PERINATAL RISK FACTORS FOR CEREBRAL PALSY IN TWIN GESTATIONS

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ABSTRACT

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Objective: This study aims to summarize and evaluate the prenatal risk factors that may contribute to the development of cerebral palsy (CP) in twin gestations.

Study Design: Observational studies examining CP in multiple gestations were identified in PubMed and systematically reviewed. The contribution of perinatal risk factors to CP and outcome discordancy were summarized.

Results: The risk of co-twins both being affected by CP is approximately 10%. Multiples may experience risk factors that are unique to multifetal gestations, including birthweight discordance, twin to twin transfusion (TTTS), and co-twin demise, all of which are more common in monochorionic (MC) and/or monozygotic (MZ) gestations and have been found to increase the risk of CP in at least one co-twin.

Conclusion: It is unclear whether specific risk factors or their co-occurrence contribute to discordancy more or less frequently. Many of the identified CP risk factors that are exclusive to twin gestations may co-occur. This co-occurrence of risk factors has the potential to play a role in which co-twin will ultimately develop CP in discordant sets. Epigenetic research focusing on CP is an emerging field and studies involving twins have the potential to offer insight into the etiology of CP in singletons.

This thesis is dedicated to my sister Joslynn.
Thank you for always inspiring me.

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KEY TO ABBREVIATIONS AND DEFINITIONS

Cerebral Palsy (CP): Lifelong motor and neurological disability due to disturbances in the developing brain.

Singleton: A gestation which results in the delivery of a single child.

Multiple Gestations/Multifetal Pregnancy: Delivery of multiple infants of the same gestation (twins, triplets, quadruplets, etc.).

Discordant: A pair in which the exposure or outcome differs.

Concordant: a pair in which the exposure or outcome is the same.

Gestational Age (GA): The length of a pregnancy, measured since the date of the mothers last menstrual cycle, usually expressed in weeks.

Preterm Birth (PTB): Birth prior to a full gestation of 37 weeks.

Low Birthweight (LBW): Weighing less than 2,500g at birth.

Monochorionic Pregnancy (MC): A twin pregnancy where both fetuses share a placenta.

Dichorionic Pregnancy (DC): A twin pregnancy where each fetus has their own placenta.

Monozygotic Pregnancy (MZ): Identical twins, resulting from one fertilized egg.

Dizygotic Pregnancy (DZ): Non-identical twins, resulting from two fertilized eggs.

Selective Fetal Growth Restriction (sFGR): Unequal sharing of placental space in MC pregnancies affecting distribution of nutrients and oxygen.

Twin to Twin Transfusion Syndrome (TTTS): An imbalance of oxygen and nutrients between co-twins, typically of MC pregnancies.

Periventricular Leukomalacia (PVL): Damage to the brain's white matter which occurs in premature children.

INTRODUCTION

Cerebral palsy (CP) is a broad term that defines lifelong motor and neurological disability which can be attributed to "non-progressive disturbances that occurred in the developing fetal or infant brain" [1]. A reliable diagnosis can be made no earlier than two to three years of age [1]. The disability presents with varying severity and is often accompanied by associated conditions such as cognitive and developmental delays [2]. In the United States, the prevalence of cerebral palsy has shown little variation since it was originally described in the mid 1800's [3]. For every 1,000 births, approximately two to three infants will experience the outcome [4, 5].

Biological pathways to congenital CP are multifactorial. About 90% of CP cases are considered congenital and originate in the perinatal period [4, 6, 7]. Around 10% of CP is post-neonatal or acquired, resulting from infection or trauma in infancy or early childhood where etiology is more readily identifiable [4, 6], and therefore not the focus of etiologic research. In term births, about a quarter of CP cases occur after newborn encephalopathy [8]; fetal stroke and malformation account for up to 5-15%, though the exact percentage is difficult to define given the complexity of the exposure [7, 9]. In preterm births, white matter damage, also known as periventricular leukomalacia (PVL), is common and has been identified in roughly 40% of CP cases [10].

Well-established risk factors associated with CP include birth prior to 37 weeks' gestation, birth weight below 2500 g, male sex, multi-fetal pregnancies, labor and delivery complications, and maternal and fetal infection [3]. Although identified risk factors have contributed to our knowledge of the etiology of CP, and some preventative practices are becoming widespread, it is difficult to find evidence of a decline in CP prevalence in the US, which has no national CP registry [11]. However, declines in CP prevalence over the past few

decades have been reported for Europe and for Australia, among both singletons and twins [12, 13]. This quite possibly reflects improvements in both the rate of preterm birth and in neonatal management of preterm births in the case of multiple gestations, which carry a higher risk of both preterm birth and CP than singletons [12, 13]. A closer examination of risk factors may illuminate how much additional CP risk is due to low gestational age and low birthweight compared to unusual factors exclusive to twins. Co-twins discordant for CP also offer an opportunity to illuminate the separate and overlapping roles of fetal and neonatal exposures that result in different CP outcomes due to similar genetics and perinatal environments.

Recent randomized controlled trials have established the efficacy of perinatal neuroprotective treatments to prevent CP, including head-cooling for encephalopathy in term births and antenatal magnesium sulfate administration during preterm labor [7, 14]. For mothers at risk of preterm delivery, magnesium sulfate reduces the risk of CP by an estimated 30% [7, 15]. While these strategies have the potential to reduce some fraction of CP that occurs in both these higher risk groups, much of CP has no proven interventions and etiology remains obscure.

Multiple gestations have increased in prevalence since 1980 due to advances in obstetric care, in vitro fertilization, and increasing maternal age at pregnancy [16-19]. In 2018, the rate of twin births in the United States was reported to be 32.6 per 1,000 births [20]. This is greater than a 70% increase since 1980 [16]. In addition to the increasing prevalence, knowledge regarding the management of multiples has led to improved outcomes [17-19]. Yet, as many as 12 in 1000 twin births will be affected by CP, a risk at least four times greater than in singletons, and increases to an even greater extent in higher order gestations [5, 17, 18, 21, 22]. These significant associations are partly due to the much greater risk for multifetal births to occur before 37 weeks and for neonates to weigh less than 2500 grams [17, 18, 23]. U.S. data reports that the percentage

of singleton preterm births was 8.24% in 2018 versus 60.32% in twin gestations [20]. The same 2018 data report that the rate of low birthweight (<2500 g) was 6.60% among singletons versus 55.62% in twins [20]. Though twins are disproportionately affected by LBW and PTB, several studies have confirmed that the greatest difference in CP rates for twins versus singletons exists among those of normal birthweight, suggesting that other risk factors aside from preterm birth and low birthweight are also at play [2, 4, 23].

Multiple gestations are often accompanied by unique risk factors that are not typically present in singleton births. These include intrauterine or infant death of one co-twin, twin to twin transfusion syndrome, birthweight discordance, chorionicity, and zygosity [2, 5, 17, 24, 25]. Multifetal pregnancies also experience a high discordance for neurodevelopmental disorders like CP [26]. Though co-twins share much of the same intrauterine and hospital birthing environment, the risk of both twins being affected by CP is approximately 10% [19, 26]. The pathway leading to outcome discordance is poorly understood and suggests that further research into this phenomenon is warranted.

Cerebral palsy research in twin gestations is limited by a relative paucity of existing research and registries. In particular, adequate postnatal information on the CP-free co-twin is sometimes lacking when CP discordancy occurs. Also, most research into CP in the context of multiple gestations compares higher order pregnancies to singletons. This focus is useful in identifying the disparity in risk between the two groups. Here, prospective studies utilizing CP registries are a strong resource. While gestational age and maternal characteristics will be nearly the same among co-twins, delivery and neonatal variables may differ. With respect to delivery characteristics, oxygen deprivation and the infant's position at time of birth may be different among co-twins, and these factors have the potential to contribute to brain injuries [7]. In terms

of neonatal characteristics, cardiovascular complications, seizure occurrence, and newborn infections may differ and contribute to adverse neurodevelopmental outcomes like CP [7]. Additionally, no previous literature has addressed all twin specific risk factors such as TTTS, birthweight discordance, chorionicity, zygosity, and co-twin demise within the same study or in outcome discordant study populations.

Although many neonatal and delivery characteristics have been previously examined in other reviews, risk factors specific to twin gestations and potentially outcome discordancy have not. Therefore, the aim of this paper is to produce a comprehensive literature review of what is currently known regarding etiology of CP in twins and to review selected risk factors that may contribute to CP discordancy. Doing so will provide a resource for future analytical studies to reference, with detailed information on relevant risk factors that should be considered. This may in turn lead to the generation of new hypotheses and provide a framework that guides future work on CP etiology.

METHODS

We used PubMed to identify literature regarding multiple gestations and CP. Given the limited research in the field, restrictions regarding publication year, study design, or geographic location were not implemented. Select search terms were utilized to ensure that all relevant studies were included (keywords: cerebral palsy, twins, discordant, chorionicity, zygosity, TTTS, FGR, neurodevelopmental outcomes, multiple births, genetic, co-twin demise, birthweight discordant). Studies were included based on a singleton or multifetal pregnancy exposure and an outcome of CP or, in certain cases, broadly defined adverse neurodevelopmental outcome. The final selection of studies included empirical analyses from hospital/registry cohorts, literature reviews, and a case report (n = 25). We begin by describing perinatal risk factors in relation to CP in singleton and multifetal gestations to provide context for the review. We then evaluate perinatal risk factors specific to CP risk in multifetal pregnancies.

RESULTS

Birthweight

Several studies suggest that the greatest disparity in CP between multiples and singletons, exists within the normal birthweight (BW) range [2, 4, 23]. A UK based registry of 5,207 twin pairs (n = 64 twin CP cases) conducted by Pharoah and Cooke in 1996 found that although the prevalence of CP generally increased as birthweight decreased, the prevalence of CP in low birthweight (LBW: <2500g) children was not significantly different when comparing twins to singletons [5]. However, the difference in prevalence among those ≥2500g was found to be 2.9 per 1000 infants, with twins being significantly more likely to have CP [5]. The same was found in 6613 children (217 twin/triplet CP cases), in a multicenter European registry by Topp *et al*. in 2004. While there was not a significant difference among LBW singletons and multiples, CP risk was 1.6 times higher among multiples of normal birthweight as compared to singletons in the same birthweight category [18]. Petterson *et al*. 1993, performed an analysis of a population-based cohort in 231,874 Western Australian births, which included 36 twin CP cases. Their findings were consistent with those stated above. CP rates in twins vs singletons <2500g were not significantly different, whereas, normal birthweight twins had a 3.8 times higher rate of CP as compared to normal birthweight singletons [27]. The takeaway message is that while decreasing birthweight is associated with increased risk of CP, multiples and singletons of low birthweight experience a comparable risk of diagnosis in the low birthweight range.

Birthweight within twin pairs may also play a role in CP etiology, as significant weight discordance is associated with an increase in CP risk [17]. Birthweight discordance among co-twins is most commonly a product of growth restriction that affects one member of the twin pair [24]. Though growth restriction also occurs in singletons, only 5-7% of singleton gestations will

experience this risk factor in contrast to 12-47% of twin gestations [28]. Additionally, monochorionic (MC) twins are at increased risk for growth restriction due to their shared placenta [24]. Bonellie *et al*. conducted a cohort study among 451,910 children in a Scottish based register and found that co-twins where at least one had CP had a larger discordance in birthweight compared to CP-free co-twins (14.2% vs 11.3%) [22]. While a 10% BW discordance was associated with 6.77 per 1000 CP cases, a 30% BW discordance was associated with 35 per 1000 cases [22].

Gestational Age

Because birthweight and gestational age are highly correlated, similar findings to those discussed in the previous section have been reported when comparing twin and singleton gestations. In singletons, gestational age is considered to be one of the most prominent risk factors for subsequent CP diagnosis [23]. In an Australian case-control study of 587 cases and 1,154 controls, neonates with CP were five times more likely to be born between 32- and 36-weeks' gestation than born at term [3]. In a comparison of all gestational ages, children with CP were 59.2 times more likely to be born at less than 32 weeks gestation compared to any other gestational age range [3].

However, when singletons and twins are both born prematurely at similar gestational age, they do not usually differ in CP risk. Indeed, Topp *et al*., found that twins were 1.65 times more likely to develop CP compared to singletons when limiting comparisons to infants born at term; however, no differences were observed in the preterm range [18]. Bonellie *et al*., observed similar findings using a Scottish CP register (4759 twins, 442,662 singletons). The 2005 study found that the greatest disparity in CP prevalence was among those ≥37 weeks' gestation, where twins (n = 56 CP cases) were 2.74 times more likely to have CP than singletons [22]. Though

preterm birth is a well cited risk factor for CP, these results suggest CP risk is comparable for twins and singletons until term, at which point the risk of CP is higher among multiples. The conclusion is supported by a recent review by Lorenz [21].

Zygosity

Few studies address the role of zygosity as a risk factor for CP in twin gestations, and those that do infer zygosity based on the presence of sex discordance [23, 27, 29]. Several studies acknowledge that CP-associated risk factors such as fetal demise of co-twin are more common in monozygotic (MZ) twins [4, 29]. Pharoah *et al.* (2002), examined CP rates in 17,188 twin births and estimated that like sex twins had approximately 3 times the risk of CP compared to unlike sex pairs [2]. When co-twin demise occurred, like-sex twins had a CP prevalence of 121.5 per 1000 infants (n = 48) vs 45.2 per 1000 (n = 8) infants in unlike sex pairs [2]. However, when both twins survived infancy, like-sex twins had a CP prevalence of 3.5 per 1000 (n = 44) infants vs 2.6 per 1000 (n = 16) in sex discordant pairs [29]. In short, sex concordance was only significantly associated with an increase in CP risk when co-twin demise occurred [2]. However, comparing CP rates in same sex versus sex discordant twins in which both twins survive, results in a nonsignificant difference in CP rates [27, 30]. The significance of these discrepant findings in unclear because sex discordance/concordance is an inaccurate indicator of zygosity. Indeed only an estimated 30% percent of like sex twins are estimated to be monozygotic [23].

Chorionicity

Chorionicity in twin gestations is a commonly cited risk factor for adverse neurodevelopmental outcomes like CP. Monochorionic (MC) twins have been found to be at increased risk of poor newborn outcomes compared to dichorionic (DC) gestations [25, 26]. Due

to a shared placenta, perinatal complications occur up to 6 times more frequently in MC gestations [24]. Certain outcomes are cited more frequently in MC twins, including adverse health for the surviving twin following co-twin demise [31]. It has been reported that there is a 4-fold increased risk for an adverse neurodevelopmental outcome when MC gestations experience fetal demise of one co-twin compared to DC gestations [16, 21]. MC gestations are by definition monozygotic; however, not all DC gestations are DZ as approximately 1/3 are MZ [23]. Therefore, the lack of zygosity specification in each study is a major limitation in these studies, as it is unclear whether zygosity drives the chorionicity findings.

A risk factor almost exclusively seen in MC gestations is twin to twin transfusion syndrome (TTTS), described as an imbalance of oxygen and nutrients between co-twins, usually as a result of abnormal connection between blood vessels [28]. TTTS is estimated to occur in up to 15% of MC gestations and complications of TTTS include perinatal mortality, premature birth, and adverse neurodevelopmental outcomes [32, 33]. Though prenatal surgical treatment for this condition is now common (amnioreduction; laser surgery), cerebral abnormalities still occur in approximately 6-38% of TTTS cases [33]. CP rates following TTTS have reduced to 3-12% following such intervention, but even with such treatment, infants exposed to TTTS are at increased risk for CP [21, 33]. That said, few studies address CP rates specifically and instead estimate risk for any neurological disability. Additionally, studies regarding neurodevelopmental complications of TTTS sometimes lack the appropriate follow up time to reliably diagnose CP.

Another risk factor that affects MC twins at higher rates than DC twins is selective fetal growth restriction (sFGR) compared to DC gestations [25]. This complication is defined as birthweight discordance of at least 20% between twins [34], and is associated with both perinatal mortality and cerebral abnormalities [34]. In a review by Groene at al., it was reported that sFGR

in the context of MC twins, was associated with a 6-fold increased risk of CP [34]. Additionally, several of the included studies reported that the growth restricted co-twin was more likely to have the neurological impairment [34].

Fetal or Infant Death of Co-twin

Fetal or infant death of a co-twin is a well-established risk factor for CP in twins and it is hypothesized that some CP cases in singletons may potentially be product of unknown death of co-twin in utero [31]. As stated previously, complications following co-twin demise are more common in MC gestations [21]. Among multi-fetal pregnancies, co-twin demise occurs in up to 5% of gestations and is strongly associated with adverse developmental outcomes [28]. Scher *et al*. (2002), found that in a cohort of 1,141,351 children, confirmed co-twin demise was associated with 4-6% of CP cases among twins, with a rate of 4.7 for intrauterine death vs 6.3% for postnatal deaths [17]. Additionally, CP occurred in approximately 5.4% of children (n = 28) following co-twin demise compared to less than 0.5% (n = 115) when both infants survived [17]. Petterson *et al*. also examined the difference in CP rates following confirmed co-twin demise. It was found that the rate of CP in the surviving co-twin was 96.2 per 1000 (n = 5) as compared to 12 per 1000 (n = 30) in pairs where both infants survive [27].

Genetic and Epigenetics

Although CP is not strongly familial [35], it is hypothesized that genetic influences may play a role in the etiology of CP [36-38]. Several studies in singletons have identified genetic contributions to CP etiology [38]. In a review by Lewis *et al*. (2021), it was reported that both alterations of single nucleotides and repetitions in the genome occurred in a significant number of CP cases [38]. For twins, Mohandas *et al*. (2018), performed a study on 15 CP discordant MZ

twin pairs using Australian register-based data [36]. It was revealed that epigenetic modifications in the form of DNA methylation occurred in several genes among the CP positive co-twins [36]. The 33 differentially methylated probes and the 2 differentially methylated regions which were identified are known to be associated with inflammation and cerebral dysfunction [36]. A similar study performed by Jiao *et al*. (2017) in China, further confirmed differences in DNA methylation among 4 MZ CP discordant twin pairs [37]. This study identified, in CP positive co-twins, 190 hypermethylated or hypomethylated genes known to be associated with cerebral dysfunction [37]. It is unclear what these epigenetic differences may be attributed to.

DISCUSSION

Evidence shows that singletons and multiples born preterm or LBW experience comparable risk for CP [2, 4, 5, 18, 21-23, 27]. However, twins born at term and with normal birthweights have higher rates of CP than comparable singletons, suggesting that other risk factors contribute to the etiology of CP in the 40% of twin gestations that reach term age [39], such as neonatal encephalopathy, perinatal stroke, neonatal infection, and being small for gestational age [8]. Though gestational age and birthweight are correlated, it is unclear whether gestational age is driving birthweight findings as preterm birth commonly results in LBW. Most included studies regarding birthweight utilized crude weights as opposed to birthweights adjusted for gestational age. However, Bonellie *et al*. (2005) demonstrated clear gradients of CP risk given growth restriction in twins and singletons, but especially twins [22]. This suggests that size for gestational age may be more important to consider when elucidating CP. Additionally, findings stratified by birthweight categories may be artifacts because the weight of term infants tends to vary more than the weight of premature infants. For this reason, it is likely that there are discrepancies in fetal growth findings and may explain why growth discordance over 30% is associated with the most significant risk. Regardless, birthweight alone cannot account for CP outcome discordancy among multiples.

Zygosity may influence CP risk for twin gestations due to risk factors more common among MZ gestations [4, 29]. However, many studies did not reliably determine the zygosity of twin pairs and used sex-concordance as a proxy [17]. This is a major limitation, as same-sex twins can be either MZ or DZ, leaving room for major error in zygosity classification [17]. Therefore, the reliability of zygosity as a risk factor has not been established and further research is warranted. That said, it is plausible that MZ twins may be more at risk than DZ twins because

they experience higher rates of birthweight discordance, TTTS, and co-twin demise, each of which are associated with CP.

Chorionicity appears to play a role in CP development among twin gestations. This is largely because MC pregnancies may experience more severe complications, such as TTTS, birthweight discordance, and co-twin demise [21, 23, 24, 28]. DC gestations may also experience these complications, however, the literature that exists suggests that adverse neurodevelopmental outcomes occur only 1% of the time among DC pregnancies [21], compared to 20% of MC pregnancies [24]. In the context of co-twin demise, it is theorized that when it occurs in MC gestations, that blood flow to the survivor may become obstructed with material from the demised fetus [31]. Another theory suggests that blood flow continues to the demised fetus, therefore reducing blood flow to the survivor and resulting in hypoxia [31]. DC gestations are less likely to be affected by fetal growth restriction which contributes to birthweight discordance and [40] neurological impairment [40, 41]. However, these finding may be confounded by zygosity, given that MC twins are by definition MZ. Therefore, more research is needed to evaluate the how zygosity may contribute to adverse perinatal outcomes.

TTTS often predisposes twin gestations to complications such as birthweight discordance or co-twin demise. However, even in the absence of said conditions, CP and other adverse neurodevelopmental outcomes have been found to occur [21]. Due to the abnormal flow of blood and oxygen between co-twins caused by TTTS, CP discordancy is common as the condition results in a donor and recipient twin [32]. In the event that both twins are live born, the donor twin is commonly found to be small for gestational age, and recipient survivors have a 0-33% prevalence of adverse neurodevelopmental outcomes such as CP [34]. If only the recipient twin survives birth, co-twin demise places at least an estimated 10-30% increased risk on the

larger/recipient co-twin for adverse neurological outcomes [23, 34, 42]. Both donor and recipient have similar risk for neurological impairment; however, co-twin demise following TTTS is considered to be higher risk and termination of pregnancy is sometimes recommended if in the early stages of gestation [22, 42]. TTTS studies tend to focus on all adverse neurodevelopmental complications of TTTS. Therefore, studies are rarely CP specific.

Both birthweight discordance and co-twin demise also occur independently of TTTS, and the most severe complications occur when discordance is >30% [41]. Non-TTTS studies have conflicting findings on whether the larger or the smaller co-twin is at higher risk for adverse neurodevelopmental outcomes [34]. The underlying reason for the conflicting evidence is whether the study accounted for demise of the co-twin. If both twins survive birth, then the smaller of the two twins is at greater risk for adverse neurodevelopmental outcomes due to the risk associated with low birthweight and preterm birth [34, 41]. However, birthweight discordance is often complicated by fetal demise of one co-twin, and typically it is the smaller twin who dies in utero or during infancy [17, 41]. This therefore leaves the larger surviving twin at risk for complications, such as CP [17, 34]. Additionally, co-twin demise is associated with greater risk for CP than birthweight discordance alone [17, 27].

Studies examining epigenetic changes among MZ CP discordant co-twins offers insight into CP etiology. However, the field is still emerging, leaving few studies with poorly understood findings. Additionally, studies regarding CP epigenetics are limited in sample size which may yield unreliable results. While studies in singleton populations are useful, utilizing twins offers significant control for environmental and genetic confounders. This is especially true in the use of MZ twins, as they are the most similar genetically and in their prenatal environment, allowing them to serve as each other's matched case/control. Mohandes *et al*.

(2018) and Jiao *et al*. (2017), both examined CP discordant MZ twin pairs for epigenetic changes in the form of DNA methylation [36, 37]. Findings indicate that though twins share many of the same genetic and environmental exposures, the twin who developed CP displayed epigenetic changes in genes associated with cerebral dysfunction and inflammation. It is unclear whether brain inflammation precedes or follows the development of CP, but inflammatory processes are commonly implicated in CP etiology [36]. It should be noted, however, that though CP is not strongly familial, the matter at present is largely unsettled.

Overall, many of the identified CP risk factors which are exclusive to twin gestations often co-occur. For example, it is unlikely that zygosity nor chorionicty contribute to CP risk independently. In CP discordant twins, it is unclear whether co-occurrence of risk factors contributes to discordancy more or less frequently. While studies did not specifically address discordancy or which co-twin experiences greater risk, inferences may be made based on risk factor results. Ultimately, more research is needed on the subject and a case-control design with detailed information on both the CP-free and CP-positive co-twins could illuminate risk factors that contribute to outcome discordancy and a better understanding of CP etiology among both multiple gestations and singletons. Unfortunately, a resource with detailed information on discordant co-twins is not readily available and few studies utilizing this design exist in the literature. Those that do often lack appropriate details on relevant variables due to use of caregiver reporting, potentially introducing bias.

There are common limitations among CP studies in general and in CP studies involving twin populations. First and foremost, defining CP is both challenging and has changed over time. Therefore, each study or register may use different CP criteria which may introduce inconsistency in findings across studies. Studies also varied for inclusion of participants.

Additionally, certain studies quantified risk as any adverse neurodevelopmental outcome rather than CP specifically.

When quantifying CP risk in twins, it is important to rerun analyses following the inclusion and exclusion of twins experiencing co-twin demise. Estimates of CP prevalence in twins will be affected by the inclusion or exclusion of pairs experiencing co-twin demise. Excluding such pairs may underestimate CP prevalence in the study sample, since survivors of those pairs are at much increased risk of developing CP [2]. Additionally, by running analyses both including and excluding twin pairs who experience co-twin demise, it will illuminate whether a particular risk factor is still important in CP cases affected by the strong impact of co-twin demise. Finally, many of the included studies used register-based data as this is the most efficient way to compare singleton and twin populations. However, registers are limited in the specific information that they contain relevant to CP twin etiology. For instance, information on co-twin survival is crucial in identifying CP risk among twins. This includes specific information on whether intrauterine or infant co-twin demise occurred. However, register based data does also have strengths, especially when population based where selection bias is unlikely, and that appropriate follow up time exists to accurately diagnose CP cases.

CONCLUSION AND FUTURE DIRECTION

It is unclear why a decline in CP prevalence has been observed in Europe and Australia yet has been unrealized in the Unites States. It has been hypothesized that these declines in other areas of the world are largely reflecting better management of preterm birth and neonatal management [12, 13]. While this may be true, it is unclear whether this decline is driven by a decrease in CP among singletons and multi-fetal gestations or only one of the populations. For this reason, future research stratified by a singleton or multi-fetal status which examines the trends of CP prevalence in populations where a decline has been realized may be beneficial.

Twin gestations are more susceptible than singletons to adverse neurodevelopmental outcomes such as CP partially based on their higher risk for low birthweight and preterm birth. However, the greatest disparity in CP rates among multiples and singletons is among those of normal birthweight and born at full-term gestation. This suggests that other risk factors are contributing to the bulk of CP risk in twin gestations. It is difficult to disentangle the impacts of chorionicity and zygosity on CP risk because they often co-occur with other factors associated with this risk (e.g., TTTS, birthweight discordance, co-twin demise).

The same is true for pregnancies affected by TTTS, because birthweight discordance and co-twin demise are common in this context. Children born to pregnant people affected by these complications often experience adverse neurodevelopmental outcomes, such as CP. Since the risk of both twins being affected by CP is just around 10% [19, 26], the birth outcome of the pregnancy has the potential to give insight on which co-twin is at greater risk. If both twins survive infancy, the smaller twin may be at greater risk for CP. In the event of co-twin demise, the larger twin may be at risk, as the smaller twin more commonly dies in utero. However, it

should again be noted that TTTS studies tend to focus on all neurodevelopmental disturbances and it is unclear how it specifically contributes to CP discordance among twin pairs.

Though MZ twins have similar genetic and environmental exposures, environmental risk factors may disproportionately affect one co-twin leaving them susceptible to epigenetic changes. Further research is warranted in the area to determine what environmental risk factors contribute to the increased risk for epigenetic changes in only one co-twin and if these changes simply reflect inflammation due to brain injury or if the epigenetic changes are contributing to the brain injury itself. Epigenetic research focusing on CP is an emerging field and studies involving twins have the potential to offer insight in the etiology of CP in singletons. Comparing co-twins offers an opportunity to illuminate the separate and overlapping roles of maternal, fetal, and infant exposures that result in a CP outcome in cases/controls with similar genetics and perinatal environments.

Research in CP outcome discordancy in twin gestations is rare; however, it serves as a unique opportunity to develop knowledge of CP etiology in more than just multiples due to the ability to disentangle and illuminate the often-overlapping risk factors, using well matched cases and controls. For this reason, future research should aim to examine each of the mentioned risk factors in CP discordant twin populations. It is also likely that other risk factors are at play aside from TTTS, birthweight discordance, and co-twin demise. For example, fetal and infant infectious exposures, postnatal complications, and newborn medical management may distinguish CP discordant co-twins.

Many of the identified CP risk factors which are exclusive to twin gestations may co-occur. It is unclear whether co-occurrence of risk factors contributes to discordancy more or less

frequently. However, this co-occurrence of risk factors has the potential to play a role in which co-twin may develop CP in discordant sets.

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