Complex dystonia with nodular heterotopias and paramagnetic brain deposits due to biallelic *ARFGEF2* variants

M. Percetti[1,2], G. Saccardin[3,4], D. Fontanesi[3,4], S. Coniglio[3,4], R. Del Bo[1], M. Magni[1], S. Pagliarani[5], D. Scalabrini[1], A. Di Fonzo[2], E. Monfrini[2]

[1] Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy. [2] Neurology Unit, Department of Neuroscience and Mental Health, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. [3] Department of Biomedical, Metabolic, and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy. [4] Neurology Unit, Department of Neuroscience and Head and Neck, Civile Baggiovara Hospital, Azienda Ospedaliera-Universitaria of Modena, Modena, Italy. [5] Department of Neuroscience and Mental Health, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

Background: Biallelic variants in *ARFGEF2* are a genetic cause of periventricular nodular heterotopia, clinically characterized by developmental delay, childhood-onset epilepsy, and, less frequently, dystonia.
The aim of the study was to describe two adult siblings who sought medical attention for long-standing dystonic movements and to determine the possible underlying genetic cause.

Methods: All subjects were evaluated by neurologists with expertise in movement disorders. Genomic DNA and RNA were extracted from peripheral venous blood with standard procedures. Exome sequencing was conducted in the two affected siblings. Sanger sequencing was used for confirmation of the variants of interest and for phasing through parents' analysis. cDNA was obtained through reverse transcription and used for splicing analysis.

Results: A 40-year-old woman (**II.III**, ***Fig. 1***) sought medical attention for a long-standing history of involuntary movements of her upper limbs. At the age of three, she developed a slight but progressive unsteady gait due to the abnormal posture of her right foot. Involuntary movements generalized and a diagnosis of cerebral palsy with choreoathetosis and mild developmental delay was made. Her last neurological examination showed a generalized dystonia with prominent oromandibular involvement, dystonic head tremor, dystonic gait, convergent strabismus, and bilateral hypoacusis. No history of epilepsy was reported. Brain MRI (***Fig. 2***) showed bilateral subependymal nodular heterotopias and globus pallidus interna (GPi) iron depositions, while CT scan detected mild brain calcifications within the GPi and the right centrum semiovale. Standard EEG was unremarkable. Her younger brother (**II.II**) presented a similar but milder phenotype, with intellectual disability, generalized dystonia, and hypoacusis.
The **c.1525+3A>G** and **c.2843T>A (p.Leu948His)** variants of ***ARFGEF2*** were identified in both siblings on exome sequencing and confirmed by Sanger (***Fig. 3***). Analysis of the parents showed that the missense variant was inherited from the father, whereas the intronic variant was inherited from the mother. cDNA analysis demonstrated that the c.1525+3A>G variant altered splicing by **exon 11 skipping**, leading to the synthesis of a truncated protein (***Fig. 4-5***). No pathogenic variants in genes associated with dystonia, brain iron accumulation, or brain calcifications were identified.

Conclusions: Multimodal neuroimaging could be useful in guiding the interpretation of genetic variants in cases with complex dystonia. Milder phenotypes of pediatric syndromic diseases could come at the attention of the adult neurologist. Based on these observations, ***ARFGEF2* should be included in genetic panels for dystonia**.

Figure 1. **Family tree.**

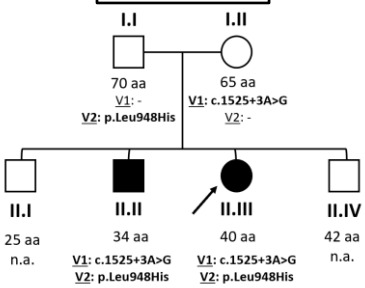
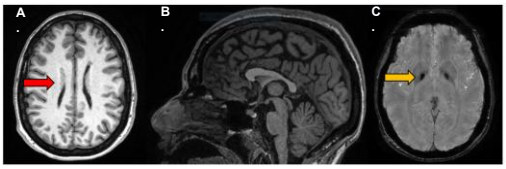


Figure 2. **Abnormal findings from brain MRI of the proband (II.III).**



A. Bilateral periventricular subependymal nodular heterotopias.
B. Mild thinning of the corpus callosum.
C. Bilateral GPi iron deposition.

Figure 3. **Electropherograms of the identified variants in *ARFGEF2*.**

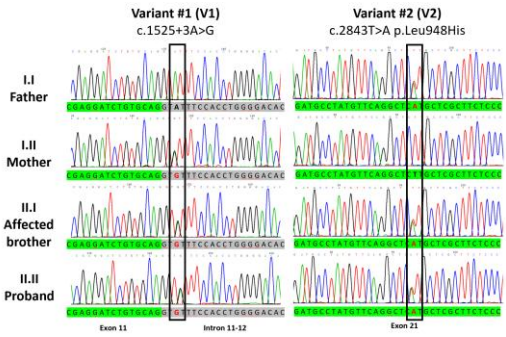


Figure 4. **cDNA analysis.**

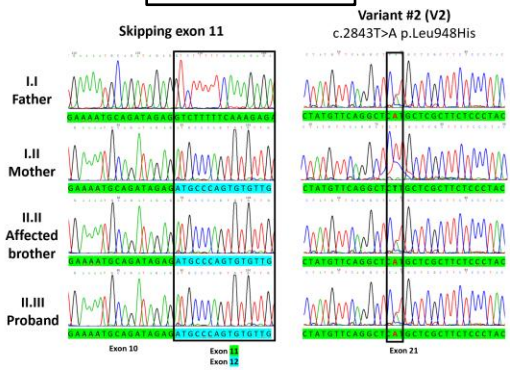
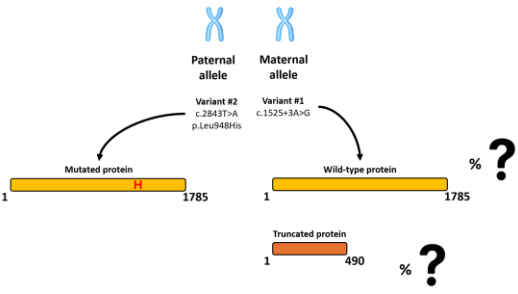


Figure 5. **Molecular consequences of *ARFGEF2* variants.**



Gene ***ARFGEF2*** (MIM# 605371), Transcript ID: ENST00000371917.5, RefSeq: NM_006420.3