

Expanding the Clinical Spectrum of *UBTF*-Related Neurodevelopmental Disorder

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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 tetra paresis, acquired microcephaly, and severe cognitive deficit complicated by pseudobulbar syndrome characterized by involuntary and uncontrollable outbursts of laughing, dysphagia requiring tube feeding, and nocturnal hypoventilation 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 biopterin.

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

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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.²⁻⁴ 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.²

In this study, we report clinical, metabolic, and neuroradiologic findings of 2 additional cases from unrelated families, which expand the clinical phenotype of *UBTF1*-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 samples⁵; and the molecular genetics data including exome sequencing of the 2 patients and segregation analysis in the 2 unrelated families.

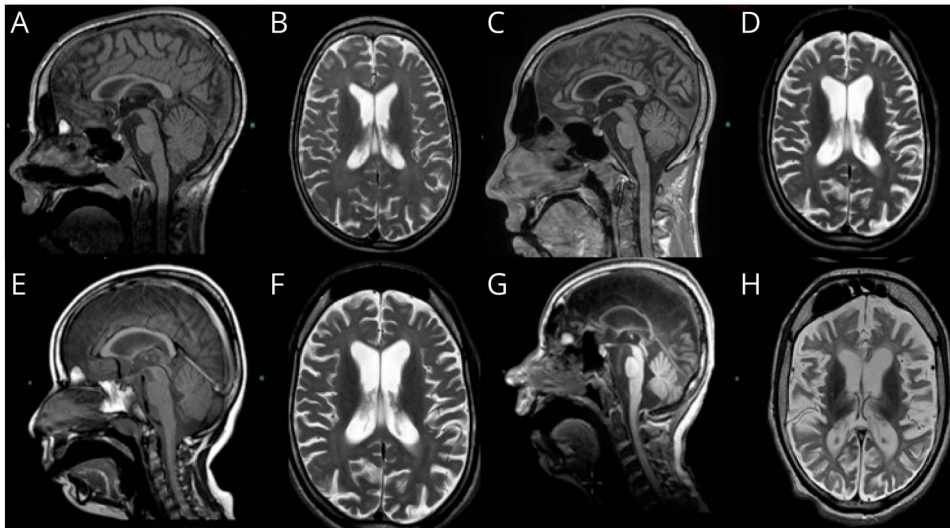
Results

Patient 1 is a 16-year-old boy, born at term after an uneventful pregnancy and spontaneous 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](https://www.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 neurodevelopment 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' conduction 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

Figure 1 Brain MRI Findings in Patient 1 (A–D) and Patient 2 (E–H)



Patient 1: Brain MRI at the first evaluation at 8 years of age showing in mid-sagittal T1-weighted (A) and axial T2-weighted (B) images, increased T2 signals in the periventricular supratentorial and deep white matter, thin corpus callosum, and supratentorial atrophy. Follow-up brain MRI at 16 years of age showing in mid-sagittal T1-weighted (C) and axial T2-weighted (D) images an increase in hyperintensity in T2-weighted white matter with moderate worsening in brain atrophy. Patient 2: Brain MRI at the first evaluation at 4 years of age showing in mid-sagittal T1-weighted (E) and axial T2-weighted (F) images modest cerebral atrophy and hyperintensity in T2-weighted images in the periventricular supratentorial and deep white matter and thin corpus callosum; Follow-up brain MRI at 13 years of age showing in mid-sagittal T1-weighted (G) and axial T2-weighted (H) images 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 signal in T2-weighted images and thalami signal hyperintensity in T2-weighted images.

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).

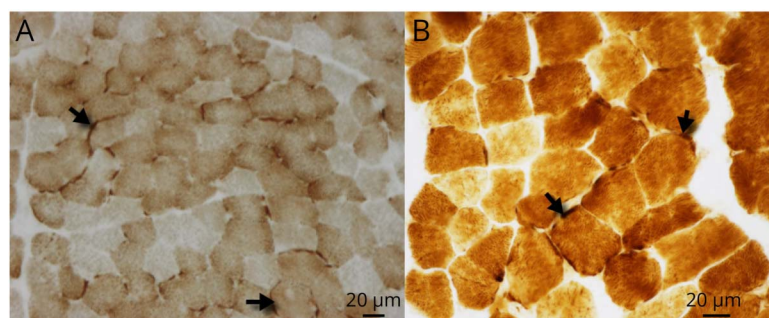
After inconclusive diagnostic analysis with a next-generation multigene panel, the genetic investigation was expanded to WES, and an *in silico* panel analysis of genes associated with severe pediatric disorders revealed the presence of a heterozygous

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

Figure 2 Cytochrome Histochemical Activity in Patient 1 (A) and Patient 2 (B) Muscle Specimens Revealing Subsarcolemmal Rims



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 pathogenetic 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 I 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, *Ubt1*^{-/-} mouse is embryonic lethal while *Ubt1*^{+/-} 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 UBF 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.² 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.

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Disclosure

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References

- Sanij E, Hannan RD. The role of UBF in regulating the structure and dynamics of transcriptionally active rDNA chromatin. *Epigenetics*. 2009;4(6):374-382. doi:10.4161/epi.4.6.9449

Appendix (continued)

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- Toro C, Hori RT, Malicdan MCV, et al. A recurrent de novo missense mutation in UBTF causes developmental neuroregression. *Hum Mol Genet*. 2018;27(4):691-705. doi:10.1093/hmg/ddx435
- Bastos F, Quinodoz M, Addor MC, et al. Childhood neurodegeneration associated with a specific UBTF variant: a new case report and review of the literature. *BMC Neurol*. 2020;20(1):17. doi:10.1186/s12883-019-1586-x
- Ikeda C, Kawarai T, Setoyama C, Orlacchio A, Imamura H. Recurrent de novo missense variant E210K in UBTF causes juvenile dystonia-parkinsonism. *Neurol Sci*. 2021;42(3):1217-1219. doi:10.1007/s10072-020-04758-y
- Cenacchi G, Peterle E, Fanin M, Papa V, Salaroli R, Angelini C. Ultrastructural changes in LGMD1F. *Neuropathology*. 2013;33(3):276-280. doi:10.1111/neup.12003
- Wilfert AB, Sulovari A, Turner TN, Coe BP, Eichler EE. Recurrent de novo mutations in neurodevelopmental disorders: properties and clinical implications. *Genome Med*. 2017;9(1):101. doi:10.1186/s13073-017-0498-x
- Edvardson S, Nicolae CM, Agrawal PB, et al. Heterozygous de novo UBTF gain-of-function variant is associated with neurodegeneration in childhood. *Am J Hum Genet*. 2017;101(2):267-273. doi:10.1016/j.ajhg.2017.07.002
- Agostini M, Romeo F, Inoue S, et al. Metabolic reprogramming during neuronal differentiation. *Cell Death Differ*. 2016;23(9):1502-1514. doi:10.1038/cdd.2016.36
- Sedláčková L, Laššuthová P, Štěrbová K, et al. UBTF mutation causes complex phenotype of neurodegeneration and severe epilepsy in childhood. *Neuropediatrics*. 2019;50(1):57-60. doi:10.1055/s-0038-1676288