Expanding the Clinical Spectrum of UBTF-Related Neurodevelopmental Disorder

Andrea Pietra, MD,* Flavia Palombo, PhD,* Melania Giannotta, MD, Monica Maffei, MD, Claudio Fiorini, PhD, Roberta Costa, PhD, Giovanna Cenacchi, MD, PhD, Valerio Carelli, MD, PhD, Duccio Maria Cordelli, MD, PhD, Antonella Pini, PhD, and Caterina Garone, MD, PhD

Neurol Genet 2023;9:e200098. doi:10.1212/NXG.0000000000200098

Abstract

Objectives
UBTF1 gene encodes for Upstream Binding Transcription Factor, an essential protein for RNA metabolism. A recurrent de novo variant (c.628G>A; p.Glu210Lys) has recently been associated with a childhood-onset neurodegenerative disorder characterized by motor and language regression, ataxia, dystonia, and acquired microcephaly. In this study, we report the clinical, metabolic, molecular genetics and neuroimaging findings and histologic, histochemical, and electron microscopy studies in muscle samples of 2 patients from unrelated families with a neurodevelopmental disorder.

Methods
Data were retrospectively analyzed by medical charts revision.

Results
Patient 1, a 16-year-old boy, presented a childhood-onset slowly progressive neurodegenerative disorder mainly affecting language skills, behavior, and motor coordination. Patient 2, a 22-year-old woman, presented with a severe and rapidly progressive disease with dystonic tetraparesis, acquired microcephaly, and severe cognitive deficit complicated by pseudobulbar syndrome characterized by involuntary and uncontrollable outbursts of laughing, dysphagia requiring tube feeding, and nocturnal hypventilation treated with noninvasive ventilation. Both patients carried the recurrent previously described UBTF1 de novo variant and had signs of mitochondrial dysfunction at muscle biopsy. The metabolic profile of patient 2 also revealed a decrease in CSF biotin.

Discussion
These case reports add new insights to the UBTF1 disease spectrum instrumental to improving the diagnostic rate in neurodevelopmental disorders.

Introduction
Upstream Binding Transcription Factor, OMIM *600673 (UBTF) gene encodes for 2 isoforms of the upstream binding factor (UBF), UBTF1 and UBTF2, able to form homodimers and heterodimers and plays an essential role in RNA transcription within the nucleolus.1 Specifically, UBTF1 regulates ribosomal RNA transcription by RNA polymerase I, whereas UBTF2 regulates mRNA transcription by RNA polymerase II. A recurrent monoallelic de novo

*These authors contributed equally to the manuscript as first authors.

From the UO Genetica Medica (Andrea Pietra), IRCCS Azienda Ospedaliero-Universitaria di Bologna; Department of Medical and Surgical Sciences (Andrea Pietra, C.G.), Alma Mater Studiorum University of Bologna; IRCCS Istituto delle Scienze Neurologiche di Bologna (F.P.), Programma di Neurogenetica; IRCCS Istituto delle Scienze Neurologiche di Bologna (M.G., Antonella Pini, C.G.), UOC Neuropsichiatria dell’et`a Pediatrica; IRCCS Istituto delle Scienze Neurologiche di Bologna (M.M., C.F.), Programma di neuroradiologia con tecniche ad elevata compl`esit`a; Department of Biomedical and Neuromotor Sciences (R.C., G.C., V.C., D.M.C.), Alma Mater Studiorum University of Bologna; and UO Anatomia (G.C.), Istologia Patologica, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by Italian Minister of Research.

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.
missense variant c.628G>A (p.Glu210Lys) causes a neurodevelopmental disorder characterized by motor, speech, language, and cognitive regression in early childhood progressively leading to severe cognitive deficit, loss of milestones, pyramidal and extrapyramidal signs, and behavioral dysfunction. This variant pathogenicity is due to the UBF gain of function responsible for a marked increase in the expression of pre-RNA and 18S rRNA.

In this study, we report clinical, metabolic, and neuroradiologic findings of 2 additional cases from unrelated families, which expand the clinical phenotype of UBTFI-related disease.

Methods

We retrospectively revised the following: clinical features; neuroimaging studies; metabolic investigations including urine, plasma, CSF; histologic, histochemical, and electron microscopy studies in muscle biopsy samples; and the molecular genetics data including exome sequencing of the 2 patients and segregation analysis in the 2 unrelated families.

Results

Patient 1 is a 16-year-old boy, born at term after an uneventful pregnancy and cesarian delivery. No consanguinity nor family history for neurodevelopmental disorders was reported. Psychomotor development was normal up to 5 years of age when he presented with attention deficit, difficulties in relationships with pairs, and coarse movements. At 6 years of age, he had a progressive global regression in cognitive, behavioral, and motor skills. He progressively developed a complex clinical picture characterized by ataxia, nystagmus, oculomotor apraxia, severe cognitive deficit, dysarthria, stuttering, self-injurious episodes, dysmetria, and dysgraphia. A decline in cognitive functions, more prominent in the language and speech skills, and a worsening of the extrapyramidal symptoms’ severity were observed during the follow-up. At 15 years of age, he also presented with episodes of obsessive-compulsive behavior. At the last evaluation at 17 years of age, he was still able to walk with support but with an ataxic gait; he had severe sialorrhea but was still able to orally feed himself; he presented with difficulties articulating every single word because of verbal fluency impairment; upper limb movements were functionally impaired by severe dysmetria; he also presented with subacute onset of additional symptoms of movement disorders with dystonia and parkinsonism for which he started treatment with levodopa with a definitive improvement at 4 months of follow-up (Video 1); IQ assessed with Wechsler Intelligence Scale for Children–type IV (WISC-IV) scale confirmed a severe cognitive deficit. Signs or symptoms in other organs/apparatus were not reported. A first brain MRI was performed at 8 years of age showing increased T2 signals in the periventricular supratentorial and deep white matter, thin corpus callosum, and supratentorial atrophy (Figure 1, A and B). A follow-up brain MRI at 16 years of age showed an increase in hyperintensity in T2-weighted white matter with moderate worsening in brain atrophy (Figure 1, C and D). Metabolic analyses of arylsulfatase A, CSF and plasma lactic acid, plasma, and urinary amino acids, acylcarnitine, and urinary organic acid were normal. Histologic and histochemical study of muscle biopsy specimen displayed a few eosinophilic subsarcolemmal accumulations, mild diameter variability of the fibers and isolated vesicular nuclei at hematoxylin-eosin staining (data not shown), a focal increase in oil red O staining (data not shown), and several fibers with a subsarcolemmal increase in cytochrome c oxidase (COX) reaction (Figure 1A). The ultrastructural analysis revealed mitochondrial alterations compatible with the light microscopy findings: accumulation of abnormal large swollen mitochondria, irregularly shaped with hypodense matrix and aberrant residual cristae (eFigure 1, links.lww.com/NXG/A642). Mitochondrial respiratory chain enzyme activities in muscle homogenate were within normal ranges (data not shown).

Patient 2 is a 22-year-old woman, born at term after an uneventful pregnancy and cesarian delivery. No consanguinity nor family history for neurodevelopmental disorders was reported. After nonspecific febrile illness, a global neurodevelopmental regression occurred in the second year of life with arrest in motor and language skills, abnormal behavior with irritability, gait unbalance with difficulty climbing stairs, frequent falls, and cranial circumference growth delay. At 6 years of age, the clinical course was complicated by axial asymmetric recurrent dystonic episodes lasting some days compromising the ability to walk and sialorrhea. At 9 years of age, she developed tetraparesis with parkinsonian rigidity and limb dystonia and pseudobulbar syndrome characterized by involuntary and uncontrollable outbursts of laughing lasting several minutes. She lost the ability to walk at 10 years. Dysphagia was progressive and required percutaneous gastrostomy (PEG) tube feeding at 18 years of age. She also presented with nocturnal hypoventilation treated with non-invasive ventilation. Awake and sleep EEG recordings showed no epileptiform or periodic discharges and nerves’ conductions were normal. At 22 years of age, she completely lost verbal communication, but she partially reacted to stimuli in the family context (Video 2). A first brain MRI, when she was 4 years of age, showed signs of modest cerebral atrophy and hyperintensity in T2-weighted images in the periventricular supratentorial and deep white matter and thin corpus callosum (Figure 1, E and F). Follow-up brain MRI at the age of 13 years showed signs of progressive cortical and subcortical atrophies, initial signs of cerebellar atrophy, and brainstem atrophy, with further thinning of the corpus callosum and higher hyperintensity of supratentorial white matter and thalami in T2-weighted images (Figure 1, G and H). Clinical and neuroimaging findings were suggestive of metabolic neurodegeneration. Metabolic analyses showed a decrease in biopterin in the CSF and a slight increase in plasma lactic (2.4; n.v. 0.3–1.3 mmol/L) and pyruvic acid (0.11; n.v. 0.03–0.08 mmol/L) levels, while white cell enzymes activities, plasma
sialotransferrin and vitamin E, plasma and urinary amino acids and organic acids, urinary purine, pyrimidine, and mucopolysaccharides were in the normal range. Muscle biopsy showed a modest subsarcolemmal increase at Gomori trichrome staining in numerous fibers, observed also with succinate dehydrogenase staining (data not shown), displaying a normal COX activity (Figure 2B). Mitochondrial respiratory chain activity in muscle homogenate detected a partial reduction in complex I activity (10.3; n.v. 13-24).

After inconclusive diagnostic analysis with a next-generation multigene panel, the genetic investigation was expanded to WES, and an in silico panel analysis of genes associated with severe pediatric disorders revealed the presence of a heterozygous missense variant, c.628G>A [p.Glu210Lys], in the UBTF gene (NM_014233.4). According to the American College of Medical Genetics classification, the c.628G>A was classified as pathogenic with the PM2, PP2, PP3, and PP5 (PM = moderate evidence of pathogenicity; PP = supporting evidence of pathogenicity) criteria. Segregation analysis showed that the c.628G>A have arisen de novo in both families, definitely confirming the diagnosis of a UBTF1-related disorder.

**Discussion**

Neurodevelopmental disorders (NDD) are a heterogeneous group of disorders including many neurodegenerative and...
neurometabolic diseases and presenting with delay or loss of acquired milestones based on the underlying pathogenetic mechanism. Currently, WES with trio analysis increasingly reveals pathogenic recurrent de novo variants in genes encoding proteins responsible for the early commitment or maturation of the neural lineage further expanding the genetic complexity of NDD.6

In 2017, one of these recurrent de novo variants was recognized in the UBTF1 gene as a single heterozygous change (c.628G>A) in a highly conserved amino acid (p.Glu210Lys) located within the second HMG-box. (High-Mobility Group box) homology box domain was first demonstrated to cause UBF gain-of-function activity with a consequent aberrant rRNA metabolism. This variant was associated with childhood-onset neurodegenerative disorders.7 Since then, 14 patients have been reported whose main clinical features are summarized in eTable 1 (links.lww.com/NXG/A643).3,4,7-9 Our additional case reports contribute to defining UBTF1-related NDD as a clinical spectrum ranging from a milder (patient 1) to a more severe (patient 2) phenotype. Patient 1 presented with motor, behavioral, and language regression, but he was still able to walk and orally feed himself at the last examination (16 years of age). Patient 2 presented instead with a more severe rapidly progressive clinical deterioration with highly disabling dystonic tetraparesis and severe language and cognitive impairment complicated by the need for PEG tube feeding and nocturnal ventilation. Moreover, she presented with pseudobulbar syndrome that was never reported in the UBTF1 NDD patients’ cohort. MRI confirmed that UBTF1 NDD involve gray and white matter including the cerebellum and basal ganglia. Brainstem atrophy, never reported in patients with de novo pathogenic variants in UBTF1, also occurred in patient 2, responsible for severe dysphagia. The metabolic profile showed a decreased level in biopterin in patient 2 as also reported by Ikeda et al.,4 qualifying this as a potential biomarker of UBTF1 NDD. A metabolomic analysis of all UBTF1 NDD patients’ cohort may be appropriate to fully disclose the underlying profile. We also showed for the first time that mitochondrial metabolism might be also involved because the muscle biopsy of both patients displayed signs of compensatory mitochondrial proliferation with subsarcolemmal rims at cytochrome histochemical activity, abnormal ultrastructure in 1 case, as well as slightly reduced respiratory complex 1 activity. This suggests a potential contributing role of mitochondrial dysfunction in the UBTF1 NDD mechanism.

UBF plays an essential role in the early stage of neurodevelopment. Either overexpression or abolished activity of UBF is responsible for a neurodegenerative process as demonstrated in vivo and in vitro models. Neuronal expression of human UBTF1 was lethal in Drosophila spp. Whereas tissue-specific expression in the eye caused a small-eye phenotype with loss of photoreceptor development. Similarly, Ubtf−/− mouse is embryonic lethal while Ubf−/− displayed only mild motor and behavioral dysfunction in adulthood.2 Pathogenicity of the recurrent human variant p.Glu210Lys in the UBTF1 gene was demonstrated because of an overexpression of the UBF protein. Of interest the knockdown of UBTF in 3T3 cells was associated with the upregulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), a master regulator of mitochondrial biogenesis. Similarly, PPARGC1A was upregulated in UBTF1 p.Glu210Lys human fibroblasts.7 We speculate that the UBTF1 disease mechanism affects mitochondrial function and leads to activation of compensatory upregulation of PPARGC1A and increased mitochondrial biogenesis, as seen in muscle biopsies from our patients. The role of this rewiring of mitochondrial function and biogenesis requires further investigations but possibly fits with the spectrum of clinical features of these patients.

In conclusion, our study expands the clinical, metabolic, and neuroradiologic findings of UBTF1 NDD contributing to improving its clinical definition, suggesting mitochondrial dysfunction as a new direction for further investigations and as a potential therapeutic target.

Acknowledgment
F. Palombo and V. Carelli were supported by the Italian Ministry of Health with the "Ricerca Corrente" funding; C. Garone was supported by the Minister of Italian Research with the Rita Levi Montalcini Award; C. Garone, V. Carelli, and D.M. Cordelli were supported by #NEXTGENERATIONEU (NEGU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) - A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022); M. Maffei, M. Giannotta, V. Carelli, and A. Pietra are part of the European Reference Network for neuromuscular diseases; The authors also thank Dr. Luca Soliani and Ilaria Pettenuzzo for kindly providing patients’ videos.

Study Funding
Ricerca corrente of Italian Minister of Health- Rita Levi Montalcini Award from the Italian Minister of Research-#NEXTGENERATIONEU (NEGU) funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) - A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022).

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

Publication History
Received by Neurology: Genetics April 3, 2023. Accepted in final form August 3, 2023. Submitted and externally peer reviewed. The handling editor was Alexandra Durr, MD, PhD.
## Appendix
### Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrea Pietra, MD</td>
<td>UO Genetica Medica, IRCCS Azienda Ospedaliero-Universitaria di Bologna; Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Italy</td>
<td>Drafting/revision of the article for content; including medical writing for content; major role in the acquisition of data</td>
</tr>
<tr>
<td>Flavia Palombo, PhD</td>
<td>IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di Neurogenetica, Italy</td>
<td>Major role in the acquisition of data; analysis or interpretation of data</td>
</tr>
<tr>
<td>Melania Giannotta, MD</td>
<td>IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Neuropsichiatria dell’età Pediatrica, Italy</td>
<td>Major role in the acquisition of data</td>
</tr>
<tr>
<td>Monica Maffei, MD</td>
<td>IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di neuroradiologia con tecniche ad elevata complessità, Italy</td>
<td>Major role in the acquisition of data</td>
</tr>
<tr>
<td>Claudio Fiorini, PhD</td>
<td>IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di neuroradiologia con tecniche ad elevata complessità, Italy</td>
<td>Major role in the acquisition of data</td>
</tr>
<tr>
<td>Roberta Costa, PhD</td>
<td>Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum University of Bologna, Italy</td>
<td>Major role in the acquisition of data</td>
</tr>
<tr>
<td>Giovanna Cenacchi</td>
<td>Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum University of Bologna; UO Anatomia, Istologia Patologica, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy</td>
<td>Analysis or interpretation of data</td>
</tr>
</tbody>
</table>

## References


Expanding the Clinical Spectrum of *UBTF*-Related Neurodevelopmental Disorder
Andrea Pietra, Flavia Palombo, Melania Giannotta, et al.
*Neurol Genet* 2023;9;
DOI 10.1212/NXG.0000000000200098

This information is current as of November 15, 2023

Updated Information & Services
including high resolution figures, can be found at:
http://ng.neurology.org/content/9/6/e200098.full.html

References
This article cites 9 articles, 0 of which you can access for free at:
http://ng.neurology.org/content/9/6/e200098.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
MRI
http://ng.neurology.org/cgi/collection/mri

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 Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.