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CASE SERIES

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Significant phenotypic variability in a multigenerational family with an *NFIA* missense mutation: Case series and review of the literature

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Key Clinical Message

We report the first multigenerational family with *NFIA*-related disorder from a missense variant. This case highlights the condition's phenotypic variability and the need for genetic testing when an initial diagnosis fails to explain all symptoms.

KEYWORDS

corpus callosum hypoplasia, developmental delay, macrocephaly, *NFIA*-related disorder, nuclear factor I

1 | BACKGROUND

Nuclear factor I/A (NFIA) is part of a larger family of proteins, the nuclear factor I (NFI) proteins, that function as cellular transcription factors. NFIA is encoded by the *NFIA* gene, which is located at 1p31.3.[1,2] Truncation or deletion of *NFIA* causing haploinsufficiency can result in an *NFIA*-related disorder.[2] Deletion of NFIA often occurs as part of a multigenic microdeletion, although 20 cases resulting from smaller intragenic deletions or point mutations have been reported.[3–10]

Clinical manifestations of *NFIA*-related disorder are highly variable, but almost always include abnormalities, such as hypoplasia, of the corpus callosum. Macrocephaly and nonspecific but distinct facial features have also been reported. Some patients have also experienced urinary tract defects, including vesicoureteral reflux and hydronephrosis. Mutations in *NFIA* are often sporadic, but they can be inherited in an autosomal dominant pattern. The general mechanism of disease is haploinsufficiency of a single *NFIA* allele, although missense mutations have been reported.[2,3,5–7]

The prognosis of *NFIA*-related disorder is not well known. This disorder is likely underdiagnosed and underreported, making it difficult to determine if life span is affected.[2] Management targets the specific manifestations present. Patients who are diagnosed with *NFIA*-related disorder are typically evaluated for brain/neurologic abnormalities through imaging and EEG. Patients may also be screened for developmental delays and behavioral abnormalities, GI/feeding abnormalities, renal anomalies, ophthalmologic abnormalities (such as strabismus), and craniofacial abnormalities.[2]

In this report, we present three members of a family with bigenerational *NFIA*-related disorder (a mother and two children). Genetic testing has revealed a likely pathogenic variation NM_001134673.4 (NFIA):(c.362G > C) in the *NFIA* gene causing a missense substitution (p.R121P) in the NFIA protein. This family presents with relatively minor developmental delay compared with previously reported cases of *NFIA*-related disorder, but relatively more severe musculoskeletal complications, thus representing an expansion of the clinical phenotype.

2 | CASE PRESENTATION

2.1 | Patient 1

Patient 1 is a 6-year-old male with a history of seizures, hypotonia/hypermobility, and developmental delay. His hypotonia/hypermobility (Figure 2A) were initially suspected to be due to Ehlers-Danlos Syndrome (EDS), and, in fact, he was clinically diagnosed with EDS at around age 2. However, EDS was insufficient to explain all of the patient's clinical features. Specifically, renal ultrasound showed bilateral enlarged kidneys, brain MRI showed hypoplasia of the corpus callosum (Figure 3), and he was diagnosed with autism spectrum disorder and attention deficit hyperactivity disorder. This patient also exhibits multiple unusual physical features including macrocephaly, bifid uvula, preaxial polydactyly, bifid great toes (Figure 2B), flat feet, lumbarization of the S1, and a sacral dimple. Based on this complex constellation of features, exome sequencing (ES) was ordered for this patient at age 3 through GeneDx, which was initially reported as normal.[11] A reanalysis was requested at age 5 where a likely pathogenic variation (c.362G > C) in the *NFIA* gene causing a missense substitution (p.R121P) in the NFIA protein.

This variant was not found in large population cohorts, was predicted by computer models to disrupt protein structure or function and was in the same site as another variant categorized as pathogenic (p.R121C) in ClinVar (VarID:265253).[12] This variant is in the DNA binding domain of the NFIA protein.

A timeline of the events in this case can be found in Figure 1.

2.2 | Patient 2

Patient 2 is a 31-year-old female who is the mother of Patients 1 and 3. She also has one other child who is currently asymptomatic and does not share the *NFIA* variant. Patient 2 has a history of anxiety, bipolar disorder, major depressive disorder, PTSD, renal calculi, spinal stenosis, mild scoliosis, and chronic muscle and joint pain. Relevant family history includes a diagnosis of rheumatoid arthritis in her father, but Patient 2 tested negative for RA as well as several other autoimmune disorders. Initially, this patient's muscle and joint symptoms were attributed to suspected fibromyalgia or Ehlers-Danlos Syndrome. She was clinically diagnosed with EDS after her son (Patient 1) was, and she underwent molecular genetic testing for further investigation for further evaluation of heritable connective tissue disorders. Her initial genetic testing found a variant of uncertain significance (c.9412G > A) in the *TNX-B* gene. However, her son was not found to share this variant, which raises suspicion for an etiology of their hypermobility symptoms besides EDS. Patient 2 shares some dysmorphic features with her son, including macrocephaly, bifid uvula, and flat feet. After identifying the *NFIA* variant in her son, Patient 2 was also tested and found to share his *NFIA* mutation. It is unclear whether this variant originated de novo in Patient 2 since her mother had negative evaluation for the mutation, but her father is not available for testing.

2.3 | Patient 3

Patient 3 is a 10-year-old female who has a history of seizure-like activity beginning at age 3, developmental delay, autism spectrum disorder, and hypotonia/hypermobility. Patient 3 initially underwent genetic testing for Fragile X Syndrome, as well as a chromosomal microanalysis, at age 3. No abnormalities were identified by this testing. At age 8, Patient 3 was evaluated by genetics and found to have macrocephaly, bifid uvula, and flat feet. By this time, the *NFIA* variant had already been identified in her brother and mother (Patients 1 and 2, respectively), and testing was ordered for her, with results showing that she also shares the familial *NFIA* variant. A recent lumbar MRI performed at age 10 showed mild thecal sac narrowing, likely related to a congenital stenosis, at L4-L5 and L5-S1, as well as mild-to-moderate neural foraminal narrowing, also at L4-L5 and L5-S1.

3 | DISCUSSION

This report presents three cases of *NFIA*-related disorder. These three family members share the same single nucleotide missense variant. These represent the first reported cases of familial missense mutations in *NFIA*. Previously reported single nucleotide variations involving *NFIA* are summarized in Table 1, organized by type of variation.

A genotype–phenotype analysis of Table 1 reveals a potential association between the type of mutation and ventriculomegaly. Ventriculomegaly was present in all recorded patients with nonsense or frameshift variations in *NFIA*, regardless of location, but only some patients with missense variations. Additionally, Chiari I malformation was noted in only two patients, both of whom had nonsense mutations. No other features appeared to show a pattern related to mutation type.

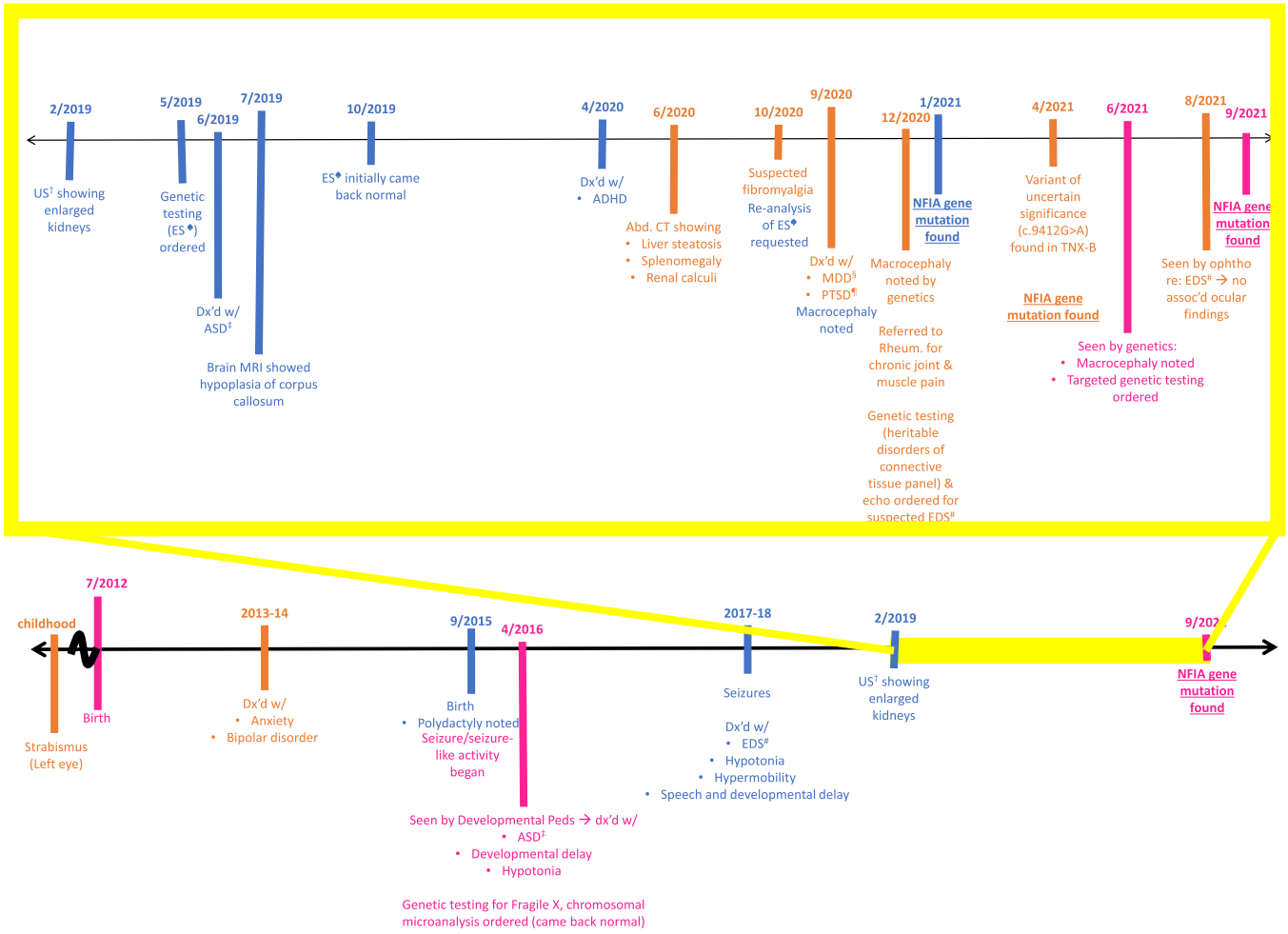

FIGURE 1 A time line of events in this case. Blue = Patient 1; Orange = Patient 2; Pink = Patient 3.†Ultrasound; ‡Autism spectrum disorder; §Major depressive disorder; ¶Post-traumatic stress disorder; #Ehlers-Danlos Syndrome; ♠Exome Sequencing.

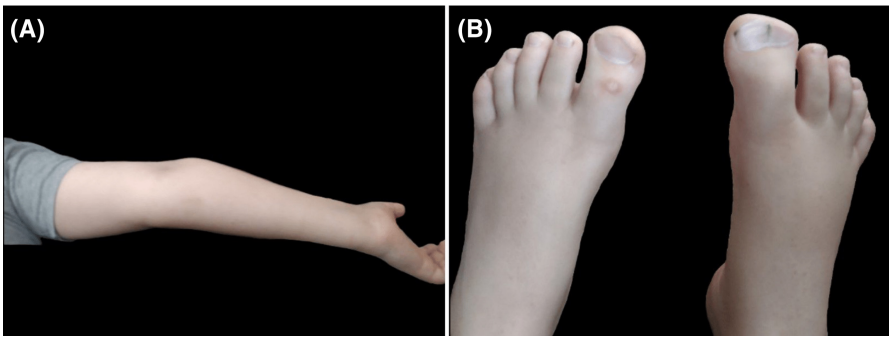

FIGURE 2 (A) an example of Patient 1's joint hypermobility, and (B) Patient 1's bifid great toes.

While the three patients reported here each exhibit several of the well-known features of *NFIA*-related disorder, the common denominator among them is muscular hypotonia/joint hypermobility. Because these features are relatively nonspecific, and because the distinction between them can be ambiguous, this family went through numerous other potential diagnoses before reaching the conclusion of an *NFIA*-related disorder. We feel there is reason to believe that the *NFIA* mutation is, indeed, to blame for their musculoskeletal abnormalities. This is because (1) the most likely alternative diagnosis, EDS, does not fully account for all of the family's ongoing problems, and (2) while Patient 2 (the mother) has a variant of uncertain significance in *TNX-B*, the children do not share this variant, making it a less likely culprit in the mother's case, and ruling it out as the cause in the children's case.

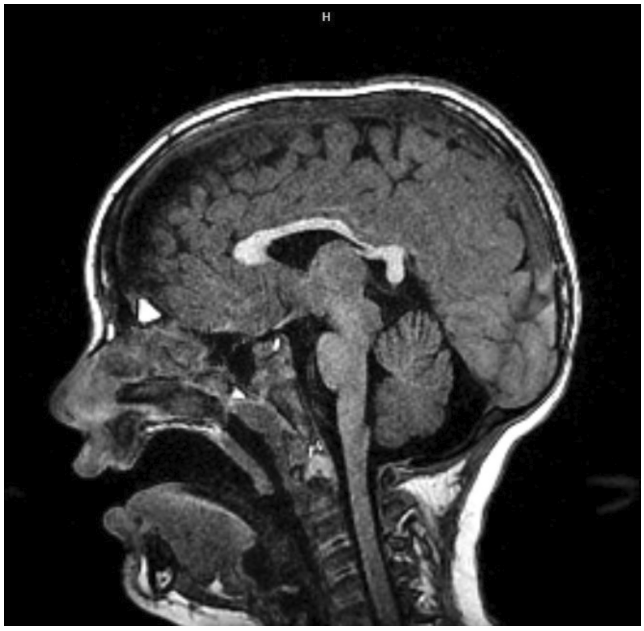

FIGURE 3 MRI of Patient 1's brain showing hypoplastic corpus callosum.

Although the function of NFIA itself in muscle development has not been well characterized, the related protein NFIX has been shown to play a crucial role in the transition from embryonic muscle tissue to fetal muscle tissue via direct activation of fetal muscle-specific genes.[13] Funk et al.[14] described the interaction of NFI proteins with myogenin, which is known to play a role in the differentiation of skeletal muscle. Rossi et al.[15,16] described the contribution of NFIX to muscle regeneration, even finding therapeutic benefit in silencing NFIX in mice with muscular dystrophy.

NFIX and NFIA come from the same family of proteins. They have very similar structures, particularly in their DNA-binding regions.[17] Further, they share the same DNA recognition sequence (5′-TTGGCNNNNNGCCAA-3′).[17] The mRNA for both are found in skeletal muscle.[18] Given the similarities between NFIX and NFIA, it is possible that NFIA shares a similar role in promotion of muscle development and regeneration. The hypotonia present in patients with *NFIA*-related disorder may therefore be explained by defective muscle differentiation, development,

TABLE 1 All previously reported cases of NFIA-related disorder caused by single nucleotide variations and short indels organized by type of variation.

Reference	Zenker et al.[3]	Reported Here - Patient 1	Reported Here - Patient 2	Reported Here - Patient 3	Uehara et al.[5]	Iossifov et al.[6]	Ogura et al.[7]
Sex and age (years)	Male, 5	Male, 6	Female, 31	Female, 9	Female, 6	Male, 14 months	Male, 10
Country of origin		USA	USA	USA	Japan	Japan	Japan
Genetic Change (all heterozygous)	c.361C > T	c.362G > C	c.362G > C	c.362G > C	c.373A > G	c.373A > G	c.1184C > T
Amino acid changes	p.Arg121 Cys	p.Arg121Pro	p.Arg121Pro	p.Arg121 Pro	p.Lys125Glu	p.Lys125Glu	p.Thr395Met
Abnormal corpus callosum	+	+	−	−	+	+	−
Ventriculomegaly or hydrocephalus	+	−	−	−	+	−	−
Macrocephaly	+	+	+	+	−	+	−
Developmental delay	+	+	−	+	+	+	+
Dysmorphic features	Prominent forehead, underdeveloped midface, and low-set ears	Bifid uvula, bifid great toes, flat feet, sacral dimple, large R piezogenic papule, and preaxial polydactyly	Bifid uvula, high-arched palate, flat feet, eversion of feet, and piezogenic papules	Bifid uvula, flat feet, and marmota-type birthmark on shoulder	High hairline, small eyes, anteverted nares, depressed nasal bridge, broad columella, thin upper lip, and high-arched palate	High hairline, thick eyebrows, short nose, anteverted nares, long philtrum, thin upper lip vermilion, and retrognathia	Thick eyebrows, anteverted nares, thick vermilion border, open mouth, fused tooth, and low-set ears
Chiari I malformation	−	−	−	−	ND	ND	ND
Seizures	−	+	−	+	ND	ND	ND
Urinary tract defects	−	+	−	−	ND	−	ND
Hypotonia/ hypermobility	−	+	+	+	−	−	+

and/or regeneration due to a lack of fully functioning NFIA transcription factor.

As a transcription factor, NFIA is involved in the regulation of several cellular processes.

Beyond its role in brain development and possible muscle regulation, potential roles of NFIA have also been identified in asthma and allergy susceptibility,[19] as well as bipolar disorder.[20]

Due to the wide-ranging functions of NFIA, and therefore the variety of manifestations with which *NFIA*-related disorder can present, it can often be difficult to identify. This family underwent significant diagnostic workup, including multiple rounds of genetic testing, before *NFIA*-related disorder was recognized. Obtaining the correct diagnosis is helpful for a number of reasons. Although it does not necessarily change the treatment, it is meaningful to the family to have a more definitive explanation of the source of and explanation for their symptoms. Additionally, the knowledge that this family has *NFIA*-related disorder, although its mechanisms and manifestations are not fully understood, is of importance to the care team of this family, as they can better predict what other issues may arise and offer earlier screening for them. Finally, the identification of this diagnosis is good for the scientific community as a whole, as it provides more cases of *NFIA*-related disorder from which information about the manifestations and prognosis can be gleaned. Therefore, rare genetic conditions such as *NFIA*-related disorder should be included on the differential when evaluating patients with seemingly unrelated constellations of clinical features. However, given the lack of specificity of symptoms, ES may be required to identify people with this rare condition.

This case is a lesson in how rare gene disorders (such as *NFIA*-related disorder) can present similarly to, or even coexist with, other conditions. The genetic variant in the family presented here may have never been discovered had it not been for the requested reanalysis of Patient 1's ES results. The discovery made therein of a hypoplastic corpus callosum led to a chain of events that came to explain the family's symptoms more fully, drawing connections between seemingly unrelated problems like their

	Revah-Politi et al. (2017)			Zhang et al.[4]	Zenker et al. (2019)	Revah-Politi et al.[9]	Negishi et al.[10]	Wongkittichote et al.[8]
Male, 5	Female, 7	Female, 35	Male, 6	Male, 3 months	Male, 18	Female, 18	Male, 5	Male, 13 months
USA	USA	USA	USA	China		USA	Japan	USA
c.112C > T	c.205C > T	c.205C > T	c.205C > T	c.220C > T	c.1051C > T	c.159_160dupCC	c.1094delC	c.819-1G > A
p.Arg38 Ter	p.Arg69 Ter	p.Arg69Ter	p.Arg69Ter	p.Arg74Ter	p.Arg351Ter	p.Gln54ProfsTer49	p.Pro365 HisfsTer32	
+	+	−	+	+	−	+	+	+
+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+
+	+	−	+	+	+	+	+	+
ND	Frontal bossing and high forehead	−	Proximal insertion of thumbs, hemangioma, hypopigmented macule, frontal bossing, high forehead, low anterior hairline, widow's peak, and prominent occiput	Hypertelorism, slightly pointed chin, broad forehead, and large ears	Long face, facial asymmetry, prominent forehead, thin eyebrows, underdeveloped midface, and thin upper vermilion	Small hands and feet	High forehead and thin upper lip	Frontal bossing, facies w/ full lips, and clefted chin
−	+	+	−	−	−	−	−	ND
−	+	+	−	−	−	+	−	+
+	+	−	ND	−	−	−	+	+
−	−	−	+	−	+	+	−	+

developmental delay, musculoskeletal issues, macrocephaly, and more. It is a cautionary tale that one should always remember to dig deeper if the most "likely" answer does not fully explain the patient's symptoms.

AUTHOR CONTRIBUTIONS

Peyton Paschell: Formal analysis; investigation; writing – original draft. **Christina Laukaitis:** Conceptualization; investigation; methodology; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests in relation to this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

Approval of the Carle Foundation Hospital Institutional Review Board Ethics committee is not required for case series of 3 patients or fewer.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

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