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RESEARCH ARTICLE

Mendelian etiologies identified with whole exome sequencing in cerebral palsy

Maya Chopra[1,*], Dustin L. Gable[2,3,*], Jamie Love-Nichols[4], Alexa Tsao[5], Shira Rockowitz[6,7], Piotr Sliz[6], Elizabeth Barkoudah[8], Lucia Bastianelli[9], David Coulter[8], Emily Davidson[2,10], Claudio DeGusmao[8], David Fogelman[11], Kathleen Huth[2], Paige Marshall[8], Donna Nimec[11], Jessica Solomon Sanders[12], Benjamin J. Shore[9], Brian Snyder[9], Scellig S. D. Stone[13], Ana Ubeda[11], Colyn Watkins[9], Charles Berde[14], Jeffrey Bolton[8], Catherine Brownstein[15], Michael Costigan[16], Darius Ebrahimi-Fakhari[8,6], Abbe Lai[8], Anne O'Donnell-Luria[6,15], Alex R. Paciorkowski[17], Anna Pinto[8], John Pugh[18], Lance Rodan[8,15], Eugene Roe[8], Lindsay Swanson[8], Bo Zhang[8], Michael C. Kruer[19], Mustafa Sahin[1], Annapurna Poduri[8] & Siddharth Srivastava[8]

[1]Department of Neurology, Rosamund Stone Zander Translational Neuroscience Center, Boston Children's Hospital, Boston, Massachusetts, USA
[2]Department of Pediatrics, Division of General Pediatrics, Boston Children's Hospital, Boston, Massachusetts, USA
[3]Child Neurology Residency Training Program, Boston Children's Hospital, Boston, Massachusetts, USA
[4]Department of Genetics, Seattle Children's Hospital, Seattle, Washington, USA
[5]Marsh & McLennan Agency, White Plains, New York, USA
[6]The Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, Massachusetts, USA
[7]Division of Molecular Medicine, Boston Children's Hospital, Boston, Massachusetts, USA
[8]Department of Neurology, Boston Children's Hospital, Boston, Massachusetts, USA
[9]Department of Orthopedics, Boston Children's Hospital, Boston, Massachusetts, USA
[10]Division of Developmental Medicine, Boston Children's Hospital, Boston, Massachusetts, USA
[11]Department of Physical Medicine and Rehabilitation, Spaulding Rehabilitation Hospital, Boston, Massachusetts, USA
[12]Department of Neurology, Denver Children's Hospital, Denver, Colorado, USA
[13]Department of Neurosurgery, Boston Children's Hospital, Boston, Massachusetts, USA
[14]Department of Anesthesiology, Boston Children's Hospital, Boston, Massachusetts, USA
[15]Division of Genetics and Genomics, Boston Children's Hospital, Boston, Massachusetts, USA
[16]Department of Neurobiology, Boston Children's Hospital, Boston, Massachusetts, USA
[17]Departments of Neurology, Pediatrics, Biomedical Genetics, and Neuroscience, University of Rochester Medical Center, Rochester, New York, USA
[18]Department of Neurology, Bernard and Millie Duker Children's Hospital, Albany Medical Center, Albany, New York, USA
[19]Department of Neurology and Pediatrics, Phoenix Children's Hospital, Phoenix, Arizona, USA

Correspondence
Siddharth Srivastava, Department of Neurology, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115. Tel: +1 617 355 6388; Fax: +1 617 730 0284; E-mail: siddharth.srivastava@childrens.harvard.edu

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Abstract

Objectives: Cerebral palsy (CP) is the most common childhood motor disability, yet its link to single-gene disorders is under-characterized. To explore the genetic landscape of CP, we conducted whole exome sequencing (WES) in a cohort of patients with CP. **Methods**: We performed comprehensive phenotyping and WES on a prospective cohort of individuals with cryptogenic CP (who meet criteria for CP; have no risk factors), non-cryptogenic CP (who meet criteria for CP; have at least one risk factor), and CP masqueraders (who could be diagnosed with CP, but have regression/progressive symptoms). We characterized motor phenotypes, ascertained medical comorbidities, and classified brain MRIs. We analyzed WES data using an institutional pipeline. **Results**: We included 50 probands in this analysis (20 females, 30 males). Twenty-four had cryptogenic CP, 20 had non-cryptogenic CP, five had CP masquerader classification, and one had unknown classification. Hypotonic-ataxic subtype showed a difference in prevalence across the classification groups ($p = 0.01$). Twenty-six percent of participants (13/50) had a pathogenic/likely pathogenic variant in 13 unique genes (*ECHS1*, *SATB2*, *ZMYM2*, *ADAT3*, *COL4A1*, *THOC2*, *SLC16A2*, *SPAST*, *POLR2A*, *GNAO1*, *PDHX*, *ACADM*, *ATL1*), including one patient with

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*Contributed equally.

two genetic disorders (*ACADM*, *PDHX*) and two patients with a *SPAST*-related disorder. The CP masquerader category had the highest diagnostic yield (n = 3/5, 60%), followed by the cryptogenic CP category (n = 7/24, 29%). Fifteen percent of patients with non-cryptogenic CP (n = 3/20) had a Mendelian disorder on WES. **Interpretation**: WES demonstrated a significant prevalence of Mendelian disorders in individuals clinically diagnosed with CP, including in individuals with known CP risk factors.

Introduction

Cerebral palsy (CP) is the most common childhood-onset motor disability, affecting more than 700,000 individuals in the United States alone.[1] CP is defined as a "group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to nonprogressive disturbances which occurred in the developing fetal or infant brain."[2] As with other neurodevelopmental disorders (NDDs), CP is a descriptive term that does not depend on etiology and can be due to various causes. Established CP risk factors include prematurity[3] and periventricular/intraventricular hemorrhage, the latter of which is associated with spastic diplegia.[4,5] Perinatal asphyxia causing hypoxic ischemic encephalopathy (HIE) can lead to CP (e.g., spastic quadriplegic CP due to cerebral hypoperfusion in watershed distributions, or dyskinetic CP due to basal ganglia injury), accounting for less than 12% of cases.[6] Maternal and fetal infection may also play a role in the pathogenesis of CP,[7–9] and perinatal stroke confers risk for hemiplegic CP.[10]

Approximately 20% of individuals with CP have no clear etiological explanation based on review of perinatal risk factors and are classified as having "cryptogenic CP."[11] Accumulating evidence suggests that some cases of cryptogenic CP are associated with chromosomal copy number variants and single gene disorders. Pathogenic copy number variations have been identified in 10%–20% of individuals with CP[12–14], especially those with dysmorphic features and nonmotor comorbidities.[11] In a whole exome sequencing (WES) study in patients with a CP diagnosis (confirmed using standard criteria), 14% of cases had a potentially disease-causing genetic alteration; this study did not differentiate between cryptogenic versus non-cryptogenic cases.[15] A study performed on a more select population, individuals with CP born at full term without specific findings on MRI, revealed a pathogenic or likely pathogenic (P/LP) variant in 9/17 (53%) cases.[16] New data have provided statistical and functional evidence that single gene variants can lead to CP phenotypes.[17] A systematic review of inborn errors of metabolism presenting with CP identified 54 treatable conditions.[18] Finally, some individuals present with symptoms that mimic CP, yet they do not meet CP diagnostic criteria due to, for example, the presence of regression or progressive symptoms; these are referred to as "CP masqueraders".[19]

Despite initial progress, the current evidence regarding genetic causes of CP is limited, partly because past conventional wisdom considered CP to be predominantly acquired. Additionally, many prior studies did not differentiate or compare findings between cryptogenic CP, non-cryptogenic CP, and CP masqueraders. As a result of these limitations, the full breadth of the genetic landscape of CP is unknown, not only as it pertains to the cryptogenic CP and CP masquerader groups, but also to the group of individuals with *known* CP risk factor(s) who may *also have* an underlying genetic disorder, which could confer vulnerability to adverse perinatal events or contribute to a more complex phenotype.

To address these questions, we report the results of comprehensive phenotyping and WES analysis on a prospective cohort of 50 unrelated individuals with CP (both cryptogenic and non-cryptogenic) or CP masqueraders.

Methods

Participant recruitment/selection

We enrolled participants in the Boston Children's Hospital (BCH) CP Sequencing Study, approved by the BCH Institutional Review Board, to conduct phenotyping and WES for individuals with CP and CP masqueraders (Fig. 1). The BCH CP Sequencing Study is part of the Children's Rare Disease Cohorts (CRDC) initiative, which integrates genomic, research, and clinical data to facilitate pediatric precision medicine.[20]

Participant referrals for this study were from (1) neurodevelopmental clinicians experienced with evaluation of CP (JSS), (2) neurologists (DC, CMD, EB, SS), orthopedists (BJS, BS, CW), physiatrists (DF, DN, AU), nurse practitioners (PM, LB), complex care pediatricians (ED, KH), or neurosurgeons (SSDS) associated with the Boston Children's Hospital CP Center who have extensive experience in CP. These physicians had clinically evaluated referred participants. Participants provided informed consent.

Some elements of the study were prospective, while others were retrospective. Recruitment, enrollment, WES,

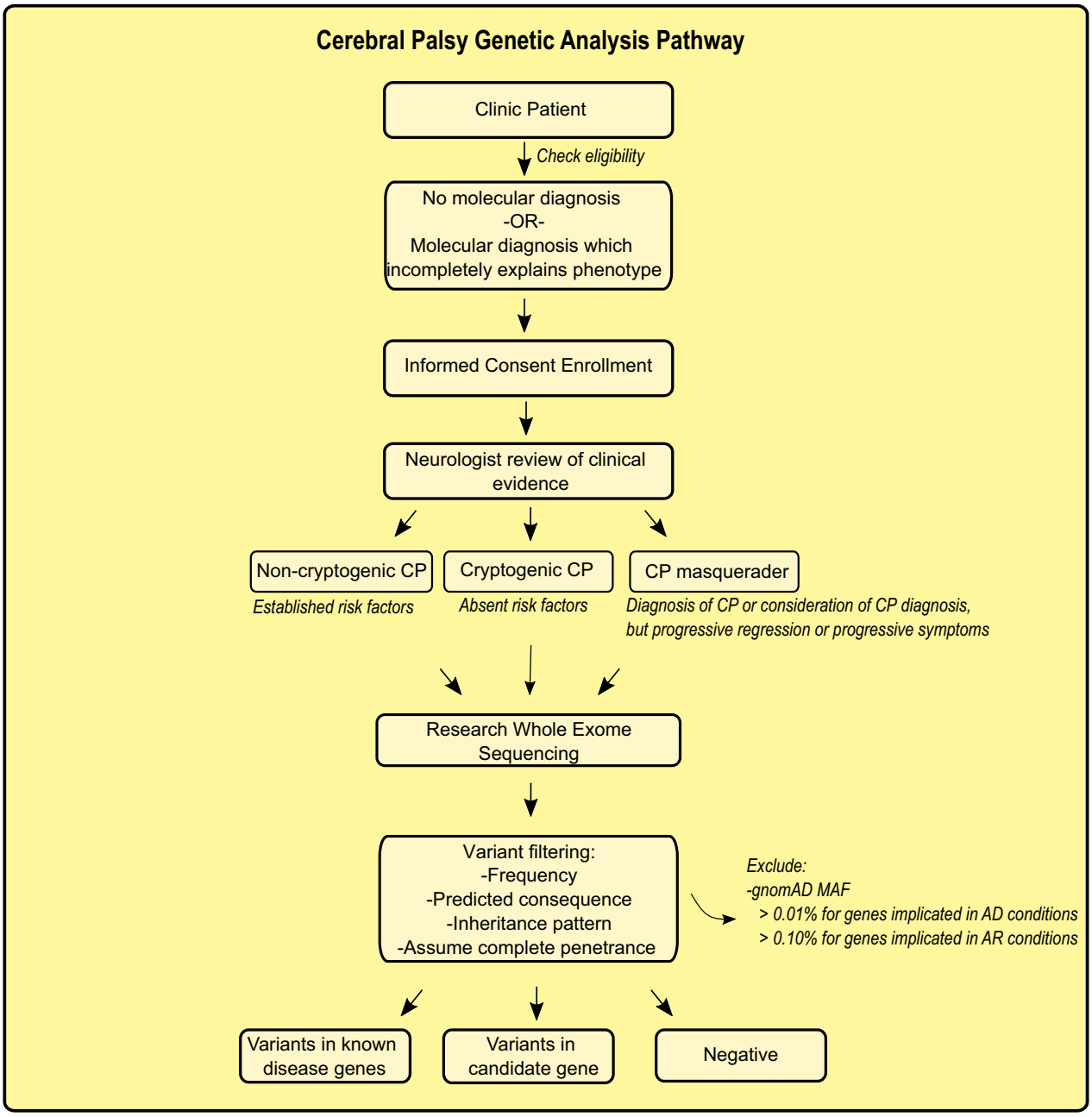

Figure 1. Research pipeline for exome sequencing analysis in this cohort.

and categorization of the participant as having CP or CP masquerader was prospective. Retrospective elements included classification of CP as cryptogenic/non-cryptogenic CP, as well as delineation of CP motor phenotype, medical comorbidities, and neuroimaging profile.

Inclusion/exclusion

Participants could be of any age or sex. We included (1) participants who fulfilled standard criteria for CP[21], and (2) participants who had been previously diagnosed with CP or could be considered to have CP, but review of their presentation revealed developmental regression, progressive symptoms, or other findings inconsistent with the definition of CP (referred to as "CP masquerader" here). The diagnosis of CP or classification of CP masquerader was verified for each participant (SS). We excluded participants with a known molecular diagnosis that completely explained their neurodevelopmental presentation.

Classification of CP: cryptogenic, non-cryptogenic, or CP masquerader

For patients meeting criteria for CP, we classified their etiology into two categories: cryptogenic and non-cryptogenic. If the participant had *any* predefined risk factors, based on review of the medical records, we designated the case as non-cryptogenic CP. If the participant had *no* predefined risk factors, we designated the case as cryptogenic CP. Risk factors considered were as follows: (1) prematurity (≤32 weeks); (2) periventricular/intraventricular hemorrhage; (3) intracranial hemorrhage; (4) perinatal stroke; (5) evidence of other acute perinatal event (such as acute onset of decreased fetal movements); (6) hypoxic ischemic injury; (7) kernicterus; (8) fetal infection; (9) maternal infection at delivery leading to sepsis in the mother; (10) neonatal infection leading to sepsis; (11) neonatal respiratory arrest; (12) neonatal cardiac arrest; (13) hydrocephalus; (14) traumatic brain injury. This list represents a modification of a recently outlined set of risk factors.[17] We added: intracranial hemorrhage; evidence of other acute perinatal event; kernicterus; maternal infection at delivery leading to sepsis in the mother; and neonatal infection leading to sepsis. We did not include: major brain malformation or brain calcifications, since these can be associated with an underlying genetic etiology.

Delineation of CP motor phenotype, medical comorbidities, and neuroimaging profile

We verified the motor phenotype of all affected individuals based on direct examination of the patient by one of the study's co-authors. Based on this exam, we designated motor phenotype with one of the following primary patterns: spastic hemiplegic, spastic diplegic, spastic quadriplegic, dyskinetic, or hypotonic-ataxic.

We determined the presence of medical comorbidities based on review of the individual's medical records by SS. We designated an affected participant as having multiple comorbidities if there were two or more of the following features (as previously delineated in the context of CP[22,23]): seizures/epilepsy, severe visual impairment, severe auditory impairment (i.e., documented permanent hearing loss), communication impairment (best estimate based on documented communication abilities), and G-tube dependence. We could not accurately ascertain intellectual disability (ID) based on review of documentation. We characterized clinical brain MRI (when available) using a classification system.[23] If there were multiple scans performed, we chose the latest study available for review. MRI images and reports were reviewed systematically (by SS) in order to label each participant's MRI with one of the eight primary patterns: (1) deep gray matter injury; (2) white matter injury; (3) white matter and cortical injury; (4) deep gray matter/white matter/cortical injury; (5) focal lesion; (6) cortical malformation; (7) normal study; (8) other.

DNA isolation and WES sequencing

DNA was isolated from probands (and both parents if available) via blood or buccal samples. WES was performed, as previously reported,[20] using the Illumina NovaSeq6000 platform (GeneDx, Gaithersburg, Maryland) on DNA extracted from these samples using IDT xGen probes. The average coverage across the WES was 100x and more than 95% of targets were covered at 20x. All sequencing data passed-specific minimal quality control requirements, including pass-filtered sequencing yield of 4GB, thresholds for mapping percent to hg19 (>95%), target coverage at 10× (90%, 97%–98% typical), mean target coverage (50×, average 100–120×), duplicate read percentage (<30%, <10% typical), and read-quality metrics (80% Q30).

Through the CRDC, FASTQ files were uniformly processed through a standard Sentieon variant calling pipeline and passed-specific quality control requirements, as previously described.[20] Read alignment, read depth calculation, realignment, recalibration, and variant calling were performed by Sentieon v201808.0391: BWA, HSMetricsAlgo, WGSMetricsAlgo, markduplication and Realigner, QualCal, Haplotyper, and GVCFtyper91. Verifybamid 1.1.392 was used to check contamination and GATK 4.1.2.093 to count reads in bins. Genuity Science GORpipe 4.3.061 converted other variant data to genomically ordered relational (GOR) format and annotated variants with VEP 96.294 and custom tools. The sequencing data were integrated and combined with phenotypic and research data in a genomics learning system (GLS). The GLS enables automated variant classification and prioritization, as well as phenotype extraction via natural language processing (NLP) of clinical notes.

Variant prioritization and interpretation

We prioritized variants by consensus within the analytic team which were (1) coding and/or canonical splice-site; (2) rare with a maximum allele frequency of 0.0001 (0.001 for recessive models) in the population database gnomAD (http://gnomad.broadinstitute.org) as accessed June 2020–June 2021; (3) variants predicted as truncating (including frameshift, stop-gain, and stop-loss), splice site, missense, or inframe indels using Variant Effect Predictor (VEP) classification from NextCODE, and if relevant, using SIFT[24] and PolyPhen-2[25] for evidence of

pathogenicity; (4) variants in genes previously implicated in neurodevelopmental/neurological disorders including CP through review of the published literature; (5) variants in genes highly intolerant to loss-of-function or missense variation in the general population based on data from gnomAD (pLI >0.9 and/or Z score for missense variation >3.09).

We assessed for inherited and de novo variants accounting for de novo/autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive models. Inheritance patterns were considered alongside the variant for established disease genes. The analytic team, led by a clinical geneticist (MC), performed a clinical chart review (including, when available, documentation of dysmorphisms, family history, and previous investigations) for each case in parallel to WES analysis to further evaluate/prioritize variants identified on this pipeline.

Variants in established disease genes

We classified variants according to American College of Medical Genetics and Genomics (ACMG) criteria.[26] For P/LP variants, GeneDx directly sequenced probands (and parents, if available) and provided a Clinical Laboratory Improvement Amendments (CLIA) report with the variant's ACMG classification (clinical confirmation). For each P/LP variant, following a thorough literature review, the analytic team determined whether the variant explained the presentation of the proband, and if so, whether this explanation was complete.

We clinically confirmed some variants of unknown significance (VUS) based on compelling rationale such as phenotypic specificity and/or potential for further investigations. We used the ACMG classification decision from the clinical laboratory as the final classification for P/LP variants and for VUS submitted for clinical validation.

Variants in candidate disease genes

We identified variants in candidate genes, which we defined as genes with functional or biological relevance to CP or other NDDs without published studies asserting a human disease-gene relationship.

Other sequencing methodologies

There were two participants with variants not identified by our pipeline whose results we have reported here. Both of these participants had undergone clinical WES by GeneDx Laboratory (Gaithersburg, MD) with methods as previously reported[27] and were included in this cohort because they met criteria at the time of enrollment.

Statistical analysis

We used exact test to compare prevalence of categorical variables across the three classification groups (cryptogenic CP, non-cryptogenic CP, and CP masquerader). We used the Kruskal–Wallis test to compare continuous variables across the three classification groups. As indicated, we converted a categorical variable into multiple dichotomous variables, each representing one of the possible values of the categorical variable. We set $p < 0.05$ as the threshold for statistical significance. Value after $\pm$denotes standard deviation.

Results

Demographics

Demographic characteristics of the probands ($n = 50$) are shown in Table 1. There were 20 females and 30 males (average age 10.1 $\pm$ 8.1 years). Among these 50 individuals, 24 (48%) had cryptogenic CP, 20 (40%) had non-cryptogenic CP, 5 (10%) had a CP masquerader classification, and one (2%) was not classifiable (due to limited perinatal history). Across the three classification groups, there were no significant differences in sex (exact test, $p = 0.33$), age (Kruskal–Wallis test, $p = 0.29$), or race (exact test, $p = 0.08$).

Motor phenotype and CP-related medical comorbidities

Across the entire cohort, the primary motor phenotypes of the probands were as follows: spastic diplegic ($n = 19/50$, 38%), spastic quadriplegic ($n = 16/50$, 32%), hypotonic-ataxic ($n = 8/50$, 16%), dyskinetic ($n = 5/50$, 10%), and spastic hemiplegic ($n = 2/50$, 4%). In comparing the number of individuals with each of these motor phenotypes versus classification group (cryptogenic CP, non-cryptogenic CP, CP masquerader), the only motor phenotype which showed a statistically significant difference across the three classification groups was the hypotonia-ataxic subtype, which only occurred in the cryptogenic CP group (exact test, $p = 0.01$; Table 2A).

We evaluated medical comorbidities associated with CP in this cohort. Hearing loss occurred in 8% ($n = 4/50$). Communication impairment was the only feature out of the five CP medical comorbidities assessed that showed differences in prevalence across the three classification categories (exact test, $p = 0.01$) with the highest prevalence in the non-cryptogenic category. A higher percentage of probands in the non-cryptogenic CP category had multiple medical comorbidities ($n = 11/20$, 55%) compared to probands in the cryptogenic CP category ($n = 6/$

Table 1. Demographic characteristics of the probands in the cohort.

	Non-cryptogenic CP (n = 20)	Cryptogenic CP (n = 24)	CP masquerader (n = 5)	Entire cohort (n = 50)	p value
Age at enrollment in years [mean(SD)]	9.3 (6.32)	8.44 (6.47)	15.25 (9.92)	10.09 (8.09)	0.29
% Female (n)	45% (n = 9)	29% (n = 7)	60% (n = 3)	40% (n = 20)	0.33
Family type					
% Trio (n)	50% (n = 10)	58% (n = 14)	40% (n = 2)	52% (n = 26)	0.77
% Quad (n)	0% (n = 0)	8% (n = 2)	0% (n = 0)	4% (n = 2)	0.59
% Duo (n)	45% (n = 9)	29% (n = 7)	40% (n = 2)	36% (n = 18)	0.58
% Proband-Only (n)	5% (n = 1)	0% (n = 0)	20% (n = 1)	6% (n = 3)	0.09
% Other (n)	0% (n = 0)	4% (n = 1)	0% (n = 0)	2% (n = 1)	1
Race					
% White (n)	80% (n = 16)	58% (n = 14)	60% (n = 3)	66% (n = 33)	0.28
% Black or African American (n)	15% (n = 3)	0% (n = 0)	0% (n = 0)	6% (n = 3)	0.14
% Asian (n)	0% (n = 0)	12% (n = 3)	0% (n = 0)	8% (n = 4)	0.32
% Other (n)	0% (n = 0)	4% (n = 1)	20% (n = 1)	4% (n = 2)	0.2
% Unknown (n)	5% (n = 1)	21% (n = 5)	20% (n = 1)	14% (n = 7)	0.27
% Unable to answer (n)	0% (n = 0)	4% (n = 1)	0% (n = 0)	2% (n = 1)	1

There is one individual (female, proband-only, Asian) with an unknown classification who is included in the "entire cohort" group.

Table 2. Motor features and medical comorbidities of the probands.

	Non-cryptogenic CP (n = 20)	Cryptogenic CP (n = 24)	CP masquerader (n = 5)	Entire cohort (n = 50)	p value
(A) Primary motor phenotype					
% spastic diplegic (n)	30% (n = 6)	42% (n = 10)	60% (n = 3)	38% (n = 19)	0.44
% spastic quadriplegic (n)	45% (n = 9)	25% (n = 6)	20% (n = 1)	32% (n = 16)	0.38
% spastic hemiplegic (n)	10% (n = 2)	0% (n = 0)	0% (n = 0)	4% (n = 2)	0.36
% dyskinetic (n)	15% (n = 3)	0% (n = 0)	20% (n = 1)	10% (n = 5)	0.1
% hypotonic ataxic (n)	0% (n = 0)	33% (n = 8)	0% (n = 0)	16% (n = 8)	0.01
(B) Medical comorbidities					
% Multiple medical comorbidities (n)	55% (n = 11)	25% (n = 6)	20% (n = 1)	36% (n = 18)	0.09
% Epilepsy (n)	45% (n = 9)	25% (n = 6)	20% (n = 1)	32% (n = 16)	0.38
% Severe visual impairment (n)	35% (n = 7)	12% (n = 3)	0% (n = 0)	20% (n = 10)	0.14
% Severe auditory impairment (n)	20% (n = 4)	0% (n = 0)	0% (n = 0)	8% (n = 4)	0.07
% Communication impairment (n)	75% (n = 15)	29% (n = 7)	40% (n = 2)	48% (n = 24)	0.01
% G-tube dependence (n)	30% (n = 6)	12% (n = 3)	0% (n = 0)	18% (n = 9)	0.22

(A) Primary motor phenotypes of the probands. There is one individual with dyskinetic CP with an unknown classification who is included in the "entire cohort" group. (B) CP-related medical comorbidities seen in the probands. There is one individual without multiple comorbidities with an unknown classification who is included in the "entire cohort" group. For CP masquerader category, primary motor phenotype refers to the phenotype the patient was diagnosed with or that most closely describes the patient's motor presentation.

24, 25%) or CP masquerader category (n = 1/5, 20%) (exact test, p = 0.09; Table 2B).

MRI classification

There were 44 probands with brain MRIs available for review, detailed in Table 3. In the cryptogenic CP category (n = 21 with MRIs available for review), the most common MRI pattern was "normal" (n = 7/21, 33%). In the non-cryptogenic CP category (n = 17 with MRIs available for review), the most common MRI pattern was bilateral white matter injury (n = 5/17, 29%).

Exome sequencing

We performed WES analysis in 26 trios, 18 duos, three proband only, two quads (parents + two affected children), and one other family type (proband, affected sibling, affected mother). Among the trios, there was one family with multiple affected male relatives on the maternal side.

Table 3. Classification of MRI pattern of the probands based on a previous scoring system. We labeled each participant's MRI with one of the eight primary patterns: (1) deep gray matter injury; (2) white matter injury; (3) white matter and cortical injury; (4) deep gray matter/white matter/cortical injury; (5) focal lesion; (6) cortical malformation; (7) normal study; (8) other. There is one individual with normal MRI and an unknown classification who is included in the "entire cohort" group.

	Non-cryptogenic CP (n = 17)	Cryptogenic CP (n = 21)	CP masquerader (n = 5)	Entire cohort (n = 44)	p value
% Focal insult (n)	12% (n = 2)	0% (n = 0)	0% (n = 0)	5% (n = 2)	0.37
% White matter injury (bilateral) (n)	29% (n = 5)	10% (n = 2)	40% (n = 2)	20% (n = 9)	0.16
% White matter and cortical injury (n)	0% (n = 0)	5% (n = 1)	0% (n = 0)	2% (n = 1)	1
% Deep gray matter injury (n)	12% (n = 2)	0% (n = 0)	20% (n = 1)	7% (n = 3)	0.14
% Near total brain injury (n)	24% (n = 4)	0% (n = 0)	0% (n = 0)	9% (n = 4)	0.05
% Malformation (n)	6% (n = 1)	29% (n = 6)	0% (n = 0)	16% (n = 7)	0.12
% Normal (n)	6% (n = 1)	33% (n = 7)	40% (n = 2)	25% (n = 11)	0.05
% Other (n)	12% (n = 2)	24% (n = 5)	0% (n = 0)	16% (n = 7)	0.51

Pathogenic/likely pathogenic variants in known disease genes

There were 13 probands (n = 13/50, 26%) who had P/LP variants in 14 established disease genes altogether, including 13 unique genes. The overall yield for Mendelian disorders for individuals with CP (cryptogenic CP + non-cryptogenic CP) was 23% (10/44). See Table 4 (additional phenotypic details in Table S1). One individual with consanguineous parents had homozygous P/LP variants in two different genes implicated in known autosomal recessive disorders (*ACADM* and *PDHX*, respectively). This individual's presentation was most consistent with the *PDHX* variants, and there were no reported hypoglycemic crises (as would be expected due to the *ACADM* variants). Most variants were de novo heterozygous/hemizygous variants in genes associated with autosomal dominant (*SATB2, ZMYM2, COL4A1, GNAO1, ATL1, POL2RA*) and X-linked (*SLC16A2, THOC2*) disorders. There were two patients with heterozygous *SPAST* variants (pathogenic in one, likely pathogenic in the other) whose inheritance we could not confirm. In addition to the individual with two autosomal recessive disorders, there was one participant with consanguineous parents who had homozygous pathogenic founder variants in *ADAT3*. There was one individual with compound heterozygous P/LP variants in *ECHS1,* corresponding to an autosomal recessive mode of inheritance.

We determined that the genetic diagnosis sufficiently explained the CP phenotype in all probands except for two patients in the non-cryptogenic CP category. The individual with the *SATB2* variant had consistent facial features and laryngeal cleft, but it is unclear whether this variant played a role in the patient's presentation of hemiplegia in the setting of likely acute perinatal event (neonatal compartment syndrome, intracranial hemorrhages). The individual with the variant in *ZMYM2* (recently implicated in congenital anomalies of the kidneys and urinary tract, nonspecific NDD, and other variable features[28]) had multiple structural malformations including anorectal malformation, but no renal malformation; it is not clear to what extent this variant contributed to the HIE leading to spastic quadriplegia.

We compared the presence of at least one genetic disorder (binary variable) to classification group, primary motor phenotype, multiple medical comorbidities, and MRI pattern. Of the 13 probands with a molecular diagnosis, seven were in the cryptogenic CP category, three were in the CP masquerader category, and three were in the non-cryptogenic CP category. The CP masquerader category was associated with the highest yield of Mendelian disorders on WES (3/5, 60%), followed by the cryptogenic CP category (7/24, 29%), and lastly by the non-cryptogenic CP category (3/20, 15%) (exact test, p = 0.10). The motor phenotype associated with the highest prevalence of a genetic disorder was spastic hemiplegic (1/2, 50%), followed by spastic quadriplegic (5/16, 31%), hypotonic-ataxic (2/8, 25%), spastic diplegic (4/19, 21%), and dyskinetic (1/5, 20%) (exact test, p = 0.91); it is worthwhile to note the small numbers in some of these categories. The prevalence of a genetic disorder was lower in those with multiple medical comorbidities (3/18, 17%) compared to those without multiple medical comorbidities (10/32, 31%), but this was not statistically significant (exact test, p = 0.33). Among the different MRI patterns, the patterns associated with having a pathogenic/likely pathogenic variant on WES were: focal insult (2/2, 100%), white matter and cortical injury (1/1, 100%), deep gray matter injury (2/3, 67%), malformation (3/7, 43%), normal (4/11, 36%), other (1/7, 14%), and bilateral white matter injury (0/9, 0%) (exact test, p = 0.01); again, it is worthwhile to note the small numbers in some of these categories.

Table 4. Pathogenic and likely pathogenic variants identified in the cohort, along with information about the patients harboring the variants.

Patient	Analysis	Age (years)	Sex	CP Classification	Primary Motor Phenotype	Gene	Variant	Inheritance	Zygosity	Clinically confirmed[1]	ACMG classification
3	Trio	1.61	F	Masquerader	dyskinetic	*ECHS1*	NM_004092.3: c.458A > G (p.Tyr153Cys); NM_004092.3: c.161G > A (p.Arg54His)	Maternally inherited, Paternally inherited	Compound heterozygous	Yes	Likely pathogenic, pathogenic
5	Trio	0.95	M	Non-cryptogenic	spastic hemiplegic	*SATB2*	NM_015265.3:c.715C > T (p.Arg239Ter)	De novo	Heterozygous	Yes	Pathogenic
7	Trio	0.99	F	Non-cryptogenic	spastic quadriplegic	*ZMYM2*	NM_003453.4: c.2843dup (p.Glu949ArgfsTer11)	De novo	Heterozygous	Yes	Pathogenic
14	Trio	8.95	M	Cryptogenic	spastic diplegic	*ADAT3*	NM_138422.2: c.430G > A (p.Val144Met)	Maternally and paternally inherited	Homozygous	Yes	Pathogenic
21	Trio	21.01	M	Non-cryptogenic	spastic quadriplegic	*COL4A1*	NM_001845.4:c.443G > A (p.Gly148Glu)	De novo	Heterozygous	Yes	Likely pathogenic
36	Duo	18.23	F	Cryptogenic	spastic quadriplegic	*THOC2*	NM_001081550.1: c.1550A > G (p.Tyr517Cys)	De novo	Heterozygous	Yes	Pathogenic
40	Duo	23.41	M	Cryptogenic	spastic quadriplegic	*SLC16A2*	NM_006517.4:c.148G > T (p.Glu50Ter)	De novo	Hemizygous	Yes	Pathogenic
42	Duo	15.88	M	Masquerader	spastic diplegic	*SPAST*	NM_014946.3:c.1168A > G (p.Met390Val)	Not maternally inherited	Heterozygous	Yes	Pathogenic
44	Trio	9.27	M	Cryptogenic	hypotonic-ataxic	*POLR2A*	NM_000937.4:c.3922 T > A (p.Tyr1308Asn)	De novo	Heterozygous	Yes	Likely pathogenic
46	Trio	2.91	F	Cryptogenic	spastic diplegic	*GNAO1*	NM_020988.2:c.625C > T (p.Arg209Cys)	De novo	Heterozygous	Yes	Pathogenic
47	Proband	27.12	F	Masquerader	spastic quadriplegic	*SPAST*	NM_014946.3:c.1085C > T (p.Ser362Phe)	Unknown	Heterozygous	Yes	Likely pathogenic
49	Trio	5.98	F	Cryptogenic	hypotonic-ataxic	*PDHX*	NM_003477.2:c.1345del (p.Leu449Ter)	Maternally and paternally inherited	Homozygous	Yes	Likely pathogenic
						ACADM	NM_000016.4:c.799G > A (NP_000007.1: p.Gly267Arg)	Maternally and paternally inherited	Homozygous	Yes	Pathogenic
50	Trio	1.92	M	Cryptogenic	spastic diplegic	*ATL1*	NM_015915.4:c.756C > A (p.Asn252Lys)	De novo	Heterozygous	Yes	Pathogenic

Abbreviations: F, female; M, male.

[1]Confirmation included CLIA-certified direct gene sequencing.

Variants of uncertain significance in known disease genes, variants in candidate genes, and variants identified by other means

Notable VUS include the following: (1) compound heterozygous variants in *ALG1* in an individual with spastic diplegia and N-glycan profile showing mild increases of Hex1GlcNAc2 and NeuAc1Hex1HexNac2 suggesting possible deficiency of mannosylation; (2) maternally inherited *PAK3* variant in a male with spastic diplegia and thin corpus callosum, whose pedigree shows multiple males with NDD presentations in an X-linked pattern and with segregation testing underway (Table S2). There were two patients with variants of interest in candidate genes (*CASKIN1, AGAP1*), detailed in Table S3. The participant with a VUS in *ITPR1* had undergone clinical testing 2 years prior to our study identifying this variant, which, upon review, still meets ACMG criteria for a VUS classification.

For two probands, variants were identified on clinical WES, but not by our analysis pipeline. For the individual with the *PAK3* variant, there was insufficient coverage of the region in our analysis. For the individual with the *THOC2* variant, the variant was called on the research pipeline, but not prioritized, as analysis was performed as a duo (in contrast to trio analysis performed with clinical WES).

Among those with P/LP variants, retrospective review revealed that two participants (one with compound heterozygous *ECHS1* variants, one with homozygous *ADAT3* variant) had previously undergone clinical testing which identified the disease-causing variants not known at the time of enrollment and exome variant analysis but identified independently on our pipeline. For three other participants with P/LP variants (two with a *SPAST* variant and one with *SLC16A2* variant), clinical testing performed in parallel to or after our research-based analysis independently identified the variant.

Discussion

Our work supports the notion that a substantial proportion of individuals with CP have an underlying monogenic disorder. This idea is counter to conventional medical teaching that a diagnosis of CP precludes the presence of a genetic disorder. This misconception was highlighted in a survey to physician members of the AACPDM (American Academy of Cerebral Palsy and Developmental Medicine) and the CNS (Child Neurology Society) demonstrating considerable variability in the diagnosis of CP in hypothetical scenarios involving patients with a nonprogressive motor disability (meeting criteria for CP) who had a genetic etiology.[29]

In our cohort, we identified a monogenic disorder in 26% (13/50) of cases (cryptogenic CP + non-cryptogenic CP + CP masquerader) and 23% (10/44) of individuals who met diagnostic criteria for CP (cryptogenic CP + non-cryptogenic CP). This yield is comparable to recent studies, the results of which vary depending on the ascertainment method and patient characteristics. The prevalence of P/LP single nucleotide variants in prior CP sequencing studies has ranged from 14% (patients meeting criteria for CP[15,17]) to 31% (patients referred to a clinical sequencing lab with a diagnosis of CP determined by the referring clinicians[30]) to 53% (patients with CP born at full term without specific findings on MRI[16]) to 100% (patients with ataxic CP[31]).

The heterogeneity of the genetic findings in our cohort likely reflects the fact that a wide range of genes have been implicated in presentations of CP. For example, in six previously published WES studies on patients with CP, P/LP variants were collectively present in 515 out of 1913 patients with CP (26%), spanning 248 unique genes[15,16,30,32–34]. Genes represented relatively more frequently included *CTNNB1*, *SPAST*, *GNAO1*, *TUBA1A*, *TUBB4A*, *STXBP1*, *KIF1A*, and *COL4A1*. It should be noted that these studies have involved heterogeneous cohorts with varying characteristics (in terms of use of trios, CP subtype, and cryptogenic classification).

One of the strengths of our study was systematic phenotyping (using variables including cryptogenic/non-cryptogenic/CP masquerader classification, primary motor phenotype, medical comorbidities, and neuroimaging profile), which enhances translational applicability of our findings into CP clinics. For example, classifying a patient into one of the three categories—representing the types of patients seen in a CP clinic–can help rank the likelihood of an underlying genetic disorder. The yield of an underlying monogenic disorder was highest in the CP masquerader category, followed by the cryptogenic CP category, and lastly the non-cryptogenic CP category. Our analysis was insufficiently powered to generate a statistical model/rubric for the prediction of the presence or absence of a genetic disorder using our phenotyping measures. Moving forward, expanding the prospective cohort with comprehensive evaluation of these specific phenotypes in relation to genotypes will better inform a clinical paradigm to guide genetic diagnosis in CP.

The finding of a monogenic disorder in three participants with non-cryptogenic CP warrants further discussion. These individuals (with variants in *COL4A1, ZMYM2, SATB2*) had risk factors to justify classification as non-cryptogenic CP (perinatal stroke in the case of the individual with *COL4A1* variant; acute perinatal event in the case of individual with the *SATB2* variant; and HIE in the case of the individual with the *ZMYM2* variant).

However, in addition to these CP risk factors, each of these individuals had features suggestive of an underlying genetic syndrome. For the individual with the *COL4A1* variant, in addition to spastic quadriplegic CP, epilepsy, ID, previous infarct, cerebellar hemorrhage, and cerebral atrophy, there were extra-neurological symptoms concerning for a genetic syndrome, including posterior embryotoxon, optic pathway hypoplasia, patent ductus arteriosus, bicuspid aortic valve, hypospadias, and cryptorchidism, consistent with *COL4A1*-related disorder (OMIM #175780). The individual with the *SATB2* variant had distinctive facial features, laryngeal cleft, global developmental delay, hemiplegia, and hemiatrophy of his left arm thought to be secondary to an acute perinatal event (with clinical history notable for intrauterine growth restriction, decreased fetal movement at 36 weeks gestation, neonatal compartment syndrome, and bilateral intracranial hemorrhages). While *SATB2*-related disorder (#OMIM 612313) explains the global developmental delay, facial features, and laryngeal cleft, its role in his CP presentation is not clear. Finally, the individual with the de novo truncating variant in *ZMYM2* had clinical and neuroimaging features consistent with HIE, including spastic quadriplegia and restricted diffusion in the bilateral thalami and globus pallidi on newborn MRI. She had a complex phenotype with multiple malformations including tethered cord, ascending aortic dilatation, and imperforate anus (but normal renal imaging). Loss of function of *ZMYM2* has only recently been implicated in syndromic renal and urinary tract anomalies, non-specific NDDs, and other features, but the role of this variant in our patient's HIE is not clear. Although there is no evidence that the *SATB2* and *ZMYM2* variants were directly causative of the CP presentations in the respective cases, such scenarios prompt the question of *whether, and to what extent, damaging variants in Mendelian disease genes contribute to vulnerability to perinatal insults that may have led to CP*. Data from larger cohorts of well-phenotyped individuals with non-cryptogenic CP will be required to address this question.

Currently, there are no consensus guidelines to recommend which patients with CP should undergo a genetic evaluation. Our cohort yields some preliminary data which could inform an approach to genomic evaluation for individuals with CP. We note that every individual in our cohort with a monogenic disorder identified on WES had at least one of the following features: (1) classification as cryptogenic CP; (2) classification as CP masquerader; (3) syndromic features/systemic malformations; (4) consanguineous parents. Based on these findings, we provide a framework for evaluating patients with cryptogenic CP/non-cryptogenic CP/CP masquerader, suggesting that the presence of the following features should warrant genetic evaluation (Fig. 2):

1 Features from the history: absence of perinatal risk factors; mismatch between perinatal history and motor severity/symptoms; consanguinity; more than one affected family member; progressive or regressive course; presence of congenital anomalies.
2 Features from prior investigations: normal brain MRI; mismatch between perinatal history and MRI findings; unexplained biochemical/metabolic disturbances.
3 Features from the examination: dysmorphic features; physical/systemic malformations; mismatch between the MRI pattern and motor phenotype.

In addition to recommending that individuals should undergo assessment of these factors upon initial CP evaluation, we also suggest regular re-review/screening of these parameters, particularly for disease progression/regression which may become apparent at a later timepoint. The identification of an etiological basis should not change the diagnosis of the overall descriptive term, CP.[35] Furthermore, the benefits of a genetic diagnosis are numerous: for patients and their families, a genetic diagnosis can provide an end to a diagnostic odyssey that may have lasted for several years; impact reproductive planning; inform prognosis, in some cases by identifying a progressive disorder in contrast to a nonprogressive condition; prompt further disease monitoring or systemic surveillance; and point to medication changes or potential natural history and clinical trial eligibility.[36]

Our work underscores the need to challenge and revisit basic assumptions in the CP scientific community, starting with basic definitions. There is wide variability in understanding and application of diagnostic criteria for CP by clinicians.[37] In the research context, there are multiple definitions of cryptogenic versus non-cryptogenic CP[11,17]. Currently, there is no standard, accepted definition for CP masquerader. The issue is complicated by the fact that some progressive disorders that can appear static at first, or in some patients may not be progressive at all. For a given disorder, phenotypic variability may be explained by different effects of variants on the protein or by other phenotypic modifiers (genetic, epigenetic, or environmental). For the sake of rigor in future CP studies, and precision for clinicians and families, clear consensus is needed about the definition of CP and its etiological classification.

There were three participants (with variants in *ECHS1*, *ADAT3*, and *THOC2*) in whom clinical testing prior to study enrollment had revealed the P/LP variant of interest. This did not bias or affect our research analysis interpretation, given that we were unaware of these findings at the time of analysis. The presence of previous highly relevant genetic testing reports, buried within the electronic medical records, highlights the need for better accessibility

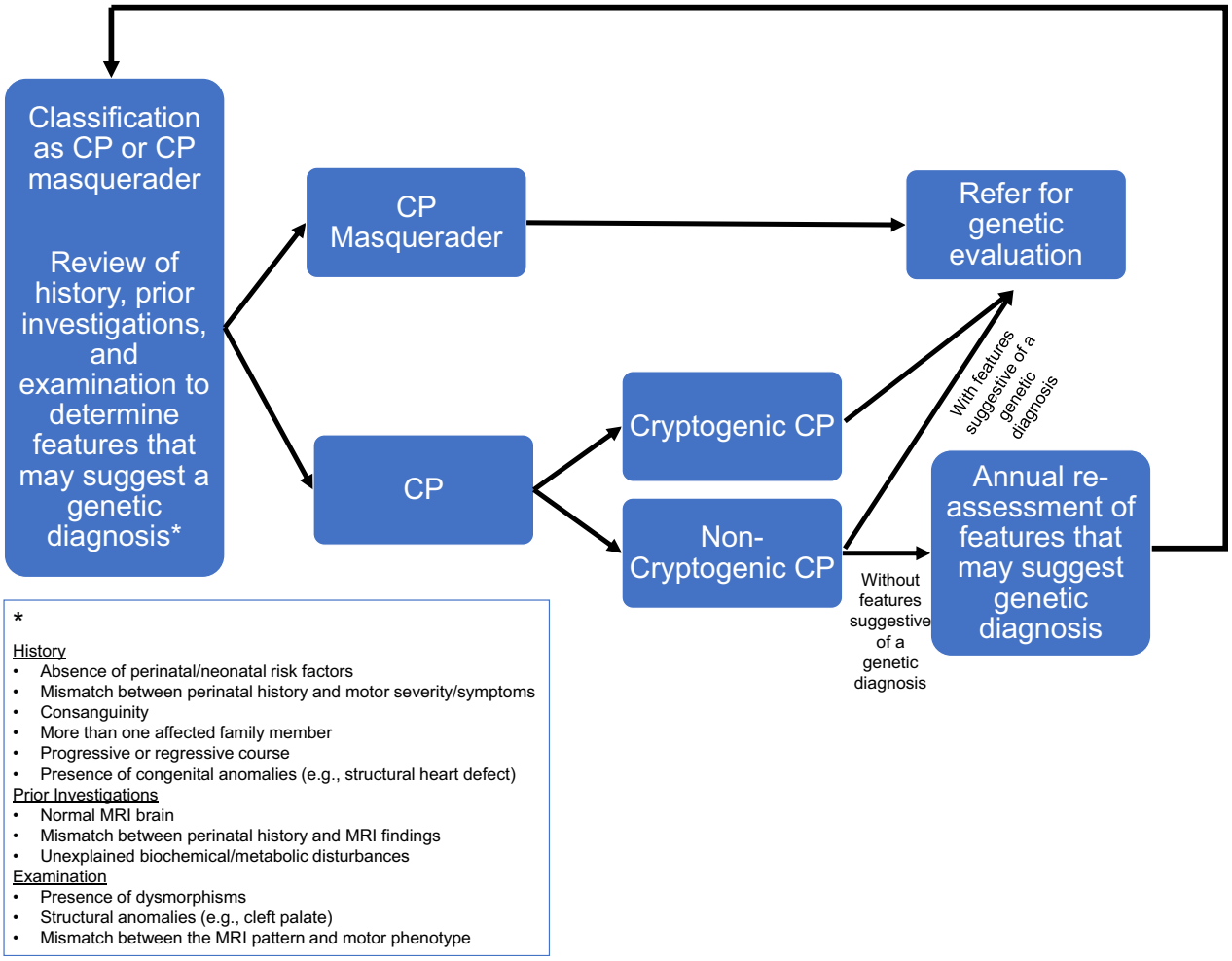

Figure 2. Proposed framework for consideration of genetic testing in patients with CP.

of genomic results to all providers who are part of a patient's team.

Limitations

The major limitation of our study was that copy number variant (CNV) analysis, detection of mosaicism, and mitochondrial DNA variant analysis was not part of our analysis pipeline. Given the likely role of pathogenic CNVs in some individuals with CP, a future pipeline that combines CNV calling with WES analysis has the potential of yielding even higher diagnostic rates for uncovering genetic disorders in the CP population. Indeed, as there is increased accessibility of whole genome sequencing, we anticipate an even broader understanding of the genomic landscape of CP. Second, for some patients in this cohort, the relation between the genetic diagnosis and CP phenotype was unclear. Third, our cohort size was relatively small, limiting generalizability of our findings.

Conclusions

In summary, we demonstrate a significant contribution of Mendelian disorders in individuals with CP, including in some patients who had known CP risk factors which may have been sufficient to explain the CP. On the basis of our findings, we propose basic guidelines for identifying patients who should be considered for genomic evaluation. Establishing genetic diagnoses stands to improve patient, family, and provider understanding of etiology, and potentially management of thousands of individuals with CP.

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Author Contributions

MC – conception and design of the study; acquisition and analysis of data; drafting a significant portion of the manuscript or figures. DG – acquisition and analysis of data; drafting a significant portion of the manuscript or figures. JLN – conception and design of the study. AT – acquisition and analysis of data. SR – acquisition and analysis of data. PS – acquisition and analysis of data. EB – acquisition and analysis of data. LB – acquisition and analysis of data. DC – acquisition and analysis of data. ED – acquisition and analysis of data. CDG – acquisition and analysis of data. DF – acquisition and analysis of data. KH – acquisition and analysis of data. PM – acquisition and analysis of data. DN – acquisition and analysis of data. JSS – acquisition and analysis of data. BJS – acquisition and analysis of data. BS – acquisition and analysis of data. SSDS – acquisition and analysis of data. AU – acquisition and analysis of data. CW – acquisition and analysis of data. CB – acquisition and analysis of data. JB – acquisition and analysis of data. CB – acquisition and analysis of data. MC – acquisition and analysis of data. DEF – acquisition and analysis of data. AL – acquisition and analysis of data. AOL – acquisition and analysis of data. Anna P – acquisition and analysis of data. Alexander P – acquisition and analysis of data. JP – acquisition and analysis of data. LR – acquisition and analysis of data. ER – acquisition and analysis of data. LS – acquisition and analysis of data. BZ – acquisition and analysis of data. MCK – acquisition and analysis of data. MS – conception and design of the study. Annapurna P – conception and design of the study. SS – conception and design of the study; acquisition and analysis of data; drafting a significant portion of the manuscript or figures.

Conflict of Interest

DEF: DEF has received grants from CureAP4 Foundation, CureSPG50 Foundation, BPAN Warriors Foundation, Thrasher Research Fund, NIH/NINDS, Tom Wahlig Foundation, Astellas Pharmaceuticals, Mitobridge Inc., The Manton Center for Orphan Disease Research. Received royalties from Cambridge University Press. Received consulting fees from Alcimed Inc. Received honoraria from International Parkinson and Movement Disorder Society. MCK: MCK has received grant support from PTC Therapeutics; consulting fees from PTC Therapeutics, Aeglea, Merz; participated on board for Aeglea and Merz. AP: AP has received support from the Boston Children's Hospital Translational Research Fund. SS: SS has received grants from NIH/NINDS; consulting fees from GLG, Guidepoint (which connected to a client, Fortress Biotech), Novartis, ExpertConnect, Orchard Therapeutics.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Additional phonotypic details of patients in the cohort with pathogenic/likely pathogenic variants in different genes, as well as expected phenotype of those disorders.

Table S2. Variants requiring ongoing evaluation, along with information about the patients harboring the variants.

Table S3. Variants in candidate genes identified in this cohort, along with information about the patients harboring the variant.

