce spikes without epileptiform brain discharges. Brainstem evoked auditory responses (BEARs) were absent on both ears despite stimulation up to 105 dB (figure, A). EMG performed at 2 months of life showed a persistent contraction without myotonia. Patient 1 died at 4 months of cardiorespiratory arrest. WES revealed the homozygous NM_001321967.1:c.1070_1071del p.(His357Argfs*15) variant in *ATAD1* confirmed by Sanger sequencing. This variant has been previously reported in *ATAD1*-related disorder[8] with thorough functional studies. Analysis of the parents showed that they were heterozygous carriers.

Patient 2 (video 2, links.lww.com/NXG/A324, figure, B) was a boy born 5 years before patient 1 to healthy consanguineous parents. He was born full term by spontaneous vaginal delivery with normal parameters. Tremor, weak sucking, and continuous crying were noticed during the first hours of life. Patient 2 was hospitalized because of repeated episodes of oxygen desaturation at the 10th hour of life. Clinical examination showed generalized stiffness, truncal hypotonia, absent spontaneous movements, and brisk tendon reflexes. He needed nasogastric tube feeding as well as oxygen therapy and

Figure Pedigrees, electrophoregrams, and BEAR
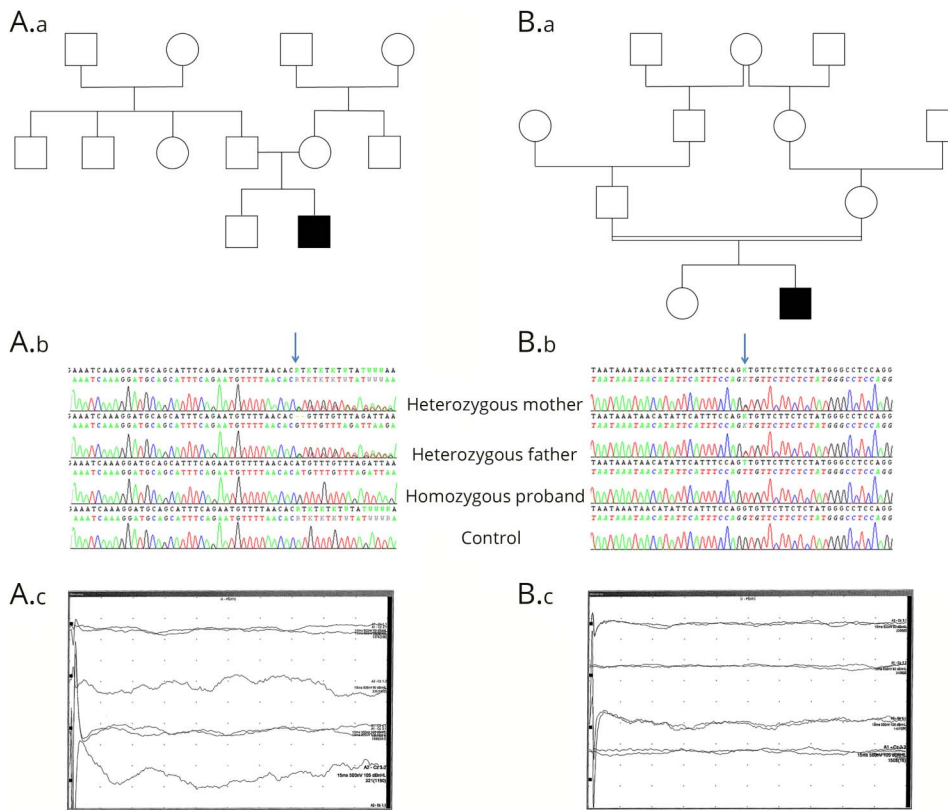
(A) Pedigree (A.a) and electrophoregrams (A.b) showing the homozygous p.(His357Argfs*15) variant in the proband and the heterozygous state of his parents. BEARs were absent with stimulation up to 90 and 105 dB (A.c). (B) Pedigree (B.a) of patient 2 and electrophoregrams (B.b) showing the homozygous p.(Gly128Val) variant in the proband and the heterozygous state of his parents. BEARs were also absent in patient 2 with stimulation up to 90 and 105 dB (B.c). BEAR = brainstem evoked auditory response.

intermittent NIV because of persistent hypoventilation. Tonic seizures began when he was 3.5 months. He died at age 6 months of a cardiorespiratory arrest. Three brain MRIs showed a moderate left ventriculomegaly. EEG recordings were initially normal (at days 3 and 29) and then showed an unstable, slow background activity without organization from the age of 3.5 months. BAER showed no responses on both ears despite stimulation up to 105 dB (figure, B). WES analysis revealed the homozygous Chr10(GRCh37):g.89544427C>A, NM_001321967.1:c.383G>T, p.(Gly128Val) variant in *ATAD1* confirmed by Sanger sequencing. This variant is present on all coding refseq transcripts, is absent from control databases (Exome Variant Server, Exome Aggregation Consortium, and gnomAD), affects a highly conserved amino acid of the protein in the AAA+ ATPase domain, and is considered as disease causing with all the prediction algorithms we have tested. Both parents were heterozygous carriers of the variant.

Discussion

ATAD1 encodes for thorase, an AAA+ ATPase protein discovered in 2010 in primary rat cortical neurons.[11] Thorase plays a role in mitochondria and peroxisomes as a quality control of membrane proteins and regulates surface expression of brain α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors. At the synaptic level, thorase induces the internalization of AMPA receptor by disassembling the subunits GLUR2 and GRIP1. Thus, thorase modulates central neurotransmission, which explains the neurologic phenotype of individuals unable to produce normal thorase species.

Six patients with biallelic *ATAD1* mutations belonging to 3 different families have been reported in the literature to date (table).[8–10] As for patient 2 of the present report, one of these diagnoses was made retrospectively, based on the identification of the distinctive *ATAD1*-related neurologic syndrome,[9] which demonstrates the uniqueness of the condition. The phenotype is characterized by a neonatal onset of stiffness of limbs associated with truncal hypotonia or hypertonia. Unlike other causes of neonatal hypertonia, including hyperekplexia, these newborns have no spontaneous movements and display poor facial expression. Clinical examination induces limb tremor and exacerbation of hypertonia, but no exaggerated startle response to sensory stimuli. These newborns need intensive care from the first days of life because of poor sucking and respiratory distress/hypoventilation. This vital distress is the main cause of their early death (3–8 months). Of note, the only 2 patients alive at age 9 months underwent tracheostomy.[10]

Most of these patients (6/8) do not have eye contact despite normal ophthalmologic examination and normal

Table Features of 8 patients with biallelic *ATAD1* variants and ATAD1-related disorder

	Ahrens-Nicklas et al.		Piard et al.			Wolf et al.	Present report		Total
	IV-6	IV-3	Patient 1	Patient 2	Patient 3		Patient 1	Patient 2	
Origin	Kuwait	Kuwait	France	France	France	NA	Tunisia-Algeria	France	
Sex	Male	Male	Male	Male	Female	Female	Male	Male	
Age at onset	Birth	Birth	Birth	Birth	Birth	Birth	Birth	Birth	Birth 8/8
Sucking difficulties	NA	NA	No	Yes	No	Yes	Yes	Yes	4/6
Respiratory distress	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8/8
Peripheral hypertonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8/8
Axial hypotonia	No	No	No	No	Yes	No	Yes	Yes	3/8
Absent spontaneous movements	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8/8
Pyramidal signs	Yes	No	Yes	No	No	Yes	Yes	Yes	5/8
Tremor	No	No	Yes	Yes	No	Yes	Yes	Yes	5/8
Clinical seizures	Yes	Yes	Yes	No	No	No	No	Yes	4/8
Visual contact	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent 8/8
Inguinal hernia	Yes	Yes	No	Yes	Yes	No	No	Yes	5/8
Umbilical hernia	No	No	No	Yes	No	No	No	Yes	2/8
Age at death	Alive at 9 mo (tracheostomy)	Alive at 9 mo (tracheostomy)	5 mo	3 mo	6 mo	8 mo	4 mo	6 mo	Mean 5.3 mo; median 5.5 mo
Initial brain MRI	Normal (2 mo)	Normal (2 mo)	Normal	NA	Myelination delay (1 d)	Normal	Normal	Ventriculomegaly (8 d, 1.5 mo)	Normal 5/7
Repeated brain MRI	Progressive loss of volume (9 mo and 20 mo)	Normal (5 mo)	NA	NA	NA	NA	Normal	Pallidum hypersignal (4 mo)	
Proton MR spectroscopy	Normal	Normal	NA	NA	NA	NA	Normal	Normal	
EEG initial	NA	NA	Abnormal background and epileptic discharges	NA	Normal	Normal	Normal	Normal	Abnormal 1/5

Table Features of 8 patients with biallelic *ATAD1* variants and *ATAD1*-related disorder *(continued)*

| | Ahrens-Nicklas et al. | | | | Piard et al. | Wolf et al. | Present report | | Total |
	IV-6	IV-3	Patient 2	Patient 3	Patient 1		Patient 1	Patient 2	
EEG follow-up (age)	Hypsarrhythmia (9 mo)	Abnormal background (2.5 mo)	NA	NA	NA	NA	Abnormal background (2 mo)	Abnormal background + tonic seizure (2 mo)	Abnormal 4/4
Fundus oculi	NA	NA	NA	NA	Normal	NA	Normal	Normal	3/3
BEAR	(Unreactive to sound)	NA	NA	NA	NA	Absent	Absent	Absent	3/3
ATAD1 variant	c.826G>T				c.1070_1071del	c.162G>C	c.1070_1071del	c.383G>T	
Type of variant	Nonsense				Frameshift	Missense	Nonsense	Missense	
Protein level	p.(E276X)				p.(His357Argfs*15)	p.(Gln54His)	p.(His357Argfs*15)	p.(Gly128Val)	

Abbreviations: BEAR = brainstem evoked auditory response; NA = not available.

electroretinogram/visual evoked potential recordings (present report). This persists throughout their lifetime and is likely related to the severity of their developmental encephalopathy. Their poor reactions to stimuli may be aggravated by hearing impairment, as suggested by absent BEAR in our 2 patients and in a previous one.[9] This may be related to a deficit of AMPA receptor–dependent neurotransmission of the signals arising from cochlear hair cells.[12]

Repeated clinical examinations of these newborns show severe encephalopathy during their entire lifetime. All these features make a strong difference with other conditions associated with neonatal stiffness, i.e., hyperekplexia,[1,2] Crisponi syndrome,[6] *SCN4A*-related SNEL syndrome,[7] and *GRIA4*-related encephalopathy.[5] Only the *BRAT1*-related encephalopathy coined as "lethal neonatal rigidity and multifocal seizure syndrome" bears comparison with the *ATAD1*-related phenotype.[3,4,13] Refractory epilepsy is usual in the former, and although seizures are not rare in the latter (4/8), the epilepsy is severe enough to suggest epileptic encephalopathy in a minority of them (2/8). Thus, the term "hyperekplexia 4" phenotype ascribed to *ATAD1* mutations seems to be inaccurate.

The 8 patients reported so far belong to 4 families carrying 4 different *ATAD1* variants. All of these variants are homozygous, and the only recurrent variant is the p.(His357Argfs*15) frameshift identified in patient 1 with unrelated parents and in Piard et al.[8] All 4 parents with this frameshift in the heterozygous state are of Tunisian descent, with those reported in Piard et al. being related. This variant is very rare, reported 3 times at the heterozygous state in the gnomAD database (allele frequency 1.2e-5), but the origin of the carriers is not mentioned in the database. This variant is either a true recurrent variant or a variant found in 2 patients with an unidentified common Tunisian ancestry. We also report the novel p.(Gly128Val) missense variant. This variant is the second missense causing *ATAD1*-related disorder, together with p.(Gln54His).[9] The exact consequence of this change is unknown for now, but its pathogenicity is highly likely according to all tested prediction algorithms and given the uniqueness of the syndrome. The first pathogenic variant ever reported is a nonsense [p.(E276X)[10]], which suggested that the loss of thorase function is the cause of *ATAD1*-related disorder. This was substantiated by studies showing that the in vitro overexpression of thorase results in a decreased expression of cell surface AMPA receptors, whereas the loss of thorase expression induces the surface expression of these receptors and an excess of AMPA-mediated current.[14] These data are in line with the clinical involvement compatible with glutamate overload. One cannot exclude, however, peroxisomal dysfunction in patients with *ATAD1*-related disorder. Other functional studies showed that the mutant thorase with the Tunisian variant impairs the recycling of glutamate receptors at the cell surface, acting as a gain-of-function–inducing variant.[8] Further investigations are

needed to clarify this issue, especially since Ahrens-Nicklas et al.[10] recommended the use of perampanel, an AMPA receptor antagonist, in these patients.

Publication history

Appendix Authors

Name	Location	Contribution
Roxane Bunod, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition and analysis of data and drafted the manuscript
Diane Doummar, MD	Hôpital Armand Trousseau, Paris, France	Revised the manuscript critically
Sandra Whalen, MD	Hôpital Armand Trousseau, Paris, France	Revised the manuscript critically
Boris Keren, MD, PhD	GH Pitié-Salpêtrière, Paris, France	Major role in acquisition of data
Sandra Chantot-Bastaraud, MD	Hôpital Armand Trousseau, Paris, France	Acquisition of data and drafted the manuscript intellectually
Kim Maincent, MD	Hôpital de Pédiatrie et de Rééducation, Bullion, France	Major role in acquisition of data
Marie-Charlotte Villy, MD	GH Pitié-Salpêtrière, Paris, France	Drafted the manuscript
Michèle Mayer, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition of data
Diana Rodriguez, MD, PhD	Hôpital Armand Trousseau, Paris, France	Drafted the manuscript intellectually
Lydie Burglen, MD, PhD	Hôpital Armand Trousseau, Paris, France	Drafted the manuscript intellectually
Pierre-Louis Léger, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition of data

Appendix (continued)

Name	Location	Contribution
François Kieffer, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition of data
Isabelle Martin, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition of data
Delphine Héron, MD	GH Pitié-Salpêtrière, Paris, France	Drafted the manuscript intellectually
Julien Buratti	GH Pitié-Salpêtrière, Paris, France	Major role in acquisition of data
Arnaud Isapof, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition and analysis of data
Alexandra Afenjar, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition and analysis of data
Thierry Billette de Villemeur, MD	Hôpital Armand Trousseau, Paris, France	Major role in acquisition of data
Cyril Mignot, MD, PhD	GH Pitié-Salpêtrière, Paris, France	Major role in acquisition and analysis of data and drafted the manuscript

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