Etiology of Severe Microcephaly in Infants: A Multinational Surveillance Study

Daniel S. Farrar, MPH,[1,a] Rachel L. Knowles, MD, PhD,[2,3,a,b] Carlos Nunez, PhD, MPH,[4,5,a] Gareth Baynam, MBBS, PhD,[6,7,8] Ari Bitnun, MD, MSc,[9,10] Mavis Duncanson, MBChB, PhD, MPH,[11] Richard M. Lynn, BSc, MSc,[2] Charlotte Moore Hepburn, MD,[12,13] Anne Morris, MBBS, MPH,[4,5,14] Clarissa Oeser, PhD,[15] Richard Pebody, MBChB, PhD,[16] Ameenat Lola Solebo, MBBS, PhD,[2,17] Elizabeth J. Elliott, MD, MPhil, MBBS,[4,5,14,b] Shaun K. Morris, MD, MPH[1,9,10,18,b]

abstract

BACKGROUND AND OBJECTIVES: Severe microcephaly, or head circumference at least 3 standard deviations below the mean for age and sex, is a rare condition with diverse etiology, making diagnosis challenging. Following the 2015 to 2016 Zika virus outbreak, surveillance studies in Australia, Canada, New Zealand, and the United Kingdom and Ireland were conducted to monitor severe microcephaly. We describe the etiology, clinical features, and diagnostic investigations of severe microcephaly among children aged younger than 1 year.

METHODS: We pooled reports of patients with severe microcephaly detected through 4 national active surveillance studies, through the International Network of Paediatric Surveillance Units. Incident cases were reported voluntarily between June 2016 and October 2018 by networks of pediatricians totaling more than 8000 members. Etiology was categorized as genetic (confirmed/suspected), acquired (infection, ischemia/hypoxia, prenatal alcohol exposure, placental insufficiency), or unknown. Anonymized data were pooled and analyzed using descriptive statistics.

RESULTS: Overall, the cases of 118 patients with severe microcephaly were analyzed, including 59 from the United Kingdom and Ireland, 34 from Canada, and 25 from Australia ($n < 5$ cases from New Zealand were not analyzed). Median age at diagnosis was 17 days (IQR 1–119), and mean head circumference-for-age Z-score was −4.0 (SD 1.1). Genetic causes were determined for 50% ($n = 59$) vs 18% acquired ($n = 21$) and 32% unknown ($n = 38$). Common investigations included brain magnetic resonance imaging (70%), DNA microarray (69%), brain ultrasonography (53%), and cytomegalovirus screening (48%).

CONCLUSIONS: At least one-half of severe microcephaly cases are attributable to genetic causes. One-third had unknown etiology, highlighting a need for a systematic approach to diagnostic investigation, including genomic sequencing and brain imaging for all children with severe microcephaly.

[1]*Centre for Global Child Health, The Hospital for Sick Children, Toronto, Ontario, Canada;* [2]*Population Policy and Practice, UCL Great Ormond Street Institute of Child Health, London, United Kingdom;* [3]*Public Health Commissioning and Operations, NHS England, London, United Kingdom;* [4]*The University of Sydney, Faculty of Medicine and Health, Discipline of Child and Adolescent Health, Sydney, New South Wales, Australia;* [5]*The Australian Paediatric Surveillance Unit, Sydney, New South Wales, Australia;* [6]*Western Australian Register of Developmental Anomalies, King Edward Memorial Hospital Perth, Perth, Western Australia, Australia;* [7]*Telethon Kids Institute, The University of Western Australia, Perth, Western Australia, Australia;* [8]*Rare Care Centre, Perth Children's Hospital, Perth, Western Australia, Australia;* [9]*Division of Infectious Diseases, The Hospital for Sick Children, Toronto, Ontario, Canada;* [10]*Department of Paediatrics, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada;* [11]*Department of Paediatrics and Child Health, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand;* [12]*Division of Paediatric Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada;* [13]*Institute of Health Policy, Management and Evaluation, University of Toronto, Toronto, Ontario, Canada;* [14]*Kids Research, The Sydney Children's Hospitals Network (Westmead), Sydney, New South Wales, Australia;* [15]*Immunisation and Vaccine Preventable Diseases Division, UK Health Security Agency, Colindale, London, United Kingdom;* [16]*Epidemic and Emerging Infections, UK Health Security Agency, Colindale, London, United Kingdom;* [17]*Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom; and* [18]*Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada*

[a]*Authors contributed equally;* [b]*Co-senior authors.*

WHAT'S KNOWN ON THIS SUBJECT: Severe microcephaly is a rare condition that can develop prenatally or in infancy. The causes of severe microcephaly are heterogeneous, and many cases have unknown etiology, suggesting a need for protocols to facilitate systematic investigation to improve diagnosis and management.

WHAT THIS STUDY ADDS: Among 118 severe microcephaly cases in this multinational study, 50% were attributable to genetic causes, 18% were acquired, and 32% had unknown etiology, with variability in investigations. There is considerable potential to enhance and standardize diagnostic investigations for severe microcephaly.

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INTRODUCTION

Microcephaly is a rare anomaly of the central nervous system that can manifest during fetal development or infancy.[1] Microcephaly became a matter of public health interest following an outbreak of congenital Zika virus (ZIKV) infection first reported in Brazil in 2015.[2] The rapid spread of ZIKV to several countries and the unusual increase in the reporting of infants born with microcephaly led the World Health Organization (WHO) to declare an international public health emergency in 2016.[3] In response to the outbreak and the paucity of population-based data for children with microcephaly, members of the International Network of Paediatric Surveillance Units (INoPSU) including Australia, Canada, New Zealand, and the United Kingdom and Ireland initiated surveillance for severe microcephaly, defined as a head circumference of at least 3 SDs below the mean for age and sex.[4–7] These studies identified similar national incidence rates of severe microcephaly between 4.0 and 5.5 cases per 100 000 live births and provide a basis for investigating the etiology and clinical practices associated with severe microcephaly in a dataset representative of contemporary populations in high-income settings.

The etiology of severe microcephaly is heterogeneous. Causes include genetic disorders, brain injury, congenital infections, prenatal exposure to teratogens, and severe deprivation (eg, maternal malnutrition and placental insufficiency).[8,9] Severe microcephaly may present in isolation or in association with other birth defects, and children with this condition can experience considerable morbidity including neurodisability.[8,9] Microcephaly can also be classified as either proportionate (symmetric) or disproportionate (asymmetric), although standardized definitions of proportionality do not exist. Practice guidelines loosely define proportionate microcephaly as having similar percentiles for head circumference, weight, and length/height,[10,11] whereas disproportionate microcephaly describes head circumference measurements or Z-scores that are discordant from either weight or length/height values, although exact thresholds differ across studies.[9,11–14] There is conflicting evidence regarding the association between proportionality, etiology, and neurodevelopmental prognosis.[10]

The aim of this study was to compare the clinical and etiologic features of infants with severe microcephaly identified by 4 national public health surveillance units with equivalent methodologies and to provide additional insights into the potential for improving diagnosis, clinical and developmental management, and future prevention strategies.

METHODS

Study Design and Population

We conducted secondary data analysis of cases of severe microcephaly using data collected prospectively through 4 national public health surveillance networks, collaborating through INoPSU.[15] Specifically, national studies were conducted through the Australian Paediatric Surveillance Unit (APSU; June 2016–July 2018), British Paediatric Surveillance Unit (BPSU; October 2017–October 2018), Canadian Paediatric Surveillance Program (CPSP; June 2016–May 2018), and New Zealand Paediatric Surveillance Unit (NZPSU; August 2016–July 2019). Similar active surveillance methods were used in each program. National networks of pediatricians and pediatric subspecialists, totaling over 8000 members across the 4 programs, were contacted each month and asked to report incident cases of conditions of interest, including microcephaly or severe microcephaly. Pediatricians who reported any cases were sent a case report form and were asked to voluntarily provide detailed data through review of case notes or medical records. Each national program has authority to collect health information for surveillance purposes without patient consent. Data were reported and collected anonymously by participating physicians. Results of the APSU,[4,5] BPSU,[7] and CPSP[6] studies have been published, and concurrent surveillance of congenital Zika syndrome is reported elsewhere.[6,16]

Children aged younger than 1 year were eligible to be reported if they met criteria defined by head circumference or head circumference-for-age Z-scores (HCAZ), which were overlapping but nonidentical between studies. The APSU monitored all microcephaly with HCAZ < -2 SD. The BPSU, CPSP, and NZPSU monitored severe microcephaly but applied different HCAZ entry criteria, as described in Supplemental Table 1. Notably, all studies included children aged younger than 1 year with HCAZ < -3 SD. For this secondary data analysis, we included children aged younger than 1 year and whose head circumference measurement met country-specific anthropometric criteria for severe microcephaly. Notably, fewer than 5 eligible cases from New Zealand were reported during the study period and were excluded from the analysis to prevent patient disclosure in accordance with national privacy policies.

Study Definitions

Standardized case report forms were used by each program, and although not identical, the ascertainment of many data elements was consistent across the 4 programs. We report only data elements collected in at least 2 programs. We describe infant characteristics including demographics, gestational age at birth, anthropometry at birth and microcephaly diagnosis, clinical manifestations by the time of diagnosis, and the etiology of severe microcephaly. HCAZ, recumbent length-for-age Z-scores (LAZ), and weight-for-age Z-scores (WAZ) were calculated using the INTERGROWTH-21st standards or WHO Child Growth Standards, as appropriate.[17,18] Maternal characteristics included prenatal comorbidities and exposures, travel history during pregnancy or preconception,

and prior pregnancy loss. We also describe familial history of congenital anomalies, and investigations conducted to identify an underlying diagnosis or exposure. In some instances, we combined heterogeneous data elements that ascertained similar concepts (eg, "any prenatal alcohol consumption" in APSU and CPSP vs "treated for alcohol problems during pregnancy" in BPSU), described further in Supplemental Table 2.

Physician reporting of underlying etiology varied both within and between surveillance programs. Therefore, for each program, investigator teams with expertise across multiple specialties adjudicated the clinical, diagnostic, and epidemiologic history of all cases to ensure that the etiologic classification was standardized and internally consistent. At the outset of this multinational study, an investigator working group met to establish a consensus set of etiologic categories. Etiology could not be determined for all cases by a common set of reviewers, given that privacy rules prohibited the sharing of full patient data between programs. We therefore relied upon adjudication previously conducted for each country-specific study (Supplemental Table 3), with only minor changes to harmonize the etiologic categories to those established for this study. The investigator working group further discussed examples of etiological classification for specific diagnoses to ensure consistency between programs. Here, we classified the etiology of severe microcephaly as attributable to confirmed or suspected genetic causes, hypoxia or ischemia, infection, placental insufficiency, prenatal alcohol exposure, or unknown causes. Given small group sizes, we further aggregated hypoxia or ischemia, infection, placental insufficiency, and prenatal alcohol exposure as "acquired" causes in the analysis, as recommended in the American Academy of Neurology (AAN) guidelines.[10] Although classification of acquired causes was consistent across countries, there were differences in the adjudication of genetic causes (Supplemental Table 3). The APSU study required abnormal genetic testing to be classified as a genetic cause, whereas the BPSU and CPSP studies did not. In addition to abnormal genetic testing, the CPSP study considered cases with a family history of congenital anomalies plus specific dysmorphic features as genetic, whereas the BPSU study considered cases with a family history of congenital anomalies or consanguinity as genetic. Given the constraints of data sharing activities for this study, we considered this group as "confirmed or suspected" genetic causes in the analysis. Confirmed genetic etiologies were further classified by pattern of inheritance, guided by Orphanet.[19]

Microcephaly proportionality was assessed using definitions published by Noyola et al[13] and von der Hagen et al[9] (later adapted by de Magalhães-Barbosa et al[12] for severe microcephaly). Noyola et al defined proportionate microcephaly as HCAZ − WAZ ≥ −2 and disproportionate microcephaly as HCAZ − WAZ < −2. de Magalhães-Barbosa et al defined proportionate microcephaly as HCAZ, WAZ, and LAZ < −3 SD and disproportionate microcephaly as HCAZ < −3 SD with either WAZ or LAZ ≥ −3 SD. We adapted this definition such that disproportionate microcephaly included cases meeting country-specific HCAZ criteria with WAZ < −3 SD and excluded LAZ criteria due to high missingness in length measurements.

Statistical Analysis

Aggregated categorical data and anonymized continuous data were shared with 1 central contact for analysis. We summarized data using means with SD, medians with IQR, or frequencies with percentages. Summary statistics were reported among all complete cases and stratified by etiology (genetic, acquired, or unknown) or jurisdiction (Australia, Canada, or United Kingdom and Ireland). Between-group differences were analyzed using Student *t* tests, analysis of variance, Wilcoxon rank-sum tests, Kruskal-Wallis tests, χ^2 tests, or Fisher exact tests, as appropriate. All statistical tests were 2-tailed, used a statistical significance threshold of $\alpha = .05$, and did not account for multiplicity. Some frequencies between 1 and 4 were masked as "<5" in accordance with British and Canadian privacy policies, whereas some larger frequencies were presented as ranges to prevent back-calculation of frequencies below 5. Data sharing agreements were approved with the explicit understanding that any disseminated materials would abide by these ethical and legal policies. The frequency of missing data was reported using footnotes, or complete case denominators were explicitly reported. The analysis was conducted using Microsoft Excel (Microsoft) and Stata version 18.5 (StataCorp LLC).

RESULTS

In total, 118 children aged younger than 1 year met country-specific criteria for severe microcephaly, including 59 in the United Kingdom and Ireland (50%), 34 in Canada (29%), and 25 in Australia (21%; Figure 1). Infants were diagnosed with severe microcephaly at a median age of 17 days (IQR 1–119 days); 58 were diagnosed at birth (49%) vs 60 later in infancy (51%; Table 1). Among the 60 cases identified later in infancy, 9 (15%) had confirmed microcephaly at birth ($-2 \leq$ HCAZ < -3), 21 (35%) did not have microcephaly at birth (HCAZ > -2), and 30 (50%) had no head circumference measurements at birth. A higher percentage of cases were diagnosed at birth in Australia (n = 14/25, 56%) and Canada (n = 22/34, 65%) than in the United Kingdom and Ireland (n = 22/59, 37%) ($P = .03$; Supplemental Table 4). The mean head circumference at birth was 29.2 cm (SD 2.8), with mean HCAZ of −3.1 (SD 1.6). At the time of severe microcephaly diagnosis, the respective means of HCAZ, WAZ, and LAZ were −4.0 (SD 1.1), −1.8 (SD 1.2), and −1.7 (SD 1.4). Weight at microcephaly diagnosis was reported for most cases

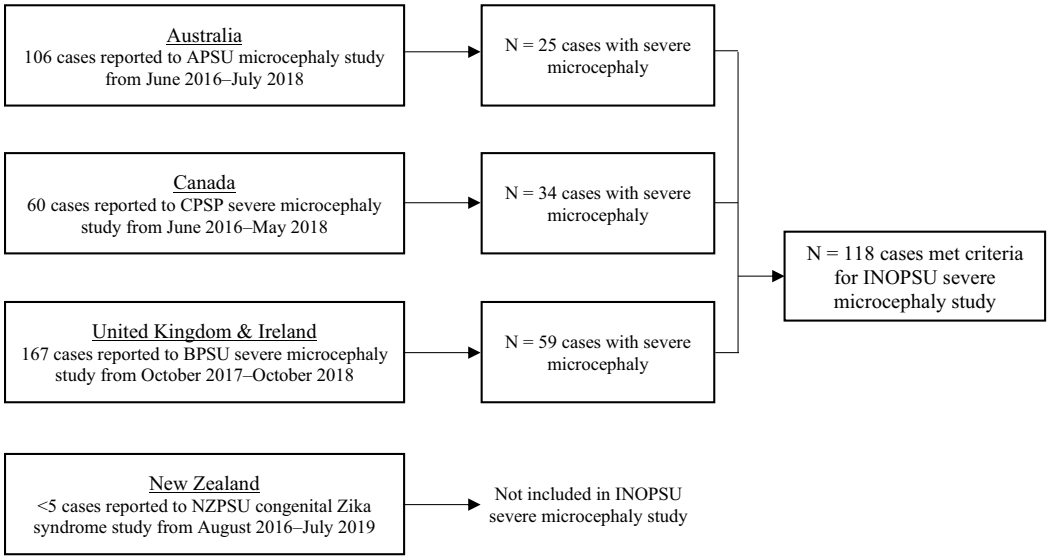

FIGURE 1.
Flowchart of cases included in the INOPSU severe microcephaly study. New Zealand cases were not included in the INOPSU study to prevent back-calculation and disclosure of frequencies <5.
Abbreviations: APSU, Australian Paediatric Surveillance Unit; BPSU, British Paediatric Surveillance Unit; CPSP, Canadian Paediatric Surveillance Program; INOPSU, International Network of Paediatric Surveillance Units; NZPSU, New Zealand Paediatric Surveillance Unit.

(n = 111/118, 94%), although length measurements were less often reported (n = 68/118, 58%). Microcephaly proportionality definitions yielded discordant findings. According to Noyola et al's definition, which measured the difference between HCAZ and WAZ, 49% (n = 51/104) of cases were disproportionate. Conversely, 87% (n = 90/104) were disproportionate using de Magalhães-Barbosa et al's definition, which measured the concordance between HCAZ and WAZ thresholds.

A confirmed or suspected genetic etiology was determined for 59 cases (50%, including n = 26 confirmed and n = 33 suspected), followed by infection (n = 8, 7%), hypoxia/ischemia (n = 8, 7%), and other acquired causes (n = 6, 5%) (Figure 2 and Supplemental Table 5). The etiology remained unknown among 38 cases (32%). Fewer children were classified with a genetic etiology in Australia (n = 6/25, 24%) than in Canada (n = 18/34, 53%) and the United Kingdom and Ireland (n = 35/59, 59%, $P = .01$) (Supplemental Table 4). These findings were inversely associated with genetic testing, with DNA microarray more common in Australia (n = 22/25, 88%) than in Canada (n = 22/31, 71%) and the United Kingdom and Ireland (n = 35/59, 59%). Differences in ascertainment of genetic cases were in part attributable to country-specific etiologic classification methods, whereby the United Kingdom, Ireland, and Canada considered suspected genetic cases based on familial history, associated congenital anomalies, or clinical dysmorphisms (n = 25 and n = 6 suspected genetic cases in the United Kingdom and Ireland and Canada, respectively), whereas Australia considered only genetic disorders known to affect head size (ie, no suspected cases) (Supplemental Table 3). Relatedly, more cases in Australia had an unknown etiology (60% vs 32% in Canada and 20% in the United Kingdom and Ireland). Among cases with unknown etiology, 63% (n = 24/38) had genetic testing and imaging performed, 21% (n = 8/38) had only genetic testing or only imaging conducted, and 16% (n = 6/38) had neither genetic testing nor imaging performed. Among infectious etiologies, fewer than 5 cases were attributable to ZIKV. Acquired causes of microcephaly were more likely than genetic causes to be associated with preterm birth (48% of acquired [n = 10/21] vs 26% of genetic causes [n = 15/59], $P = .07$), although they had similar mean WAZ at birth (−0.9 [SD 1.5] for acquired vs −1.2 [SD 1.3] for genetic, $P = .42$). Mean birth HCAZ was significantly lower for genetic (−3.4 [SD 1.5]) than acquired causes (−2.4 [SD 2.0], $P = .046$) (Table 1). Etiology was not associated with disproportionate microcephaly using either definition considered.

Eye abnormalities or vision impairment were common at the time of diagnosis (n = 40/100, 40%) and were more prevalent among genetic (n = 29/52, 56%) than acquired causes (n < 5/18, <28%, $P = .02$) (Table 2). Neuromuscular features including hypotonia (n = 18/58, 31%) and spasticity (n = 14/57, 25%) were also common. One-half (n = 49/94, 52%) of mothers had a fetal anomaly identified on prenatal ultrasonography, including a greater share in Canada (n = 24/32, 75%) than in Australia (n = 2/8, 25%) and in the United Kingdom and Ireland (n = 23/54,

TABLE 1. Anthropometric and Birth Characteristics by Etiology of Severe Microcephaly

Characteristic	All Cases (N = 118)	Etiology of Severe Microcephaly			
		Genetic (N = 59)	Acquired (N = 21)	*P* Value	Unknown (N = 38)
Age at diagnosis (days), median (IQR)	17 (1–119)	1 (1–108)	31 (1–126)	.20	37 (1–140)
Identified at birth, n (%)	58 (49)	32 (54)	9 (43)	.37	17 (45)
Identified later in infancy, n (%)	60 (51)	27 (46)	12 (57)		21 (55)
Sex, n (%)[a]				.39	
Female	64 (55)	34 (59)	10 (48)		20 (53)
Male	53 (45)	24 (41)	11 (52)		18 (47)
Premature birth, n (%)[a]	38 (32)	15 (26)	10 (48)	.07	13 (34)
Gestational age (weeks), median (IQR)	35.0 (28.0–36.0)	35.3 (34.0–36.0)	29.5 (26.0–35.0)	.01	35.7 (31.0–36.0)
Multiple birth, n/N (%)	5/91 (5)	—	—	—	—
Birth measurements, mean (SD)					
Head circumference (cm)[b]	29.2 (2.8)	29.3 (2.4)	28.8 (3.7)	.47	29.2 (2.9)
Birthweight (kg)[c]	2.35 (0.84)	2.48 (0.81)	2.03 (0.86)	.04	2.32 (0.85)
Length (cm)[d]	46.0 (3.9)	46.9 (4.2)	45.8 (3.6)	.49	45.2 (3.5)
Birth measurement Z-scores, mean (SD)					
HCAZ[b]	−3.1 (1.6)	−3.4 (1.5)	−2.4 (2.0)	.046	−2.9 (1.3)
WAZ[e]	−1.3 (1.3)	−1.2 (1.3)	−0.9 (1.5)	.42	−1.5 (1.3)
LAZ[f]	−1.2 (1.7)	−0.9 (1.9)	−1.0 (1.4)	.90	−1.7 (1.4)
Z-scores at diagnosis, mean (SD)					
HCAZ	−4.0 (1.1)	−4.0 (1.2)	−4.1 (1.3)	.67	−4.1 (1.0)
WAZ[g]	−1.8 (1.2)	−1.8 (1.1)	−1.7 (1.0)	.91	−1.8 (1.3)
LAZ[h]	−1.7 (1.4)	−1.5 (1.4)	−1.8 (1.2)	.47	−2.1 (1.4)
Disproportionate microcephaly at diagnosis, n/N (%)					
Definition 1: SM with WAZ ≥ -3 SD[i]	90/104 (87)	48/53 (91)	16/19 (84)	.43	26/32 (81)
Definition 2: SM with HCAZ − WAZ < -2[j]	51/104 (49)	29/53 (55)	8/19 (42)	.35	14/32 (44)

Abbreviations: HCAZ, head circumference-for-age Z-score; LAZ, length-for-age Z-score; WAZ, weight-for-age Z-score.
[a] N = 117 (58 genetic, 21 acquired, 38 unknown); gestational age described among preterm births only.
[b] N = 93 (47 genetic, 16 acquired, 30 unknown).
[c] N = 111 (55 genetic, 19 acquired, 37 unknown).
[d] N = 49 (22 genetic, 8 acquired, 19 unknown).
[e] N = 110 (54 genetic, 19 acquired, 37 unknown).
[f] N = 50 (22 genetic, 8 acquired, 20 unknown).
[g] N = 101 (53 genetic, 19 acquired, 29 unknown).
[h] N = 68 (35 genetic, 12 acquired, 21 unknown).
[i] Definition of disproportionate microcephaly adapted from von der Hagen et al[9] and de Magalhães Barbosa et al.[12]
[j] Definition of disproportionate microcephaly from Noyola et al.[13]

43%, $P = .003$) (Supplemental Table 6). One-quarter of mothers experienced comorbidities during pregnancy (n = 26/108, 24%), more so in Australia (n = 13/25, 52%) than Canada (n = 6/34, 18%) and the United Kingdom and Ireland (n = 7/49, 14%, $P = .001$). Gestational or pregestational diabetes (n = 8/115, 7%) and vascular complications (eg, hypertension, placental insufficiency, or preeclampsia placental insufficiency; n = 6/106, 6%) were the most prevalent complications. During pregnancy, 14% of mothers smoked (n = 13/96), 6% consumed alcohol or were treated for alcohol misuse problems (n = 6/103), and 5% used illicit drugs (n = 5/104). A family history of microcephaly was reported for 9 cases (n = 9/93, 10%), including 5 first-degree relatives (ie, parents or siblings) (Table 2). Consanguinity was reported for 18 cases (n = 18/82, 22%), including 14 first-cousin relatives.

Brain magnetic resonance imaging (MRI) (n = 82/118, 69%) and DNA microarray (n = 79/115, 69%) were the most common diagnostic investigations conducted (Table 3). Brain imaging also included ultrasonography (n = 62/118, 53%) and computed tomography (CT) (n = 17/118 14%), with multiple modalities conducted in 41% of cases (n = 48/118). MRI and CT scans frequently

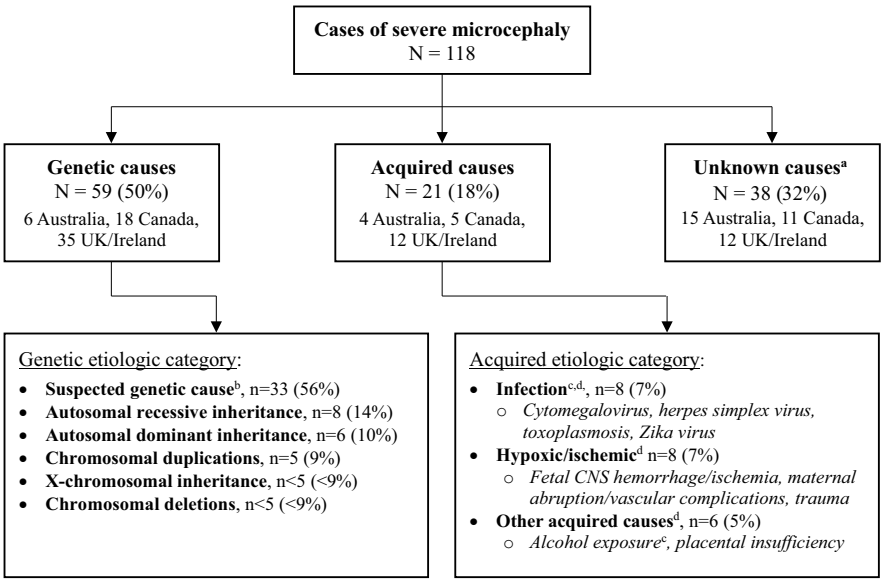

FIGURE 2.
Etiology of severe microcephaly.
[a]Includes one case diagnosed with craniosynostosis, but of unknown etiology.
[b]Includes n = 19 determined based on clinical consensus of familial history, associated congenital anomalies, and/or clinical dysmorphisms; and n = 14 where genetic testing identified a syndrome that was not previously associated with microcephaly. Suspected genetic causes were only classified in Canada and the United Kingdom and Ireland—Australian cases were only classified as genetic based on confirmed chromosomal defects or single gene disorders known to affect head size.
[c]One case was identified as having a multifactorial cause (alcohol exposure and infection) and is counted twice. Therefore, all acquired subgroups equal sum to 22 rather than 21 (ie, the total number of acquired cases).
[d]Frequencies for all specific diagnoses among acquired cases were n < 5 and are therefore not reported.

yielded abnormal results (n = 66/82 [80%] and n = 14/17 [82%], respectively). Using Noyola et al's definition, abnormal brain imaging (CT, MRI, or ultrasonography) was more common among cases with disproportionate (n = 41/49, 84%) than proportionate microcephaly (n = 30/51, 59%, $P = .006$) (Supplemental Table 7). Cytomegalovirus screening was conducted for one-half of all cases (n = 57/118, 48%), either as part of toxoplasmosis, rubella, cytomegalovirus, and herpes simplex virus screening or separately. ZIKV screening was less common (n = 12/118, 10%).

DISCUSSION

Key Findings

National public health surveillance studies of severe microcephaly can provide unique insights into its etiology and population-based impact, with the potential for improving diagnosis, management, and prevention initiatives. This pooled analysis included 118 cases identified by 3 population-based surveillance studies undertaken within a 3-year period (2016–2018). One-half of all cases had a confirmed or suspected genetic basis, 18% were pre- or perinatally acquired, and in one-third, the etiology was unknown. Eye abnormalities and vision impairment were more likely to be associated with genetic than other etiologies, whereas hearing impairment, neuromuscular abnormalities, and seizures were associated with all etiologies. The median age at diagnosis was 17 days, with a mean HCAZ of −4.0 (SD 1.1) and >2 Z-scores discrepant from the mean WAZ and LAZ. Approximately 57% of cases were diagnosed with any microcephaly at birth (49% with severe microcephaly), whereas 21% lacked birth head circumference measurements. This lack of longitudinal information emphasizes a need for more systematic birth measurement to monitor HCAZ trajectories, including record linkage across specialties (eg, obstetrics and pediatrics).

Etiology

Although studies have emphasized the importance of etiologic diagnosis to improve prognosis and offer appropriate interventions and family support, the cause of microcephaly is often difficult to ascertain.[9,20,21] In von der Hagen et al's 11-year case review of 680 microcephaly cases from 2 German hospitals, one-third were diagnosed at birth and two-thirds postnatally.[9] In contrast, more than one-half of Australian and Canadian cases were diagnosed at birth vs 37% in the United Kingdom and Ireland. In the German study, 42% of children had a confirmed or suspected genetic cause, 29% were attributable to peri- or postnatal

TABLE 2. Clinical Features and Pregnancy Genetic Exposures

Characteristic, n/N (%)	All Cases		Etiology of Severe Microcephaly						
			Genetic		Acquired			Unknown	
	%	n/N	%	n/N	%	n/N	*P* Value	%	n/N
Infant clinical features									
Eye abnormalities/vision impairment	40	40/100	56	29/52	<28	<5/18	.02	17–30	5–9/30
Floppiness/hypotonia	31	18/58	38	9/24	<56	<5/9	.68	20–36	5–9/25
Muscle spasticity	25	14/57	<22	<5/23	56	5/9	.08	20–36	5–9/25
Hearing impairment	21	18/86	23	10/44	<38	<5/13	1.00	17–31	5–9/29
Seizure(s)	19	20/108	22	12/54	<28	<5/18	.75	14–25	5–9/36
Joint contractures	5	6/114	10	6/59	0	0/18	.33	0.0	0/37
Hepatosplenomegaly	<6	<5/90	—	—	—	—	—	—	—
Rash	<5	<5/91	—	—	—	—	—	—	—
Pregnancy and fetal complications									
Abnormal fetal ultrasonography	52	49/94	57	30/53	38	6/16	.18	52	13/25
Intrauterine growth restriction	41	46/111	49	28/57	42	8/19	.60	28	10/35
Any maternal pregnancy complication	24	26/108	15	8/55	31	5/16	.13	35	13/37
Maternal prenatal exposures									
Alcohol, illicit drugs, or smoking	19	19/98	10–18	5–9/50	33–60	5–9/15	.02	<15	<5/33
Prescribed medications	15	15/100	14	7/51	<36	<5/14	.32	<14	<5/35
Smoking	14	13/96	14	7/51	<33	<5/15	.45	<17	<5/30
Alcohol[a]	6	6/103	—	—	—	—	—	—	—
Illicit drug use	5	5/104	—	—	—	—	—	—	—
Traveled during pregnancy or preconception									
Mother traveled	33	13/40	—	—	—	—	—	—	—
Father or sexual partner traveled[b]	26	9/34	—	—	—	—	—	—	—
Family history									
Mother had prior miscarriage	25	10/40	—	—	—	—	—	—	—
Any parental consanguinity	22	18/82	29–37	15–19/52	<38	<5/13	.16	<29	<5/17
Any congenital anomalies	20	9/46	22–39	5–9/23	0	0/5	.55	<28	<5/18
Any microcephaly	10	9/93	10–18	5–9/56	0	0/15	.19	<18	<5/28
Mother had prior stillbirth	0	0/37	—	—	—	—	—	—	—

[a] Measured as receiving treatment for alcohol or illicit drugs in the United Kingdom.
[b] Collected as the father in Canada, and mother's sexual partner in Australia.

brain injury, and 41% had an unknown cause.[9] These findings are comparable to our population-based analysis, with genetic causes predominant and supporting the frequent use of genetic testing including DNA microarray and karyotype. We found a lower proportion of cases with unknown causes compared with von der Hagen et al. Nevertheless, one-third of our cases were unknown, and one-half of genetic cases lacked specific diagnoses, suggesting there is significant room to improve and standardize the diagnostic work-up of severe microcephaly, including advanced genetic testing (ie, whole exome or whole-genome sequencing). Implementation of more comprehensive rare disease coding (eg, Orphacodes[22]) can further facilitate better understanding of the underlying genetic causes of microcephaly.

Investigations

The AAN practice parameters[10] recommend neuroimaging as a central investigation, particularly for disproportionate microcephaly. Brain MRI is considered more sensitive than CT or ultrasonography, although repeated imaging may be needed. Clinicians in our studies performed brain MRI in approximately 70% of cases and identified abnormalities in 80% of those infants. This was similar to von der Hagen et al, where 72% of children received either brain MRI or ultrasonography, with 76% of MRIs yielding abnormal findings.[9] Our analysis demonstrated variations in neuroimaging practice between countries, which may be related to access, cost, or clinical preference for different imaging modalities. AAN practice parameters do not

TABLE 3. Testing Modalities for Etiological Investigations

Characteristic, n/N (%)	All Cases		Country					
			Australia		Canada		United Kingdom and Ireland	
	%	n/N	%	n/N	%	n/N	%	n/N
Brain imaging[a]								
MRI scan	69	82/118	76	19/25	71	24/34	66	39/59
Abnormal results	80	66/82	63	12/19	88	21/24	85	33/39
Ultrasonography scan	53	62/118	32	8/25	47	16/34	64	38/59
Abnormal results	55	34/62	25	2/8	50	8/16	63	24/38
CT scan	14	17/118	4	1/25	18	6/34	17	10/59
Abnormal results	82	14/17	100	1/1	100	6/6	70	7/10
>1 brain imaging modalities[a,b]	41	48/118	20	5/25	32	11/34	54	32/59
EEG	39	35/89	—[c]	—[c]	43	13/30	37	22/59
Genetic testing								
DNA microarray	69	79/115	88	22/25	71	22/31	59	35/59
Karyotype	40	19/47	35	7/20	44	12/27	—[3]	—[3]
Infection screening[a,d]								
CMV	48	57/118	64	16/25	59	20/34	36	21/59
Toxoplasmosis	38	45/118	48	12/25	53	18/34	25	15/59
Rubella	36	42/118	52	13/25	47	16/34	22	13/59
HSV	26	31/118	36	9/25	41	14/34	14	8/59
Syphilis	14	17/118	40	10/25	<15	<5/34	<8	<5/59
VZV	14	17/118	20	5/25	26	9/34	5	3/59
ZIKV	10	12/118	16	4/25	18	6/34	3	2/59
HIV	5	6/118	—	—	—	—	—	—

Abbreviations: CMV, cytomegalovirus; CPSP, Canadian Paediatric Surveillance Program; CT, computed tomography; EEG, electroencephalogram; HSV, Herpes simplex virus; MRI, Magnetic resonance imaging; VZV, Varicella zoster virus; ZIKV, Zika virus.

[a] Negative responses could not be distinguished from missing responses in Australian (brain imaging, infection screening) and Canadian data (infection screening); therefore, total country denominators were used to calculate proportions. Proportions may therefore be underestimated.

[b] Includes CT, MRI, and/or ultrasonography.

[c] Data not collected in national study.

[d] In some CPSP cases, reports indicated results from an overall TORCH screen. If specific results were not indicated, we assumed results applied to the pre-specified options of CMV, HSV, rubella, toxoplasmosis, and VZV.

recommend routine electroencephalogram (EEG) or ophthalmological examination, instead suggesting these investigations in the setting of clinical concerns.[10] In our study, EEGs were performed on 39% of infants, and separately, 40% had eye or vision abnormalities identified through ophthalmological exam, suggesting that these investigations were clinically productive.

Microcephaly Proportionality

Assessment of whether head circumference and body size (weight and/or length) are proportionate is frequently mentioned as part of microcephaly investigation.[9,20] However, there is no consistent definition of proportionality in current practice guidelines or other studies.[9–11,13,14] Whereas Dolk did not find an association between disproportionate microcephaly and worse neurodevelopmental delay, Sells reported higher academic achievement in children whose microcephaly was proportionate to body size.[23,24] In our analysis, the definitions adapted from Noyola et al and de Magalhães-Barbosa et al yielded highly different estimates of disproportionate microcephaly (49% and 87%, respectively). Using Noyola et al's definition, which measured the difference between HCAZ and WAZ, disproportionate microcephaly was associated with increased likelihood of abnormal brain imaging. Conversely, de Magalhães-Barbosa et al's definition was not associated with etiology, in keeping with research using similar threshold-based definitions.[9,14,25] Although proportionality was not associated with etiology, a standardized difference-based definition applicable to all microcephaly cases regardless of severity, and which encourages measurement of both weight and length (frequently not recorded in our study), could plausibly lead to greater clinical utility.

Strengths and Limitations

As severe microcephaly is a rare disorder, large population-based datasets are uncommon, and this secondary data analysis represents an important contribution to our understanding of this condition. Our analysis is based on public health surveillance data with good case ascertainment and is representative of contemporary national populations and real-world clinical management in high-resource nations, and therefore our findings are likely to be robust and generalizable to similar settings. The sharing of data collection methods between research teams during the study design phase, and the overlap in timing of surveillance, ensured that individual datasets were suited to a pooled analysis and to comparisons between countries. Nevertheless, there were differences between national approaches including the use of local growth charts, case review methods to determine etiology, and ascertainment of certain data elements. Given that full clinical data could not be shared with one central group to determine etiology, differences in country-specific classification were inevitable. Specifically, the APSU study considered only confirmed chromosomal or single gene mutations to classify cases as having a genetic etiology, whereas the BPSU and CPSP studies considered patterns of dysmorphic features and familial history in addition to genetic testing to classify cases as genetic (considered as "suspected" genetic etiologies in this study). Our study therefore likely underestimates the proportion of cases attributable to genetic causes, specifically in Australia. Consideration of suspected genetic cases via clinical consensus in epidemiologic studies can guide future research and recommendations for diagnostic investigations, but, importantly, is not conclusive in determining an etiology. The phrasing of certain data elements, such as prenatal alcohol consumption, was inconsistent given real-world differences in epidemiology between countries (ie, "any use" in Australia and Canada vs "treatment for alcohol problems" in the United Kingdom and Ireland), and therefore certain exposures may have been underestimated. As surveillance data were provided by clinicians from their own or hospital medical records without further examination or investigation of affected children, some analyses are limited by missing data. Moreover, restrictions on data sharing between countries and safeguards on patient confidentiality have limited the reporting of low-frequency events such as specific genetic diagnoses.

CONCLUSION

This contemporary multicountry analysis of severe microcephaly highlights ongoing uncertainty about the etiology and prognosis of this condition. Although public health surveillance was initiated in response to the ZIKV epidemic, very few cases of Zika-associated severe microcephaly were identified. Our findings, however, can serve as a point of reference to assess changes in presentation of severe microcephaly, should emerging infections such as congenital ZIKV or Oropouche virus present concern in the future.[26] Our findings underline the need for greater standardization of diagnostic investigations to improve the etiological classification of severe microcephaly, optimize clinical and neurodevelopmental management, and inform future preventive strategies.

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ABBREVIATIONS

AAN: American Academy of Neurology
APSU: Australian Paediatric Surveillance Unit
BPSU: British Paediatric Surveillance Unit
CPSP: Canadian Paediatric Surveillance Program
CT: Computed tomography
CMV: Cytomegalovirus
EEG: Electroencephalogram
HCAZ: Head circumference-for-age Z-score
HSV: Herpes simplex virus
INoPSU: International Network of Paediatric Surveillance Units
LAZ: Length-for-age Z-score
MRI: Magnetic resonance imaging
NZPSU: New Zealand Paediatric Surveillance Unit
VZV: Varicella zoster virus
WAZ: Weight-for-age Z-score
WHO: World Health Organization
ZIKV: Zika virus

Address correspondence to: Shaun K. Morris, MD, MPH, Centre for Global Child Health, The Hospital for Sick Children, 555 University Ave, Toronto, ON M5G 1X8, Canada. shaun.morris@sickkids.ca

Mr Farrar and Dr Nunez planned and conducted the statistical analysis and drafted the initial manuscript. Dr Knowles planned and conducted the statistical analysis, drafted the initial manuscript, and was principal investigator of the British Paediatric Surveillance Unit (BPSU) study. Drs Duncanson, Elliott, and S. Morris were principal investigators of the New Zealand Paediatric Surveillance Unit, Australian Paediatric Surveillance Unit (APSU), and Canadian Paediatric Surveillance Program (CPSP) studies, respectively, and provided oversight on the project. Drs Baynam, Bitnun, Lynn, Moore Hepburn, A. Morris, Oeser, Pebody, and Solebo contributed to the design, implementation, and interpretation of the APSU, BPSU, and CPSP studies as well as this secondary analysis. All authors contributed edits to the manuscript, approved the final manuscript as submitted, and agree to be accountable for all aspects of the work. Deidentified case-level data cannot be made available.

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REFERENCES

1. Woods CG, Parker A. Investigating microcephaly. *Arch Dis Child.* 2013;98(9):707–713. PubMed doi: 10.1136/archdischild-2012-302882
2. Panchaud A, Stojanov M, Ammerdorffer A, Vouga M, Baud D. Emerging role of Zika virus in adverse fetal and neonatal outcomes. *Clin Microbiol Rev.* 2016;29(3):659–694. PubMed doi: 10.1128/CMR.00014-16
3. Gulland A. Zika virus is a global public health emergency, declares WHO. *BMJ.* 352:i657. PubMed doi: 10.1136/bmj.i657
4. Nunez C, Morris A, Jones CA, et al. Microcephaly in Australian children, 2016–2018: national surveillance study. *Arch Dis Child.* 2021;106(9):849–854. PubMed doi: 10.1136/archdischild-2020-320456
5. Nunez C, Morris A, Elliott EJ. National surveillance of severe microcephaly in Australia. *Acta Paediatr.* 2023;112(8):1734–1737. PubMed doi: 10.1111/apa.16846
6. Morris SK, Farrar DS, Miller SP, et al. Population-based surveillance of severe microcephaly and congenital Zika syndrome in Canada. *Arch Dis Child.* 2021;106(9):855–861. PubMed doi: 10.1136/archdischild-2020-320968
7. Knowles RL, Solebo AL, Sampaio MA, et al. Incidence, aetiology and neurodisability associated with severe microcephaly: a national surveillance study. *Arch Dis Child.* 2023;108(3):211–217. PubMed doi: 10.1136/archdischild-2022-324311
8. Graf WD, Le Pichon JB, Bittel DC, Abdelmoity AT, Yu S. Practice parameter: evaluation of the child with microcephaly (an evidence-based review): report of the quality standards subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology.* 2010;74(13):1080–1081. PubMed doi: 10.1212/WNL.0b013e3181d5e077
9. von der Hagen M, Pivarcsi M, Liebe J, et al. Diagnostic approach to microcephaly in childhood: a two-center study and review of the literature. *Dev Med Child Neurol.* 2014;56(8):732–741. PubMed doi: 10.1111/dmcn.12425
10. Ashwal S, Michelson D, Plawner L, Dobyns WB; Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Practice parameter: Evaluation of the child with microcephaly (an evidence-based review) [RETIRED]: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology.* 2009;73(11):887–897. PubMed doi: 10.1212/WNL.0b013e3181b783f7
11. Soares de Araújo JS, Regis CT, Gomes RGS, et al. Microcephaly in north-east Brazil: a retrospective study on neonates born between 2012 and 2015. *Bull World Health Organ.* 2016;94(11):835–840. PubMed doi: 10.2471/BLT.16.170639
12. de Magalhães-Barbosa MC, Prata-Barbosa A, Robaina JR, Raymundo CE, Lima-Setta F, da Cunha A. Prevalence of microcephaly in eight south-eastern and midwestern Brazilian neonatal intensive care units: 2011–2015. *Arch Dis Child.* 2017;102(8):728–734. PubMed doi: 10.1136/archdischild-2016-311541
13. Noyola DE, Demmler GJ, Nelson CT, et al; Houston Congenital CMV Longitudinal Study Group. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. *J Pediatr.* 2001;138(3):325–331. PubMed doi: 10.1067/mpd.2001.112061
14. Adachi K, Romero T, Nielsen-Saines K, et al. Early clinical infancy outcomes for microcephaly and/or small for gestational age Zika-exposed infants. *Clin Infect Dis.* 2020;70(12):2663–2672. PubMed doi: 10.1093/cid/ciz704

15. Grenier D, Elliott EJ, Zurynski Y, et al. Beyond counting cases: public health impacts of national Paediatric Surveillance Units. *Arch Dis Child.* 2007;92(6):527–533. PubMed doi: 10.1136/adc.2006.097451

16. Oeser C, Aarons E, Heath PT, et al. Surveillance of congenital Zika syndrome in England and Wales: methods and results of laboratory, obstetric and paediatric surveillance. *Epidemiol Infect.* 2019;147:e262. PubMed doi: 10.1017/S0950268819001535

17. Villar J, Cheikh Ismail L, Victora CG, et al; International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st). International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet.* 2014;384(9946):857–868. PubMed doi: 10.1016/S0140-6736(14)60932-6

18. World Health Organization. WHO child growth standards. *Dev Med Child Neurol.* 2009;51(12):1002. doi: 10.1111/j.1469-8749.2009.03503.x

19. Rath A, Olry A, Dhombres F, Brandt MM, Urbero B, Ayme S. Representation of rare diseases in health information systems: the Orphanet approach to serve a wide range of end users. *Hum Mutat.* 2012;33(5):803–808. PubMed doi: 10.1002/humu.22078

20. Hanzlik E, Gigante J. Microcephaly. *Children (Basel).* 2017;4(6):47. PubMed doi: 10.3390/children4060047

21. Becerra-Solano LE, Mateos-Sánchez L, López-Muñoz E. Microcephaly, an etiopathogenic vision. *Pediatr Neonatol.* 2021;62(4):354–360. PubMed doi: 10.1016/j.pedneo.2021.05.008

22. Orphanet. Procedural document on the Orphanet nomenclature and classification of rare diseases. June 2023 – Version 05. Accessed May 26, 2025. https://www.orpha.net/orphacom/cahiers/docs/GB/eproc_disease_inventory_R1_Nom_Dis_EP_05.pdf

23. Dolk H. The predictive value of microcephaly during the first year of life for mental retardation at seven years. *Dev Med Child Neurol.* 1991;33(11):974–983. PubMed doi: 10.1111/j.1469-8749.1991.tb14813.x

24. Sells CJ. Microcephaly in a normal school population. *Pediatrics.* 1977;59(2):262–265. PubMed doi: 10.1542/peds.59.2.262

25. Silva AA, Barbieri MA, Alves MT, et al. Prevalence and risk factors for microcephaly at birth in Brazil in 2010. *Pediatrics.* 2018;141(2):e20170589. PubMed doi: 10.1542/peds.2017-0589

26. das Neves Martins FE, Chiang JO, Nunes BTD, et al. Newborns with microcephaly in Brazil and potential vertical transmission of Oropouche virus: a case series. *Lancet Infect Dis.* 2025;25(2):155–165. PubMed doi: 10.1016/S1473-3099(24)00617-0