FULL-LENGTH ORIGINAL RESEARCH

How should children with West syndrome be efficiently and accurately investigated? Results from the National Infantile Spasms Consortium

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Summary

Objective: To prospectively evaluate the etiology of new-onset infantile spasms and evaluate the yield of genetic and metabolic investigations in those without obvious cause after initial clinical evaluation and magnetic resonance imaging (MRI).

Methods: Twenty-one U.S. pediatric epilepsy centers prospectively enrolled infants with newly diagnosed West syndrome in a central database. Etiology and investigations performed within 3 months of diagnosis were documented.

Results: From June 2012 to June 2014, a total of 251 infants were enrolled (53% male). A cause was identified in 161 (64.4%) of 250 cases (genetic,14.4%; genetic-structural, 10.0%; structural-congenital, 10.8%; structural-acquired, 22.4%; metabolic, 4.8%; and infectious, 2.0%). An obvious cause was found after initial clinical assessment (history and physical examination) and/or MRI in 138 of 161, whereas further genetic and metabolic studies were revealing in another 23 cases. Of 112 subjects without an obvious cause after initial evaluation and MRI, 81 (72.3%) had undergone genetic testing, which showed a causal abnormality in 23.5% and a variant of unknown significance in 14.8%. Although metabolic studies were done in the majority (serum, 79.5%; urine, 69.6%; and cerebrospinal fluid [CSF], 38.4%), these revealed an etiology in only five cases (4.5%). No correlation was found between type of health insurance (public vs. private) and either genetic or metabolic testing.

Significance: Clinical evaluation and MRI provide a specific diagnosis in 55% of children presenting with West syndrome. We propose that a cost-effective workup for those without obvious cause after initial clinical evaluation and MRI includes an array comparative genomic hybridization (aCGH) followed by an epilepsy gene panel if the microarray is not definitive, serum lactate, serum amino acids, and urine organic acids.

KEY WORDS: Infantile spasms, West syndrome, Pediatric, Diagnostic test assessment, Observational cohort.

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West syndrome is the most common epileptic encephalopathy in the first 2 years of life.[1–3] It consists of a triad of (1) epileptic spasms, (2) hypsarrhythmia on interictal electroencephalography (EEG), and (3) developmental arrest or psychomotor delay.

Traditionally, infantile spasm etiology was classified as *symptomatic* or *cryptogenic*.[4] *Symptomatic* spasms had a clear underlying cause and/or significant developmental delay preceding seizure onset, whereas the term *cryptogenic* was used if no underlying cause was found and normal development preceded the spasms. The term *idiopathic* was used to describe patients with normal development at onset, normal examination and neuroimaging, and a hypsarrhythmia pattern without focal abnormalities.[5] However, the most recent proposal by the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE) proposes replacing these terms with specific etiologic categories including genetic, structural/metabolic, and unknown.[6]

With modern neuroimaging and advanced genetic testing, the proportion of children with defined causes for their spasms is increasing. In the United Kingdom Infantile Spasms Study (UKISS), 61% had a proven etiology, 33% had no identified etiology, and 6% were incompletely investigated.[7] Details of specific metabolic and genetic testing were not reported for children without an obvious etiology at presentation.

We used the National Infantile Spasms Consortium (NISC) database to determine the etiologies for a large, contemporary, prospective cohort of children with newly diagnosed infantile spasms. We identified the proportion of patients with an obvious cause after initial clinical evaluation and magnetic resonance imaging (MRI). For those patients without obvious cause, we evaluated the yield of genetic and metabolic investigations carried out in the first 3 months after diagnosis.

Methods

NISC is part of the Pediatric Epilepsy Research Consortium (PERC), a network of 38 U.S. pediatric epilepsy academic centers. For this prospective, observational study, 21 centers enrolled newly diagnosed children with infantile spasms, aged 2 to 24 months, between June 1, 2012 and June 1, 2014. Included children had a history consistent with epileptic spasms and EEG showing hypsarrhythmia or modified hypsarrhythmia. Children were also included if the EEG did not meet criteria for hypsarrhythmia, but had background slowing, multifocal spikes, and electroclinical spasms. Children with early infantile epileptic encephalopathy were excluded. Choice of diagnostic investigations was determined by the treating neurologist.

Individual centers began patient entry at the time of local institutional review board (IRB) approval (June 2012 through December 2013). Each patient's parent or guardian provided written informed consent. Data were entered at the time of recruitment and updated 3 months after initial diagnosis. Thus, this study evaluates defined etiologies and investigations performed during the first 3 months after diagnosis. Data collected included underlying etiology; significant prenatal, perinatal, and postnatal history; and results of neuroimaging, genetic, and metabolic studies performed prior to or during the first 3 months after diagnosis. Because all children were assessed by a pediatric neurologist, we assumed each had undergone a careful neurologic and general examination, including skin examination with a Wood's lamp and funduscopy, although did not record specific findings of those examinations.

Determination of etiology

Etiology was coded by two independent reviewers into *etiology class* and most likely *specific etiology(ies)* based on the etiologies entered by site investigators, along with significant pre-, peri-, and postnatal complications; prior provoked seizures; and results of investigations. When the reviewers' coding differed, a third reviewer was consulted to reach final consensus.

Etiology class was divided into eight subgroups (Table 1), along with *specific etiology*, based on results of all recorded investigations. Infants with spasms of unknown cause were subdivided into those with normal development versus those with definite or suspected delay.

If there was a disparity between the diagnosis entered by the site investigator and the result of a specific investigation, the final diagnosis coded reflected the test result. If the

Table 1. Etiologic categories

Genetic: Genetic disorders that were felt to be likely causes of epilepsy, but were not associated with structural brain changes on imaging (i.e., Down syndrome)
Genetic-structural: Genetic disorders that result in structural brain changes, which result in epilepsy (i.e., tuberous sclerosis, *DCX* mutation)
Structural-congenital: Malformative changes in the brain without a documented genetic etiology (i.e., focal cortical dysplasia, schizencephaly)
Structural-acquired: Structural brain change resulting from some type of injury or tumor. Included here are both perinatal (i.e., periventricular leukomalacia, intraventricular hemorrhage, and neonatal hypoxic-ischemic injury) or postnatal (i.e., ischemia, trauma, and tumor) etiologies
Metabolic: Metabolic disorder causing brain dysfunction leading to seizures. These conditions are nearly all inborn errors of metabolism, and many have a genetic inheritance. Disorders due to an inborn error of metabolism with resultant structural brain changes were coded as metabolic (i.e., *POLG1* or Walker-Warburg syndrome)
Immune: Documented immunologic disorder that results in perturbation of brain function with seizures
Infectious: Documented or highly suspected brain infection resulting in epilepsy (i.e., TORCH infection, HIV)
Unknown: No known etiology found for seizures

history or investigations suggested but did not confirm a different etiology, the site investigator was contacted for clarification.

Investigations within 3 months of diagnosis of infantile spasms

The specific genetic, metabolic, and immune studies were coded by two independent reviewers, and where disagreement occurred, a third reviewer adjudicated the result.

Genetic testing

The type of test (karyotype, array comparative genomic hybridization (aCGH), targeted single nucleotide polymorphism array, specific gene testing, epilepsy panel, whole exome/genome sequencing, mitochondrial single nucleotide polymorphism array, and mitochondrial panel) and results were recorded. Results were classified as normal, clearly abnormal, or variants of uncertain significance (VUS). Clear abnormalities were genetic mutations that were indicated to be pathogenic based on the lab report and/or review of the medical literature.

Metabolic testing

Results of testing for glucose, calcium and other electrolytes, and renal and liver function, were not recorded, as these are performed routinely. Instead, specific metabolic studies requested from blood/serum, urine, and cerebrospinal fluid (CSF), and their results were noted (normal/non-significant changes vs. abnormal).

Immune testing

Immune studies, including CSF oligoclonal bands, serum, and CSF paraneoplastic panels were recorded.

Data analysis

The percentage of subjects in each etiologic group and those undergoing specific investigations are presented.

We defined two groups, based on whether an obvious cause for spasms was found after the initial clinical evaluation and brain MRI. Children who had (1) a diagnostic abnormality on MRI, (2) dysmorphic features highly suggestive of a particular diagnosis (i.e., Down syndrome), or (3) a previously diagnosed condition that is strongly associated with infantile spasms (i.e., tuberous sclerosis, perinatal brain injury, and so on) were defined as having an obvious cause at diagnosis. Those lacking these three criteria were defined as having no obvious cause at diagnosis.

Cognitive and motor development were defined as normal, clearly abnormal, or suspect based on the medical record. The relationship between (1) an obvious cause at diagnosis and (2) abnormal development at initial presentation and the ordering of genetic or metabolic testing was assessed.

We stratified our cohort to assess the effect of insurance type on ordering of investigations in infants without obvious cause at diagnosis. Insurance types included public (publicly funded health care, e.g., Medicaid or other state insurance), private (e.g., health care insurance through a private health maintenance organization or preferred provider organization), and other (e.g., self-pay, uninsured, or other).

Potential predictors of ordering particular investigations were compared between groups, using chi-square and *t*-tests.

Results

Between June 2012 and June 2014, a total of 251 children with newly diagnosed infantile spasms were prospectively recruited from 21 U.S. pediatric epilepsy centers. Baseline data were entered for all subjects; however, 3-month data were missing in 20 (8.0%) of 251. Demographics are shown in Table 2. Mean age at onset of spasms was 7.1 months (standard deviation [SD] 3.6, range 0.1–22.7), and 134 (53.4%) were male.

Of these 251 children, 249 (99.2%) had West syndrome and 2 (0.8%) had been diagnosed previously with early myoclonic encephalopathy, which evolved to West syndrome. Forty-five children had prior provoked seizures, including 23 (9.2%) with acute symptomatic neonatal seizures, 3 (1.2%) with febrile seizures, and 19 (7.6%) with other provoked seizures.

Table 2. Demographic details of cohort with spasms (N = 251)

Male sex	134 (53.4%)
Ethnicity	
Hispanic	29 (11.4%)
Non-Hispanic	183 (71.8%)
Unknown	43 (16.9%)
Race	
Caucasian	159 (63.3%)
African American	32 (12.7%)
Asian	8 (3.2%)
Native Hawaiian or Pacific Islander	2 (0.8%)
First Nations	1 (0.4%)
Other	24 (9.6%)
Unknown	25 (10.0%)
Mean age at spasms onset (SD, range) in months	7.1 (SD3.6, range 0.1–22.7)
Gestational age	
<28 weeks	10 (4.0%)
28 to <32 weeks	11 (4.4%)
32 to <36 weeks	26 (10.4%)
≥36 weeks	199 (79.3%)
Unknown	5 (2.0%)
Prenatal or perinatal complications (3 unknown)	113/248 (45.6%)
Insurance type	
Public	93 (37.1%)
Private	112 (44.6%)
Self-Pay/Uninsured	1 (0.4%)
Other	3 (1.2%)
Unknown	42 (16.7%)

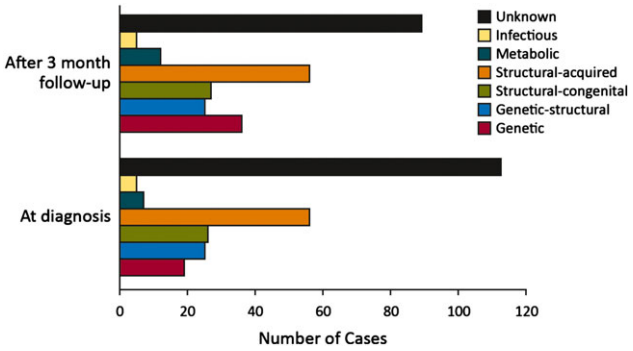

Figure 1.
Etiological Class at diagnosis, and after 3 months of follow-up.
Epilepsia © ILAE

Etiology of infantile spasms

Etiology data were missing for one subject (Fig. 1, Table S1). Figure 1 lists the causes divided into etiologic categories and Table S1 lists details of specific causes, including type of structural, genetic, or metabolic disorder found. An *obvious cause at diagnosis* was present in 138 (55.2%) of 250 cases. Following investigations performed within 3 months of initial presentation, an underlying etiology was found in another 23 cases. Thus, 64.4% of patients had a known etiology for spasms: genetic alone, 14.4%; genetic-structural, 10.0%; structural, 33.2% (congenital, 10.8%; acquired, 22.4%); metabolic, 4.8%; infection, 2.0%; and unknown, 35.6%.

Down syndrome was the most common genetic cause (N = 15), accounting for 42% of purely genetic etiologies, and tuberous sclerosis accounted for nearly half of genetic-structural etiologies (N = 12). Of the structural-acquired causes, 33 (58.9%) of 56 were a result of perinatal brain injury.

Causes were further defined as pre-, peri-, and postnatal. Prenatal causes, including genetic, genetic-structural, structural-congenital etiologies, and toxoplasmosis, other (syphilus, varicella-zoster, parvovirus B19), rubella, cytomegalovirus and herpes infection (TORCH) infections accounted for 93 (57.8%) of those with known etiologies. Perinatal causes, including perinatal structural-acquired causes, were causal in 39 cases (24.2%). Postnatal causes, including tumor, trauma, metabolic disease, postnatal infection, ischemia, or stroke accounted for 29 cases (18%).

Investigations for spasms

Neuroimaging

Neuroimaging study details were available for 249 children. Brain MRI was obtained in 218 (87.6%), either prior to spasm onset, due to preexisting neurologic abnormalities, or in the 3 months after diagnosis (171 with dedicated seizure protocol and 47 using routine, nonseizure protocols). MRI evaluations were ordered but not completed in another six subjects. Of the 25 without MRI, 9 had confirmed genetic causes, 10 had structural causes confirmed on prior computed tomography (CT) or cranial ultrasound, and 6 had unknown cause.

Of the 171 cases who had MRI with dedicated seizure protocols, 70 (40.9%) showed causal abnormalities (35 malformations of cortical development, 27 acquired structural brain abnormalities, 5 changes suggestive of an inborn error of metabolism, and 3 TORCH infection) and 9 (5.3%) showed minor, noncausal changes (2 remote small cerebellar hemorrhage, 2 delayed myelination, 1 mild white matter volume loss in child later found to have a genetic etiology, 1 mild atrophy, 1 old, small, bifrontal subdural hemorrhage, 1 mega cisterna magna, and 1 developmental cyst). Of the 47 cases with MRI without dedicated seizure protocol, 39 (83.0%) showed causal abnormalities (15 malformations of cortical development, 1 with protein-O-mannosyltransferase 1 (POMT1) Walker-Warburg, 21 acquired structural brain abnormalities, and 2 TORCH infection) and none were reported to show nonspecific changes.

Genetic investigations

Genetic studies were performed on 141 children (56.2%), of whom 32 (12.7%) had studies prior to onset of spasms (Fig. 2A,B). Compared to children with an obvious cause at diagnosis, those without an obvious cause at diagnosis were more likely to undergo aCGH ($p < 0.001$), epilepsy gene panel ($p = 0.001$), and karyotype ($p = 0.019$).

Among those with an obvious etiology at diagnosis, all causal mutations detected were associated with their obvious etiology, including six children with malformations of cortical development, five with tuberous sclerosis, one with Leigh disease, and two with dysmorphisms in keeping with a specific genetic syndrome (Pretzel syndrome and Schinzel-Giedion syndrome).

Karyotype. Karyotype was done in 32 children (12.7%), with abnormalities found in 17 (85%) of 20 cases tested prior to spasm onset (15 had Down syndrome), versus zero of 12 after onset.

Array comparative genomic hybridization array (aCGH). aCGH was performed in 87 children (34.7%). No diagnostic abnormalities were seen in cases tested prior to spasm onset, but definite abnormalities were reported in 12 (15%) and VUS in 9 (11%) tested after spasm onset.

Targeted chromosomal single-nucleotide polymorphism (SNP). Targeted chromosomal single-nucleotide polymorphism (SNP) analysis was performed in four cases (1.6%) and abnormalities were found in two (50%), confirming Williams syndrome in one and lissencephaly 1 (LIS1) in the other.

Targeted single gene testing. Targeted single gene testing was performed in 24 cases (9.6%). Abnormalities were seen

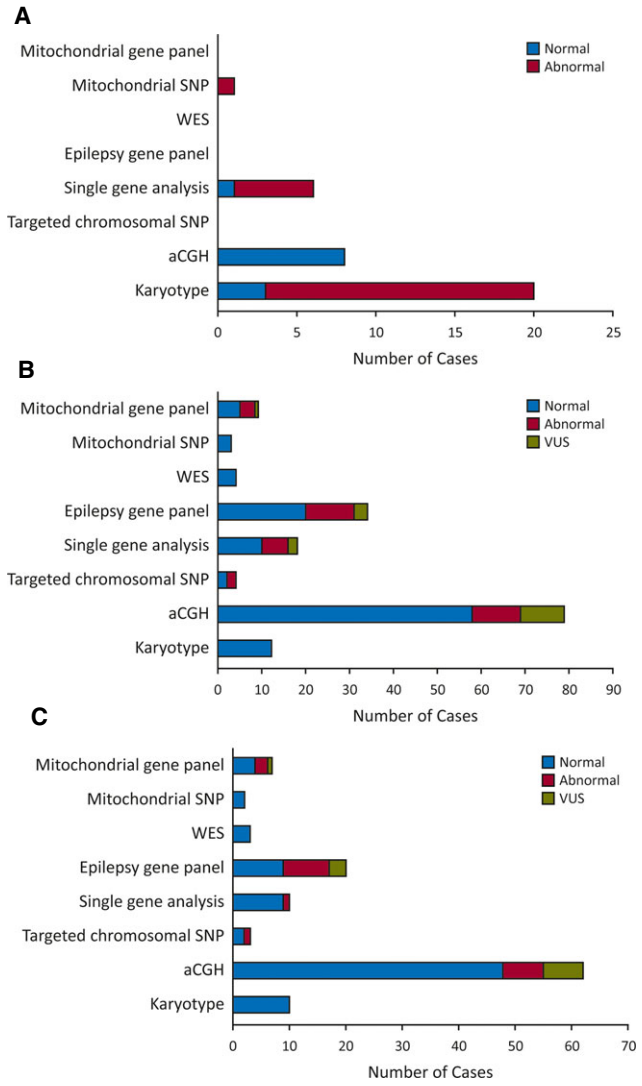

Figure 2.
Yield of genetic testing. (**A**) Prior to diagnosis of spasms. (**B**) Entire cohort; within 3 months after diagnosis of spasms. (**C**) Children without obvious cause at diagnosis.
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in five cases (83.3%) tested prior to spasm onset, confirming mutations in doublecortin (*DCX*) (N = 1), kirsten rat sarcoma viral oncogene (*KRAS*) with Noonan syndrome (N = 1), *POMT1* with Walker-Warburg (N = 1), neurofibromatosis type 1 (*NF1*) (N = 1), and polyhydramnios, megalencephaly and symptomatic epilepsy (*PMSE*) with Pretzel syndrome (N = 1). Six (33.3%) of those tested after spasm onset showed confirmatory mutations: tuberous sclerosis (TSC) 1 or 2 (N = 3), glycine decarboxylase (GLDC) in a child with nonketotic hyperglycinemia (N = 1), PMSE in Pretzel syndrome (N = 1), and set-binding protein 1 (SETBP1) in Schinzel-Giedion syndrome (N = 1). Single gene testing also showed two VUS.

Epilepsy gene panel. Epilepsy gene panels were obtained in 34 cases (13.5%), showing diagnostic abnormalities in 11 (32.4%): cyclin-dependent kinase-like 5 (*CDKL5*) (N = 3), voltage gated potassium channel 3 (*KCNQ3*) (N = 2), *TSC2* (N = 2), one each with syntactin binding protein (*STXBP1*), KAT8 regulatory NSL complex subunit 1 (*KANSL1*), polymerase gamma 1 (*POLG1*), and sodium channel 1A (*SCN1A*), and *VUS* in 3.

Whole exome/whole genome sequencing. Four children (1.6%) underwent either whole exome or whole genome sequencing, all with normal results.

Targeted mitochondrial SNP. Four children (1.6%) underwent targeted mitochondrial SNP. The single study done prior to spasm onset confirmed a diagnosis of Leigh disease, whereas all three studies done after spasm onset were normal.

Mitochondrial gene panel. Nine children (3.6%) underwent a mitochondrial gene panel. Results were abnormal and diagnostic in three cases (33.3%; two pyruvate dehydrogenase mutation, one *ATP6* mutation). One *VUS* was detected.

Overall yield of genetic testing in children without an obvious cause at diagnosis

Of the 112 children *without obvious cause at diagnosis*, 81 (72.3%) had undergone genetic testing, which showed a causal abnormality in 19 (23.5%), and a VUS in another 12 (14.8%) (Fig. 2C). The diagnostic yield was zero (0%) of 10 for a karyotype, 7 (11.3%) of 62 with an aCGH, one (33.3%) of 3 for targeted chromosomal SNP analysis, one (11.1%) of 9 for targeted single gene analysis, 8 (30.8%) of 26 for an epilepsy gene panel, zero (0%) of 3 for whole exome/genome sequencing, zero (0%) of 2 for a mitochrondrial SNP analysis, and 2 (28.6%) of 7 for a mitochondrial gene panel.

Causal abnormalities tended to be more likely, with increased severity of delay, being detected in 2 (8.3%) of 24 with normal development, 4 (23.5%) of 17 with suspect development, and 13 (32.5%) of 40 with clearly abnormal development ($p = 0.09$).

We found no significant difference between type of health insurance (public vs. private) and number of children undergoing any type of genetic testing, including karyotype ($p = 0.77$), aCGH ($p = 0.14$), targeted single gene analysis ($p = 0.41$), and epilepsy gene panel ($p = 0.51$).

Among children still classified with *unknown* etiology 3 months after the diagnosis of spasms, 46 (51.7%) of 89 underwent aCGH, 17 (19.1%) of 89 an epilepsy gene panel, 4 (4.5%) of 89 mitochondrial genetic testing, and 3 (3.4%) of 89 whole exome/genome sequencing.

Specialized metabolic investigations

Details on metabolic investigations ordered were reported in all children, with the specific metabolic studies

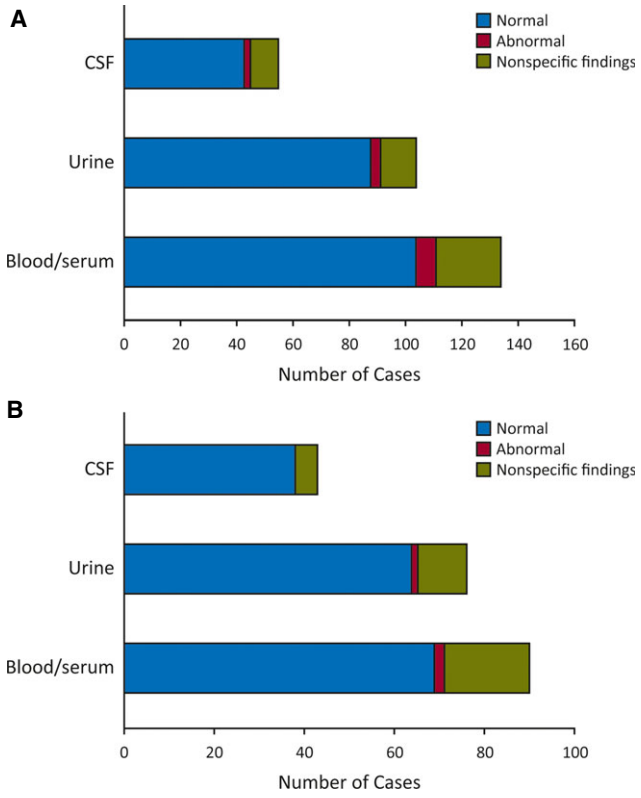

Figure 3.
Yield of metabolic testing. (**A**) Entire cohort; testing done prior to spasm onset or within first 3 months after diagnosis. (**B**) Children without obvious cause at diagnosis.
Epilepsia © ILAE

ordered listed in Table S2 (Fig. 3). Of the 12 children with a documented metabolic cause for spasms, 3 were diagnosed prior to spasm onset (one POLG1, one POMT1, and one congenital hyperinsulinism and hypothyroidism). Four exhibited MRI findings highly suggestive of metabolic disease at the time of presentation, with spasms, all confirmed with further studies (two Leigh syndrome, one lactic acidosis, and one nonketotic hyperglycinemia).

Blood/serum studies. One hundred thirty-four children (53.4%) underwent blood/serum metabolic testing, and of these, a median of 4 (interquartile range 2–5) studies were performed. Investigations were normal in 104 (77.6%) of 134, showed nonspecific findings in 23 (17.1%) of 134, and were clearly abnormal in 7 (5.2%) of 134. Of the abnormal studies, six showed markedly elevated lactate (one POLG1, one complex 1 deficiency, one Leigh syndrome, and three lactic acidosis), one elevated lactate and pyruvate and an abnormal lactate/pyruvate ratio (lactic acidosis), and five abnormal amino acids (one methylmalonic aciduria, two lactic acidosis, one Leigh syndrome, and one nonketotic hyperglycinemia). One child had an elevated insulin level and low thyroid function, in keeping with congenital hyperinsulinism and hypothyroidism.

Urine studies. One hundred four children (41.4%) underwent urine metabolic testing, and of these, a median of 1 (interquartile range 1–2) studies were performed. Results were normal in 88 (84.6%) of 104, showed nonspecific findings in 13 (12.5%) of 104, and were abnormal in 3 (2.9%) of 104, one of whom had increased methylmalonic acid on urine amino acids, and one with Leigh syndrome and another with lactic acidosis, both of whom had marked abnormalities on urine organic acids.

CSF studies. Fifty-five children (21.9%) underwent CSF metabolic testing, with a mean of 4 (interquartile range 1–5) studies performed. Results were normal in 43 (78.2%) of 55, showed nonspecific findings in 10 (18.2%) of 55, and were abnormal in 2 cases (3.6%) (one elevated glycine due to nonketotic hyperglycinemia, one elevated lactate with POLG1).

Overall yield of metabolic testing in children without an obvious cause at diagnosis

Children *without an obvious cause at diagnosis* were more likely than those *with a known cause* to undergo blood/serum (89/112 vs. 44/138, $p < 0.001$), urine (78/112 vs. 28/138, $p < 0.001$), and CSF metabolic testing (43/112 vs. 12/138, $p < 0.001$) (Fig. 3B). Of this group (N = 112), metabolic studies revealed an etiology in only five cases (one complex 1 deficiency, one pyruvate dehydrogenase deficiency, two lactic acidosis, and one methylmalonic aciduria), and serum lactate, pyruvate, amino acids, and urine organic acids were the only metabolic studies showing abnormalities. CSF testing did not reveal a metabolic etiology in any of the patients undergoing such testing after spasm presentation.

Developmental delay at presentation was not predictive of higher likelihood of undergoing metabolic testing or of greater yield of testing. There was no correlate between insurance type (private vs. public) and undergoing further blood/serum ($p = 0.90$), urine ($p = 0.24$), or CSF ($p = 0.28$) metabolic testing, or in the number of metabolic studies performed on blood/serum ($p = 0.86$), urine ($p = 0.83$), or CSF ($p = 0.35$).

Of children still classified as *unknown etiology* after 3 months, metabolic studies were done in the majority (blood/serum in 83.1%, urine in 70.8%, and CSF in 42.7%).

Immunologic investigations

Five children underwent immunologic studies (oligoclonal banding-4, paraneoplastic panel-1, and anti-*N*-methyl-D-aspartate [NMDA] receptor antibody testing-1) and all were normal.

Discussion

In this prospective registry of newly diagnosed infantile spasms, just over half (55.2%) had an obvious etiology iden-

tified after initial clinical assessment and brain MRI. Many of these children exhibited preexisting neurologic problems and had undergone testing prior to spasm onset. The proportion of children with an obvious etiology is lower in our sample than suggested by a recent consensus report, which states that after a thorough clinical history, examination, EEG, and MRI, an etiologic diagnosis is possible in 70% of children with infantile spasms, and of the remaining 30% who undergo further metabolic or genetic investigations, a defined cause is found in less than half.[1] Among children in our study without an obvious cause at the time of diagnosis of spasms, just 21% yielded a definitive etiology established within 3 months. Our results are similar to the those of the UKISS, in which 61% of children with spasms had a proven etiology when followed up to 14 months of age.[7] However, 6% of cases in the UKISS were not adequately investigated for an underlying cause, the majority of whom had not undergone brain imaging.

Similar to the UKISS results, we found that prenatal causes were most common, accounting for 57.8% of known etiologies in our cohort and 49.6% in the United Kingdom cohort.[7] Of prenatal causes, we identified genetic etiologies in approximately two thirds, with or without cerebral malformations, which highlights the important, causal role of genetics in this cohort of children. Parallel to the UKISS cohort, we found that perinatal etiologies were causal in approximately one fourth of known causes, with the most common etiologies being hypoxic-ischemic encephalopathy and brain injury of the preterm infant. This underscores the importance of optimal prenatal and perinatal care in epilepsy prevention. Postnatal causes accounted for 18.0% of known causes in our group, compared to only 6.3% in the UKISS cohort.

Of those patients who underwent imaging studies, brain MRI demonstrated causal abnormalities in half of infants with new-onset spasms, and thus is an appropriate and necessary first-line investigation in cases without a defined etiology. Our data show that MRI detected 86.2% (119/138) of the known causes at initial presentation, and 73.2% (119/161) of causes at 3 months of follow-up. Of 112 cases without an obvious cause at presentation, in our study 105 (94%) had either undergone MRI or had an MRI pending. Of the remaining seven children, etiology remained unknown in six after 3 months; their workup was considered incomplete as they lacked an adequate neuroimaging study. The recent guidelines from the ILAE Subcommittee for Pediatric Neuroimaging recommend special sequences including sagittal, axial, and coronal T_2-weighted sequences in addition to a three-dimensional dataset in children younger than age 2 years, as immature myelination limits the ability to identify cortical malformations.[8] Furthermore, if the initial MRI is normal, and seizures persist, an MRI may be repeated at 6-month intervals to detect cortical dysplasia, and certainly after age 24–30 months when myelination is more mature.

What is the yield of investigations in children without an obvious cause after initial clinical evaluation and MRI?

Genetic and metabolic testing was performed in the majority of our cases *without obvious cause at diagnosis*. Of the 72% of cases undergoing genetic testing, a specific genetic diagnosis was reached in 24% and VUS were reported in another 15%. The highest yield genetic study was the epilepsy gene panel, which resulted in an etiologic diagnosis in 31%, but was done in <25% of cases. aCGHs were performed in 55% and revealed a causative etiology in 11%. More than one half of those mutations affected the 15q region, which has been previously recognized as an important locus for many types of epilepsy and intellectual disability.[9,10] Although the yield of detecting an etiologic diagnosis was just over 25% with mitochondrial gene panels, these were performed in only a small number of children, most of whom had other metabolic findings suggestive of a mitochondrial disorder.

Our results suggest that genetic testing, particularly with an aCGH and epilepsy gene panel are high yield—the combination of these two tests provided a definitive diagnosis in >40% of children presenting with new-onset spasms *without an obvious cause* after initial clinical evaluation and MRI.

Other studies have emphasized the importance of careful genetic evaluation in children with epileptic encephalopathies.[11–13] Paciorkowski et al.[14] recently classified important genes predisposing to infantile spasms, based on their pathogenic mechanisms. Increasingly, outside of rare and very specific clinical scenarios, epilepsy gene panels are preferred over targeted gene testing, as they allow for testing of a large number of genes at a cost of approximately $5,000 USD, which is comparable to targeted testing of only one to two individual genes (approximately $1,000–3,000 USD per gene). Whole exome sequencing has been shown to be high yield in unexplained infantile spasms[15]; however, as only a minority of children in our cohort underwent this study, we cannot comment on the yield of such testing.

Despite the high yield of genetic testing in this setting, our findings suggest that genetic investigations remain underutilized in many patients. Somewhat surprisingly, we did not find any difference in ordering of genetic tests based on insurance type. This could be related to state-to-state differences in public insurance coverage for genetic testing; differing relationships between genetics and neurology clinicians; the practice of sending costly testing during inpatient admissions to avoid direct billing of families; or other undetermined factors. Still, we speculate that difficulties with insurance approval, which occur both with public and private insurance, contribute substantially to this underutilization. Discovering a genetic etiology is important for several reasons. First, it provides a cause and prevents ongoing, invasive and expensive diagnostic investigations. Second, it may increase understanding of the pathogenesis and potentially provide avenues for future, more targeted and better

therapies, or even preventive strategies. Third, it may have an effect on genetic counseling for future pregnancies.

Metabolic studies were performed in the majority of children without an obvious cause, but the yield of such testing was relatively low, with definitive diagnoses reached in only 4.5%. Diagnostic abnormalities were found only on serum lactate, pyruvate, amino acids, and urine organic acid testing. All four children with findings suggestive of neurometabolic disorders on initial MRI, but who had not been previously diagnosed with a metabolic condition, were found to have metabolic diagnoses on further testing, underscoring the importance of targeted evaluation in this subgroup. Although a recent study from China reported a higher rate (22%) of inborn errors of metabolism in infants with newly diagnosed spasms,[16] neuroimaging was abnormal in 62% of reported cases, and their cohort included disorders which are diagnosed by routine newborn screening in the United States, such as phenylketonuria and biotin deficiency.

Our study has some important strengths and limitations. Our sample is large, contemporary, and performed in a wealthy country in which most children have excellent access to investigations. We had representation of patients in multiple regions of the United States; however, the profile of etiologies and testing might not be generalizable to children cared for in other geographic locations outside the United States. Our study did not have a population-based design, and our sample was derived from children treated at tertiary pediatric epilepsy centers, which may enrich for some of the rarer causes of infantile spasms. Still, our patient population reflected many of the most commonly reported etiologies.

Genetic and metabolic testing protocols were determined by the individual treating clinicians without a mandated testing protocol. This allowed for evaluation of a wide variety of tests. However, there was substantial practice variability between our 21 centers regarding genetic and metabolic testing. We are unable to account for the attitudes of the individual clinical teams toward, and/or rationale for, ordering specific tests, and we were not able to discuss families' preferences for specific testing strategies.

Finally, only data from the first 3-month follow-up were analyzed. Because 3-month follow-up data were missing in 8% of cases, and genetic testing results often take 3 months or longer, it is probable that we are missing genetic etiologies that would be apparent with longer and more complete follow-up. Repeat, dedicated MRIs using seizure protocol may also identify subtle cortical dysplasias as myelination progresses in the developing brain.

Based on the results of our contemporary, large, multicenter U.S. study, we propose that a cost-effective workup in children presenting with newly diagnosed West syndrome, without an obvious cause after clinical history, physical examination, and MRI should include aCGH, followed by an epilepsy gene panel if the microarray is not definitive; serum lactate; serum amino acids; and urine organic acids. If these studies are normal, whole exome sequencing could be considered; however, larger cohort studies will be needed to evaluate diagnostic yield in this setting.

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serves on the Laboratory Accreditation Board for Long Term (Epilepsy and Intensive Care Unit) Monitoring, on the Council of the American Clinical Neurophysiology Society, on the American Board of Clinical Neurophysiology, as an Associate Editor for *Seizure*, as Contributing Editor for *Epilepsy Currents*, and as an Associate Editor for *Wyllie's Treatment of Epilepsy* 6th edition. He is part of pending patent applications to detect seizures and to diagnose epilepsy. He receives research support from the American Epilepsy Society, the Epilepsy Foundation of America, the Epilepsy Therapy Project, PCORI, the Pediatric Epilepsy Research Foundation, CURE, Danny-Did Foundation, HHV-6 Foundation, Lundbeck, Eisai, and Upsher-Smith. Dr. Nordli is a section editor for *UpToDate*, and has received support from NIH and CURE. Dr. Sánchez Fernández is funded by a grant for the study of Epileptic Encephalopathies from "Fundación Alfonso Martín Escudero" and by the HHV6 Foundation. The remaining authors have no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Appendix I
Pediatric Epilepsy Research Consortium (PERC)

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Specific primary etiology of spasms (N = 250; one missing data).

Table S2. Metabolic studies performed prior to spasm onset or within the first 3 months after presentation.

Table S3. Proposed list of current genes affiliated with infantile spasms.