

# Cerebral palsy: causes, pathways, and the role of genetic variants

Alastair H. MacLennan, MD, FRANZCOG; Suzanna C. Thompson, MBBS, FRACP; Jozef Gecz, PhD

Two international expert task forces addressed cerebral palsy (CP) causation in 1999<sup>1</sup> and 2003.<sup>2</sup> In 2014, the American Congress of Obstetricians and Gynecologists and the American Academy of Pediatrics, with many international consultants, updated these reports but chose to focus on neonatal encephalopathy and a variety of neurological outcomes rather than discuss CP causation specifically and did not directly address the ramifications of litigation following a diagnosis of CP.<sup>3</sup> Recent findings published after the 2014 report have identified likely causative genetic variants associated with CP cases and this review contributes to updating clinicians.

CP is a heterogeneous condition with multiple causes; multiple clinical types; multiple patterns of neuropathology on brain imaging; multiple associated developmental pathologies, such as intellectual disability, autism, epilepsy, and visual impairment; and more recently multiple rare pathogenic genetic variations (mutations). CP would be better named “the cerebral palsies” given that within the CP clinical spectrum there are many causal pathways and many types and degrees of disability. These various pathways and etiologies have each resulted in a nonspecific nonprogressive disorder of

Cerebral palsy (CP) is heterogeneous with different clinical types, comorbidities, brain imaging patterns, causes, and now also heterogeneous underlying genetic variants. Few are solely due to severe hypoxia or ischemia at birth. This common myth has held back research in causation. The cost of litigation has devastating effects on maternity services with unnecessarily high cesarean delivery rates and subsequent maternal morbidity and mortality. CP rates have remained the same for 50 years despite a 6-fold increase in cesarean birth. Epidemiological studies have shown that the origins of most CP are prior to labor. Increased risk is associated with preterm delivery, congenital malformations, intrauterine infection, fetal growth restriction, multiple pregnancy, and placental abnormalities. Hypoxia at birth may be primary or secondary to preexisting pathology and international criteria help to separate the few cases of CP due to acute intrapartum hypoxia. Until recently, 1-2% of CP (mostly familial) had been linked to causative mutations. Recent genetic studies of sporadic CP cases using new-generation exome sequencing show that 14% of cases have likely causative single-gene mutations and up to 31% have clinically relevant copy number variations. The genetic variants are heterogeneous and require function investigations to prove causation. Whole genome sequencing, fine scale copy number variant investigations, and gene expression studies may extend the percentage of cases with a genetic pathway. Clinical risk factors could act as triggers for CP where there is genetic susceptibility. These new findings should refocus research about the causes of these complex and varied neurodevelopmental disorders.

**Key words:** causes, cerebral palsy, DNA variants, epidemiological risk factors, genetic variants, genomics, heterogeneity, whole exome sequencing

posture and movement control. Thus, CP should be considered as a descriptive term for affected individuals, with each case receiving adequate consideration of an underlying etiology. There has been little change in the prevalence of this diagnosis throughout the world, where

population data are available.<sup>1</sup> It remains around 2-2.5/1000 births. Although there have been small statistical fluctuations in the CP rates among children born preterm, the rates of CP at term remain stable.<sup>4</sup> New interventions such as head or body cooling in selected

From the Australian Collaborative Cerebral Palsy Research Group at the Robinson Research Institute, the University of Adelaide, Adelaide, Australia (Dr MacLennan); and Department of Paediatric Neurology, Adelaide Women's and Children's Hospital (Dr Thompson) and Neurogenetics Research Program (Dr Gecz), School of Pediatrics and Reproductive Health, the University of Adelaide, Adelaide, Australia.


Received Jan. 28, 2015; revised May 11, 2015; accepted May 15, 2015.

These studies have been supported by grants from the Australian National Health and Medical Research Council (1041920 and 1019928), Cerebral Palsy Foundation, Tenix Foundation, Women's and Children's Research Foundation, and Robinson Research Institute Foundation.

The authors report no conflict of interest.

Corresponding author: Alastair H. MacLennan, MD, FRANZCOG. [alastair.maclennan@adelaide.edu.au](mailto:alastair.maclennan@adelaide.edu.au)

0002-9378 • Crown Copyright © 2015 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>). • <http://dx.doi.org/10.1016/j.ajog.2015.05.034>

 Click Supplemental Materials under the article title in the online Table of Contents

cases with acute hypoxia have yet to significantly lower overall rates. Only a small percentage of cases are associated solely with acute intrapartum hypoxia.<sup>5-8</sup> Despite this, many cases are mislabeled as due to birth asphyxia.

### Birth asphyxia

“Birth asphyxia” is an outdated term that may wrongly convey that a baby born with signs of fetal and neonatal compromise must have undergone an acute hypoxic event in late labor and/or birth. These clinical signs may also be present when there has been much longer-standing fetal compromise with possible secondary hypoxia near delivery.<sup>1</sup> Similarly, the term “hypoxic ischemic encephalopathy” has been replaced by the term “neonatal encephalopathy” as the large majority of newborn infants showing signs of encephalopathy does not have objective proof of acute hypoxia or ischemia at birth, but have other causes of perinatal compromise such as infectious or genetic.<sup>9</sup> Of note, only 13% of term babies who exhibit neonatal encephalopathy are later diagnosed with CP.<sup>10</sup>

At birth, nonspecific signs of fetal compromise such as meconium-stained amniotic fluid, nonreassuring fetal

heart rate patterns, low Apgar scores, and neonatal encephalopathy could all be associated with either acute intrapartum timing or chronic long-standing timing of the pathologies (ie, beginning before labor and during pregnancy). The same signs can be caused by not only hypoxia and/or ischemia, but also by other factors such as infection, placental and umbilical vessel thrombosis, or an altered fetal inflammatory response.<sup>1</sup> Very recent studies suggest that many cases of CP are associated with genetic alterations (mutations) that may either directly cause CP or contribute to susceptibility to CP.<sup>11,12</sup> As yet, they are not detectable antenatally or preventable.

### International consensus criteria to identify severe acute intrapartum hypoxia

There is now increasing evidence that babies given a “birth asphyxia” label due to clinical signs such as low Apgar scores often do not have primary asphyxia.<sup>13,14</sup> Many such babies are in ill health due to longer-standing problems. Acute or chronic hypoxia can cause a metabolic acidosis in the blood of the newborn and this has to be objectively measured in umbilical arterial blood gases at birth to

ascertain if clinically severe hypoxia is contributing to the poor condition of the newborn. When metabolic acidosis is proven to be present, this is evidence of either acute hypoxia beginning in labor or chronic hypoxia (ie, long-standing compromise in pregnancy beginning before labor). Secondary asphyxia in labor is not necessarily the initial cause of the brain injury but may be a subsequent result of the established neuropathological process. International consensus criteria have been published and refined to help define cases where neuropathology may have become established only in labor and birth.<sup>1,2</sup> These 9 criteria have helped recognize the few cases of severe de novo acute intrapartum hypoxia (Table 1). These criteria, as a group, have been well verified.<sup>15</sup> The first 4 essential criteria have a high but not individually perfect correlation (94-100%) in acutely asphyxiated neonates. The 5 nonspecific timing criteria were individually less predictive, but were to be assessed together, and their consensus helps understand the likely timing of the neuropathology. In 2014, a third consensus statement similarly examined neonatal encephalopathy, rather than CP, and largely supported the criteria that define a

TABLE 1

#### International consensus criteria to determine a severe acute hypoxic event as a potential cause of cerebral palsy

Essential criteria to show presence of hypoxia at birth are:

1. A metabolic acidosis at birth (pH <7.00 and Base Excess <-12).
2. Early moderate to severe neonatal encephalopathy.
3. Cerebral palsy of spastic quadriplegic or dyskinetic type.
4. Exclusion of other identifiable causes of cerebral palsy, eg, coagulation or genetic disorders, infectious conditions, intrapartum pyrexia, antepartum hemorrhage, prematurity, intrauterine growth restriction, tight nuchal cord, complications of multiple pregnancy.

Five nonspecific criteria collectively point toward acute or chronic causes of hypoxia.

If most are met they suggest timing of neuropathology near delivery. If most are not met they suggest longer-standing pathological process. These criteria are:

5. Sentinel (signal) hypoxic event sufficient to cause sudden severe hypoxia in healthy fetus, eg, cord prolapse, antepartum hemorrhage, ruptured uterus.
6. Sudden sustained fetal heart rate bradycardia from that event.
7. Apgar score <4 after 5 min.
8. Signs of multisystem failure in neonate.
9. Early (within 5 d) neuroimaging signs of edema and intracranial hemorrhage.

In 2003, American College of Obstetricians and Gynecologists/American Academy of Pediatrics Cerebral Palsy Expert Task Force<sup>2</sup> updated 1999 criteria<sup>1</sup> on basis of published evidence to that date. Task force agreed with 1999 task force criteria and added fourth essential criterion was necessary, ie, that no major chronic cause of neuropathology should be present if intrapartum acute asphyxial cause was to be presumed. Also acute de novo intrapartum event severe enough to be associated with cerebral palsy would cause Apgar scores to remain at ≤3 after 5 min of birth. Lastly, it only accepted evidence of severe metabolic acidosis from arterial umbilical samples taken at birth, as blood gases can improve or worsen in first hour depending on successful or problematic neonatal resuscitation.

MacLennan. Cerebral palsies: new insights and causes. *Am J Obstet Gynecol* 2015.

causative intrapartum neuropathological event.<sup>3</sup>

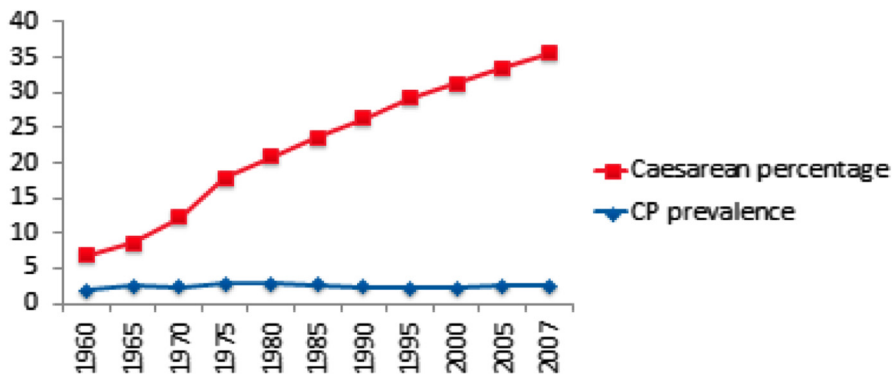
### Intrapartum cardiotocography

It is important to understand the great limitations of intrapartum electronic fetal heart rate monitoring, and in particular its inability to reduce or prevent CP. Cochrane systematic reviews of relevant randomized controlled trials of electronic fetal heart rate monitoring show no reduction in CP rates with its use compared to intermittent auscultation of the fetal heart.<sup>16</sup> Continuous electronic fetal heart rate monitoring was introduced in the 1960s, without prior testing in randomized controlled trials, partly in the belief that it would allow early recognition of acute fetal compromise and in particular hypoxia. It was hoped that this would reduce intrapartum brain injury, because of the long-standing assumption and belief that many cases of CP were due to preventable acute hypoxia beginning in labor. However, fetal heart rate is a very indirect and poor measure of past and present fetal brain function and damage.

Intrapartum cardiotocography has a very high false-positive rate and with the pressures of obstetric litigation in many countries, birth attendants and especially individuals giving private care, who cannot be in continuous attendance, often opt for cesarean delivery. Defensive obstetrics, often in response to uncertain cardiotocographic interpretation, has contributed to a large increase in cesarean delivery rates without a change in CP rates (Figure 1). The quantum of claims following CP is very high in countries such as the United States, Australia, and the United Kingdom. English health trusts paid £482 million for “maternity negligence” coverage in 2012 through 2013 equating to a fifth of all spending on maternity services.<sup>17</sup> A small group of expert witnesses for the plaintiff regularly opine that the cause of the CP was birth asphyxia that was recognizable in labor and preventable by earlier delivery. To challenge such nonevidence-based opinion, it is time for obstetric colleges and academic scientific societies in this field

FIGURE 1

### Six-fold increase in cesarean delivery without change in CP prevalence



Prevalence of CP/1000 births compared with cesarean rates over the past 50 years.<sup>68</sup>

CP, cerebral palsy.

MacLennan. *Cerebral palsies: new insights and causes*. *Am J Obstet Gynecol* 2015.

to spell out that cardiotocography: (1) cannot detect the timing of the onset of neuropathology; (2) cannot determine a time when it would be reversible and then irreversible; and (3) cannot be used to determine that earlier delivery by cesarean delivery “on the balance of probabilities” would have prevented the CP outcome.<sup>18</sup> Courts should find that junk science is inadmissible in determining CP causation and prevention.<sup>19</sup>

### Clinical risk factors for CP during pregnancy

There is increasing scientific evidence that CP is usually associated with long-standing intrauterine pathology like genetic mutations and probable environmental triggers such as bacterial and viral intrauterine infection, intrauterine growth restriction (IUGR), antepartum hemorrhage, tight nuchal cord, and threatened miscarriage.<sup>20</sup> It can be difficult to pinpoint adverse pregnancy factors in retrospect, many years after birth, that individually or together might have triggered the pathways to the neuropathology.

### Preterm delivery

Preterm delivery is a major risk factor for CP and is seen in approximately 35% of all cases, and the risk increases the lower the viable gestational age.<sup>20,21</sup> The risk of subsequent CP <33 weeks' gestation is 30 times higher than among

those born at term and is approximately 70/1000 deliveries.<sup>7</sup> The prevalence of CP is highest in children born <28 weeks' gestational age (111.8/1000 neonatal survivors; 82.25/1000 live births) and declines with increasing gestational age, being 43.15/1000 live births between 28-31 weeks, 6.75/1000 between 32-36 weeks, and 1.35/1000 for those born >36 weeks.<sup>22</sup> The mechanisms and pathways to the neuropathology of CP may differ from term babies, although associated risk factors such as infection, genetic variations, and growth restriction are likely to contribute.

### Coexisting congenital anomalies

The prevalence of congenital anomalies in children with CP is much higher than in the general population and most are cerebral, such as schizencephaly and hydrocephaly.<sup>23</sup> Noncerebral malformations are also increased, such as cardiac, musculoskeletal, and urinary.<sup>24</sup> In a case-control study of 494 singleton infants with CP born >35 weeks' gestation included on the Western Australian Register of Developmental Anomalies and 508 matched controls, birth defects (42.3%) and fetal growth restriction (16.5%) were more strongly associated with CP than potentially asphyxial birth events (8.5%) and inflammation (4.8%).<sup>25</sup> Birth defects had the largest association with CP in that study in both

term and preterm babies. Growth-restricted babies with birth defects were at special risk of CP. The strong association with congenital abnormalities suggests possible genetic factors although congenital infections, nutritional disorders, and teratogenic influences all contribute to maldevelopment.

**Intrauterine infection**

There are many probable antenatal causes of white-matter damage and risk factors for CP (Table 2). Some of these causes include damage acquired following perinatal infection (ie, maternal infection that affects the fetus and its brain during pregnancy and/or labor or in the neonatal period).<sup>26</sup> Viral or bacterial infections may be relatively silent during pregnancy and not recognized clinically at the time and the placenta is often discarded without histological examination for inflammatory pathology. Maternal reports of fever or infection during pregnancy are significantly associated with an increased risk of CP in our recent large Australian case-control study.<sup>27</sup> Evidence of intrauterine infection, evidenced by histological chorioamnionitis in the placenta and membranes or intrapartum pyrexia, is associated with a 4-fold increase in CP (odds ratio 3.8; 95% confidence interval, 1.5–10.1) in term infants.<sup>28</sup>

**Abnormal fetal inflammatory response and thrombophilia**

Another possible related cause and mechanism of CP is an abnormal

inflammatory response in the fetus and the neonate.<sup>29,30</sup> An excessive or abnormal rise in cytokines (due to genetic predisposition or mutations) following infection and an inflammatory response, which is part of the body’s normal defense mechanism against infection or toxins, may cause an autoimmune type of attack on the fetal or neonatal developing nerve cells. The prematurely delivered baby’s immature brain is even more vulnerable to these proinflammatory cytokines. In previous genetic association studies, hereditary thrombophilia (the methylenetetrahydrofolate reductase cC677T mutation and the prothrombin gene c20210G>A variant) and some cytokine polymorphisms (interleukin-6, interleukin-8, tumor necrosis factor, and mannose binding lectin) were associated with an increased risk of CP.<sup>31,32</sup>

**Intrauterine growth restriction**

IUGR is associated with up to a 10- to 30-fold increase in the risk of CP at term.<sup>20,33</sup> In particular, spastic CP increases with the degree of fetal growth restriction. Our large epidemiological study of Australian children and normal controls clearly confirms IUGR as a major risk factor for CP.<sup>27</sup> The risk of CP increased from values <20th percentile and the risk escalated greatly in babies under the third percentile (Figure 2).

IUGR can be due to many known and unknown causes, but usually reflects poor implantation and poor placenta-tion from genetic, anatomical (eg, uterine fibroids, congenitally abnormal

uterus, abnormal placental site), or pathological (eg, preeclampsia, diabetes, systemic lupus) causes. IUGR increases in late pregnancy when growth velocity should be at its greatest and fetal demand may outstrip placental and maternal supply. This usually creates an asymmetrical growth restriction where the baby is lighter than its length suggests. This gives a low ponderal index (birth-weight/length<sup>3</sup> × 100) and can suggest unsuspected late growth restriction.<sup>34</sup> Use of the ponderal index and customized weight for gestation charts improve the recognition of growth restriction at birth and the risk of subsequent CP.<sup>35</sup>

When IUGR is suspected during pregnancy there are no published randomized quality data to suggest that earlier delivery reduces the risk of CP. It is not possible to detect or predict when in pregnancy the neuropathology of CP begins, becomes established, and is irreversible. A growth-restricted fetus may show signs of possible fetal compromise during labor. This can reflect reduced capacity/reserves to withstand the normal stresses of labor, established neurological and ongoing fetal compromise, or both. It is not possible to distinguish between these timings.

**Multiple pregnancy**

Multiple pregnancy increases CP risk 2-fold in each twin. In vitro fertilization twins each have >4-fold risk (9.5/1000), giving another reason to encourage single embryo transfer in fertility programs.<sup>36</sup>

**Tight nuchal cord at delivery**

Potentially chronic asphyxiating conditions, chiefly a tight nuchal cord, increase the risk of spastic quadriplegic CP. A large population-based study, noted that a tight umbilical cord around the fetal neck, requiring cutting before delivery of the shoulders, or a true umbilical knot increased the risk of spastic quadriplegia 18-fold (odds ratio, 18; 95% confidence interval, 6.2–48).<sup>37</sup> Tight cords may prevent or slow descent of the head into the pelvis in late pregnancy and may cause intermittent ischemia and hypoxia during Braxton

**TABLE 2**  
**Epidemiologic and genetic risk factors for cerebral palsy**

- Preterm delivery
- Coexisting congenital anomaly (maldevelopment)
- Probable genetic causes
- Bacterial and viral intrauterine infection
- Altered fetal inflammatory or thrombophilic response (perinatal stroke)
- Fetal growth restriction
- Higher-order pregnancy, risk greater with monozygosity and in vitro fertilization
- Tight nuchal umbilical cord
- Prolonged shoulder dystocia
- Placental pathology, eg, chorioamnionitis, funisitis, villitis
- Inborn errors of metabolism
- Male:female ratio 1.3:1

MacLennan. Cerebral palsies: new insights and causes. *Am J Obstet Gynecol* 2015.

Hicks contractions prior to labor. Further cord constriction and delay is likely in the second stage of labor, causing acute on chronic hypoxic signs.

### Prolonged shoulder dystocia

This can be difficult to anticipate and manage. If severe it can lead to fractures, stillbirth, or severe acute hypoxia. Predisposing causes to CP must be excluded and arterial cord gases showing a severe metabolic acidosis are needed to infer that this acute sentinel event caused the CP.

### Placental pathology

Chorioamnionitis, funisitis, and in particular necrotizing funisitis all are evidence of infection predating labor, and are associated in all epidemiological studies with an increased risk of CP.<sup>28,29,38</sup> Chronic villitis, large infarcts, fetal thrombotic vasculopathy, and meconium-associated fetal vascular necrosis are all risk factors for subsequent CP or neurological impairment.<sup>39</sup> The increased prevalence of placental and cord abnormalities in children subsequently diagnosed with CP underlines the importance of requesting placental histology and cord arterial gases in all cases where the baby is delivered in poor condition.<sup>13</sup>

### Viral infection in pregnancy

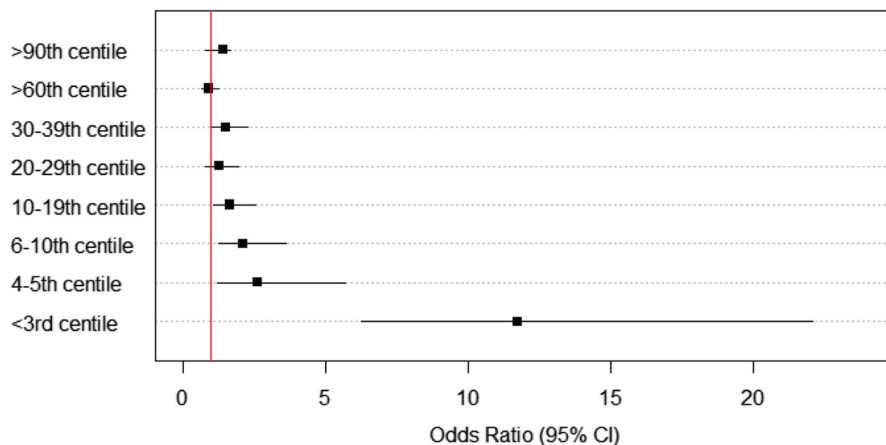
Studies using polymerase chain reaction techniques on neonatal blood spots from CP cases and controls show increased CP risk after both *Cytomegalovirus* and Epstein-Barr virus infections during pregnancy.<sup>40</sup> Epidemiological studies do not associate upper respiratory infections during pregnancy with CP, but some studies have associated increased risk with bacterial urinary tract infections.<sup>26,41</sup>

### Treatable and nontreatable inborn errors of metabolism presenting as CP

A recent systematic review of the world literature has described 67 treatable inborn errors of metabolism (IEMs) belonging to 13 different biochemical categories and >20 currently untreatable IEMs with symptoms that mimic

FIGURE 2

### Cerebral palsy risk increases with severity of fetal growth restriction



Customized birthweight centile and cerebral palsy (CP).<sup>27</sup>

CI, confidence interval.

MacLennan. Cerebral palsies: new insights and causes. *Am J Obstet Gynecol* 2015.

CP.<sup>42</sup> Individually, these inborn errors of metabolism IEMs are all very rare (1:10,000-1:250,000). Together, they may contribute to only 0.1-0.2% of cases of CP, but are important as many can be treated or controlled. New-generation sequencing is likely to facilitate new IEMs that exhibit CP-like symptoms.

### Genetic causes of CP

Genetic causes have long been suspected because of the link with congenital malformations, and increased risk in consanguineous families and monozygotic twins.<sup>43</sup> Although initially candidate gene association studies suggested that several genes may be linked to CP, the power of these studies was low and multiple comparisons weakened their validity.<sup>31,32</sup> A multivariable analysis of 39 candidate genes from single-nucleotide polymorphism association studies with CP was conducted with statistical allowance for type I error. This study did not statistically confirm previous gene associations in CP causation.<sup>44</sup> A recent study showing an association with the apolipoprotein E e3 allele speculated that those with the APOEe2 and APOEe4 alleles were more likely to die in utero.<sup>45</sup>

Previous estimates have suggested that the contribution of genetic variants to the burden of CP was about

2%.<sup>46</sup> With the advent of affordable new-generation DNA sequencing the focus of genetic investigations in CP shifted from gene association studies to the identification of the likely causal variants. Several of the currently known single-gene causes of CP have been identified through study of families with  $\geq 2$  individuals with CP, such as the *KANK1*, *AP4MI*, and *GAD1* gene mutations.<sup>47-50</sup> Until recently, though, only a few singleton cases with CP had been resolved. Cases with autosomal recessive, rare autosomal dominant, or X-linked forms have also been described.<sup>51</sup>

One example of a success of an identification of a novel gene and mutation leading to CP is the *ZC4H2* gene. With the aid of whole exome sequencing (WES) or X-chromosome exome sequencing across a large set of families with different clinical presentations, primarily intellectual disability, mutations have been identified in the zinc-finger gene *ZC4H2* in 5 different families and at least 3 singletons. Interestingly, the clinical presentations of *ZC4H2* gene mutations are broad and variable within and between families, including CP spasticity phenotype and shared comorbidities, namely intellectual disability and seizures.<sup>52</sup> Functional studies of these variants

**TABLE 3**  
**Results of WES in 98 case-parent CP trios**

**Results**

57 of cases had validated genetic variants  
 14% were deemed likely to be pathogenic by strict bioinformatic criteria  
 8 were novel genes in CP  
 5 were known disease genes with CP as a new phenotype  
 Another 44% had variants of lesser bioinformatic priority  
 All of these variants require function tests to refute or help confirm pathogenicity

Putative causative variants in 98 sporadic cerebral palsy (CP) families.<sup>12</sup>

X-Chr, X chromosome; OMIM, Online Mendelian Inheritance in Man register (Johns Hopkins University, Baltimore, MD; <http://www.omim.org/>); WES, whole exome sequencing.

MacLennan. *Cerebral palsies: new insights and causes. Am J Obstet Gynecol* 2015.

using zebrafish model showed that loss of the ZC4H2 protein function caused abnormal swimming and impaired alpha-motoneuron development. In mouse hippocampal neurons, transiently expressed ZC4H2 protein localized to the postsynaptic compartment of excitatory synapses and loss of ZC4H2 function led to reduced dendritic spine density and impaired central and peripheral synaptic plasticity.<sup>52</sup> Such follow-up functional studies are essential to confirm CP pathogenicity and better understand molecular pathways involved and provide explanation for complex and variable clinical presentations.

WES is indeed a powerful tool to identify efficiently the likely causative genetic variant. In particular, sequencing of multiple family members can reduce the number of candidate DNA variants to 1 or 2 and thus also lead to timely and precise diagnosis. This was nicely demonstrated in a family initially diagnosed with a CP-like movement disorder, where 3 members with “hereditary benign chorea” were identified to carry a 7-base pair deletion in exon 1 of the *NKX2-1* gene. The mutation is predicted to lead to a frame shift in protein translation and subsequent premature termination of *NKX2-1*

messenger RNA translation and *NKX2-1* functional haploinsufficiency.<sup>53</sup>

Recent investigations of genetic causes in a large cohort of singleton CP cases using WES shows that the proportion of the cases carrying plausible genetic mutation is much larger than previously thought. At least 14% of nearly 200 singleton cases with CP studied have been found to have a plausible genetic mutation, de novo or inherited<sup>12</sup> (Table 3). A further 44% had candidate variants that are yet to be resolved in regard to their causation of the CP. The percentage of cases with a genetic mutation is likely to rise as larger cohorts are studied, new CP genes are discovered, and whole genome sequencing is routinely performed.

Better resolution of the genetic diagnosis will also be achieved using sophisticated formulae for disease gene and disease DNA variant prioritization such as the Residual Variation Intolerance Score<sup>54</sup> and Combined Annotation-Dependent Depletion,<sup>55</sup> respectively (Figure 3). Contribution of the dosage imbalances or copy number variants (CNVs) is yet to be fully assessed. Ten of 50 singleton CP cases had potentially relevant CNVs in our pilot study.<sup>56</sup> However, all these CNVs were inherited from a healthy parent suggesting another genetic or environmental contributing factor. Such inheritance pattern is not unusual for other neurodevelopmental disorders like intellectual disability or autism. In a new study from Israel, 16 CP cases of 52 cases (31%) of unknown etiology had CNVs that were likely to be pathogenic and 12 of these were de novo.<sup>57</sup> Currently, the combination of 14% of cases with individually likely pathogenic point mutations found by WES and 20-31% with CNVs of interest gives a potential genetic contribution to causation in up to 34-45% of CP cases. This new study also reinforces the conclusions of previous CNV<sup>56</sup> and WES<sup>12</sup> studies suggesting considerable genetic heterogeneity of CP.

Sophisticated functional studies are required to validate genetic findings. A plethora of approaches using in silico tools; animal models like zebrafish, fruit fly, or mice; or stem cells

**FIGURE 3**  
**DNA variant and gene prioritization for pathogenicity**

- Type of variant (i.e. stopgain, splice, missense)  
These are likely to disrupt function.
- In silico prediction of functional effect  
Disrupt normal protein production
- Evolutionary conservation  
Regions of structural or functional importance.
- Haploinsufficiency index When a single functional copy of a gene is insufficient to maintain normal function
- Brain expression pattern RNA disruption in brain
- Known disease association (OMIM)  
Registered on Online Mendelian Inheritance in Man register



OMIM, Online Mendelian Inheritance in Man register (Johns Hopkins University, Baltimore, MD; <http://www.omim.org/>).

MacLennan. *Cerebral palsies: new insights and causes. Am J Obstet Gynecol* 2015.

(ie, patient-derived induced pluripotent stem cells) will greatly assist in these pursuits<sup>58,59</sup> (Figure 4). In this example, the ZC4H2 mutation impairs neuronal plasticity and movement control in the zebrafish model.

It is likely that at least a proportion of the CP cases will be explained by more complex genetics and not just single major gene effect. Our WES study identified likely causative genetic contributions to CP. Variants that may predispose to CP by interacting with environmental triggers have yet to be identified. Predisposing variants would likely be oligogenic or polygenic and thus additive in nature and would require genomewide association studies and gene-environment investigations on a large sample of CP cases and controls. Such variants would increase the risk of a CP phenotype and not be deterministic of CP. Established environmental risk factors for CP, such as IUGR, infection, and prematurity, may interact with predisposing genetic variants and potentiate and multiply the chance of a CP outcome.

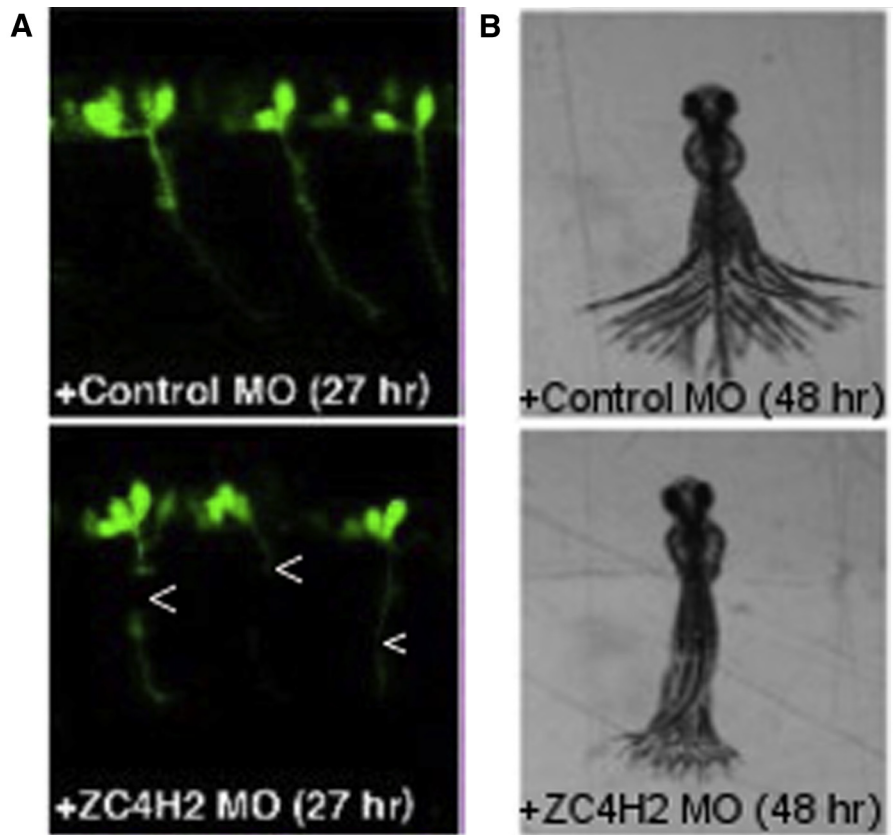
### Male sex

In most epidemiological studies, males are more at risk of CP than females: 1.3:1.<sup>5,7,27</sup> Recessive X-linked chromosome variants may contribute to this difference and males may be more vulnerable to genetic mutation (point or copy number) than females.<sup>60</sup>

### Interventions to prevent CP

Over the last 50 years, CP rates have remained the same despite major advances in obstetrics and neonatology including a 6-fold increase in cesarean delivery rates and liberal induction policies to reduce postmaturity (Figure 1). Currently there are no proven obstetric clinical policies that have been shown to reduce CP in term babies, other than head cooling in selected cases. In cases with neonatal encephalopathy, early neonatal head cooling reduces the risk of death or major neurological disability in 1 of 6 of these selected cases treated.<sup>61</sup> Policies that have been ineffectual include elective cesarean delivery, earlier emergency delivery in pregnancy, and electronic fetal heart rate monitoring.

**FIGURE 4**  
**Zebrafish ZC4H2 knockdown compromises movement and motoneuron development**



**A**, Outgrowth of spinal cord motoneurons and formation of neuromuscular junctions (*arrows*) are morphologically affected in ZC4H2 morphants, but not in controls. **B**, Superimposed images of tail movement at 48 hours postfertilization. Compared to animals injected with control MOs, ZC4H2-knockdown zebrafish (lower) show weak swimming contraction.

MO, morpholino oligonucleotide.

Adapted from Hirata et al.<sup>52</sup>

MacLennan. *Cerebral palsies: new insights and causes*. *Am J Obstet Gynecol* 2015.

Although prospective randomized control trials are not ethically possible, all observational studies show no protective effect of elective or emergency cesarean delivery for CP outcome (as opposed to neonatal encephalopathy, which is a different clinical diagnosis with often different causes).<sup>62</sup> It has not been shown that earlier rapid delivery of a fetus by cesarean delivery, when a compromised fetus is first suspected during labor in an obstetric hospital, changes the risk of a CP outcome. Audits of decision-to-delivery reaction times often do not show that cesarean

delivery can always practically and safely be achieved in <30 minutes or that rapid delivery changes the neurological outcome.<sup>63</sup>

In very preterm infants maternal magnesium sulfate infusion in labor reduces slightly the risk of CP. Numbers needed to treat to prevent 1 case = 63.<sup>64</sup>

### CP classification by causation

There is considerable debate in the international literature whether to remove the diagnosis of CP in nonprogressive cases that fulfill the clinical definition, when a genetic cause is found.<sup>65</sup> Recent

research highlights the importance of rigorous clinical assessment of all cases, with appropriate investigations, to avoid misdiagnosis (eg, progressive disorders) and to identify etiology whenever possible. One useful tool is the Developmental Brain Disorders Database (DBDB: <https://www.dbdb.urmc.rochester.edu/home>), which is a publicly available, online-curated repository of genes, phenotypes, and syndromes associated with neurodevelopmental disorders.<sup>66</sup> This database curates the genes associated with neurodevelopmental phenotypes, assembles ontology of these phenotypes from a number of sources, and develops a system of levels of evidence for gene-phenotype associations.

The CPs share a common clinical sign of nonprogressive dysfunction of posture and movement control but not a common diagnosis. Although there are many CP types, many causes, and now many associated pathogenic genetic variants, it is probably not advisable to change the clinical diagnosis of CP made by specialist pediatricians in cases that fulfill the clinical definition, when a specific cause is later apparent. The diagnosing clinician should give a full description of the type of CP, and any known underlying etiological factors. The clinical diagnosis of CP and its 5 grades of disability define the clinical problem and requirements for care. Removing the label of CP would artificially lower the prevalence of CP in specific CP registries, making historical comparisons difficult and the effect of new preventative interventions such as early detection and/or gene therapy difficult to measure. Also disability pension schemes can be based on the diagnosis of CP and level of disability. Such no-fault insurance pension schemes are efficient, cheaper, and much more equitable for all families with a CP child rather than the current inequitable de facto social welfare system of suing insured maternity services and their staff.<sup>67</sup> Future screening of current CP cases with a panel of known candidate genes proven in function studies or likely to cause CP (targeted gene resequencing) should reduce inappropriate litigation and help to diminish its

deleterious effect on the maternity services.

### Future clinical applications

The long-term goal is the prevention of CP. Targeted screening of parents for inherited causative genes, embryo pre-implantation screening, or antenatal diagnostic DNA techniques in early pregnancy are possibilities in the near future. Gene silencing and gene therapy remain a more distant and exciting prospect in the prevention of some of the CPs.

### Conclusion

The long-held belief that most or many cases of CP are due to trauma or asphyxia around the time of birth and that earlier intervention can prevent the neuropathology is not evidence based, has held back research into other pathways, and has fuelled unwarranted litigation that has had an untoward effect on modern maternity care and maternal outcomes. While it is possible that a severe acute de novo metabolic acidosis could be a rare primary cause of CP, or that intrapartum hypoxia could be a continuing or secondary cause of CP, the great likelihood is that, with the exception of uncommon causes in infancy, the pathways to the neuropathology of CP usually begin well before labor and often in earlier pregnancy. It is now important to consider possible genetic causes that may directly, or through genetic susceptibility, trigger different pathways to different neuropathologies that share the common clinical trait of a nonprogressive movement disorder diagnosed as CP (Video). In the near future, it will be possible to test for many of the putative or validated genes that have been associated with CP to date. This panel of different pathogenic genetic variations contributing to the CP spectrum is very likely to grow over the next decade, and should open a new direction into the causes of CP and challenge previous medicolegal assumptions about the culpability of the accoucheur. ■

### ACKNOWLEDGMENTS

The authors acknowledge the Australian Collaborative Cerebral Palsy Research Group.

### REFERENCES

1. MacLennan AH. A template for defining a causal relation between acute events and cerebral palsy: international consensus statement. *BMJ* 1999;319:1054-9.
2. Hankins GDV, Speer M. Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. *Obstet Gynecol* 2003;102:628-36.
3. American College of Obstetricians and Gynecologists and American Academy of Pediatrics. Neonatal encephalopathy and neurologic outcome. Washington, DC: American College of Obstetricians and Gynecologists; 2014.
4. Watson L, Blair E, Stanley F. Report of the Western Australian Cerebral Palsy Register to birth year 1999. Perth (Australia): Telethon Institute for Child Health Research; 2006.
5. Nelson KB, Ellenberg JH. Antecedents of cerebral palsy, I: univariate analysis of risks. *Am J Dis Child* 1985;139:1031-8.
6. Nelson KB, Ellenberg JH. Antecedents of cerebral palsy: multivariate analysis of risk. *N Engl J Med* 1986;315:81-6.
7. Blair E, Stanley F. Intrapartum asphyxia: a rare cause of cerebral palsy. *J Pediatr* 1988;112:515-9.
8. Strijbis EM, Oudman I, van Essen P, MacLennan AH. Cerebral palsy and the application of the international criteria for acute intrapartum hypoxia. *Obstet Gynecol* 2006;107:1357-65.
9. Badawi NK, Kurinczuk JJ, Keogh JM, et al. Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ* 1998;317:1554-8.
10. Badawi N, Felix JE, Kurinczuk JJ, et al. Cerebral palsy following term newborn encephalopathy: a population-based study. *Dev Med Child Neurol* 2005;47:293-8.
11. Moreno-De-Luca A, Ledbetter DH, Martin CL. Genetic insights into the causes and classification of the cerebral palsies. *Lancet Neurol* 2012;11:283-92.
12. McMichael G, Bainbridge MN, Haan E, et al. Whole-exome sequencing points to considerable genetic heterogeneity of cerebral palsy. *Mol Psychiatry* 2015;20:176-82.
13. Wong L, MacLennan AH. Gathering the evidence: cord gases and placental histology for births with low Apgar scores. *Aust N Z J Obstet Gynaecol* 2011;51:17-21.
14. Page FO, Martin JN, Palmer SM, et al. Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings. *Am J Obstet Gynecol* 1986;154:1306-11.
15. Phelan PP, Korst LM, Martin GI. Application of criteria developed by the task force on neonatal encephalopathy and cerebral palsy to acutely asphyxiated neonates. *Obstet Gynecol* 2011;118:824-30.
16. Alfirevic Z, Devane D, Gyte GML. Continuous cardiotocography (CTG) as a form of electronic fetal monitoring (EFM) for fetal assessment during labor. *Cochrane Database Syst Rev* 2006;3:CD006066.



17. Maternity Services in England. Report by the Comptroller and Auditor General, National Audit Office, Department of Health, United Kingdom. 2013 Summary 1.17. Available at: <http://www.nao.org.uk/wp-content/uploads/2013/11/10259-001-Maternity-Services-Book-1.pdf>. Accessed June 24, 2015.
18. Sartwelle TP, Johnston JC. Cerebral palsy litigation: change course or abandon ship. *J Child Neurol* 2015;30:828-41.
19. Daubert v Merrell Dow Pharmaceuticals Inc. Bloomberg Law. 509 US 579, 113 S Ct 2786, 125 L Ed 2d 469, 1993 US.
20. Stanley F, Blair E, Alberman E. Cerebral palsies: epidemiology and causal pathways, Vol 151. London (United Kingdom): MacKeith Press; 2000.
21. Murphy DJ, Johnson AM, Sellers S, MacKenzie IZ. Case-control study of antenatal and intrapartum risk factors for cerebral palsy in very preterm singleton babies. *Lancet* 1995; 346:1449-54.
22. Oskoui M, Coutinho F, Dykeman J, Jette E, Pringsheim T. An update on the prevalence of cerebral palsy: a systematic review and meta-analysis. *Dev Med Child Neurol* 2013;55: 509-19.
23. Game E, Dolk H, Krageloh-Mann I, Holst Ravn S, Cans C. Cerebral palsy and congenital malformations. *Eur J Paediatr Neurol* 2008;12: 82-8.
24. Blair E, Asedy F, Badawi N, Bower C. Is cerebral palsy associated with other defects other than cerebral defects? *Dev Med Child Neurol* 2007;49:252-8.
25. McIntyre S, Blair E, Badawi N, Keogh J, Nelson K. Antecedents of cerebral palsy and perinatal death in term and late preterm singletons. *Obstet Gynecol* 2013;122:869-77.
26. Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. *JAMA* 1997;278:207-11.
27. O'Callaghan ME, MacLennan AH, Gibson CS, et al. Epidemiologic associations with cerebral palsy. *Obstet Gynecol* 2011;118: 576-82.
28. Wu YW, Escobar GJ, Grether JK, Croen LA, Greene JD, Newman TB. Chorioamnionitis and cerebral palsy in term and near-term infants. *JAMA* 2003;290:2677-84.
29. Yoon BH, Romero R, Park JS, Kim SH, Choi J, Han TR. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. *Am J Obstet Gynecol* 2000;182:675-81.
30. Bashiri A, Burstein E, Mazor M. Cerebral palsy and fetal inflammatory response syndrome: a review. *J Perinat Med* 2006;34: 5-12.
31. Gibson CS, MacLennan AH, Hague WM, et al. for the South Australian Cerebral Palsy Research Group. Associations between inherited thrombophilias, gestational age, and cerebral palsy. *Am J Obstet Gynecol* 2005;193: 1437-43.
32. Gibson CS, MacLennan AH, Goldwater PN, et al. The association between inherited cytokine polymorphisms and cerebral palsy. *Obstet Gynecol* 2006;194:674.e1-11.
33. Blair EM, Nelson KB. Fetal growth restriction and risk of cerebral palsy in singletons born at least 35 weeks gestation. *Am J Obstet Gynecol* 2015;212:520.e1-7.
34. Williams MC, O'Brien WF. Cerebral palsy in infants with asymmetric growth restriction. *Am J Perinatol* 1997;14:211-5.
35. Gardosi J. Customized growth curves. *Clin Obstet Gynecol* 1997;40:715-22.
36. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012;366:1803-13.
37. Nelson KB, Grether KB. Potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight. *Am J Obstet Gynecol* 1998;179:507-13.
38. Craver RD, Baldwin VJ. Necrotizing funisitis. *Obstet Gynecol* 1992;79:64-70.
39. Redline RW. Severe fetal placental vascular lesions in term infants with neurological impairment. *Am J Obstet Gynecol* 2005;192:452-7.
40. McMichael G, MacLennan AH, Gibson G, et al. *Cytomegalovirus* and Epstein-Barr virus may be associated with some cases of cerebral palsy. *J Matern Fetal Neonatal Med* 2012;25: 2078-81.
41. Polivka BJ, Nickel JT, Wilkins JR. Urinary tract infection during pregnancy: a risk factor for cerebral palsy? *J Obstet Gynecol Neonatal Nurs* 1996;26:405-13.
42. Leach EL, Shevell M, Bowden K, Stockler-Ipsiroglu S, van Karnebeek C. Treatable inborn errors of metabolism presenting as cerebral palsy mimics: systematic literature review. *Orphanet J Rare Dis* 2014;9:197.
43. Schaefer GB. Genetics considerations in cerebral palsy. *Pediatr Neurol* 2008;15:21-6.
44. O'Callaghan ME, MacLennan AH, Gibson CS, et al. for the Australian Collaborative Cerebral Palsy Research Group. Fetal and maternal candidate SNP associations with cerebral palsy: a case-control study. *Pediatrics* 2012;129:414-23.
45. Stoknes M, Lien E, Andersen GL, et al. Child apolipoprotein E gene variants and risk of cerebral palsy: estimation from case-parent triads. *Eur J Paediatr Neurol* 2015;19:286-91.
46. Rajab A, Yoo SY, Abdulgail A, et al. An autosomal recessive form of spastic cerebral palsy (CP) with microcephaly and mental retardation. *Am J Med Genet* 2006;140:1504-10.
47. Lerer I, Sagi M, Meiner V, Cohen T, Zlotogora J, Abeliovich D. Deletion of the ANKRD15 gene at 9p24.3 causes parent-of-origin-dependent inheritance of familial cerebral palsy. *Hum Mol Genet* 2005;14: 3911-20.
48. Verkerk AJ, Schot R, Dumeé B, et al. Mutation in the AP4M1 gene provides a model for neuroaxonal injury in cerebral palsy. *Am J Hum Genet* 2009;85:40-52.
49. Lynex CN, Carr IM, Leek JP, et al. Homozygosity for a missense mutation in the 67 kDa isoform of glutamate decarboxylase in a family with autosomal recessive spastic cerebral palsy: parallels with stiff-person syndrome and other movement disorders. *BMC Neurol* 2004;4:20.
50. Hemminki K, Li X, Sundquist K, Sundquist J. High familial risks for cerebral palsy implicate partial heritable etiology. *Paediatr Perinat Epidemiol* 2007;21:235-41.
51. McHale DP, Mitchell S, Bunday S, et al. A gene for autosomal recessive symmetrical spastic cerebral palsy maps to chromosome 2q24-25. *Am J Hum Genet* 1999;64:526-32.
52. Hirata H, Nanda I, van Riesen A, et al. Mutations of *ZC4H2* are associated with intellectual disability and arthrogryposis multiplex congenita through impairment of central and peripheral synaptic plasticity. *Am J Hum Genet* 2013;93: 681-95.
53. McMichael G, Haan E, Gardner A, et al. NKX2-1 mutation in a family diagnosed with ataxic dyskinetic cerebral palsy. *Eur J Med Genet* 2013;56:506-9.
54. Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet* 2013;9:e1003709.
55. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* 2014;46: 310-5.
56. McMichael G, Girirajan S, Moreno-DeLuca A, et al. Rare copy number variation in cerebral palsy. *Eur J Hum Genet* 2013;22: 40-5.
57. Segel R, Ben-Pazi H, Zeligson S, et al. Copy number variations in cryptogenic cerebral palsy. *Neurology* 2015;84:1660-8.
58. Lawlor KT, O'Keefe LV, Samaraweera SE, et al. Double-stranded RNA is pathogenic in *Drosophila* models of expanded repeat neurodegenerative diseases. *Hum Mol Genet* 2011;20:3757-68.
59. Manzini CM, Tambunan DE, Hill RS, et al. Exome sequencing and functional validation in zebrafish identify GTDC2 mutations as a cause of Walker-Warburg syndrome. *Am J Hum Genet* 2012;91:541-7.
60. Jacquemont S, Coe BP, Hersch M, et al. A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. *Am J Hum Genet* 2014;94: 415-25.
61. Shah PS, Ohlsson A, Perlman M. Hypothermia to treat neonatal hypoxic ischemic encephalopathy. *Arch Pediatr Adolesc Med* 2007;161:951-8.
62. O'Callaghan M, MacLennan AH. Cesarean delivery and cerebral palsy: a systematic review and meta-analysis. *Obstet Gynecol* 2013;122: 1169-75.
63. Phelan JP, Ahn MO, Jáuregui I, Phelan SL, Kim C. A timely cesarean decision—incision time does not prevent fetal brain injury. *Am J Obstet Gynecol* 1999;180:S112.
64. Doyle LW, Crowther CA, Middleton P, Marret S, Rouse D. Magnesium sulfate for

women at risk of preterm birth for neuroprotection of the fetus. *Cochrane Database Syst Rev* 2009;1:CD004661.

**65.** The definition and classification of cerebral palsy. *Dev Med Child Neurol* 2007;49:1-44.

**66.** Mirzaa GM, Millen KJ, Barkovich AJ, Dobyns WB, Paciorkowski AR. The

developmental brain disorders database (DBDB): a curated neurogenetics knowledge base with clinical and research applications. *Am J Med Genet A* 2014;164A:1503-11.

**67.** Maclennan AH. A "no-fault" cerebral palsy pension scheme would benefit all

Australians. *Aust N Z J Obstet Gynaecol* 2011;51:479-84.

**68.** Cerebral Palsy Alliance Research Institute. Australian Cerebral Palsy Register. Report 2013. Available at: [https://www.cpregister.com/pubs/pdf/ACPR-Report\\_Web\\_2013.pdf](https://www.cpregister.com/pubs/pdf/ACPR-Report_Web_2013.pdf). Accessed June 24, 2015.