COGNITION, LANGUAGE, AND BEHAVIOR IN CHILDREN BORN PREMATURELY, WITH AND WITHOUT WHITE MATTER INJURY

by

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Children born prematurely are at risk for perinatal brain injuries (PBI). Both prematurity and PBI confer risk for neurodevelopmental disabilities. We investigated the linguistic and behavioral characteristics of children born prematurely, with and without perinatal brain injuries, at ages 10-15. In total, 16 children born preterm participated. Eight were born at 30-37 weeks and eight were born earlier than 30 weeks gestation. Six children reportedly had PBI and ten children reported no PBI. Clinical tools were used to measure global language skills, vocabulary, reading and comprehension and grammar. Parent questionnaires were used to assess behavior. Children born after 29 weeks of age scored significantly higher on tests for receptive vocabulary, language memory, reading and passage comprehension than children born before 30 weeks gestation ($p \leq 0.05$). Scores reported for anxiety and depression symptoms were higher for the children born before 30 weeks than scores reported for children born after 29 weeks. The mean scores of children with no PBI were significantly higher for expressive language and passage comprehension than the mean scores of children with PBI. There were no significant behavior differences between the children with and without PBI. There were no differences measured between groups, by PBI or gestational age, for tests of grammar and sentence comprehension, accuracy and reaction time. The results suggest that children with PBI may have difficulty with reading comprehension and expressive language and that gestational age and not PBI specifically, may have a greater effect on linguistics and behavior. Identifying long-term

consequences of prematurity is relevant to public health because it will help a growing population of children at risk for neurodevelopmental disabilities benefit from early intervention.

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PREFACE

Only with the help of family, friends and mentors, both near and far, could I have completed a work such as this. To these people I thank here for their unwavering support.

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1.0 PREMATURITY

The incidence of prematurity is on the rise. Full-term births decreased while total preterm births, defined as delivery before 37 gestational weeks, climbed to 12.3% with the greatest increases affecting preterm births between 34 and 36 gestational weeks (Figure 1) [1, 2].

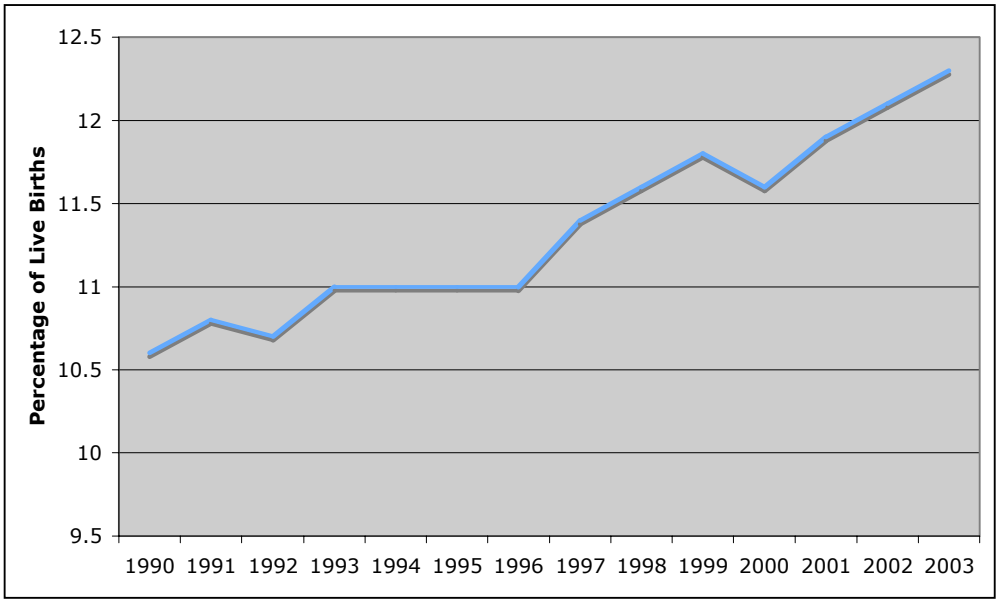


Figure 1. Incidence of preterm birth in the United States (1990-2003)

Terms used to further define prematurity include late preterm, born between 34 and 36 weeks gestation; moderately preterm, born between 32 and 36 weeks; and very preterm, born prior to 32 weeks gestation. In 2006, the March of Dimes announced that the average length of pregnancy was no longer 40 weeks. Over a span of ten years, the average gestational age at birth decreased by one week and is now 39 weeks. The majority of infants born preterm is born at 34

weeks or later and 30% are born prior to 34 weeks gestation (Figure 2). Between groups the most striking change has been an increased incidence of preterm and moderately preterm infants over the span of a decade while very preterm infant incidence remained unchanged.

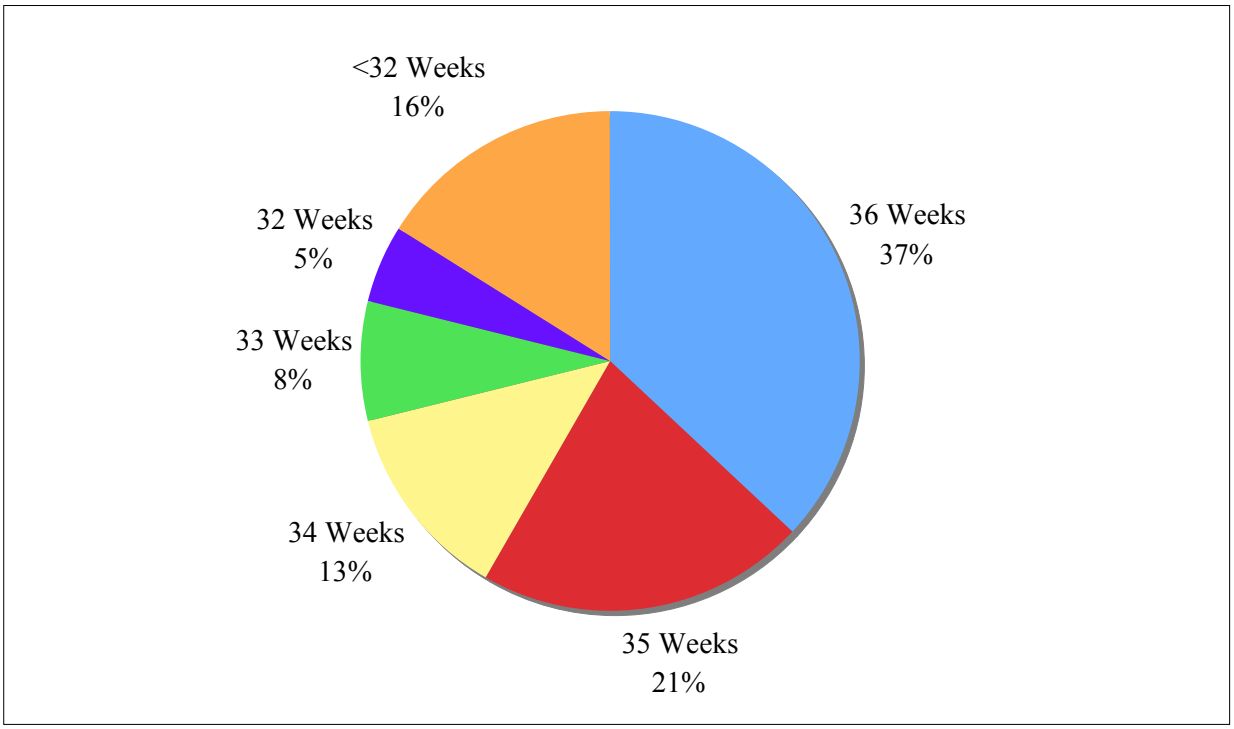


Figure 2. Preterm births in the United States (2003)

Preterm birth emerged as the leading cause of neonatal mortality in the United States during the same time period according to the National Center for Health Statistics [3, 4]. The sobering reality of this trend is that the death rate for preterm infants is fifteen times greater than for full-term infants. A 2006 study published in *Pediatrics* reviewed the leading neonatal mortalities, defined as death before one year of age, in the United States and identified what proportion of each population was born preterm [1]. In fourteen of the top 20 causes of neonatal mortality, at least 50% of the neonates were preterm and in twelve conditions greater than 80% were preterm (Table 1).

Table 1. Top 20 leading causes of neonatal death in the United States[1]

Cause of death	*No. of Infant deaths*	*% With Condition Born Preterm*
Congenital malformations, deformation and chromosomal abnormalities	5630	49.5
Disorders related to short gestation and low birth weight, not elsewhere classified	4636	93.6
Sudden infant death syndrome	2295	23.2
Newborn affected by maternal complications of pregnancy	1704	91.3
Newborn affected by complications of placenta, cord and membranes	1013	87.5
Respiratory distress of newborn	949	94.8
Accidents	940	20.2
Bacterial sepsis of newborn	753	87.1
Diseases of the circulatory system	662	42.3
Intrauterine hypoxia and birth asphyxia	582	54.0
Atelectasis	396	90.4
Neonatal hemorrhage	390	84.9
Necrotizing enterocolitis of newborn	352	94.3
Birth trauma	348	93.8
Chronic respiratory disease originating in the perinatal period	316	93.6
Septicemia	295	67.7
Homicide	289	25.7
Gastritis, duodenitis and non-infective enteritis and colitis	268	80.5
Influenza and pneumonia	260	35.9
Hydrops fetalis not attributable to hemolytic disease	195	87.0
Total	22,273	66.6

Preterm birth leads to neonatal and acute- and chronic medical, developmental and behavioral consequences. We consider these morbidities of prematurity. In the newborn period, infants born at less than 37 weeks are at greatest risk for Respiratory distress syndrome (RDS), white matter disease, necrotizing enterocolitis, patent ductus arteriosus, infection, metabolic abnormalities and nutritional deficiencies. Short-term morbidities commonly the result of

[1] Table adapted from Callaghan, W.M., et al., *The contribution of preterm birth to infant mortality rates in the United States*. Pediatrics, 2006. **118**(4): p. 1566-73.

prematurity include feeding difficulties, trouble with growth, infection, retinopathy, apnea, transient dystonia and neurodevelopmental disabilities. Prematurity can result in a range of long-term morbidities that vary in severity. Children and adults born prematurely may have cerebral palsy, incomplete growth, chronic lung disease or other special health care needs. Many will have sensory deficits, learning disabilities and behavioral problems. Some may have no lasting effects.

The short- and long-term consequences of preterm birth extend into public and personal finance. The costs of prematurity begin with initial healthcare. Infants born prematurely are more likely to have prolonged hospitalization and/or extended care in the neonatal intensive care unit than infants born full-term. Additionally, initial hospitalization costs at birth have an inverse relationship with gestational age at birth [5]. Infants born premature have more frequent acute care visits than full-term infants. The cost to employers and private insurers of infants born prematurely is dramatically increased above the care costs for a full-term infant. Table 2 summarizes the difference in healthcare costs to an employer between full- and preterm infants for the first twelve months of life.

Table 2. Employer costs of prematurity[2]

	Full-term	Preterm	
Hospital Cost	$1,210	$35,034	X29
Physician Office Visits	$1,518	$6,079	
Drug costs	$102	$497	
Total	$2,830	$41,610	X15

Long-term costs rise in association with the long-term morbidities. There may be extra educational costs, public and private, pertaining to special education if learning difficulties develop. Special health care needs can require developmental services such as early intervention, occupational, physical and speech therapy, specialized day care, respite or home care and services such as case management and counseling. So-called "hidden costs" include the potential for loss of income and additional travel and accommodation costs necessary to meet a child's special heath care needs. Not quantifiable are the emotional costs to families and parents of preterm infants. In total, the lower limit cost of care in the United States for preterm infants annually is 26.2 billion dollars [6].

The substantial impact on the United States in terms of mortality, morbidity and finance make preterm birth a national public health concern. The March of Dimes has launched a massive campaign to educate individuals and healthcare professionals about prematurity. Preconception counseling and other healthy pregnancy initiatives addressing risk factors for prematurity have been developed and continuing education programs for health professionals are

[2] Adapted from the March of Dimes Compendium on Preterm Birth. Epidemiology and Biology of Preterm Birth. Data collected from self-insured U.S. employers and analyzed by Thomson Medstat for the March of Dimes.

available free of charge. Focus has also been directed at funding additional research into epidemiologic studies on risk, genetic and environmental contributions, racial and ethnic differences, the role of infection and immune response, the effects of maternal and fetal stress and the development of clinical trials to develop effective treatments for prematurity [7]. One of the biggest steps toward addressing this public health endemic was the successful lobby for legislative support in preventing preterm birth. In 2006 the PREEMIE Act[3] was signed into law with the purpose of increasing federal support for research and education on prematurity [8].

1.1 PREMATURITY AND LOW BIRTHWEIGHT

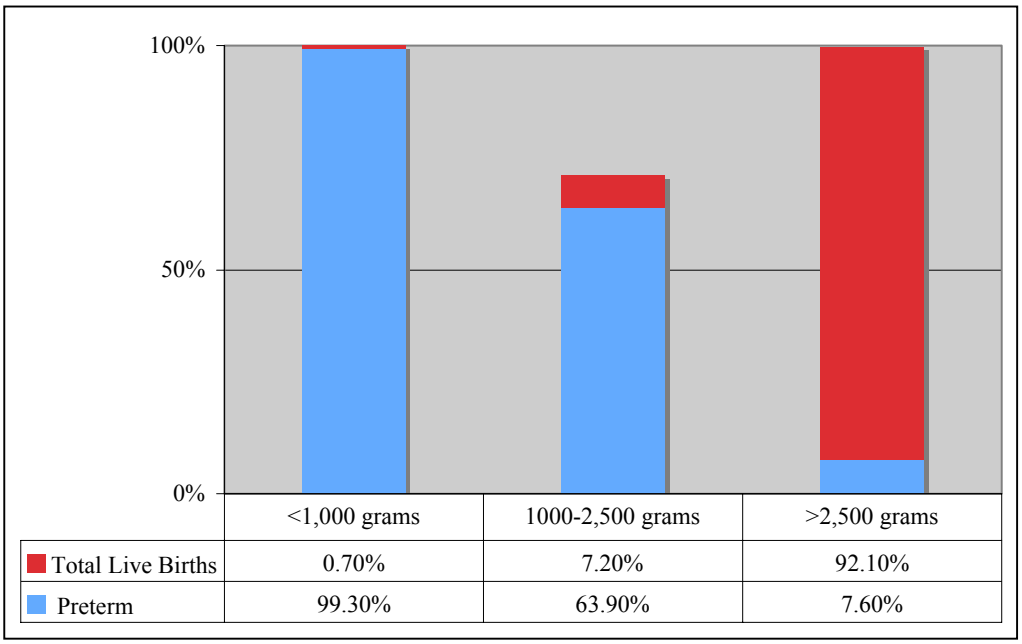
Preterm birth is correlated with low birthweight. Categories defining low birthweight have been established and are listed in Table 3.

Table 3. Categorizing birthweights

Descriptor	Birthweight (grams)
Low birthweight (LBW)	<2,500
Very low birthweight (VLBW)	<1,500
Extremely low birthweight (ELBW)	<1,000

[3] The Preemie Act was sponsored by Senator Lamar Alexander (R-Tennessee) and became Public Law No: 109-450, signed into law on December 22, 2006.

It has long been observed that the incidence of low birthweight corresponds to the incidence of preterm births. Of infants born prematurely, more than 40% are also low birthweight and two-thirds of infants born low birthweight are premature (Figure 3) [9].



	<1,000 grams	1000-2,500 grams	>2,500 grams
Total Live Births	0.70%	7.20%	92.10%
Preterm	99.30%	63.90%	7.60%

Figure 3. Birthweight and gestational age in the United States (2003)

1.2 PREMATURITY: NATURE AND NUTURE

The etiology of preterm birth is largely unknown but several environmental and biological risk factors have been identified associated with an increased risk for preterm birth.

1.2.1 Biological pathways

Biological factors contribute to preterm birth. Lockwood et al. proposed four physiological pathways for organizing the causes of prematurity[10-12]. These pathways account for discrete obstetrical risk factors previously associated with preterm birth, incorporating them within a broader physiological etiology. Accounting for virtually all preterm labor, the pathways include inflammation, activation of the maternal-fetal hypothalamic-pituitary-adrenal axis, decidual hemorrhage and uterine distension.

Inflammation during pregnancy plays a major role in preterm labor and is the first pathway proposed by Lockwood [13]. Accounting for 40% of all preterm labor, the inflammation pathway reflects the important role immunoresistance plays during pregnancy. Common infections such as pneumonia, genitourinary infections, bacterial vaginosis, periodontal disease, peritonitis and sexually transmitted diseases can affect the maternal-fetal environment and can begin a cascade of immunological responses that culminate in preterm labor. Some of these infections are amniochorionic-decidual and some are bacterial.

An infection during pregnancy ignites an inflammatory response. First to respond are the tumor necrosis factor and interleukin-1β cytokines. These cytokines become activated in the cervix, decidual and fetal membranes and result in production of prostaglandin and inhibition of prostaglandin metabolism. Affected next are the membranes that, in the presence of elevated matrix metalloproteinases and interleukin-8, begin to breakdown in conjunction with the ripening of the cervix. Increased prostaglandin can stimulate contractions that, in combination with physical changes to the cervix can result in preterm labor.

The second pathway, responsible for 30% of preterm labor, is activation of the maternal-fetal hypothalamic-pituitary-adrenal (HPA) axis. This pathway essentially describes the

physiological element that stress can have on a pregnancy [14, 15]. Both fetal and maternal stress can activate a neuroendocrine pathway that begins with the release of corticotropin-releasing hormone (CRH). Commonly associated maternal stressors include domestic violence and racism, while fetal stress is most often physiological in nature, for example, disrupted placental blood flow. These stressors result in increased levels of maternal and fetal adrenal cortisol that induce production of prostaglandin. A secondary effect of increased corticotropin-releasing hormone is increased production of estrogen. Just as inflammation incites physical changes in the cervix and contractions, the increased production of prostaglandin and estrogen also accumulate and can trigger preterm labor.

A third pathway combines decidual hemorrhage and placental abruption, two physiological factors that can result in preterm labor. Vaginal bleeding is a primary indication of decidual hemorrhage, intricately related to coagulation and clot formation. Decidual hemorrhage, seen in 20% of preterm deliveries, starts a chain of biochemical reactions involving coagulation and production of tissue factor, thrombin and proteases. These elements can lead to cervical changes, contractions and membrane rupture. Maternal factors such as marital status, education and age affect risk for decidual hemorrhage. Placental abruption describes when the placenta tears from the uterine wall, resulting in hemorrhage. Most often placental abruption leads to premature rupture of membranes. As with decidual hemorrhage, placental abruption commonly involves vaginal bleeding. Unlike decidual hemorrhage however, placental abruption is most commonly associated with maternal, behavioral risk factors such as smoking, use of cocaine, preeclampsia, and maternal trauma. Intrauterine growth retardation and certain bleeding disorders can also increase risk for placental abruption.

The fourth pathway is abnormal uterine distension and is responsible for 10% of preterm labor. This mechanism involves a physical stretching of the uterus that, with multiple other mechanisms of labor, initiates a cascade of signaling that results in production of cytokines. The stretching process begins with an increase in myometrial gap junctions that lead to activation of oxytocin receptors and subsequent prostaglandin synthesis that in turn, results in cervical changes, rupturing of membranes and contractions. This pathway to preterm labor is most commonly associated with multifetal pregnancies and polyhydramnios, conditions involving excessive intrauterine volume. Abnormalities affecting the structure of the uterus also increase risk for abnormal uterine distension, including bicornuate or T-shaped uterus.

All four pathways culminate in a final, shared pathway that results in *preterm* premature rupture of membranes before labor has begun. While premature rupture of membranes (PROM) is estimated to occur in 10% of pregnancies, only 10% are preterm premature rupture of membranes (PPROM), PROM before 37 weeks gestation. The mechanism for PROM ultimately involves increases in uterotonins that initiate contractions, prostaglandins, endothelin and oxytocin. Uterotonins also trigger expression of matrix metalloproteinases, plasminogen activator, plasmin and elastase, proteases that result in membrane rupture, cervical changes and eventual cervical dilation.

Obstetrical management of preterm labor varies widely. Labor is typically induced if PROM is diagnosed after 36 weeks gestation. In cases of PPROM, decisions regarding induction of labor depend on determination of fetal lung maturity and fetal heart rate. If induction is inappropriate due to pulmonary immaturity or problematic fetal heart rate testing, management usually involves attempts to prolong labor for as long as possible accompanied by monitoring for fetal distress and infection. Interestingly, despite the common incidence of PROM, management

strategies are inconsistent among health care providers. The use of nonstress tests versus biophysical profiles and frequency of fetal monitoring is actively debated. Also controversial is whether certain biomarkers should be used to increase early diagnosis of PROM. Also debated is the benefit of inpatient versus outpatient management [16].

Other biological risk factors of preterm birth have been identified [2, 17, 18]. Diabetes, obesity and hypertension are associated with increased risk for preterm delivery. Obesity is a well-known risk factor for diabetes and hypertension and often during pregnancy these conditions present simultaneously. Preeclampsia is also a common complication of maternal obesity during pregnancy. A study by Hedderson, Ferrara and Sacks found that diabetes in pregnancy, particularly elevated glycemia, increases risk for spontaneous preterm labor [19]. Epidemiological research conducted by Rosenberg et al. looked at maternal diabetes, preeclampsia and hypertension during pregnancy and associated adverse pregnancy outcomes [20]. The greatest risk factor for preterm birth was preeclampsia, with an adjusted odds ratio of 5.07 overall. Women with diabetes or hypertension were 2.54 and 2.34 times more likely to deliver preterm, respectively. Despite the role obesity plays in hypertension, diabetes and preeclampsia, it was low maternal weight, or being underweight, that was directly related to increased risk of preterm delivery. Obesity alone was found to be protective, with an adjusted odds ratio of 0.54. A short pregnancy interval has also been established as a risk factor for prematurity [21-24].

Preterm birth is associated with birth defects. Shaw et al. [25] conducted a large study utilizing data from the California Birth Defects Monitoring Program population registry and found that structural birth defects were associated with preterm birth. Birth defects were reported in 8.4% of deliveries at less than 31 weeks gestation while only 2.1% of full-term births

were associated with birth defects. According to the same study, the birth defects most commonly associated with preterm birth before 31 weeks, isolated or with occurring with other birth defects, were anencephaly, spina bifida, other nervous system and eye anomalies, heart, respiratory and musculoskeletal anomalies. The prevalence ratio of preterm birth before 31 weeks versus after 36 weeks was 13.8 for isolated chromosomal abnormalities. In some cases of birth defects diagnosed prenatally, induction of early labor may be planned and contribute to the prevalence of preterm births. However, this proportion is thought to be small, with the particular birth defects thought to be intricately responsible for onset of preterm labor.

1.2.2 Environmental risk factors

Several environmental factors play a role in preterm birth. Though multiple environmental associations have been identified the mechanisms have not. Less understood is how directly an affect they have on preterm labor. It is likely that multiple environmental elements impact the physiological pathways through complex interactions. Due to the intricate nature of unraveling such nuanced interactions, environmental risk factors for preterm delivery have been proposed independently based, most often, on epidemiological studies. Environmental risk factors are difficult to define due to their often diffuse and ubiquitous nature. In this analysis, non-medical risk factors will be categorized as either demographic or behavioral and environmental [18].

Preterm birth is associated with maternal age at delivery [17, 26]. Mothers younger than 19 are at an increased risk for preterm birth compared to women who deliver between the ages of 20-35, who have the lowest incidence of preterm birth. Women who deliver at age 35 or older are at an increased risk for preterm birth, similarly to young mothers. For more than a decade,

however, the proportion of teen pregnancies has decreased while incidence of preterm birth continues to climb. The recent rise in preterm birth rates are likely attributed in small part to more women having children in their mid –thirties and forties than in previous years. To compound this trend, women of advanced maternal age are more likely to become pregnant with multiples than women in their twenties and early thirties. In twin pregnancies, 50% of deliveries are preterm while the risk of prematurity for triplets is 90%.

Other significant demographic risk factors are race and socioeconomic status[17, 18]. Women who are black have higher rates of preterm birth than women who are white, Asian or Pacific Islander[27]. Women who have an adverse rather than a favorable socioeconomic profile have higher rates of prematurity. However, it is unclear whether it is the demographic or a culmination of several behavioral and environmental risk factors prevalent among these high-risk groups that contribute to the high rates of preterm birth.

Many important behavioral and environmental factors play an important role in preterm birth. Women who smoke, use alcohol or illicit drugs or use these substances in combination during pregnancy are at greater risk for preterm birth than women who abstain from use of these substances [17, 18, 28-31]. Also impacting risk for prematurity are poor or absent prenatal care, domestic violence, lack of social support, certain work environments and lack of social support. A woman who is physically abused has a higher risk of prematurity than a woman who is not abused [32]. As with many environmental and behavioral factors, there is an interaction between this risk factor, abuse, and smoking, alcohol consumption and illicit drug use during pregnancy. Results from a study by McFarlane et al. found that women who are abused during pregnancy are more likely to smoke, drink alcohol and use illicit drugs during pregnancy (Table 4) [33].

Table 4. Percent of pregnant women reporting substance use and physical abuse[4]

	Physical Abuse	Smoking	Alcohol/Illicit drug use
Overall	16	29.5	11.9
White and African American	No Abuse		20.8
	Abuse		42.1
White	No abuse	46.6	
	Abuse	59.6	
African American	No Abuse	33.7	
	Abuse	49.50%	

Women with late, limited or no access to prenatal care have high rates of prematurity as do women who have few or no social supports [34, 35]. The experience of stress during pregnancy has also been implicated in higher rates of prematurity [36, 37]. Not unlike the other behavioral and environmental risk factors, stress during pregnancy is complicated by another factor, the maternal HPA pathway to preterm labor. Also interacting with stress and the HPA pathway are demographic factors. Certain stressors such as racism are prevalent for non-white women and compound the already high risk of prematurity for women that are black who may or may not have a favorable socioeconomic demographic.

Prematurity is multifactorial. Madan et al. looked at rates of prematurity within same ethnic groups but with different socioeconomic demographics [38]. Prematurity rates increased among Mexican women with an improved socioeconomic status as compared to Mexican women with an adverse socioeconomic profile. Interestingly, the same effect was not observed when looking at variation of prematurity rates among Asian-Indian women of discordant

[4] Table constructed from results of McFarlane, J., B. Parker, and K. Soeken, *Physical abuse, smoking, and substance use during pregnancy: prevalence, interrelationships, and effects on birth weight.* J Obstet Gynecol Neonatal Nurs, 1996. **25**(4): p. 313-20.

socioeconomic profiles. This research suggests that while we see disparity in rates of preterm labor across racial and ethnic groups and by socioeconomics that additional, undefined factors may be contributing to these trends.

A major factor in the rising rates of prematurity has been the growing incidence of pregnancies conceived with assisted reproductive technology (ART). The reason for this is that both *in vitro* fertilization (IVF) and ovarian stimulation result in multiples at a much higher frequency than natural conception. In vitro fertilization is the process of removing ova from the ovaries of a woman in order to fertilize them with sperm outside of the body. Once fertilization has occurred, the newly formed embryo is transferred to the uterus where it implants and pregnancy results. Higher rates of multiples, most commonly twins, are the result of IVF because often several embryos are transferred to the uterus to maximize pregnancy rates. Ovarian stimulation is a technique that involves the use of medications such as Clomid, Gonal-F/Follistim AQ, Metformin, Bravelle, Menopur and Repronex to stimulate ovulation. The process often results in overproduction of mature eggs that can subsequently be fertilized, resulting in pregnancy of multiples. The conception of multiples using ART confers, at a minimum, the same risk for prematurity as multiples conceived naturally (Table 5) [39].

Table 5. Percentage of infants born by ART in the US and preterm ART rates, by plurality (2003)[5]

Pregnancy outcomes by plurality conceived using ART	% of total US Births	% of ART multiples born preterm
Singletons	0.6	14.7
Twins	16.4	64
Triplets or higher-order multiples	44.2	97

[5] Table adapted from Wright, V.C., et al., *Assisted reproductive technology surveillance--United States, 2003*. MMWR Surveill Summ, 2006. **55**(4): p. 1-22

The Center for Disease control reported that the ratio of higher-order multiples (triplets or more) resulting from ART more than quadrupled between 1980 and 1997 [40]. The exponential increase in higher-order multiples has no doubt made an impact on the rates of prematurity and the associated mortality and morbidity rates in the United States.

1.3 GENETICS AND PREMATURITY

The risk for preterm birth is highest among women with a previous history of prematurity. A woman with a history of preterm delivery is four times more likely to deliver preterm in subsequent pregnancies than if she had no history [41-43]. Studies investigating the familial implications for risk of preterm delivery support the presence of genetic risk factors. A study by Porter et al. found that not only was risk for preterm birth higher if the mother herself was born prematurely, but that the risk was inversely related to how preterm she was born [44]. Johnstone and Inglis found that sisters of women who had a preterm birth had a higher rate of preterm birth than sister-in-laws [45]. Twin studies have also shown that pair correlation of preterm birth is higher among monozygotic than dizygotic twins [46-48]. Differences in preterm birth incidence by race further supports a genetic element to prematurity. Previously discussed were several factors affecting risk for prematurity that also varied across race, such as socioeconomic status and hypertension. Despite these potential confounders, several studies correcting for such factors found a persistent difference in incidence between African Americans, Mexican Americans and Caucasians [49-53]. Although the genetics of prematurity

are currently not well understood, the effect medical and family history has on risk is indicative of a genetic role in preterm birth.

Research investigating the genetics of prematurity has focused primarily on inflammatory response mediators [54-58]. In particular, human polymorphism association studies of cytokines have been conducted. Discussed above, cytokines play an important role in the cascade of events that culminate in initiation of delivery, particularly in but not limited to the inflammatory pathway [56]. The most compelling studies to date have investigated tumor necrosis factor alpha, or TNF-α, a factor involved primarily in pregnancy maintenance and prolongation. TNF-α has been found to play roles in placental implantation and development, amniotic membrane growth and remodeling and downstream regulation of stimulating prostaglandin production and matrix metalloproteinase-mediated membrane degradation. These changes ultimately damage the placenta and membranes and often result in preterm labor [59-64]. Table 6 summarizes polymorphisms in TNF-α and other factors suspected to have a pathological effect related to preterm birth.

Table 6. Polymorphisms associated with prematurity[6]

Polymorphisms	*Pathologic effect*
TNF (-308A)	Increased risk for PPROM; preterm birth [57, 65]
	Children in Kenya born preterm more likely to be homozygous [66]
TNF (-488)	Increase risk of spontaneous preterm birth [66]
TNF2	Markedly increased risk of preterm birth in conjunction with vaginosis [58]
IL-1RA*2	Increased risk of preterm birth and neonatal mortality in twin gestations [67]
	Fetal homozygosity associated with increased risk of preterm birth [68]
IL-4 (-590T)	Risk of preterm birth in homo- and heterozygotes [69]
IL-6 (-174C)	Homozygosity associated with reduced risk of preterm birth [70]
VEGF (936C/T)	Associated with preterm birth [71]
Toll-like receptor 4 (299Gly)	Associated with increased risk of premature birth [72]
MMP-9	Associated with PPROM in African Americans [73]

Interleukin (IL) and matrix metalloproteinase (MMP) mediators are involved in the breakdown of membranes and ripening of the cervix that can lead to preterm labor. Several studies have been done that looked at IL-1, 4, and 6 and MMP-1, 8 and 9 for links to preterm birth risk and research continues in this area [74-76]. More recent focus has been directed at methylenetetrahydrofolate reductase enzyme (MTHFR) and Factors V, VII and VIII with the suspicion that there will be an association between inherited thrombophilias and preterm birth [77-79]. Investigation of altered genes responsible for vasodilation and regulation of placental blood flow such as α1- and β2-adrenergic receptor and human paraoxonase enzyme (PON) is ongoing [80, 81]. Future research into the genetic etiology of preterm birth will rely heavily on large population studies using microarray technology and bioinformatics to unravel the complex gene-gene and gene-environment interactions characteristic of prematurity [51, 64, 82, 83].

[6] Table based on the review DeFranco, E., K. Teramo, and L. Muglia, *Genetic influences on preterm birth.* Semin Reprod Med, 2007. **25**(1): p. 40-51.

2.0 WHITE MATTER

Preterm birth interrupts a critical period in development of white matter. The central nervous system begins to form early in embryology. Certain structures of the human brain continue to develop after birth and into child- and adulthood. This extensive interval of growth exposes the brain to a variety of time-dependent insults. White matter in the brain undergoes rapid development in the perinatal and infant period. Infants born prematurely have a higher incidence of perinatal brain injury, in particular, white matter disease. In subsequent sections I will review white matter development, disease and methods of assessing white matter disease.

2.1 WHITE MATTER DEFINITIONS

The telencephalon is the region of the brain that, in humans, is made up of the limbic system, basal ganglia, olfactory bulb and the cerebral cortex. Located just above the brainstem, the telencephalon, or cerebrum, is composed of white matter and grey matter. Grey matter is composed primarily of unmyelinated cells such as nerve cell bodies and glial (non-nerve) cells. Structures of grey matter throughout the brain include the cerebral cortex, subcortical nuclei and nuclei of the cerebellum, brainstem, spinal white matter and cranial nerves. Information in the form or sensory or motor impulses are processed in these grey matter structures and routed throughout the central nervous center.

White matter is the elaborate highway of fibers connecting the many regions of grey matter. White matter is composed of nerve cells that transmit information throughout the brain. Myelin is a phospholipid characteristic of white matter nerve cells. Wrapped around axons, the myelin functions to insulate the electrical charge of a neuron. Myelin covers the surface of axons leaving spaces called "Nodes of Ranvier.[7]" Neural impulses propagate from node to node, jumping between sheaths of myelin along the length of the axon. As a result, propagation of impulses travel faster along the myelinated fibers of white matter than unmyelinated fibers. The wealth of sensory and motor impulses processed in structures of grey matter is transmitted quickly along myelinated nerve cells of white matter throughout the central nervous system [84-87].

2.2 WHITE MATTER DEVELOPMENT

In a developing embryo the nervous system forms out of the ectoderm, one of three germ layers that give rise to all of the tissues, organs and systems in the human body. Formation of the spinal cord, nerve fibers and brain begins during the third and fourth weeks of development, a period of development that is called neurulation. During neurulation the neural plate forms and folds upon itself to form the neural tube. Further differentiation of the neural tube eventually leads to the central nervous system (CNS). The posterior segment of the neural tube becomes the spinal cord and the anterior segments become the forebrain, midbrain and hindbrain [84-87].

[7] Louis-Antoine Ranvier discovered myelin in 1878.

The development of the spinal cord originates from the neuroepithelium that composes the walls of the neural tube. Neuroepithelial cells proliferate rapidly and migrate to produce three layers called the ventricular, mantle and marginal zones. The ventricular zone is a mitotically active layer that eventually gives rise to all of the neurons and macroglia (astrocytes and oligodendrocytes) of the CNS. The mantle zone is a densely packed layer of neuroblasts. The neuroblasts are originally bipolar neurons (having one axon and one dendrite) that differentiate into multipolar neurons (having one axon and many dendrites) that associate in specific nuclei and ultimately become the gray matter. The marginal zone is predominantly cell processes of the neuroblasts of the mantle zone that becomes white matter. Proliferation and subsequent differentiation of the mantle zone result in a dorsal alar plate and ventral basal plate [84-87].

The basal plate is the motor region of the spinal cord. Neurons begin to develop in the basal plate that eventually aggregate in bundles and become the ventral roots of spinal nerves. Neural crest cells adjacent to the neural tube cluster into sensory neurons to form dorsal root ganglia. Bipolar neuroblasts produce dorsal root ganglion cells that grow into the alar plate forming the dorsal root (sensory region) of the spinal nerve and the mixed spinal nerve. These two processes combine to form pseudounipolar neurons that then divided into the dorsal ramus and ventral ramus that together supply the musculature and skin of the body. The marginal layer becomes the ascending and descending tracts (white matter) of the spinal cord and myelination of the glioblast-derived oligodendrocytes and neural crest –derived Schwann cells begins [84-87].

While the spinal cord and nerve fibers are forming, the brain is also developing. Three brain vesicles form from the anterior neural tube called the prosencephalon, mesencephalon and

rhombencephalon. The prosencephalon correlates with the forebrain, the mesencephalon with the midbrain and the rhombencephalon the hindbrain. After the fourth week of development the prosencephalon gives rise to the telencephalon and diencephalon. The rhombencephalon gives rise to the metencephalon and myelencephalon, dividing the hindbrain. These five vesicles form the principle regions of the brain. The myelencephalon forms the medulla oblongata and the metencephalon forms the pons cerebellum, and the fourth ventricle. The midbrain cerebral aqueduct forms from the mesencephalon and the thalamus, hypothalamus and third ventricle form from the diencephalons. The telencephalon results in the cerebral hemispheres and lateral ventricles [84-87].

As the structures of the brain continue to grow and neurons continue to differentiate, the tracts of the white matter spread throughout the brain. Major white matter tracts in the brain start out mostly unmyelinated but undergo significant myelination after birth. It is the elaborate network of myelinated white matter that facilitates rapid transfer of signaling throughout the brain that enables higher order neurodevelopmental processes such as language, speech and behavior. Figure 4 demonstrates the development of the corpus callosum, a well-studied major white matter tract, starting in the neonatal period and persisting into adulthood. From magnetic resonance imaging studies we have learned that gray matter is more developed prior to birth than white matter and that after birth, the proportion of white matter to gray matter increases [84-87].

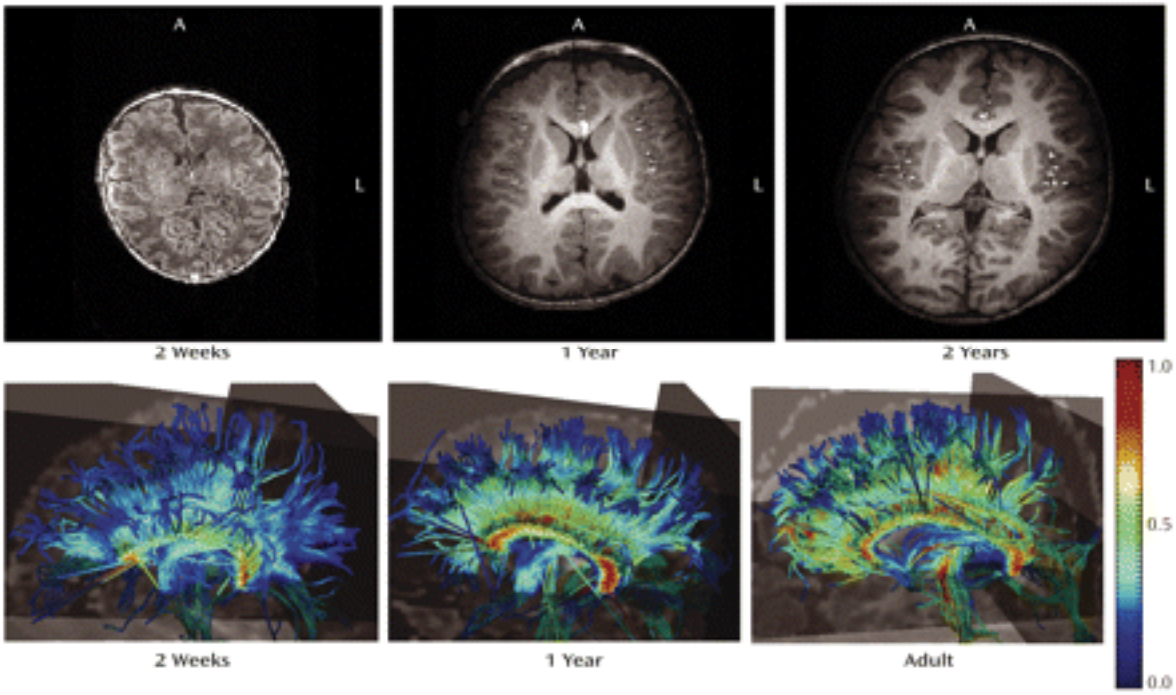


Figure 4. MR imaging of corpus callosum development[8]

In the top row of longitudinal T1-weighted magnetic resonance images of the same child (and same scale), note the dramatic increase in total brain size as well as in white matter intensity over early development. In the bottom row of diffusion tensor images, white matter tractography of a neonate, one year old, and adult shows the organization of corpus callosum white matter fibers, reflected in increasing fractional anisotropy (yellows, reds), developing with age.

Specific to the corpus callosum, we have learned that formation begins in the seventh week of gestation with a general thickening that becomes grooved and eventually closes. Axons continue to grow and cross the midline, connecting the two halves of the brain. The corpus callosum develops anteriorally to posteriorally except for the rostrum which develops last, a process that is complete around 20 weeks gestation. Even by fullterm, however, the corpus callosum is not yet mature. Looking again to Figure 4 it is clear that the corpus callosum

[8] Image from Gilmore, J.H., W. Lin, and G. Gerig, *Fetal and neonatal brain development.* Am J Psychiatry, 2006. **163**(12): p. 2046. Used with permission from the author.

continues to develop myelinated fibers into childhood, a structure vulnerable during the perinatal period and likely to be affected by preterm birth [88-90].

The development of the corpus callosum is consistent with development of other white matter structures. Generally, white matter is understood to develop from the core to the periphery and from the anterior to the posterior. White matter myelination of the central nervous system typically occurs in the sensory systems before occurring in the motor systems. Within the cerebral hemispheres, myelination of structures responsible for higher-order sensory and associative functions extends into adulthood [84]. Continued myelination following birth was evidenced in a study by Brody et al. that examined myelin in 162 autopsied infants having diverse disease at different ages [91]. Table 7 reviews the median age when mature myelin is reached for major white matter tracts in the brain based on data from this study.

Table 7. Median age when mature myelin is reached[9]

Brain region	Posterior fronto-parieto-occipital sites	Age (wks)	Anterior frontotemporal sites	Age (wks)
Internal capsule	Posterior limb	4	Anterior limb	47
Sensory radiation	Optic radiation	12	Heschl's gyrus	48
Corpus callosum	Body	20	Rostrum	47
	Splenium	25		
Central white matter	Precentral gyrus	30	Temporal lobe	79
	Posterior frontal	40	Temporal pole	82
	Posterior parietal	59	Frontal pole	79
	Occipital pole	47		

A recent study by Huang et al. chronicled typical white and gray matter development at 19-20 gestational weeks, birth and 5-6 years using diffusion tensor imaging (DTI) [92]. Overall,

[9] Table adapted from Volpe, J.J., *Neurology of the Newborn*. Fourth ed. 2001, Philadelphia: W.B. Saunders Company.

the brain increased in volume by almost 17 times between the 19-20 week and fullterm periods of gestation. From birth to 5-6 years there was a 3-4 times increase in volume. They were able to quantify the changes in white matter tract development over time by calculating the area of the structure at three different stages of development with the oldest stage (5-6 years old) representing 100% of total development. Table 8 below summarizes their findings for major white matter tracts in the brain.

Table 8. Major white matter tracts at different stages of development as a percentage of total area[10]

White matter tract	*Tract function*	*19/20 wks GA*	*Neonate*	*5/6 yrs*
Corpus callosum	Connects the left and right hemispheres	4.72%	48.85%	100%
Cingulum	Limbic system communication	10.13	63.17	100
Uncinate fasciculus	Unites the frontal and temporal lobes	8.27	64.92	100
Sagittal stratum	Contains association fibers	4.67	44.7	100
Internal capsule (anterior)	Sensory fibers connecting the cerebral cortex with the brainstem	8.46	57.99	100
Internal capsule (posterior)		4.86	46.27	100
Superior region of the corona radiata	Descending sheet of fibers involved in communicating movement	5.34	43.26	100
Superior longitudinal fasciculus	Connects the front and back of the cerebrum	0.00	19.01	100

Most of the major white matter tracts including association and projection fibers were developed enough to identify at 19-20 weeks gestation, though it is interesting that the superior longitudinal fasciculus was not detectable. By birth most of the major tracts were about 50% developed except, again, for the superior longitudinal fasciculus that by the same time, has only completed 20% of total development. This may be an important factor in considering

[10] Image adapted from Huang, H., et al., *White and gray matter development in human fetal, newborn and pediatric brains.* Neuroimage, 2006. **33**(1): p. 27-38.

susceptibility to white matter disease due to prematurity. With a shorter gestational period to develop, white matter typically developing late in the third trimester, such as the superior longitudinal fasciculus, may be more fragile than white matter tracts that began forming before 20 weeks.

2.3 WHITE MATTER DISEASE IN PRETERM INFANTS

Preterm birth disrupts this critical period of white matter development. As a result, children born prematurely are at risk for perinatal brain injuries, particularly white matter disease. Furthermore, the risk for white matter disease confers subsequent risk for neurodevelopmental disabilities. As rates of preterm birth continue to climb and overall neonatal mortality rates decline, a growing population at risk for neurodevelopmental disabilities is emerging, constituting necessary research into white matter development, disease, and treatment.

Periventricular leukomalacia (PVL) is the most common form of white matter damage seen in children born prematurely. [84] Caused by a lack of oxygen to the periventricular regions of the brain, PVL is a softening of the brain tissue adjacent to the lateral ventricles that results in necrosis of white matter. On ultrasound PVL lesions are described as cysts in the white matter. Premature infants are susceptible to this type of ischemic injury because they are at risk for hypotension, decreased blood flow, hypocarbia and compression of arterioles in the white matter by edema and hemorrhage. On a cellular level, PVL is characterized by a loss of oligodendrocytes and an increase in astrocytes.

Maternal chorioamnionitis is a secondary infection of the chorion and the amnion that has often affected children born prematurely with PVL. Many may also have cardiac and respiratory

conditions. Few symptoms of PVL are apparent at birth or are obscure. Some neonatal symptoms include hypotonia in the legs, hypertonia in the neck, apnea, bradycardia, irritability, poor feeding and seizures. Long-term effects are difficult to quantify due to many environmental and biological factors. However, many preterm infants with periventricular leukomalacia develop cerebral palsy. They are also at risk for nystagmus, strabismus and blindness, intellectual impairment and developmental delay.

Another common form of white matter lesion seen in preterm infants is periventricular hemorrhage (PVH). The subependymal germinal matrix is a region of the fetal brain that gets smaller as the brain grows. Functionally, the subependymal germinal matrix continues to produce glial cells between 20 and 32 weeks, after the majority of neuronal proliferation has ceased. The capillaries that supply the germinal matrix are delicate. This vulnerability makes the germinal matrix a site for capillary bleeding and the location for PVH. Preterm infants are unable to control the pressures of cerebral blood flow as well as fullterm infants. They can also have difficulty synchronizing breathing that in turn leads to alterations in blood pressure. Loss of cerebral auto-regulation and fluctuations in cerebral blood flow and pressure are two contributory factors that can result in PVH.

The degree of PVH is established according to radiological findings. Grades are used to distinguish types of hemorrhage, or bleeds, by severity. The system used to classify hemorrhage grade is outlined in Table 9.

Table 9. Classification of periventricular hemorrhage

Grade	Radiologic appearance- Site of Hemorrhage
Grade I (Mild)	Subependymal region and/or germinal matrix
Grade II (Moderate)	Subependymal hemorrhage with minimal filling of lateral ventricles with no or little ventricular enlargement
Grade III (Severe)	Subependymal hemorrhage with significant filling of lateral ventricles with significant ventricular enlargement
Periventricular hemorrhagic infarction (Grade IV)	Intraparenchymal venous hemorrhage

An intraparenchymal hemorrhage is a Grade IV bleed, sometimes called a periventricular hemorrhagic infarction. This is because the mechanism of injury differs from that of Grades I, II and III. A Grade IV bleed or periventricular hemorrhagic infarction results from venous infarctions typically subsequent to a less severe bleed [84].

Periventricular hemorrhage can lead to subsequent pathological conditions. Areas of necrosis (destruction of neurons in the brain) can follow PVH resulting in cysts. Hydrocephalus is commonly associated with PVH. Formation of a hemorrhage can lead to communicating hydrocephalus in which cerebral spinal fluid is malabsorbed. Obstructive hydrocephalus can also result due to result of hemorrhage obstructing the circulation of cerebral spinal fluid. A ventricular-peritoneal shunt is often placed in the brain to manage and treat hydrocephalus though placement of a shunt increases risk for a brain infection. It is also possible for fluctuating cerebral blood flow, characteristic of PVH, to effect and damage other regions of the brain. These primary sequelae of PVH correspond to increases in risk for neurological injuries, including cerebral palsy, mental retardation and seizures [84].

2.4 METHODS OF ASSESSING WHITE MATTER DISEASE

Historically, clinical diagnosis of white matter disease was based on sonography and computed tomography. Use of magnetic resonance imaging (MRI) for white matter assessment greatly improved diagnostic sensitivity, offering resolute white and gray matter contrast. However, the recent introduction of diffusion tensor imaging, a type of MRI, has unleashed new potential in capturing detailed structure and development of white matter tracts not possible with traditional MRI and other imaging technologies.

Magnetic resonance imaging is a primary technology used in medicine to visualize and assess human anatomy and diagnose physiological changes. Also referred to as nuclear magnetic resonance, MRI utilizes our understanding of how hydrogen nuclei in water and lipids behave when subjected to a steady magnetic field. Based on quantum mechanics, hydrogen atoms that typically have 1/2 spin align parallel or antiparallel to the magnetic field. The difference in energy states of the atoms in parallel or antiparallel alignment is detectable. Using polarized radio-frequency fields, the atoms can be manipulated into spin transitions between orientations that create a signal. MRI scanners pulse particular known frequencies in order to affect only certain protons in a predetermined location. Information about location and position from imaged slices can then be recorded and transformed into two- or three-dimensional images.

Magnetic resonance imaging is non-invasive and is safe because it utilizes non-ionizing radiation. The images provide soft-tissue contrast that have clinical resolutions of about 1 mm^3 but can exceed 1μm^3. Time constants that measure the time required for spin to equilibrate after pulses of frequency fields is how contrast is defined. “Time 1” or T1 weighted imaging is based on the time it takes for the nuclear spins to realign with the magnetic field. White matter appears white and grey matter appears grey. Cerebrospinal fluid is dark in color. Imaging that uses

transverse, or perpendicular energy pulses to disrupt spin is called T2-weighted imaging. Contrast for T2-weighted imaging is the reverse of T1-weighted imaging contrast. The quality of T2-weighted images are less resolute but offer greater sensitivity for certain applications such as functional MRI (fMRI) and are better at detecting recent intracranial hemorrhage. Demyelinating diseases are best studied using T2-weighted imaging.

Functional magnetic resonance imaging is used to detect changes in the brain due to neural activity. When the brain is actively working it requires more oxygen. Levels of oxygenated hemoglobin increase disproportionately resulting in a stronger signal in areas of increased activity. This type of neuroimaging is done with T2-weighted contrast.

Functional magnetic resonance imaging is a commonly used research tool that has the advantage of being non-invasive over other imaging methods. Functional MRI is usually not used for white matter, which needs to be intact, but rather cortical and subcortical grey matter. Several aspects of fMRI have been scrutinized, however. The criticism has been directed at the technology, such as the resolution of the temporal response and the theoretical models fMRI is based on. Others question the indirect relationship between what is measured (hemodynamic response) and what is inferred (neural activity); also, that hemodynamic responses may vary across regions of the brain from the responses assumed for fMRI. Additional concerns are with regard to what information fMRI produces. The images indicate the location of neural activity but not the underlying mechanisms. Despite the active debate over the use of fMRI, proponents of the technique claim that if used properly by individuals with understanding of the technology and its benefits and limitations, it can be a valuable neuroimaging tool.

Anisotropy is the physical property of something being directionally dependent.

Diffusion tensor imaging (DTI) is an application of magnetic resonance imaging technology that measures restricted diffusion of water in white matter. The structure of white matter is tract-like and directs the diffusion of water so that the anisotropy of the tracts can be measured. The result of DTI is a three-dimensional constructed image with color and brightness used to communicate structural position and direction and degree of anisotropy. One use of DTI is mapping the white matter fiber tracts in the brain but the potential for research and clinical applications extend into neuroscience and beyond.

3.0 COGNITION, LANGUAGE AND BEHAVIOR IN CHILDREN BORN PREMATURELY, WITH AND WITHOUT PERINATAL WHITE MATTER INJURY

The work presented here is the first phase of a study conducted over five years (2005-2010) by Beatriz Luna and Heidi M. Feldman at the University of Pittsburgh and Stanford University Medical School entitled "fMRI and DTI in Children with PVH/PVL [93]." The goal of the study is to relate the degree and patterns of white matter damage from PVH and PVL to linguistic and cognitive outcomes.

3.1 PURPOSE

Children born prematurely are at risk for perinatal brain injuries (PBI). Prematurity and PBI confer risk for neurodevelopmental disabilities. We investigated the linguistic and behavioral characteristics of children born prematurely, with and without perinatal brain injuries, at ages 10-15.

3.2 BACKGROUND

One of the morbidities associated with prematurity is perinatal white matter disease. Periventricular hemorrhage and periventricular leukomalacia are the most common forms of white matter disease associated with prematurity. Research has shown that children with periventricular hemorrhage and periventricular leukomalacia are at subsequent risk for neurodevelopmental disabilities. Commonly seen in children with PVH/PVL is cerebral palsy [94]. Neurodevelopmental disabilities are also common in this population, affecting cognition, language and behavior. The outcomes vary across a broad spectrum of severity with some having severe neurodevelopmental disabilities such as mental retardation and severe cerebral palsy while some exhibit mild learning disabilities or no signs of neurological affect. To further complicate the etiology of neurological disabilities are the many environmental and behavioral factors that can also contribute to language and learning development and behavior. In order to further understand the roles prematurity and white matter disease play in neurodevelopment, one aspect of development, language, was studied. Children born preterm with perinatal brain injury were expected to have differences in performance on clinical tests for language than children born preterm without perinatal brain injury.

3.3 METHODS

3.3.1 Subjects

In total, approximately 2000 families whose children received early intervention services between 1993 and 2003 were notified by mail regarding the study. The letters provided telephone and e-mail address contact information for interested families. Eight hundred letters were returned due to insufficient or outdated contact information. Almost 100 families expressed interest in participation. Sixty-five children 9 to 16 years of age were successfully screened for eligibility. Families interested in participating that had children younger than age 9 and therefore ineligible were asked to participate in a research registry for participation future studies.

In total, 16 children initiated participation. All participants were born at less than 37 weeks gestation; eight children were born at 29 weeks gestation or earlier. Ten were reported to have white matter damage at birth and six children had no reported white matter damage. Overall, six children were both born prior to 29 weeks and having white matter disease (Table 10).

Table 10. Summary of subjects

ID#	*Age*	*Sex*	*GA*	*Reported WMD*
10003	13	M	24	Yes
10013	11	F	24	Yes
10029	14	F	26	Yes
10030	14	F	26	Yes
10034	13	M	27	Yes
10037	11	M	27	Yes
10002	15	F	28	No
10005	10	M	28	No
10016	13	M	29	No
10017	12	M	29	No
10018	12	M	30	No
10021	11	M	30	No
10027	14	F	32	No
10049	11	M	34	No
10057	14	M	34	No
10058	11	F	36	No

3.3.2 Procedures

A questionnaire was administered to interested parents over the telephone to establish eligibility for their children. The domains questioned included birth, medical, developmental and behavioral history. If determined to be eligible, identifying information was collected and an assessment was scheduled. Informed consent was obtained from an accompanying parent or guardian and informed assent was obtained from participating children. Testing was administered individually and over the time course of two hours. Table 11 lists the clinical tests for language administered, the score that the test generates, and the domains assessed. Parents were asked to complete 3 questionnaires about their child's social maturity and behavioral and emotional problems [95, 96]. All of the clinical tests for language and parent questionnaires were administered and scored according to published instructions.

Table 11. Clinical tests for language

Test	Scores	Domain assessed
Peabody Picture Vocabulary Test-III [97]	Standard Score	Receptive vocabulary
Clinical Evaluation of Language Fundamentals 4 (CELF-IV) [98]	Standard Score	Global measure of language function
Woodcock-Johnson III Tests of Achievement [99]	Standard Score	Reading
Test of Receptive Grammar (TROG)-computer	Number correct; Reaction time	Grammar
Sentence Comprehension Protocol Part 1 and Part 2-computer	Number correct; Reaction time	Sentence Comprehension

3.3.3 Statistic Analysis

Two-tailed tests were used for all analyses. Independent t-tests were used to evaluate differences in performance on tests of linguistics among children with and without PBI and among children born prior to 29 weeks gestation and children born 29 weeks or later.[11] Statistical analyses were done using SPSS Software [100].

3.4 RESULTS

3.4.1 Group Analysis

Table 12 summarizes the means and frequencies for all 16 participants (all born preterm).

[11] The groups were split at the median gestational age (29 weeks).

Table 12. Total group (N=16) performance means and statistics

	GA	BW	PPVT	CELF Core	CELF Rec	CELF Exp	CELF L Mem	WJ W ID	WJ Pass Comp	WJ Word Attack	WJ Basic Reading Skills	WASI SS
N	16	16	16	8*	8*	16	8*	16	16	16	16	11**
Mean	29	1213.69	105.15	107.0	98.88	99.5	100.75	98.25	98.19	97.25	97.81	94.45
Std. Error	0.89	115.43	3.3	4.52	3.24	4.07	5.18	2.09	2.63	2.9	2.5	4.91

**This test was not available for children older than 13 years.*
***This assessment had not yet been administered to 5 of the 16 total participants.*

The groups' performances on tests of linguistics were compared using standard scores based on a normal curve (Figure 5).

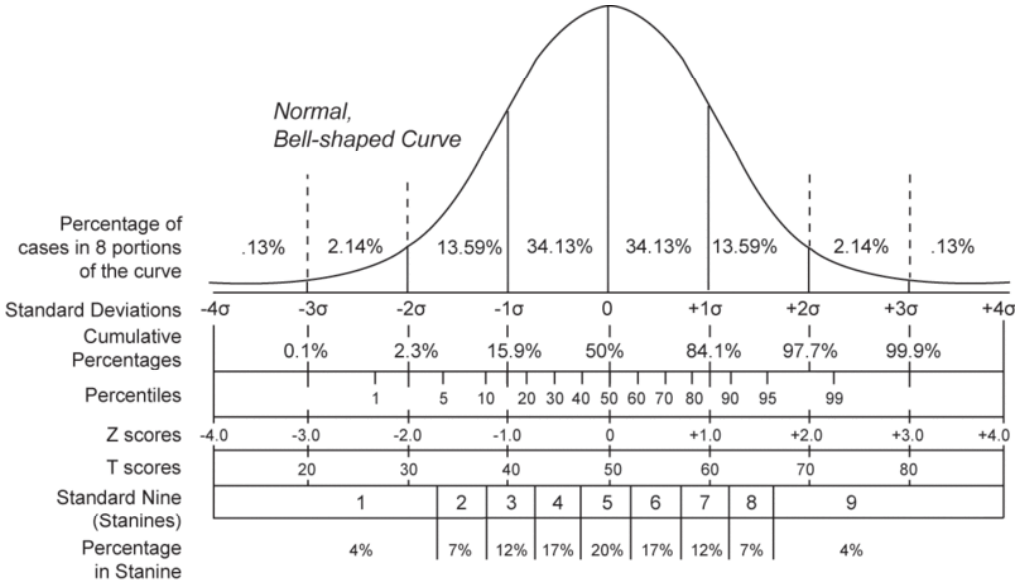


Figure 5. Normal curve and standard scores[12]

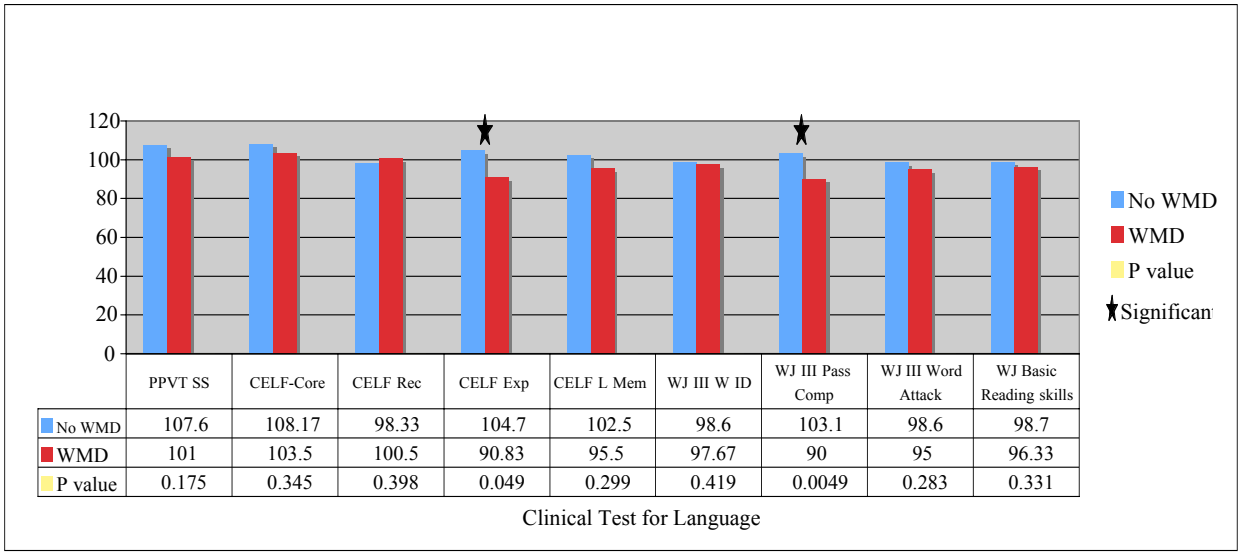
[12] Figure from *Standard score*. (2007, March 17). In Wikipedia, The Free Encyclopedia. Retrieved 13:39, March 23, 2007, from http://en.wikipedia.org/w/index.php?title=Standard_score&oldid=115714140.

The standard scores reflect performance in the general population and correlate to clinical domains of functioning (Table 13).

Table 13. Standard scores and clinical assessment of function

Standard Score	Functional assessment of performance
<70	Extremely low
70-85	Moderately low
86-99	Average-low
100	Average
101-115	Average-high
116-130	Moderately high
>130	Extremely high

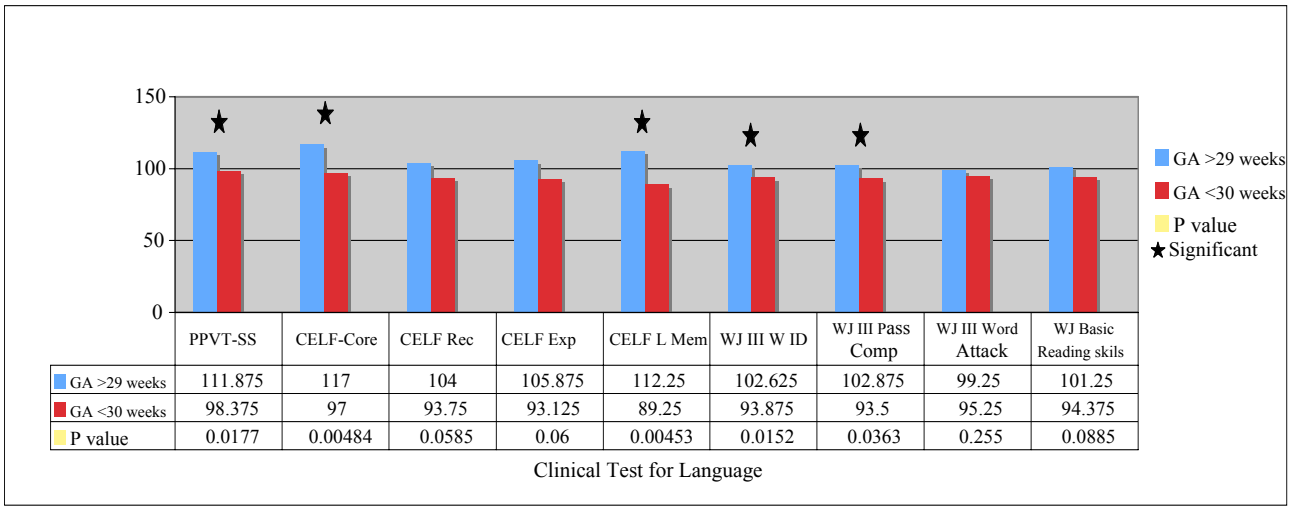
There was no difference in performance on any of the assessments by sex. A significant relationship was found between perinatal brain injury and birth before 29 weeks gestation (Fisher's Exact, p=0.007). Figure 6 summarizes the scores on the tests above for children with and without perinatal brain injury. The mean scores of the children reported to have no white matter damage were significantly higher for the expressive language and passage comprehension tests than the children with reported white matter damage. The children with no reported white matter damage had higher mean scores than those with reported damage on the receptive vocabulary test and all but one of the remaining language and reading tests (CELF subtest for receptive language), though the differences were not significant. There were no significant differences between the two groups with regard to behavior, including assessments for hyperactivity and impulsivity, inattention, anxiety, depression and conduct and opposition-defiant behaviors.



	PPVT SS	CELF-Core	CELF Rec	CELF Exp	CELF L Mem	WJ III W ID	WJ III Pass Comp	WJ III Word Attack	WJ Basic Reading skills
No WMD	107.6	108.17	98.33	104.7	102.5	98.6	103.1	98.6	98.7
WMD	101	103.5	100.5	90.83	95.5	97.67	90	95	96.33
P value	0.175	0.345	0.398	0.049	0.299	0.419	0.0049	0.283	0.331

Figure 6. Language performance and perinatal white matter damage

Figure 7 reorganizes the data, contrasting the children with regard to gestational age at birth. Children born after 29 weeks of age scored significantly higher on tests for receptive vocabulary, language memory, reading and comprehension than children born at less than 30 weeks gestation ($p \leq 0.05$). Additional trends suggest that the children born after 29 weeks gestation also performed better on the tests for receptive and expressive language. Behaviorally, the frequency of anxiety and depression symptoms reported for the children born before 30 weeks was also statistically significant.



	PPVT-SS	CELF-Core	CELF Rec	CELF Exp	CELF L Mem	WJ III W ID	WJ III Pass Comp	WJ III Word Attack	WJ Basic Reading skils
GA >29 weeks	111.875	117	104	105.875	112.25	102.625	102.875	99.25	101.25
GA <30 weeks	98.375	97	93.75	93.125	89.25	93.875	93.5	95.25	94.375
P value	0.0177	0.00484	0.0585	0.06	0.00453	0.0152	0.0363	0.255	0.0885

Figure 7. Language test performance by gestational age

All other behavioral indices were similar between the two groups. There were no differences measured between groups, by perinatal white matter disease or gestational age, for tests of grammar and sentence comprehension, accuracy and reaction time.

3.5 RESULTS- CASE STUDIES

Three pairs of subjects were matched for age and sex and one or more of the following: gestational age at birth, birthweight, and reported perinatal brain injury. Parent questionnaires and report and tester observations were used to perform a qualitative assessment of function of each matched pair. The purpose of the qualitative assessment was to supplement preliminary data analysis with a general estimate of how function might vary by gestational age at birth, birthweight or perinatal brain injury.

The International Classification of Functioning, Disability and Health (ICF) rubric was used for qualitatively assessing function. The World Health Organization developed the ICF in

2001 as a tool to assess how individuals live with a disability or health condition. Physical, individual and societal functioning are organized into domains that include learning and applying knowledge, general tasks and demands, communication, mobility, self care, domestic life, interpersonal interactions and relationships and community, social and civic life. Table # summarized the functional analysis [101, 102].

3.5.1 Pair 1 case study

This pair was selected for qualitative comparison because they are of same sex, similar ages and birthweight and could be contrasted by gestational age at birth and perinatal brain injury. Table 14 summarizes demographic information and performance and functional assessments for each subject in Pair 1.

Table 14. Functional assessment of Pair 1 case study using the ICF

Pair 1

Subject	Sex	Age	Gestational age (wk)	Birthweight (g)	Reported PBI
A	M	13	31	1360.78	-
B	M	13	28	1360.78	+

Performance on tests of linguistics

	Receptive vocabulary	Core language	Word ID	Passage Comp	Word Attack/reading	Overall reading skills	Intelligence
A	110	87	96	103	89	92	94
B	89	67	100	78	99	100	67

ICF Domain	Functional Description
Learning and Applying Knowledge	A: Not reported. B: Special Education-reading
General Tasks and Demands	A: Some problems in social relationships; some thought and attention problems; special education in school for emotional support B: Has a diagnosis of ADHD; question of Aspergers autism diagnosis
Communication	A: Not reported. B: Speech and language support in school
Mobility	A: Did not appear to have mobility issues; has a diagnosis of Cerebral palsy and receives physical and occupational therapy B: Severe cerebral palsy diagnosis and uses a wheelchair most of the time
Self Care	A: Not reported. B: Not reported.
Domestic Life	A: Does three chores and has two hobbies B: Does one chore and has three hobbies
Interpersonal Interactions and Relationships	A: Has at least three close friends and sees friends three or more times a week B: Has no close friends and sees friends less than once a week
Community, Social and Civic Life	A: Belongs to two social organizations, clubs or teams and participates in two sports B: Belongs to one club or team and participates in three sports

3.5.1.1 Pair 1 discussion

Pair 1 were two boys, both 13 years old. Subject 1A was born at a later gestational age (31 weeks) and without perinatal brain injury than 1B, born at 28 weeks with periventricular leukomalacia. The pair were similar in domains for domestic life, self care, and community, social and civic life. The pair both had some problems in the general tasks and demands domain. Subject 1B had some problems with communication and 1A received learning supports for reading. The pair differed with regard to interpersonal interactions and relationship. Subject 1B has no friends and subject 1A has several close friends that he sees often. Both boys in Pair 1 had cerebral palsy, though varied degrees of severity. Subject 1B was more severely affected and used a wheelchair for the majority of his activities and mobility and 1A had no obvious physical features of cerebral palsy and did not need assistance with mobility.

Pair 1 differed in performance scores on expressive language. Both had performance scores in the average-low range for the reading and word identification tests. Subject 1A, however, performed in the average-high range for passage comprehension, unlike the group of children with PBI and 1B. Notice that 1B's WASI score falls in the range of mental retardation on formal testing, even though his receptive vocabulary, word identification, word attack and overall reading skills are within the normal range. This demonstrates an unevenness in cognitive abilities between types of language function and supports a threshold of functioning in which liability, such as perinatal brain injury or being very preterm, has an effect on functional ability to complete higher-level language tasks such as comprehension. It should be noted that these tests were not exhaustive and inconsistent scores could also be due to something unrelated to linguistic function.

3.5.2 Pair 2 case study

This pair was selected for qualitative comparison because they are of same sex, similar ages and both have reported perinatal brain injury. They were contrasted by gestational age at birth and birthweight. Table 15 summarizes demographic information and performance and functional assessments for each subject in Pair 2.

Table 15. Functional assessment of Pair 2 case study using the ICF

Pair 2

Subject	Sex	Age	Gestational age (wk)	Birthweight (g)	Reported PBI
A	M	11	25	875	+
B	M	11	29	1162.33	+

Performance on tests of linguistics

	Receptive vocabulary	Core language	Word ID	Passage Comp	Word Attack/reading	Overall reading skills	Intelligence
A	104	109	90	81	94	92	103
B	113	121	103	103	91	96	NA*

* The WASI had not yet been administered for this subject at the time of data analysis

ICF Domain	Functional Description
Learning and Applying Knowledge	A: Receives learning support in school; in 5th grade he is at a 2nd-3rd grade level B: Not reported.
General Tasks and Demands	A: Questions of ADHD, depression and anxiety; counseled for compulsivity B: Special education in school for social skills support
Communication	A: Not reported. B: Not reported.
Mobility	A: Mild unilateral cerebral palsy B: Not reported.
Self Care	A: Not reported. B: Not reported.
Domestic Life	A: Does two chores and has at least three hobbies B: Does three chores and has at least three hobbies
Interpersonal Interactions and Relationships	A: Has two or three close friends and sees friends less than once a week B: Has four or more close friends and sees friends one or two times a week
Community, Social and Civic Life	A: Belongs to one social organization, team or club and participates in three sports B: Belongs to one social organization, team or club and participates in two sports

3.5.2.1 Pair 2 discussion

Pair 2 were two boys, both 11 years old. Subject 2A was born at an earlier gestational age (25 weeks) and lower birthweight (623.69 grams) than 2B, born at 28 weeks and weighing 1162.33 grams. One factor in addition to gestational age and birthweight that differentiate Pair 2 is that 2A has several medical conditions persisting from birth and into adolescence whereas 2B has had few heath conditions. The pairs are similar in most domains and neither boy were reported to have problems with communication, self care, domestic life, interpersonal interactions and relationships and community, social and civic life. Both boys have some reported problems in general tasks and demand though the specific issues are different. Subject 2A has some difficulty with behavior and emotional and 2B has some trouble with social skills. The boys differ slightly with mobility. The biggest functional contrast between Pair 2 is that 2A has learning and academic performance needs and 2B does not.

Similar to the group of children having reported PBI, Pair 1 scored average-low and average-high on tests of language. Subject 2B performed in the average-high range for passage comprehension while 2A had the most difficulty on passage comprehension, scoring average-low. Interesting is that Pair 2 both scored in the average-high range on several tests whereas the group scores for children born at fewer than 30 weeks were all average-low. Both boys also had little difficulty with the testing protocol, demonstrated focus and consideration of the presented tasks and were calm without evidence of inattention or hyperactivity. This may demonstrate the role environmental factors play in addition to gestational age and perinatal brain injury on performance for tests of language.

3.5.3 Pair 3 (Dizygotic Twins) case study

As with many genetic research studies, twins are used because they share common genetic and environment factors and are matched for age and gender (often). These fraternal twins were assessed at age 12 having no history of perinatal brain injury but having significantly discordant birthweights. Table 16 summarizes demographic information and performance and functional assessments for each subject in Pair 3 (Dizygotic Twins).

Table 16. Functional assessment of Pair 3 (Dizygotic Twins) case study using the ICF

Pair 3 (Dizygotic Twins)

Subject	Sex	Age	Gestational age (wk)	Birthweight (g)	Reported PBI
A	M	12	34	2381	-
B	M	12	34	1400	-

Performance on tests of linguistics

	Receptive vocabulary	Core language	Word ID	Passage Comp	Word Attack/reading	Overall reading skills	Intelligence
A	126	118	101	110	124	115	98
B	109	120	114	106	105	111	108

ICF Domain	Functional Description
Learning and Applying Knowledge	A: Above average academic performance B: Below average academic performance; no learning supports
General Tasks and Demands	A: Some problems with social relationships; received counseling for emotional problems in the past B: Some social skills problems
Communication	A: Not reported. B: Not reported.
Mobility	A: Not reported. B: Not reported.
Self Care	A: Not reported. B: Not reported.
Domestic Life	A: Does three chores and has three hobbies B: Does three chores and has three hobbies
Interpersonal Interactions and Relationships	A: Has no close friends and sees friends less than once a week B: Has one close friend and sees friends less than once a week
Community, Social and Civic Life	A: Belongs to one social organization, team or club and participates in three sports B: Belongs to one social organization, team or club and participates in two sports

3.5.3.1 Pair 3 (Dizygotic Twins) discussion

The twins were a very interesting case study. Despite the difference in birthweight, both twin 3A and twin 3B had similar function in most domains including communication, mobility, self care, domestic life and community, social and civic life. They were most different in the domains for learning and applying knowledge and general tasks and demands. Twin 3B does very well in school and Twin 3A reportedly performs below average in school. Inconsistent with this report, the twins performed similarly on tests for linguistics. Socially, the twins differed in how many friends they had and both boys had some degree of difficulty with social skills. The social differences were observed during the testing session and were most impressive for approach to testing, motivation and attitude. Twin 3A seemed motivated by obligation to participate and appeared uninterested and indifferent to performance. In contrast, his brother, Twin 3B, was excited to participate and motivated by achievement. These differences were most apparent during the computer tasks, though overall, performances on the computer tasks were similar. Twin 3A spent less time completing each computer task (on average) than 3B but made more total errors.

Overall, the similar strengths of both twins' performance are interesting for several reasons. They are siblings sharing similar environments growing up. Both had the same prenatal exposures, delivery complications and premature birth. Both had no evidence of perinatal brain injury. With these aspects shared between the twins we can look more closely at the effects birthweight has on long-term language development and behavior. Twin 3B was small for gestational age, a risk factor for neurdevelopmental delays. However, despite his low birthweight, 3B performed similarly to his twin brother who was not small for gestational age.

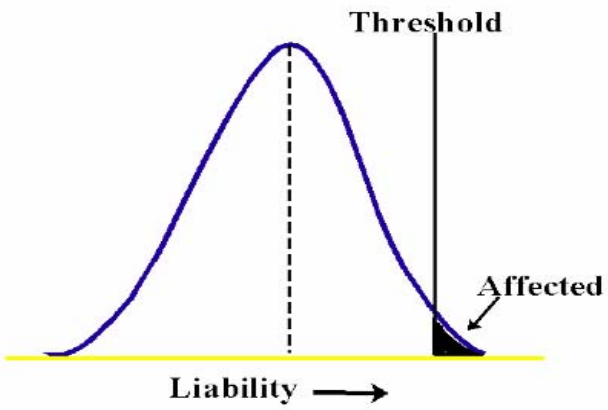
This suggests that birthweight contributed less to the twins' long-term outcomes than may be expected.

3.6 DISCUSSION

Children with PBI performed similarly to children with no PBI on tests for language, reading and grammar. The two exceptions (reading comprehension and expressive language) suggest that children with PBI may have more difficulty with certain aspects of language and reading. Reading comprehension is a task that requires incorporating several aspects of language to complete successfully. Single words must be recognized (reading), sentences must be decoded (grammar) and independent pieces must be understood and organized (processing) to achieve comprehension. Expressive language also builds on more than one language skill. First, words must be received (listening or reading). Meaning must be decoded and processed (comprehension). Finally, an appropriate response must be synthesized and returned to the environment (expression). Tests indicating no significant differences in performance between groups according to perinatal brain injury required fewer steps to complete than reading comprehension and expressive language.

One possible explanation for the significantly lower scores on complex language tests for children with perinatal brain injury is that white matter plays a role in executing complex tasks. White matter tracts are the highways that information travels along throughout the brain and between brain regions involved in language and cognition. If the fiber tracts are disrupted at birth as a result of prematurity the ability to perform multiple, simultaneous complex language

tasks may exceed a higher-function processing threshold and be compromised while the capacity to perform less complicated, basic language tasks is unaffected.



Threshold=Complex language tasks
Liability=White matter disease (and environmental, behavioral and genetic modifiers)
Affected=Language disability

Figure 8. Threshold model for complex language tasks

Based on the threshold model (Figure 8) white matter disease would impact ability to complete complex language tasks but not basic language function. When considering this model, it is important to incorporate the seemingly limitless environmental, behavioral and genetic factors that modify "liability" and the effects of white matter disease. Hoekstra et al. identified several factors that have an affect on neurodevelopment (Figure 9) [103].

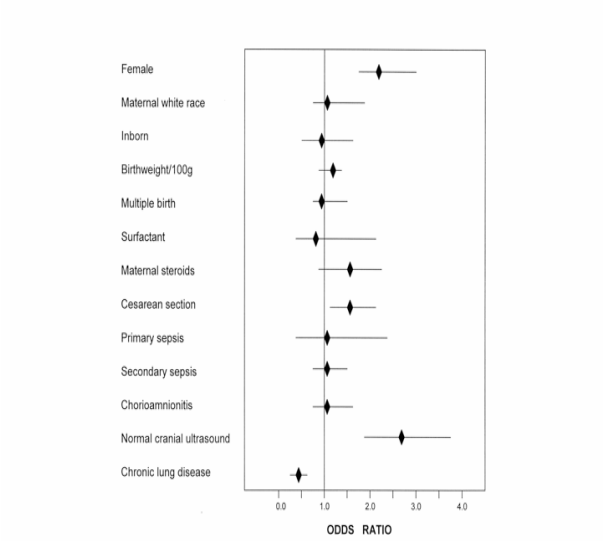


Figure 9. Factors that affect neurodevelopmental outcome[13]

More robust were the differences in performance between the children born at different gestational ages. Children born after 30 weeks had higher scores than the children born before 29 weeks on nearly every linguistic test, as well as having higher levels of depression and anxiety. This indicates that gestational age and not PBI specifically, may have a greater effect on linguistics and behavior. A study by Huppi et al. used MRI to demonstrate that healthy preterm infants had less white and gray matter differentiation and white matter myelination than fullterm infants and also had worse performance on neurobehavioral parameters of function [104]. Studies have also demonstrated an inverse relationship between gestational age and neurodevelopmental disabilities [103].

Overall, the group as a whole (N=16) performed well on tests for linguistics despite the conferred risk for neurodevelopmental disabilities associated with prematurity. Performance

[13] Image from Hoekstra, R.E., et al., *Survival and long-term neurodevelopmental outcome of extremely premature infants born at 23-26 weeks' gestational age at a tertiary center.* Pediatrics, 2004. **113**(1 Pt 1): p. e1-6.

score means fell in the average-low to average-high range. This may suggest that prematurity, with our without perinatal brain injury, may not play as significant a role in language as originally thought.

These data are preliminary and it is expected that a larger sample size may reveal differences in scores as a function of brain injury. It is also important to consider that the majority of children identified as having perinatal brain injury 1) had a diagnosis made based on MRI or cranial ultrasound, techniques less sensitive to subtle white matter changes and 2) were reported verbally by parents and not corroborated with medical records. The work presented here, *Linguistic and Behavioral Outcomes of Children Born Prematurely With and Without Perinatal Brain Injury* is the first part in a larger, five-year study consisting of three total parts. Following the initial assessment described above is a second session of assessment using clinical tests for cognition, language and executive function. The third session will use fMRI and DTI to assess brain function and white matter structure and disease, respectively.

Using DTI to better define white matter changes in children born prematurely will identify children without white matter disease that were incorrectly reported. It is expected to also identify children with white matter disease not previously diagnosed with other forms of imaging. Improved phenotypic parameters for white matter disease will increase power to measure differences between groups of children born preterm with and without white matter disease. The larger study is also expected to have more power due to a larger sample size (n=80).

4.0 PREMATURITY AND GENETIC COUNSELING: A REFLECTION

In the past eighteen months I have lost count of the number of times someone has asked me what prematurity has to do with genetic counseling. That the question arises seems to imply that many people (at least more than I have been able to keep count of) believe that prematurity and genetic counseling are unrelated. Having become accustomed to explaining what genetic counseling is to nearly every person asking about my studies, to explain further a relationship between genetic counseling and prematurity seems hardly a huge undertaking.

I will begin with the most direct answer. Preterm birth is a multifactorial condition. Reviewed exhaustively in the previous pages, environment, behavior and genetics all contribute to prematurity. Multifactorial diseases such are currently, and will continue to be, a huge focus of genetics research. These are the conditions that have the largest impact on the greatest number of people. And, because prematurity affects so many people, it is likely to affect a significant proportion of my future patient population, particularly in prenatal settings. Understanding preterm birth within and outside the context of genetics will better prepare me as a professional in healthcare.

The morbidities associated with preterm birth include acute and chronic medical conditions, problems with growth and nutrition and a broad spectrum of neurodevelopmental disabilities that include learning and behavior. I just described many of my future clients. Learning about these complicated medical and developmental conditions provided an

opportunity to enrich my understanding of a wide variety of phenotypes likely to reoccur frequently in genetics clinic.

Working on this project, I have practiced a variety of skills are used by genetic counselors on a regular basis. Participation in the clinical aspect of this research granted me the opportunity to interact with families directly and over the phone. I developed skills in administering clinical tests for language, practiced qualitative assessment of complex behaviors and learned about typically developing children in early intervention and public education settings. I performed data analyses, prepared submission for IRB approval and adhered to strict practices of confidentiality. Assuming administrative duties such as scheduling clinical assessments and communicating and coordinating research between collaborators were some of the practical skills I acquired (more valuable to genetic counseling than one may think). Fortunate to be encouraged, I developed skills for working independently and nurtured professional and personal leadership goals.

The biggest impact this work had on my development as a genetic counselor and health care provider was the result of my participation in UCLID, an interdisciplinary leadership training program centered on individuals and families of individuals with disabilities. Focused on function and family-centered care (a principle that nicely complements non-directiveness and autonomy), UCLID clinic was a rich experience that allowed me the opportunity to learn about the intricate framework of medical, educational and therapeutic services clients often access (or need assistance accessing) after they visit genetics clinic. Most importantly, I grew as an advocate for individuals with special health care needs.

"…And so you see, genetic counseling has quite a bit to do with prematurity."

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