Neurological Phenotypes of *IRF2BPL* Gene Variants: A Report of Four Novel Variants

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ABSTRACT

IRF2BPL gene variants have recently been associated to developmental disability and epilepsy in children and movement disorders in adults. So far, only few cases have been reported; here we present four novel cases identified by exome sequencing, while investigating developmental delay, adult-onset cerebellar ataxia or regression.

KEYWORDS: parkinsonian disorders, developmental disabilities, epilepsy, IRF2BPL protein, whole exome sequencing, rare diseases, HCN1 protein

Introduction

In 2018, a novel syndrome associated with pathogenic variants in *IRF2BPL* gene was delineated, which includes developmental delay, regression, epilepsy, movement disorders, ataxia, pyramidal signs, vertical gaze palsy, and dysmorphic features.[1,2] This condition was named "neurodevelopmental disorder with regression, abnormal movements, loss of speech, and seizures" – NEDAMSS (MIM #618088). It has been proposed that nonsense variants cause regression, movement disorders and severe developmental delay, whereas missense variants were associated with developmental delay and seizures.[1] We report on four new patients with novel variants of this gene.

Case 1

An 18-month-old girl presented with global developmental delay. She is the second child of a non-consanguineous couple and there is no reported family history of malformations or developmental delay.

Pregnancy was complicated by gestational diabetes after the 7th month. The child was born at 39 weeks and 1 day by c-section due to pelvic presentation. Birth weight was 3860g (p90-p97), and length 51 cm (p90-97). She was diagnosed with hip dysplasia during the neonatal period, and treated non-surgically with Pavlik harness and body cast until 6 months old. After cast removal, hypotonia became evident, with referral for neurological evaluation at the age of 8 months.

She was able to stand without support at the age of 13 months and to walk without support at 2 years. She spoke simple words at the age of 2 years and was later diagnosed with speech apraxia by a speech therapist. She never presented with epilepsy or developmental regression.

Clinical exam revealed subtle dysmorphic features such as ectropion of lower eyelids, sparse eyebrows, posteriorly rotated ears, prominent fingertip pads, long fingers, and pectus carinatum. Weight, height, and head circumference were within the normal range at the ages of 18 months and 4 years 3 months. (Figure 1).

Figure 1. Patient 1: Note sparse eyebrows, ectropion of lower palpebra.

Clinical investigation included G-band karyotype, array-CGH, electroencephalogram, abdominal ultrasound, echocardiogram, ophthalmological evaluation, auditory evoked potentials test, brain MRI; no abnormalities were found. Exome sequencing revealed a heterozygous variant classified as pathogenic on *IRF2BPL* – c.2102del; p.Asn701ThrfsTer66 (NM_024496.4). Moreover, a probably pathogenic variant was identified in another gene: *HCN1* – c.1246 C>T; p.Gln416Ter (NM_021072.4). Both variants were *de novo*.

Case 2

A 47-year-old woman presented with cerebellar ataxia and parkinsonism. Although she had gait imbalance since the age of 20, her ability to do daily activities and self-care were never been impaired before. She was prescribed levodopa at the age of 52 years, with mild response in bradykinesia.

At the age of 55, she presented with severe speech impairment and disrupted sleep pattern. Benzodiazepine was prescribed for insomnia, but induced hallucinations. Polysomnography revealed diminished REM sleep, shortening of N3 stage of sleep, and frequent episodes of waking up during the night, but did not confirm a REM sleep disorder. Currently, at the age of 57 years, she needs aid for all daily living activities and is wheelchair bound.

Information about developmental milestones was not available. However, her family denies any cognitive or motor impairments during childhood. She graduated from university and used to work as a school supervisor.

Family history was negative for cerebellar ataxia or parkinsonism. She is the only child of a non-consanguineous couple, and unfortunately no information was available for the paternal family. Her mother presented with mild intellectual disability, was diagnosed with hydrocephalus at the age of 13 years and died at the age of 52 years due to hypertension.

The first clinical evaluation of patient 2 by a medical genetics' specialist was at the age of 48 years. Her clinical exam revealed preserved strength, upper vertical gaze palsy, rigidity, bradykinesia, incoordination, hyperreflexia in upper limbs and areflexia in lower limbs, Babinski sign on the right foot, and mild dysarthria. No dysmorphic features were observed.

Memory and cognitive abilities were never formally evaluated. She performed 27 out of 30 points in the mini-mental test. She didn't report memory loss or difficulties to manage daily activities within the limits of her motor disabilities.

Figure 2. Patient 3 at 7 years of age: Upslanted palpebral fissures, epicanthus inversus over the left eye, overfolded helix, synophrys.

Her clinical investigation included molecular analysis of *SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA17, DRPLA*; dosing of chitotriosidase, vitamin E, copper, ceruloplasmin; beta-glucuronidase activity, hexosaminidase activity, plasma amino acid chromatography, Fillipin test, urinary organic acids; all tests revealed normal results. Electroneuromyography at the age of 50 years did not disclose any abnormalities, nor did brain MRI.

Exome sequencing revealed an heterozygous probably pathogenic variant on *IRF2BPL* – c.590del; p.Asn197ThrfsTer15 (NM_024496.4). Parents were not available for testing.

Case 3

A 5-year-old boy presented with global developmental delay. He was able to walk without support by the age of 14 months. Although simple words were spoken at the age of 1 year, he only used simple sentences after the age of 3 years.

He was born by c-section after an uneventful pregnancy, post-term (42 weeks), weighting 3700 g (p50), length 51 cm (p50), no intercurrences during neonatal period.

Several upper airways infections and constipation were observed until the age of 3 years. Clinical evaluation revealed hypotonia, upslanted palpebral fissures, epicanthus inversus over the left eye, hypertelorism, posteriorly rotated ears, overfolded helix, anteverted nares, synophrys, high, narrow palate, prominent fingertip pads, slender fingers, and joint laxity (Figures 2 and 3). Currently he is 10 years old and shows impaired social interactions. No seizures or regression were observed so far.
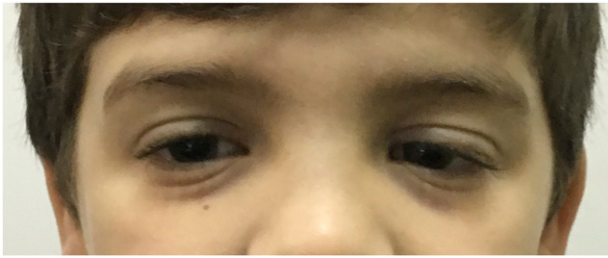
Figure 3. Patient 3 at 7 years: Upslanted palpebral fissures, epicanthus inversus over the left eye, overfolded helix, synophrys.

He is the first child of a non-consanguineous couple with no family history for malformations or developmental delay.

Clinical investigation included G-band karyotype, array-CGH, electroencephalogram, abdominal ultrasound, echocardiogram, brain MRI; all were within normal range. Exome sequencing revealed an heterozygous *de novo* variant classified as pathogenic on *IRF2BL* – c.1099 G>T; p.Glu367Ter (NM_024496).

Case 4

A 13-year-old boy presented with progressive walking impairment, gait instability and cognitive decline. The family also reports that behavioral problems became evident and school performance declined over the years. He presented tonic-clonic seizures at the age of 16 years and was started on oxcarbazepine. At the age of 17 years, he presented dysphagia and dysarthria.

He was born by c-section, at term, after an uneventful pregnancy. His birth weight was 3000 g, no other information from birth was available. The family reports motor development within the normal range and no speech issues during infancy. However, until the age of 3 years, feeding was difficult due to chewing problems.

He is the only child of a non-consanguineous couple with no family history of genetic or neurological diseases.

Clinical exam revealed ataxic gait, impairment of strength in lower limbs, and no dysmorphic features were observed. Brain and spine MRI showed no abnormalities. Electroneuromyography revealed proximal myopathy; muscular enzymes were the within normal range. Exome sequencing disclosed a pathogenic variant on *IRF2BPL* – c.474_504del, p.Ala161SerfsTer8 (NM_024496.4). We could not evaluate this variant in his parents.

Discussion

IRF2PBL variants have been associated with neurological symptoms from childhood to adulthood. Most patients reported so far had frameshift or nonsense variants. *IRF2BPL* is an intronless gene, which translates to a sequence of 796 aminoacids. Rampazzo et al.[3] suggested that this is a housekeeping gene, as it lacks a TATA-box upstream the origin of transcription.

First studies about *IRF2BPL* suggested that its function was regulation of female reproduction.[4] Recently, Marcogliese et al.[5] identified that overexpression of *IRF2BPL* downregulates expression of Wnt. Therefore, when *IRF2BPL* loses its function, Wnt is overexpressed and leads to neuronal degeneration. The majority of intronless genes encrypt receptors, regulatory and signaling molecules. Also, they escape nonsense mediated decay.[1]

We report on three new frameshift variants and a new nonsense variant that are predicted to insert a premature stop codon. Most variants reported so far are nonsense or frameshift (Figure 4). Even though pathogenic variants have been reported throughout the gene, the majority are localized in the polyglutamine tract or before the first PEST sequence of the gene.[1,2,6-13] A PEST sequence is a proline, glutamate, serine, threonine rich region that serves as signal for rapid proteolytic degradation.[14] There are 3 regions predicted to be PEST motifs in this gene (positions 201-213, 331-342 and 545-559). Interestingly, in all four patients (one reported here) who presented symptoms in adult life,[6,12,15,16] the variants were localized in this region. Whether this region represents a cluster for adult-life presentations of the disease is still not known.

In case 1 two pathogenic variants were identified, one in *IRFBPL2* gene and another on *HCN1* gene. *HCN1* gene has been associated to heat sensitive seizures, epileptic encephalopathy and generalized seizures. Some patients also present intellectual disability, global development delay, attention-deficit hyperactivity disorder, behavioral disturbances.[23] This gene is part of a family of hyperpolarization-activated cyclic nucleotide-gated channels which are expressed in the brain and the heart. It has a role in controlling spontaneous activity of cardiac rhythm and neuronal excitability among other functions.[24] Nava et al.[25] reported 6 patients with missense variants in this gene who presented epileptic encephalopathy or a Dravet-like syndrome. Interestingly, the variants identified in this study had a gain-of-function or dominant negative effect. Bonzanni et al.[26] and DiFrancesco et al.[27] reported missense variants in patients with epilepsy who did not present developmental delay or epileptic encephalopathy. Epilepsy is usually secondary to loss of inhibitory mechanisms. Nevertheless, *HCN1*-epilepsy has been associated to both loss of function and gain of function variants.[23]

As the *HCN1* nonsense variant was identified in a patient with developmental delay, at this moment we cannot define if her symptoms are secondary to *IRF2BPL* variant or the *HCN1* variant. She did not present seizures and electroencephalogram did not reveal any epileptic activity up to the age of 4 years. The laboratory classified the *HCN1* variant as likely pathogenic based on ACMG criteria (PVS1, PS2, PM2, BP5). Whether this variant represents a new variant with incomplete penetrance, age-dependent penetrance or if its only presentation is related to developmental delay is unknown at this moment.

The second patient is the oldest person reported so far. Interestingly, she presented symptoms in adult life, and unlike
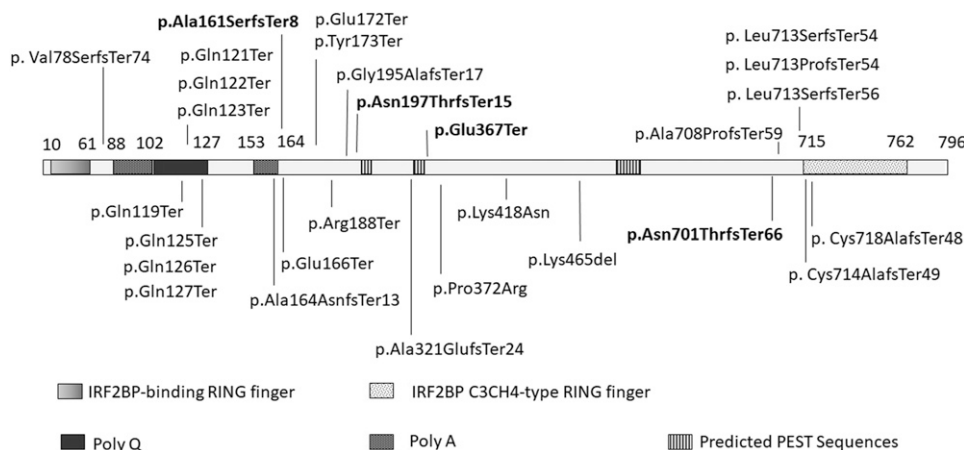
Figure 4. Schematic representation of *IRF2BPL* gene and variants reported.[1,2,7-11,13,15-22] Bold text represents variants described in this study.

the other adult patients reported, she does not seem to present with severe cognitive decline, which may be observed in the following years, as regression is an expected symptom. She has already lost many of her motor skills. Symptoms of sleep disorder were suggestive of REM sleep behavior disorder. This has been associated with α-synucleionopathy neurodegenerative diseases, neurological disorders, or antidepressant medications.[28] Diagnosis requires video polysomnography, which was not performed in our case. In the cases published so far, manifestations of sleep disorders have not been described in detail. As loss of *IRF2BPL* leads to overexpression of Wnt[5] and this pathway has been associated with insomnia,[29] it is interesting to further investigate sleep patterns in patients with NEDAMSS.

The third patient seems to have a very mild presentation of *IRF2BPL* impairment. Even though speech delay was most evident when he was very young, his intellectual abilities are evolving. Nowadays his major impairment is related to social interaction. Even though regression has not been observed in cases 3 and 4, it may still happen.

Dysmorphic features were observed in 7 out of 11 patients described by Mau-Them et al.[2] However the authors did not provide a full description of them. Shelkowitz et al.[7] mentioned a few dysmorphic features of which hypertelorism was also observed on patient 3. At this point, it is not possible to define if dysmorphic features are an important manifestation of this condition.

Epilepsy is a main feature of NEDAMSS and has been reported in more than half of the cases.[1,2,7-10,13,15-19] Some variants were even identified while screening for monogenic diseases among epilepsy patients[13,16,18] and, it has been proposed that this gene should be included in epilepsy panels. However, in none of the cases reported here, seizures were a manifestation. Therefore, NADAMSS should be considered in the differential diagnosis of neuro-developmental disorders, even in cases that seem mildly affected.

IRF2BPL should also be considered in the differential diagnosis of adult patients with parkinsonism, ataxia, and cognitive decline. Recently, this gene has been recognized as a dystonia associated gene with a generalized dystonia phenotype.[30] It is important to carefully document all clinical, laboratory and pathological findings, as this will help increase our knowledge about this condition.

Author Contributions

All authors participated equally in the acquisition, analysis and interpretation of data. Maria Angelica de F. D. de Lima drafted the manuscript and all authors reviewed this manuscript. All authors approved this version.

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REFERENCES

1. Marcogliese PC, Shashi V, Spillmann RC, et al. IRF2BPL is associated with neurological phenotypes. *Am J Hum Genet* 2018; 103(3):456-260.
2. Tran Mau-Them T, Guibaud L, Duplomb L, et al. De novo truncating variants in the intronless IRF2BPL are responsible for developmental epileptic encephalopathy. *Genet Med.* 2019;21(4):1008-1014.
3. Rampazzo A, Pivotto F, Occhi G, et al. Characterization of C14orf4, a novel intronless human gene containing a polyglutamine repeat, mapped to the ARVD1 critical region. *Biochem Biophys Res Commun.* 2000;278(3):766-774.
4. Heger S, Mastronardi C, Dissen GA, et al. Enhanced at puberty 1 (EAP1) is a new transcriptional regulator of the female neuroendocrine reproductive axis. *J Clin Invest.* 2007;117:2145-2154.
5. Marcogliese PC, Dutta D, Ray SS, et al. Loss of IRF2BPL impairs neuronal maintenance through excess Wnt signaling. *Sci Adv.* 2022;8(3):eabl5613.
6. Ganos C, Zittel S, Hidding U, et al. IRF2BPL mutations cause autosomal dominant dystonia with anarthria, slow saccades and seizures. *Parkinsonism Relat Disord.* 2019;68:57-59.
7. Shelkowitz E, Singh JK, Larson A, et al. IRF2BPL gene mutation: expanding on neurologic phenotypes. *Am J Med Genet A.* 2019;179(11):2263-2271.
8. Skorvanek M, Dusek P, Rydzanicz M, et al. Neurodevelopmental disorder associated with IRF2BPL gene mutation: Expanding the phenotype? *Parkinsonism Relat Disord.* 2019;62:239-241.
9. Ginervino M, Battini R, Nuovo S, et al. A novel IFR2BPL truncating variant is associated with endolysosomal storage. *Mol Biol Rep.* 2020;47(1):711-714.

10. Spagnoli C, Rizzi S, Salerno GG, et al. IRF2BPL gene variants: one new case. *Am J Med Genet A*. 2020;182(1):255-256.

11. Kafantari E, Andreasson S, Säll T, et al. Do variants in IRF2BPL cause both neurological disorders and keratoconus 8? *Parkinsonism Relat Disord*. 2020;79:138-140.

12. Prilop L, Buchert R, Woerz S, et al. IRF2BPL mutation causes nigrostriatal degeneration presenting with dystonia, spasticity and keratoconus. *Parkinsonism Relat Disord*. 2020;79:141-143.

13. Hong SY, Yang JJ, Li SY, et al. A wide spectrum of genetic disorders causing severe childhood epilepsy in Taiwan: a case series of ultrarare genetic cause and novel mutation analysis in a pilot study. *J Pers Med*. 2020;10(4):281.

14. Reichsteiner M, Rogers SW. PEST sequences and regulation by proteolysis. *Trends Biochem Sci*. 1996;21(7):267-271.

15. Ganos C, Biskup S, Krüger S, et al. Dystonia with aphonia, slow horizontal saccades, epilepsy and photic myoclonus: a novel syndrome? *Parkinsonism Relat Disord*. 2014;20:328-331.

16. Pisano S, Melis M, Figorilli M, et al. Neurological phenomenology of the IRF2BPL mutation syndrome: analysis of a new case and systematic review of the literature. *Seizure*. 2022;99:12-15.

17. Johannesen KM, Nikanorova N, Marjanovic D, et al. Utility of genetic testing for therapeutic decision-maing in adults with epilepsy. *Epilepsia*. 2020;61(6):1234-1239.

18. Mahdiannasser M, Rashidi-Nezhad A, Badv RS, et al. Exploring the genetic etiology of drug-resistant epilepsy: incorporation of exome sequencing into practice. *Acta Neurol Belg*. 2022;21.

19. Peng P, Kessi M, Mao L, et al. Etiologic classification of 541 infantile spasms cases: a cohort study. *Front Pediatr*. 2022;10:774828.

20. Qian XH, Liu XY, Zhu ZY, et al. Neurodevelopmental disorder caused by a truncating de novo variant of IRF2BPL. *Seizure*. 2021;84:47-52.

21. Sainio MT, Aaltio J, Hyttinen V, et al. Effectiveness of clinical exome sequencing in adult patients with difficult-to-diagnose neurological disorders. *Acta Neurol Scand*. 2022;145(1):63-72.

22. Antonelli F, Grieco G, Cavallieri F, et al. Adult onset dystonia-plus syndrome: a novel presentation of IRF2BPL associated neurodegeneration. *Parkinsonism Relat Disord*. 2022;94:22-24.

23. Kessi M, Peng J, Duan H, et al. The contribution of HCN Channelopathies in different epileptic syndromes, mechanisms, modulators, and potential treatment targets: a systematic review. *Fron Mol Neurosci*. 2022;15:807202.

24. Baruscotti M, Bottelli G, Milanesi R, et al. HCN-related channelopaties. *Pfluger Arch*. 2010;460(2):405-415.

25. Nava C, Dalle C, Rastetter A, et al. De novo mutations in HCN1 cause early infantile epileptic encephalopathy. *Nat Genet*. 2014;46(6):640-645.

26. Bonzanni M, DiFrancesco JC, Milanesi R, et al. A novel de novo HCN1 loss-of-function mutation in genetic generalized epilepsy causing increased neuronal excitability. *Neurobiol Dis*. 2018;118:55-63.

27. DiFrancesco JC, Castellotti B, Milanesi R, et al. HCN ion channels and accessory proteins in epilepsy: genetic analysis of a large cohort of patients and review of the literature. *Epilepsy Res*. 2019;153:49-58.

28. Dauvilliers Y, Schenck CH, Postuma RB, et al. REM sleep behaviour disorder. *Nat Rev Dis Primers*. 2018;4(1):1-16.

29. Sun H, Zhang J, Ma Y, Liu J. Integrative genomics analysis identifies five promising genes implicated in insomnia risk based on multiple omics datasets. *Biosci Rep*. 2020;40(9):BSR20201084.

30. Sarmiento IJK, Menacacci NE. Genetic dystonias: update on classification and new genetic discoveries. *Curr Neurol Neurosci Rep*. 2021;21(3):8.