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Heterozygous *EIF2AK2* variant causes adolescence-onset generalized dystonia partially responsive to DBS

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Eukaryotic translation initiation factor 2-alpha kinases (EIF2AK) are serine-threonine kinases involved in integrated stress response, a cytoprotective pathway which ensures adaptation of mammalian cells to stress conditions.[1] Among the four members of this protein family, EIF2AK2, also known as Protein Kinase R, is activated by double-stranded RNA (primarily during viral infections), oxidative stress, endoplasmic reticulum (ER) stress, cytokines, and growth factors.[2] By phosphorylating Eukaryotic Translation Initiation Factor 2 Subunit 1 (EIF2S1) in response to cellular stressors, EIF2AK2 negatively regulates mRNA translation and protein synthesis and induces apoptosis.[1,2]

De novo missense variants in the *EIF2AK2* gene were first linked to a complex neurological syndrome characterized by developmental delay, language impairment, various combinations of motor manifestations (including cerebellar, pyramidal, and dystonic features), and brain MRI abnormalities (encompassing dysmyelination, thin corpus callosum, and cerebral and/or vermian atrophy) in nine unrelated children in 2020.[3] Intriguingly, all individuals with *EIF2AK2* variants exhibited neurological deterioration in the context of febrile illness or infection.[3]

In 2021, Kuipers, Musacchio, and respective colleagues reported 13 individuals from six pedigrees carrying heterozygous (autosomal dominantly inherited or *de novo*) or homozygous *EIF2AK2* missense variants which cause early-onset, mostly isolated, generalized dystonia likely through a gain-of-function mechanism.[4,5]

In order to replicate the association between *EIF2AK2* mutations and isolated dystonia phenotypes, we retrieved our internal database of approximately 18,000 exomes (522 belonging to subjects recruited under the diagnostic category “dystonia”) and the 100,000 Genomes Project repository (1116 participants enrolled using the *Human Phenotype Ontology* term “dystonia”) searching for *EIF2AK2* variants previously found in dystonia patients and other rare variants.[6]

The missense variant NM_001135651(*EIF2AK2*):c.388G>C (p.Gly130Arg) was detected in the heterozygous state in a 28-year-old Algerian male who underwent whole-exome sequencing (WES) for adolescence-onset generalized dystonia with leg involvement.[7] He was healthy until age 17, when he presented with dystonic posturing of the first two left fingers. Four years later he developed right hand tremor while performing fine manual tasks. Tremor

spread to the head and contralateral hand over few months. At this time, he started experiencing chewing and gait difficulties and abnormal trunk posture on walking, which was alleviated by trunk anteflexion, walking backwards, carrying a heavy load, or running. His past medical history included myopia and scoliosis. There was no history of exposure to dopamine receptor antagonists. He was the eldest of five siblings born to non-consanguineous parents. His family history was negative for neurological disorders. On examination (Figure 1A; Video 1), he had dysarthria and generalized dystonia mainly affecting the trunk and arms. Truncal dystonia showed extensor and torsional components on walking. No parkinsonian, pyramidal, cerebellar, or cognitive signs were detected. Serum copper and ceruloplasmin, iron profile, vitamin E, brain MRI and NCS/EMG were unremarkable. Dystonia did not respond to levodopa nor trihexyphenidyl but showed a 40% improvement in severity from baseline with deep brain stimulation (DBS) of the globus pallidus internus (age 24; Video 1) over a 3-year period. Prior to WES, the patient had been tested negative for *TOR1A* and *THAP1* through single gene testing. On WES, we did not identify any other potential candidate variants in genes linked to monogenic movement disorders (MD). We acknowledge that no quantitative genetic testing was performed to rule out variants in MD-related genes potentially missed due to next-generation sequencing intrinsic limitations. Segregation analysis revealed the proband's parents did not carry the *EIF2AK2* mutation detected, which is therefore assumed *de novo* according to the American College of Medical Genetics and Genomics (ACGM) guidelines (maternity and paternity not genetically confirmed; Figure 1B).[8]

The *EIF2AK2* variant herein reported consists in a novel nucleotide change (NM_001135651:c.388G>C) causing the same amino acid substitution p.Gly130Arg previously reported in 10 affected individuals from four pedigrees who however carried a guanosine-to-adenosine substitution at the same position (NM_001135651:c.388G>A), which is due to codon redundancy.[4,5] The mutation is absent in the population database gnomAD (https://gnomad.broadinstitute.org/) and predicted benign/tolerated by most *in silico* tools, including a low Combined Annotation Dependent Depletion (CADD) score (Figure 1C). This likely reflects intrinsic limitations of pathogenicity prediction algorithms when examining regions with poor evolutionary conservation, as is the case of this amino acid residue (Figure 1B).[4] The variant is pathogenic according to ACGM guidelines.[8] No other rare *EIF2AK2* variants associated with isolated dystonia phenotypes were identified by screening the above-mentioned datasets (Supplementary File 1).

Our case further supports the inclusion of *EIF2AK2* mutation analysis in the diagnostic workup of early-onset isolated generalized dystonia, including sporadic cases.[4] Furthermore, it confirms that DBS is an effective treatment for *EIF2AK2*-associated dystonia.[4,5] Although triggers or precipitating factors were not identifiable in our patient's history and clinical course, the association between *EIF2AK2* variants and dystonia might directly link inflammatory or infectious events and phenotypic expression of dystonia in patients with causative (incompletely penetrant) or predisposing genetic makeup, thereby unveiling one of the molecular underpinnings of gene-environmental interaction in dystonia pathogenesis.[9] Defective EIF2S1 signaling pathway is the shared pathobiological mechanism on which not only *EIF2AK2* mutations but also other monogenic causes of dystonia, including DYT-*TOR1A*, DYT-*THAP1*, DYT-*PRKRA*, and DYT-*SGCE* converge, either directly or indirectly.[4,10] In addition, since the EIF2S1 pathway plays a role in regulating neuronal long-term synaptic plasticity, *EIF2AK2* variants might represent a direct link between ER stress and aberrant synaptic plasticity, which is a well-recognized pathophysiological mechanism of dystonia.[11] Finally, we speculate that *EIF2AK2*(NM_001135651) nucleotide 388 might be critical for *EIF2AK2*-related dystonia since a variant at this level segregates with the phenotype in five out of seven kindreds hitherto reported, including the present one. Further evidence is warranted to establish whether the corresponding codon or, more broadly, the second double-stranded RNA binding motif of EIF2AK2 might represent a mutational hotspot for *EIF2AK2*-associated dystonia.[4]

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Author roles

1. Research project: A. Conception, B. Organization, C. Execution;
2. Data Analysis: A. Design, B. Execution, C. Review and Critique;
3. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique.

FM: 1A, 1B, 1C, 2B, 3A
DM: 1B, 1C, 3B
MT: 1B, 1C, 3B
LAP: 1B, 1C, 3B
AV: 3B
KPB: 1C, 2C, 3B
RM: 1A, 1B, 1C, 2C, 3B
HH: 1C, 2C, 3B

Disclosures

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Ethical Compliance Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines. The authors confirm that the approval of an institutional review board was not required for this work. We confirm that we have obtained the patient consent for genetic testing on a research basis as well as for video acquisition and publication.

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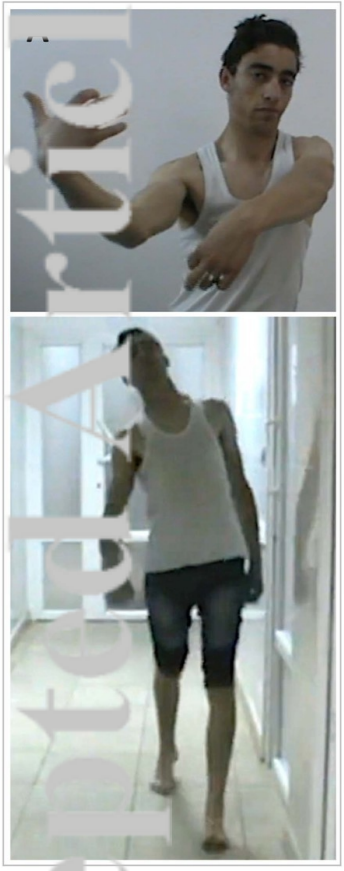
Legends:

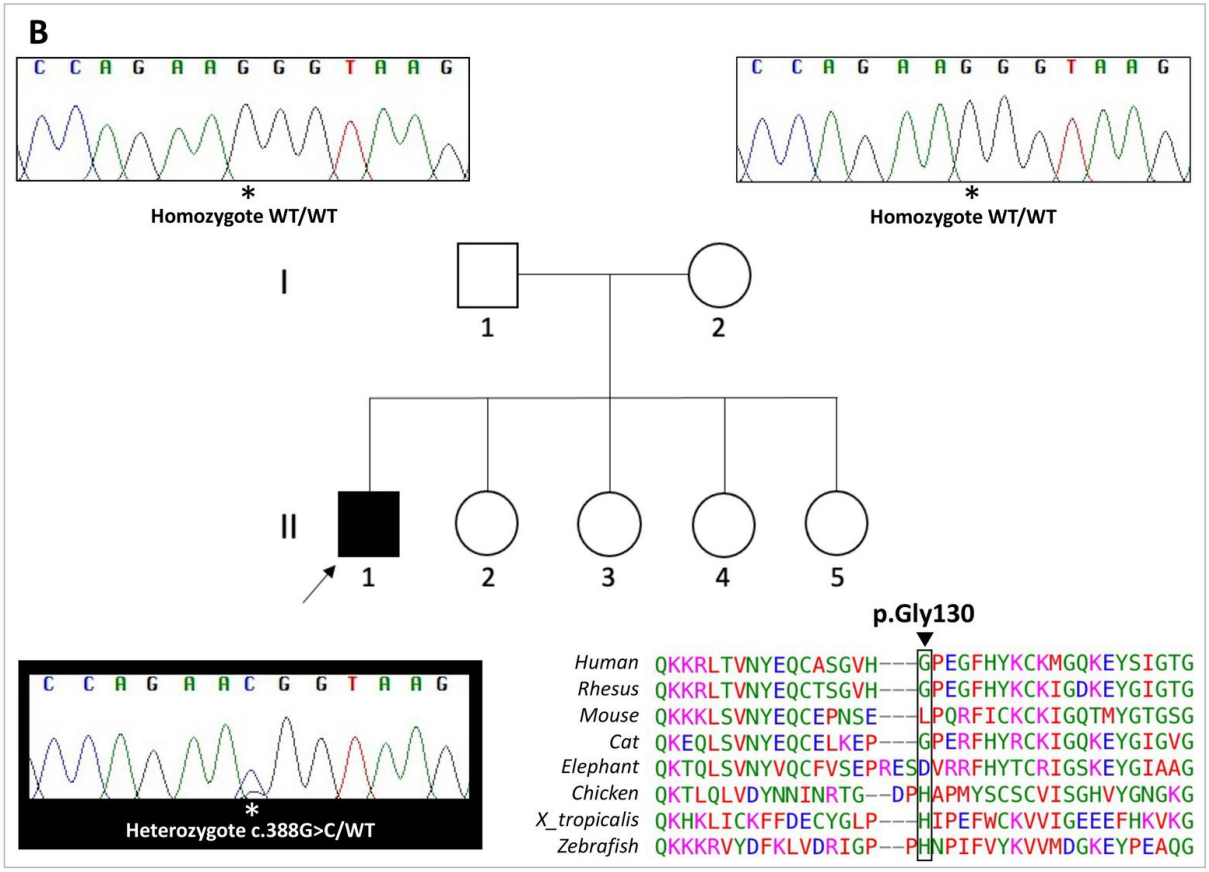
Figure 1. (A) Video frames showing the proband with prominent upper limb and truncal dystonia, the latter having extensor and torsional components on walking. (B) Family tree and segregation analysis revealing the NM_001135651(*EIF2AK2*):c.388G>C (p.Gly130Arg) missense variant occurred in the heterozygous state only in the proband (II-1). The DNA region

of interest was amplified bidirectionally using the following primers (5'→3'): F-catggggaattacataggcct and R-gtggcaccctgtactctctt, with an amplicon size of 385 base pairs. Electropherograms were analyzed using the Sequencher software package. Arrow: proband. WT: wild type. *Bottom right.* Interspecies alignment showing lack of evolutionary conservation of the amino acid involved by the variant. (C) Functional analysis of the *EIF2AK2* variant herein reported. ACMG: American College of Medical Genetics and Genomics; CADD: Combined Annotation Dependent Depletion (https://cadd.gs.washington.edu/snv); HSF: Human Splicing Finder (https://hsf.genomnis.com/home); MutationTaster (http://www.mutationtaster.org/); MutPred2 (http://mutpred.mutdb.org); PolyPhen-2: Polymorphism Phenotyping v2 (http://genetics.bwh.harvard.edu/pph2/); PROVEAN: Protein Variation Effect Analyzer (http://provean.jcvi.org/index.php); SIFT: Sorting Intolerant From Tolerant (http://sift.bii.a-star.edu.sg).

Video 1. *First segment*. The proband (age 23) presented with dysarthria and dystonia mainly affecting the trunk and arms. Truncal dystonia worsened on walking, with extensor and torsional components. Dystonic posturing of the upper limbs while writing. *Second segment.* The proband (age 24) with truncal dystonia. *Third segment.* The proband (age 27) after undergoing DBS of the globus pallidus internus at age 24 showed improvement of dysarthria, upper limb and truncal dystonia, and gait.

Supplementary File 1. Rare *EIF2AK2* variants identified by screening our internal exome database and the rare disease sub-cohort of the 100,000 Genomes Project.





C

Variant	CADD	PolyPhen-2	SIFT	PROVEAN	MutationTaster	MutPred2	HSF	ACMG
EIF2AK2 GRCh38:2-37[illegible]41554-C-G NM_00[illegible]651:c.388G>C NP_0011291[illegible]:p.Gly130Arg	1.867	**HumDiv:** Benign, score: 0.045 **HumVar:** Benign, score: 0.029	Tolerated Score: 0.862	Neutral Score: 1.86	Polymorphism	**Pathogenic** **Score: 0.544**	No significant impact on splicing signals	**Pathogenic**

MDC3_13371_Figure 1.jpg