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RESEARCH ARTICLE

Genomic Diagnoses for Ectopic Intracerebral Calcifications

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Abstract

Background and Objectives

Ectopic intracerebral calcifications (EICs) in the basal ganglia, thalamus, cerebellum, or white matter are seen in a variety of disease states or may be found incidentally on brain imaging. The clinical significance and proportion of cases attributable to an underlying genetic cause is unknown.

Methods

This retrospective cohort study details the clinical, imaging, and genomic findings of 44 patients with EICs who had no established diagnosis despite extensive medical workup.

Results

In total, 15 of 44 patients received a diagnosis through genomic testing explaining their calcifications, and 2 more received a diagnosis that has not been previously associated with EICs. Six of the 15 were found to have one of the 4 genes (*PDGFB*, *PDGFRB*, *SLC20A2*, and *XPR1*) conventionally associated with the phenotypic term "idiopathic basal ganglia calcifications."

Discussion

These findings support the use of genomic testing for symptomatic patients with EICs.

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Glossary

EICs = ectopic intracerebral calcifications; **ES** = exome sequencing; **GS** = genome sequencing; **PFBC** = primary familial brain calcification; **UDN** = Undiagnosed Disease Network; **UDPICS** = Undiagnosed Disease Program Integrated Collaboration System.

Introduction

Intracranial calcifications are a common finding on neuroimaging. Some intracranial calcifications might be considered normal variation while in other cases calcifications might be excessive or in unexpected locations. Abnormal calcifications might be uncovered incidentally, for example, in the context of imaging for head trauma or other neurologic conditions. Here, we use the term ectopic intracerebral calcifications (EICs) to reference the radiographic finding of calcifications, usually bilateral, involving a combination of structures including in the basal ganglia, thalamus, cerebellum, and/or subcortical white matter. While this is sometimes used synonymously with terms such as primary brain calcification, idiopathic basal ganglia calcinosis, striatopallidodentate calcinosis, calcinosis nucleorum, or Fahr syndrome,[1,2] the definitions of these terms are often ambiguous and we use EIsC to include cases of calcifications without basal ganglia involvement, asymmetric brain calcifications, and areas not classically associated with "Fahr syndrome". A variety of presentations including ataxia, movement disorders, and neuropsychiatric symptoms have been associated with brain calcifications; however, there is no clear correlation between the location, size, and underlying etiology of EICs with the presence, nature, and severity of clinical symptoms.[3-5]

Although most cases are found incidentally with no attributed cause, EICs can be associated with both underlying genetic and acquired disorders or be seen in individuals without apparent related illness.[6,7] Major acquired causes of brain calcifications include endocrine disturbances involving calcium homeostasis, parathyroid or vitamin D metabolism, vascular malformation, angiopathies, inflammatory disorders, neoplasms, and infections. Intracerebral calcifications have also been reported in the setting of many genetic disorders ranging from 22q11 deletion syndrome, tuberous sclerosis complex, and Cockayne syndrome among others.[6] More recently, studies on idiopathic basal ganglia calcifications sometimes known as primary familial brain calcifications (PFBCs), defined by kindreds with a dominantly inherited form of EIC, identified pathogenic variants in *PDGFB*, *PDGFRB*, *SLC20A2*, and *XPR1* as the underlying molecular cause of around half of all familial cases.[8] Currently there is no guideline for genetic testing in EICs, and genetic testing is not considered standard of care. Previous studies of genetic testing for familial EICs were done by sequential targeted testing or panels involving the 4 common genes with a yield of around 54%.[9,10] The yield of genetic testing in patients with EICs, without affected family members, after acquired causes have been ruled out, and the optimal strategy for testing is currently unknown.

The Undiagnosed Disease Program was created in 2008 and expanded to the Undiagnosed Disease Network (UDN) in 2014 to take an integrated clinical and genomic approach in evaluating patients with unexplained diseases.[11] Applications are reviewed with the goal of identifying those with no diagnosis despite comprehensive standard-of-care evaluation and are likely to generate new knowledge about disease pathogenesis. Accepted patients undergo extensive evaluation on a clinical and research basis for deep phenotyping and genomic testing. To date, over 1800 patients have been evaluated with more than 500 having received a diagnosis. The Undiagnosed Disease Program Integrated Collaboration System (UDPICS) was created to support collaborative translational research on rare diseases between UDN sites.[12]

We present genetic and clinical findings of 44 patients evaluated through the UDN who had EICs on imaging and underwent genomic sequencing analysis.

Methods

We conducted a retrospective review of patients evaluated through the UDN between 2008 and 2021 with a phenotype including EIC and had completed genomic testing. Relevant cases were identified through a search of standardized Human Phenotype Ontology based on input of phenotype information recorded in our secured database, UDPICS. The keywords "cerebral calcifications" and "cerebellar calcifications" were used to identify cases. No additional cases were identified using the term "intracranial calcifications." Medical and imaging records were then reviewed by designated investigators in the UDN protocol. Cases were excluded from this study based on the following criteria: no calcifications were found on review of imaging, the patient withdrew from the study, or genomic testing was not completed. Exome vs genome sequencing was done based on availability at time of evaluation. In some cases, genome sequencing was done after negative exome sequencing.

Standard Protocol Approvals, Registrations, and Patient Consents

Approval for this study was obtained under the designated and previously published IRB.[13] All patients were enrolled on protocol 76-HG-0238 or 15-HG-1030. Written informed consent was obtained from all participants.

Data Availability

Deidentified clinical data and gene-level information are available individually on request.

Results

A total of 63 study participants with EICs were identified based on our database search. Age at presentation ranged from birth to 70 years. Twenty-one of our cases first reported symptoms after turning 18 years while the rest presented in childhood. Nineteen patients were excluded because of lack of intracranial calcifications, absent genomic testing results, or withdrawal from the study. Of the remaining 44 patients, 24 (55%) were female and 20 (45%) were male. Ten patients had at least one known affected first-degree relative. All participants had extensive medical evaluation and did not have evidence for an acquired cause of EIC.

Calcifications were found on head computerized tomography in every case. Representative images are shown in Figure. The most common site of EIC was the basal ganglia (39, 89%) while the cerebellum (21, 48%), thalami (12, 27%), and subcortical white matter (16, 36%) represented other common sites of involvement. Calcifications in the central pons (4, 9%) cases and periventricular white matter (2, 5%) were less commonly seen.

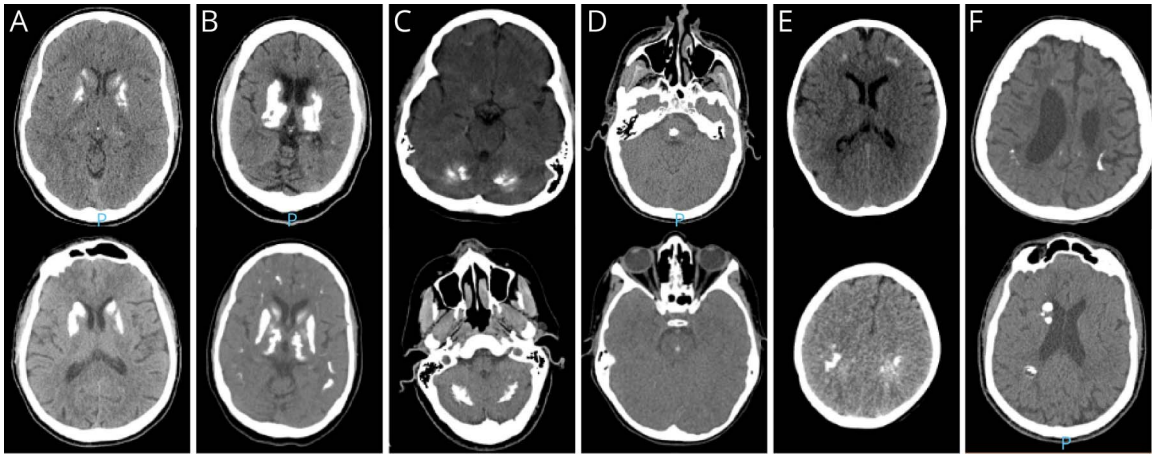
Detailed clinical findings are presented in Table. All but one case (evaluated based on family history) had at least one neurologic or psychiatric symptom, though the severity and nature of their findings varied. Every patient who received a genetic diagnosis was symptomatic. Twenty-seven (61%) individuals in our cohort had extrapyramidal signs such as spasticity, parkinsonism, or dystonia while 3 individuals had no neurologic symptoms. A wide spectrum of other findings such as vertigo, paresthesia, headaches, or cognitive decline were also present. Psychiatric symptoms were noted in 26 (59%) of our cases with anxiety disorder or major depressive disorder being the most common diagnoses. Age at symptom onset varied from infancy to the seventh decade.

Genomic testing method and diagnostic results are presented in Table. Twenty-four patients underwent exome sequencing (ES), 9 underwent genome sequencing (GS), and 10 received ES, followed by GS. One patient was diagnosed with XMEN based on reanalysis of previous ES and therefore not sequenced by the UDP.

Most (33, 75%) cases were tested in conjunction with at least one other family member. Fifteen patients were found to have a pathogenic or likely pathogenic variant in a gene that explained their EICs. Six patients were found to be heterozygous for a variant in *SLC20A2* (MIM 158378), 2 were compound heterozygous in *SNORD118* (MIM 616663), 2 were heterozygous in *IFIH1* (MIM 6060951), 1 case was heterozygous in *CSF1R* (MIM 164770), 1 was hemizygous in *MAGT1* (MIM 300715), 1 was heterozygous in *PDGFB* (MIM 190040), 1 was compound heterozygous in *PANK2* (MIM 606157), and 1 was compound heterozygous in *RNASEH2B* (MIM 610326). Two individuals had pathogenic variants in genes that partially explained their symptoms but did not explain their EIC. One patient with dystonia was found to have a *TOR1A* variant and diagnosed with Torsion dystonia type 1 (MIM 128100). Another patient had a known diagnosis of Gaucher disease (MIM 230800) at the time of enrollment in the UDP that was seen again, but this was not thought to explain the EIC. One case received a diagnosis on genome sequencing after negative exome sequencing. This was due to a pathogenic variant in *SNORD118*. The other case attributable to variants in *SNORD118* had not undergone previous exome sequencing.

The diagnostic rate was 11 of 33 (33%) for those in whom additional family members were sequenced, including a rate of 7 of 24 (29%) for families with at least complete trios including both parents. The diagnostic rate was 3 of 10 (30%)

Figure Representative Images of Calcifications



Selected computed tomography (CT) images showing calcifications in the (A) basal ganglia, (B) basal ganglia and thalamus, (C) cerebellum, (D) pons, (E) subcortical white matter, and (F) periventricular white matter. Diagnosis associated with each image (Top, Bottom): A (ND, ND), B (ND, ND), C (ND, ND), D (*SNORD118*, ND), E (ND, *IFIH1*), F (*CSF1R*, *SNORD118*). ND = no diagnosis found.

Table Summary of Genetic and Clinical Findings

Patient ID	Location of calcification	Sex	Age at symptom onset	Family history	Neurologic phenotype	Psychiatric phenotype	Final diagnosis	Genetic tests done	Molecular findings[a]
1	BG, Th, Cer	Female	70	None	None	Impulsivity, intrusive compulsive thoughts		Exome	—
2	PVWM	Female	30	None	Right hemiparesis, speech apraxia, dysarthria, pseudobulbar affect, exaggerated startle, generalized spasticity	Depression	Hereditary diffuse leukoencephalopathy with spheroids 1	Exome	NM_005211.3(*CSF1R*):c.2381T>C (p.Ile794Thr)
3	BG, Cer	Female	<1	None	Global developmental delay, spasticity, moderate intellectual disability	None		Exome	—
4	BG	Male	Unknown	None	None	None		Exome	—
5	BG, Th, Cer	Male	62	None	Paresthesia and hemiparesis	Depression		Exome	—
6	SWM	Female	<1	Adopted	Global developmental delay, dysarthria, macrocephaly/ hydrocephalus, sensorineural hearing loss, LE hypotonia	None		Genome and exome	—
7	BG, Th	Female	50	None	Headaches, vertigo, facial sensation diminished on L side.	Anxiety		Exome	—
8	BG, Th, Cer, SWM	Female	59	Mother, brother, and one son	Vertigo, ataxia, headaches, hand tremors, paresthesia, memory impairment	None	Familial primary brain calcification	Genome	NM_006749.4(*SLC20A2*):c.935-2A>G
9	BG, Cer, SWM	Female	8	None	Ataxia, memory impairment, brisk reflexes, hyperacusis,	Obsessive compulsive behavior Anxiety	Familial primary brain calcification	Exome	NM_006749(*SLC20A2*):c.1723G>T (p.E575X)
10	BG, Th, SWM	Male	56	Son	Paresthesia and pain. Migraines. Positional vertigo	None	Familial primary brain calcification	Exome	NM_006749(*SLC20A2*):c.136C>T (p.Q46X)
11	Cer, PVWM, pons, external capsule	Male	14	None	Progressive spasticic hemiparesis, focal epilepsy, numbness of the L side, L-sided clonus	Emotional expressivity more than usual	Labrune syndrome	Genome	NR_033294.1(*SNORD118*):n.3C>T/NR_ 033294.1(*SNORD118*):n.20C>T
12	BG	Female	36	None	Cognitive decline, choreiform movements, left foot drag	None		Genome and exome	—
13	BG, Th, Cer, SWM, pons	Female	52	None	Dysarthria, paresthesia, gait instability, headaches, dysphagia	Poor concentration, anxiety		Single exome, duo genome	—

Continued

Table Summary of Genetic and Clinical Findings *(continued)*

Patient ID	Location of calcification	Sex	Age at symptom onset	Family history	Neurologic phenotype	Psychiatric phenotype	Final diagnosis	Genetic tests done	Molecular findings[a]
14	BG	Male	Unknown	Mother, maternal uncle, and maternal grandmother	None	Anxiety	Familial primary brain calcification	Genome	NM_006749.4(*SLC20A2*):c.935-2A>G
15	Cer	Male	15	None	Cognitive decline	Depression, anxiety	Gaucher type 1 (known before referral)	Genome	NM_001005741.2(GBA):c.1192C>T (p.Arg398Ter)/ NM_001005741.2(GBA):c.1171G>C (p.Val391Leu)
16	BG, Cer, SWM	Male	15	Mother and maternal grandfather	Headaches	Anxiety	Familial primary brain calcification	Exome	NM_006749(*SLC20A2*):c.1375G>T (p.E459X)
17	BG, Cer, SWM	Female	20	None	Hyporeflexia, progressive hemifacial spasm, limb myoclonus, dementia	Depression		Genome	—
18	BG,Th, Cer, SWM	Male	22	Mother and brother	Dysarthria, ataxia, dysmetria, myoclonus, parkinsonism	Bipolar disorder	XMEN syndrome MAGT1-CDG c.414C>A, p.Y138X)	Exome (reanalysis)	NM_032121.5(MAGT1):c.414C>A (p.Y138X)
19	BG	Female	34	Mother and son	Headaches	None	Familial primary brain calcification	Exome	NM_006749(*SLC20A2*):c.1375G>T (p.E459X)
20	BG	Female	40	None	Patient lost her sense of smell, taste, pain, and temperature.	Anxiety/depression, episodes of uncontrollable crying.	Idiopathic basal ganglia calcification 5	Genome	NM_002608.3(*PDGFB*):c.598C>T (p.R200X)
21	BG, Cer, SWM	Male	38	Mother with BG calcification	Cogwheel rigidity paresthesia, hyperreflexia, memory impairment	Depression		Genome	—
22	BG, Cer, SWM	Male	67	None	L-sided weakness, dysarthria, bilateral postural tremor, dysmetric saccades	None		Genome	—
23	BG	Female	13	Mother and brother	Headaches, muscle pain, generalized weakness, lethargy, absence seizures, progressive memory loss, dysautonomia	Auditory and visual hallucinations, depression, anxiety, anorexia		Genome	—
24	BG	Female	39	None	Chorea, vertical gaze palsy, hypophonia, dystonia, parkinsonism	Decline in executive function. Mild deficits in memory/visuospatial abilities.		Genome and exome	—
25	BG, Th, Cer, SWM	Male	32	None	Dysmetria, dystonia, chorea. Dysdiadochokinesis, restless legs	Anxiety		Exome	—

Continued

Table Summary of Genetic and Clinical Findings *(continued)*

Patient ID	Location of calcification	Sex	Age at symptom onset	Family history	Neurologic phenotype	Psychiatric phenotype	Final diagnosis	Genetic tests done	Molecular findings[a]
26	BG, Cer, pons	Female	<1	None	Global developmental delay, epilepsy, choreoathetosis, infantile spasms, hypotonia, microcephaly, cortical blindness	None		Exome	NM_005677.3(*COLQ*):c.391A>G (p.Lys131Glu) - Variant of Uncertain Significance/NM_005677.3(COLQ):c.749C>T (p.Pro250Leu) - Variant of Uncertain significance
27	BG	Female	12	None	Dysarthria, bulbar weakness, involuntary and hard movements, tremor, brisk reflexes, ataxia	Impulsive disorder, anxiety, depression	Pantothenate kinase–associated neurodegeneration	Exome	NM_153638.2(*PANK2*):c.1561G>A (p.G521R)/NM_153638.2(PANK2):c.1583C>T (p.T528M)
28	BG, SWM	Male	<1	Brother	Developmental regression, hyperreflexia, exaggerated startle, spastic paraplegia	None	Aicardi-Goutieres syndrome type 2	Exome	NM_024570.3(*RNASEH2B*):c.529G>A, p.Ala177Thr (homozygous)
29	BG	Female	18	None	Migraines, bilateral carpel tunnel syndrome	None		Exome	—
30	BG	Male	1	Adopted	Dystonia, dyslexia, migraines w/visual aura, writer's cramp	ADHD		Exome	—
31	BG, Th, Cer	Female	43	None	Dysarthria, dystonia, dysphagia, headaches, staring spells, ataxia, tremor, upgoing toes.	Anxiety, impulsivity		Exome	—
32	BG, Th, Cer	Male	12	None	Tic disorder, impairment of attention/executive function, headache, brain fog	Anxiety disorder, panic attacks, ADHD		Genome and exome	—
33	BG	Male	<1	None	Hypertonia, tremor, severe developmental delay	None		Exome	—
34	BG	Female	Childhood	None	Generalized dystonia	None	TOR1A dystonia	Exome	NM_000113.2(TOR1A):cc.904_906delGAG (p.Glu303del)
35	BG, Th, Cer	Female	52	None	Mild cognitive impairment, ataxia	Anxiety, depression, agoraphobia		Genome and exome	—
36	BG, Cer	Female	30	None	Short-term memory loss, executive dysfunction, word-finding difficulty, parkinsonism	Obsessive compulsive disorder		Exome	—
37	BG, SWM	Male	1	None	Global developmental delay, microcephaly, hypotonia, dystonia	None	Aicardi-Goutieres syndrome type 7	Exome	NM_022168.3(IFIH1):c.1009A>G (p.R337G)
38	BG, SWM	Male	4	Two brothers	Migraine, episodic ataxia, limb dystonia	Anxiety		Genome and exome	—

Continued

Table Summary of Genetic and Clinical Findings *(continued)*

Patient ID	Location of calcification	Sex	Age at symptom onset	Family history	Neurologic phenotype	Psychiatric phenotype	Final diagnosis	Genetic tests done	Molecular findings[a]
39	BG, pons, SWM	Male	3	None	Migraines, vertigo, torticollis, abnormal coordination and balance	Anxiety disorder		Genome and exome	—
40	BG, SWM	Male	12	None	Bilateral optic disc edema	None		Genome and exome	—
41	Cer	Female	<1	None	Ataxia, dystonia, spasticity, dysarthria, bradykinesia, axonal peripheral neuropathy	She has repeated several years of elementary and high school.		Genome and exome	—
42	BG, SWM	Female	10	None	LE weakness and atrophy with spasticity, dystonic posturing, brisk reflexes, upgoing toes	None	Aicardi-Goutieres syndrome type 7	Exome	NM_022168.3(*IFIH1*):c.2342G>A (p.G781E)
43	BG, Th, Cer	Female	28	None	Spasticity, dysarthria, dysphagia, cervical dystonia, urinary and bowel incontinence, pseudobulbar affect	None	Labrune syndrome	Genome and exome	NR_033294(SNORD118):n.-6G>A/NR_033294(*SNORD118*):n.131C>G
44	BG	Male	<1	Sister affected	Hypotonia, global developmental delay	None		Genome and exome	—

Abbreviations: ADHD = attention deficit and hyperactivity disorder; BG = basal ganglia; Cer = cerebellum; PVWM = periventricular white matter; SWM = subcortical white matter; Th = thalamus.
[a] Findings presented are pathogenic or likely pathogenic by ACMG criteria unless otherwise stated.

when only the proband was sequenced. The diagnostic rate was 7 of 12 (58%) when an additional family member was affected and 8 of 32 in simplex cases (25%).

Four individuals in our cohort were found to have variants of interest in *PDGFB* (MIM190040), *PGDFRB* (MIM 173410), *XPR1* (MIM 605237), or *APP* (MIM104760) all of which are genes associated with EIC though these variants did not meet ACMG criteria to be classified as pathogenic or likely pathogenic.

Discussion

We present the results of comprehensive genomic testing in patients with EICs regardless of family history. Our cohort consisted of 55% female and 45% male patients, which is consistent with previous studies reporting a higher prevalence of basal ganglia calcifications in female patients.[14] The overall diagnostic yield of genomic testing for EICs was 34% in our study. 46% (7 of 15) of patients who received a diagnosis developed symptoms in adulthood. This mirrors the overall cohort where 47% of patients presented after age 18 years and suggests that age at symptom onset does not predict pretest probability of having an underlying genetic etiology for EICs.

Our cohort represents individuals who have features consistent with a rare disease and sought extensive medical evaluation and thus is not reflective of the general population of patients with EICs. Because a significant portion of patients with EICs are asymptomatic and therefore may not ever seek medical attention, our results likely overestimate the diagnostic yield of genomic testing on the general EIC population after secondary EIC has been ruled out. In addition, those who are referred have typically undergone extensive prior testing for both acquired and genetic etiologies, which implies an above average level of access to care as well as medical literacy. There are several reasons patients with known EIC genes may not have been identified in our cohort before inclusion in this study. First, genetic testing is currently not standard of care in all cases of EICs. Second, the clinical significance of EICs is not well understood; the phenotypes associated with this finding range from asymptomatic to more prototypical neurologic syndromes such as neuropsychiatric syndromes or movement disorders. As such, brain imaging may not have been indicated in all cases before research evaluation or EICs may have been seen as an incidental finding in some of our participants rather than an anchor for diagnosis. Finally, clinical genomic analysis pipelines are commonly phenotype rather than brain imaging-driven and EIC-causing variants may not have been considered or reported by previous testing laboratories because of the lack of clear clinical correlation between EICs and the patient's presenting symptoms.

Of the 9 patients with an affected first-degree relative, 3 (33%) had one of the 4 canonical familial calcification genes, which is lower than the 55% yield in previous published reports.[10] This may reflect ascertainment bias in our cohort because patients with EICs are more likely to be tested for these 4 genes, and positive cases would be less likely to be referred to the UDN. Our most common pathologic finding in this cohort was *SLC20A2*, which was previously reported to account for around 40% of all primary familial brain calcification cases[9] and was present in 4 of our familial cases in this cohort. Of the remaining 2, one received a diagnosis of XMEN (MIM 300853) and the other received a diagnosis of Aicardi-Goutieres syndrome type 7 (MIM 610181). While sequencing of additional family members, especially parents, has previously been shown to increase diagnostic yield of genomic testing, it did not substantially change the diagnostic yield in this cohort in the absence of a positive family history. This likely reflects a large contribution of familial rather than de novo variations toward disease prevalence.

Nine of 33 (27%) in sporadic cases of EIC in our cohort received a diagnosis. Three cases, 2 with *SLC20A2* and 1 with *PDGFB*, had classic primary familial brain calcification genes. The remaining patients received diagnosis of Labrune syndrome (MIM 614561), Aicardi-Goutieres syndrome type 7 (MIM 615846), PKAN (MIM 234200), or leukoencephalopathy with spheroids 2 (MIM 221820). Although some of these patients had nonspecific findings unrelated to calcifications such as growth retardation in one patient with Aicardi-Goutieres syndrome and leukoencephalopathy in one patient with Labrune syndrome, many of these cases did not exhibit classic signs associated with their disease such as cysts, spheroids, or clear iron deposition which likely contributed to their referral to the UDN. These cases were unlikely to have been diagnosed without genomic testing, highlighting the heterogeneity of disease presentations and potential pitfalls of relying on clinical findings to perform targeted genetic testing. Notably, given that *SNORD118* is a noncoding RNA, this may be missed on exome sequencing but would be detected on genome sequencing.

Among the 29 cases with EIC who did not receive a genomic diagnosis, 4 were found to be heterozygous for a possibly causative variant in a canonical gene associated with familial brain calcifications. Further research is necessary to determine whether these variants are causative. In addition, 3 more individuals were found to have pathogenic variants that explained their clinical presentation but not their EICs. One patient with bilateral basal ganglia calcifications and childhood-onset dystonia was found to be heterozygous for the known pathogenic deletion in *TOR1A* (MIM 605204) causative of DYT1. By definition, DYT1 primary dystonia is not expected to have structural brain abnormalities, perhaps suggesting this patient might have a blended phenotype with a yet uncharacterized cause of EICs. Two others had complex clinical presentations, one with pathogenic variants in *GBA* (MIM 606463) and one with potentially causative variants in *COLQ* (MIM 603033) which is undergoing further evaluation. These diagnoses would explain some, but not all, of the respective probands' phenotypes. For these cases, ambiguity

remains if EICs are a rare feature of these disorders or if these individuals have blended phenotypes from more than one condition and/or gene-gene interactions which might result in EICs.

Of the 39 individuals in our cohort who had bilateral calcifications in the basal ganglia consistent with the classic description "idiopathic basal ganglia calcification" (MIM PS213600), 12 received a diagnosis on genomic testing of which 6 had a pathogenic change in a gene associated with this phenotype. Of the remaining 5 cases that did not have basal ganglia involvement, 3 received a genomic diagnosis. None of the 3 were found to have one of the canonical genes which supports the inclusion of basal ganglia involvement as a criterion for this entity. There were no clear correlations between our genetic findings with clinical symptoms or age at presentation; however, because of the nature of the UDN, atypical presentations are likely overrepresented in our cohort, and asymptomatic forms of IBG are excluded by the nature of the UDP program.

The causative genes we identified are involved in a variety of physiologic and cellular processes including ribosomal RNA processing, prostaglandin transport, magnesium transport, platelet and vascular endothelial growth, coenzyme A biosynthesis, DNA replication, and innate immunity. The wide range of functions of pathogenic changes that can lead to EICs points to this phenotype being at the convergence of disturbances in multiple pathways. More research is needed to understand how these processes interact and contribute to abnormal calcium deposition.

In conclusion, we present genomic, clinical, and imaging findings in 44 patients who had EICs and did not have a diagnosis after extensive routine workup. Extrapyramidal movement disorders were the most common clinical finding, and the basal ganglia was the most common site of calcification on imaging. We identified a genetic diagnosis through genomic testing in 25% of sporadic cases and 58% of cases with a similarly affected first-degree relative (dominant EIC). Because our cohort is not representative of all patients with EICs, more research needs to be done to determine the clinical utility of genetic testing in EICs and the generalizability of our findings. Our findings suggest that genetic testing should be considered for patients with neurologic or nonspecific neuropsychiatric symptoms along with EICs. Most of our solved cases were not due to one of the 4 genes associated with PFBC, even when the classically described basal ganglia calcifications were present. These findings support the use of genomic testing rather than targeted or panel testing. The presence of Labrune syndrome, caused by a small nonprotein coding element, in our cohort supports the use of genome rather than exome sequencing.

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Appendix Authors

Name	Location	Contribution
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Thomas Cassini, MD	National Human Genome Research Institute	Including medical writing for content; major role in the acquisition of data; analysis or interpretation of data; additional contributions (in addition to one or more of the above criteria)
Daniel Benavides, BS	National Human Genome Research Institute	Major role in the acquisition of data; analysis or interpretation of data; additional contributions (in addition to one or more of the above criteria)
Anusha Ebrahim, BA	National Human Genome Research Institute	Major role in the acquisition of data; analysis or interpretation of data; additional contributions (in addition to one or more of the above criteria)
David Adams, MD, PhD	National Human Genome Research Institute	Major role in the acquisition of data; analysis or interpretation of data; additional contributions (in addition to one or more of the above criteria)
Camilo Toro, MD	National Human Genome Research Institute	Including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data; additional contributions (in addition to one or more of the above criteria)

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