Published in final edited form as:

Early Hum Dev. 2009 November ; 85(11): 719–725. doi:10.1016/j.earlhumdev.2009.08.060.

The ELGAN Study of Brain and Related Disorders in Extremely Low Gestational Age Newborns

O'Shea TM, MD, MPH¹, Allred EN, MS², Dammann O, MD^{2,3,4}, Hirtz D, MD⁵, Kuban KCK, MD, SMEpi⁶, Paneth N, MD, MPH⁷, and Leviton A, MD² for the ELGAN study Investigators

¹Department of Pediatrics (Neonatology), Wake Forest University Health Sciences, Winston-Salem, NC

²Neuroepidemiology Unit, Children's Hospital of Boston, Boston, MA

³Department of Pediatrics (Newborn Medicine), Floating Hospital for Children at Tufts Medical Center, Boston, MA

⁴Perinatal Neuroepidemiology Unit, Departments of Gynecology and Pediatrics, Hannover Medical School, Germany

⁵National Institute of Neurological Disorders and Stroke, Bethesda, MD

⁶Department of Pediatrics (Pediatric Neurology), Boston Medical Center, Boston, MA

⁷Department of Epidemiology, Michigan State University, East Lansing, MI

Keywords

prematurity; developmental disability; autism spectrum disorder; autism; cerebral palsy; chorioamnionitis; pregnancy; preeclampsia

Introduction

In the late 1990's, we designed and laid the foundations for a study intended to advance our understanding of what contributes to brain damage in extremely low gestational age newborns (ELGANs). Our planning considered the following:

1. A model of brain damage in the preterm newborn

We postulate that the preterm newborn is at very high risk of brain damage, for at least three reasons.

First, the very processes that lead to preterm delivery can contribute to brain damage.(1-3) These processes, which are likely inflammatory, involve the fetus,(4) are complex,(5-10) probably persist for days if not weeks,(11) and need not be initiated by microorganisms.(12)

© 2009 Elsevier Ireland Ltd. All rights reserved.

Correspondent: Department of Pediatrics Wake Forest University Health Sciences Medical Center Blvd Winston-Salem, NC 27157
Phone 336-716-252 FAX 336-716-2525 moshea@wfubmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interests: The authors declare that they have no conflicts of interests.

Originally our focus was on inflammatory exposures associated with preterm labor and pre-labor premature rupture of membranes, but has since been expanded to the processes that impair fetal growth as well.

Second, the developmental processes during the 23rd through 27th weeks of gestation might be among the most vulnerable. The transformation of oligodendrocyte precursor to oligodendrocyte is one maturational process that appears particularly vulnerable.(13) Another is the migration of neuron precursors from the germinal plate to their final destination.(14, 15) In addition, excitatory neurotransmitter pathways are up-regulated in the immature brain, apparently to facilitate neuronal migration, division, and organization, and the development of synapses and synaptic networks.(16) This heightened excitatory state enhances the vulnerability of the brain to excitotoxic injury from inflammatory or metabolic disorders.

Third, ELGANs are born before they can synthesize adequate amounts of proteins normally provided by the placenta/mother. These proteins, many of which satisfy criteria for being called neurotrophins because they promote the differentiation/maturation of neurons and oligodendroglia, have the capacity to protect these cells against perturbation/adversity.(17, 18)

The combination of a potentially damaging exposure, easily disturbed developmental processes, and the lack of protection against the disturbances provoked by the damaging exposure are what we think make the developing brain so vulnerable.

2. Biomarkers

Biomarkers are objectively measured cellular or molecular characteristics that may be useful for studies of risk and causation. The ELGAN Study biomarkers include organisms recovered from the placenta parenchyma, placenta histologic characteristics, and blood proteins in maternal blood, umbilical cord blood, and postnatal blood obtained 1, 3, 5, 7, 14, 21, 28 days after birth.

3. Highest risk

The risk of cerebral palsy and developmental dysfunctions increases with decreasing gestational age.(19) Thus, the study was limited to infants deemed to be at highest risk (<28 weeks).

4. A sample defined by gestational age not birth weight

Birth-weight-defined eligibility for a study results in an overrepresentation of infants who are growth restricted but gestationally older.(20) This distortion hinders drawing inferences about maturational processes. We chose the name ELGAN to emphasize the gestational-age-defined sample.

5. Antenatal enrollment

Because of our interest in inflammatory exposures associated with preterm labor and pre-labor premature rupture of membranes, maternal blood and placenta were important sources of biomarker data. Antenatal enrollment allowed us to request permission for placentas and pre-delivery maternal blood, which otherwise could not have been available to us. Consequently, at most institutions, mothers were enrolled shortly after they were admitted to the hospital and before delivery. To these women we said “We hope you deliver well past the 28th week and do not become a participant. If you do deliver early, however, we want you to be part of our study.”

Some institutions did not approve of enrolling mothers prior to delivery. One of them came up with a compromise that several other institutions implemented, including those that enrolled grávida antenatally. These institutions informed all women that placentas of babies born prematurely are routinely saved for several days, so that should she enroll in a study of placentas, a piece would be used, but otherwise the placenta would be discarded.

6. Large numbers, multiple centers

Because of the large number of biomarkers to be studied, the range of outcomes, and the multiple potential (and interactive) risk factors, a large sample was needed for sufficient statistical power. Having many centers participate would also increase the generalizability of results. By enrolling newborns at 14 institutions, the ELGAN Study achieved large numbers (close to 1500) and generalizability.

7. Homogenous outcomes

A long-standing assumption is that all cerebral palsy is the same. The epidemiologic literature, however, suggests that the three most common clinical forms of cerebral palsy in preterm newborns, diplegia, quadriplegia, and hemiplegia, are not equivalent etiologically (21,22). Rather than aggregating possibly heterogenous outcomes in an effort to increase statistical power, we have chosen to define homogenous entities in order to increase validity. (23) (24)

8. Multiple disciplines

As the design for the ELGAN Study became more complex, the need grew for expertise in diverse disciplines. We had experience in epidemiology, statistics, neonatology, and neurology. Neonatologists were the site principal investigators and perinatologists were also involved. Moreover, important scientific questions required the expertise of sonologist/radiologists, pathologists, ophthalmologists, developmentalists, and lab directors with expertise in immunology, bacteriology, endocrinology, and molecular biology.

9. Highest quality data

We created data collection instruments based on forms of proven value from a prior study (25-33) and instituted data quality control procedures that were also of proven value <http://www.fstrf.org/intro.html>. Colleagues who provided subjective data (sonologists/radiologists, pathologists, ophthalmologists, and and those who assessed motor and developmental functions at age two years) participated in exercises to minimize inter-observer variability.

10. Communication

To assure frequent communication at multiple levels, the study personnel were connected through a hub and spoke arrangement. The principal investigator communicated frequently with three hub directors who, in turn, communicated frequently with principal investigators at 3 to 5 study sites. These site principal investigators then communicated frequently with study coordinators, sonologist/radiologists, pathologists, ophthalmologists, and developmentalists. On several occasions each year, a steering committee comprised of the principal investigator, the National Institutes of Health project officer, the three hub directors, a neonatologist representing each hub, and the study coordinators, met. Each year the site principal investigators met with the principal investigators and hub directors. To reduce costs, these meetings took place at the Pediatric Academic Societies meetings, which many of the investigators ordinarily attend.

11. Manuscript preparation

Inclusiveness is one of the core values of the study leadership. When data collection was complete, we encouraged all investigators to participate in data analyses and manuscript preparation related to topics in which they are interested. At this writing, more than 100 individuals have co-authored published manuscripts arising from the ELGAN Study; and five junior faculty, who had not directly participated in the ELGAN study, were provided the opportunity to be lead authors. (34-56)

Results thus far

1. Overview

Of the mothers approached, 85% gave consent for participation in our study. The ELGAN cohort is described in Table 1. Of the 1506 newborns enrolled, 1222 (81%) survived until discharge and 1201 (80%) survived to 2 years corrected age. Of the 1506 infants enrolled, 1445 had had at least one set of protocol ultrasound scans. All ultrasound scans were ordered by clinicians for clinical purposes. The protocol specified procedures for obtaining and reading the scans. For study purposes, the only scans considered were those obtained between the first and fourth day (first protocol scan), between the fifth and fourteenth day (second protocol scan), and between the fifteenth and 40th day (third protocol scan). First scans were obtained for 1123 infants, second scans for 1302 infants, and third scans for 1268 infants. Follow up assessments of health and development were obtained at about 24 months adjusted age. Attrition in the first two years was modest, with information obtained at the two-year assessment available for 83% to 91% of eligible children, depending on the outcome of interest.

We have completed collection and editing of the ELGAN Study data. Once the data were collected, we needed to understand the meaning of what we found. For example, we recovered bacteria from placenta parenchyma of 67% of vaginal deliveries following labor, 45% of Cesarean deliveries following labor, and 24% of Cesarean deliveries for preeclampsia.(53) To rely on these data for etiologic studies of white matter damage, cerebral palsy, and other developmental dysfunctions, required us to have some sense of how much of these differences reflect biology, and how much might reflect contamination. Consequently, data analysis and manuscript writing followed a linear pattern, with assessments of data quality preceding data analyses of primary and secondary outcomes.

We have also evaluated the antecedents and correlates of other variables examined in prior etiologic studies of small babies, including placenta histologic characteristics,(34-36) disorders that led to preterm delivery,(50) diagnoses used by the sonologists,(40) congenital microcephaly,(45) hypotension therapy,(47) nutrition and growth,(56) SNAP-II and SNAPPE-II,(38) and the autism spectrum disorder screening instrument we used (Modified Checklist for Autism in Toddlers; M-CHAT).(44) These analyses resulted in separate manuscripts. Only when we had a sense of some of the meaning of each of these variables did we feel comfortable using them in etiologic studies of brain disorders. Analyses of other variables constituted a search for potential confounders (some of which were antecedents) and were incorporated in etiologic reports.

2. Efforts to enhance data quality

Ultrasound-defined outcomes—To minimize variability of ultrasound interpretations, we held conference calls with study radiologists to discuss aspects of images prone to different interpretations, and arranged for all ultrasound scans to be read by two independent readers who were not provided clinical information (40). One reader was at the institution of the infant's birth; the second, at another ELGAN study institution. When these two readers differed in their recognition of intraventricular hemorrhage, moderate/severe ventriculomegaly, a parenchymal

echodense lesion, and a parenchymal echolucent lesion, the films were sent to a third (tiebreaking) reader who did not know what the earlier readers reported.

We cannot directly assess the validity of ultrasound observations of white matter abnormalities, but we have assessed inter-reader agreement. Inter-reader agreement was lower for echodense lesions ($\kappa = 0.32$) than for echolucent lesions ($\kappa = 0.68$) and ventriculomegaly ($\kappa = 0.62$), suggesting that the latter two findings are less subject to misclassification bias. Based on concordance readings, 24% of all children had an IVH, 12% had moderate/severe ventriculomegaly, 16% had an echodense lesion and 8% had an echolucent lesion.

Neurological findings—To standardize the quality of data about the neurological findings, examiners attended a one-day workshop, viewed a training video, and then classified neurological findings illustrated on an assessment video.(43) On the initial set of inter-observer variability assessments, 89% of the responses agreed with the gold standard responses. Following annotated feedback to the examiners about the two items that had a 60% correct rate, the agreement rate rose to 93%.(43)

To minimize expectation bias, we concealed the child's medical history from the examiner and only 4% of examiners indicated at the time of the examination that they had knowledge of the child's brain-imaging studies. Findings from the neurological exam served as the basis for an algorithm that classified infants into one of four groups: no cerebral palsy, diplegic cerebral palsy, hemiplegic cerebral palsy, and quadriplegic cerebral palsy.(42)

3. The importance of studying contemporary cohorts

One of our findings illustrates how secular trends in care practices might diminish the relevance, over time, of some research studies. When we computed growth velocity, measured as grams of weight gained/kg of body weight/day between postnatal days 7-28, we found that only 30 of the 1195 babies had a growth velocity below 10 gms/kg/day, the rate of growth targeted by most nutrition guidelines. In our sample, the median growth velocity was 23.2 gms/kg/day, well above the goals most authors have recommended.(56) Thus an exposure of interest historically (low growth velocity) appears now to occur infrequently.

4. The importance of studying multi-center cohorts

Another of our findings illustrates the importance of multi-center studies to increase generalizability of research findings. Among the 1387 infants in our sample who survived the first postnatal week, infants treated for hypotension tended to have lower mean arterial pressures than infants who were not, but uniform thresholds for treatment were not apparent.(47) Institutions varied greatly in their tendency to offer any treatment, but neither the lowest mean arterial pressure on the day of treatment nor other characteristics of the infants accounted for center differences in treatment.

To account for the possibility that infants born at a particular hospital are more like each other than like infants born at other hospitals, we often include a hospital cluster term in logistic regression models.(57) We continue to explore how to make the most informative use of "center variations" in care practices.

5. Information conveyed by gestational age

In the ELGAN cohort we found support for the concept that gestational age is a complex variable that conveys information about multiple processes that influence infant outcome.(58) Among these processes are those that lead to preterm birth (e.g., intrauterine infection). ELGANs born to mothers with preterm labor were more likely to be born at 23 weeks and less likely to be born at 27 weeks than those born to mothers with preeclampsia.(50) Another

process reflected by gestational age could be termed “immaturity”. In the ELGAN cohort, lowest mean blood pressure decreased with decreasing gestational age, as did the proportion of infants who received treatments to increase blood pressure.(47) Further, measures of physiological instability decreased with advancing gestational age.(38,59) A third set of processes, those that might confer protection against brain damage (e.g., thyroxine, anti-oxidants), have not yet been evaluated. Finally, gestational age conveys information about developmentally-regulated structural changes, apparently explaining why we found that the risks of pulmonary deterioration after the first week of life,(49) early and persistent pulmonary dysfunction,(49) and chronic lung disease(60) each decreased with increasing gestational age.

Our studies of the Score for Acute Neonatal Physiology (SNAP) illustrate the importance of assessing, when possible, each gestational age-related process. SNAP was initially developed as an illness severity score to help predict mortality in newborns.(61) Limited to the first 12 postnatal hours, it appears to provide information about the risk of ultrasound lesions. After adjustment for gestational age, high values (high predicts death) of two variations of SNAP, SNAP-II and SNAPPE-II(62) predicted moderate/severe ventriculomegaly and an echolucent lesion in the white matter as well as both MDI and PDI scores <55.(37,38) No SNAP-II or SNAPPE-II cut-off predicted any cerebral palsy diagnosis with statistical significance. The approximately doubling of risk of hemiparetic cerebral palsy seen with some SNAP-II and SNAPPE-II cutoffs did not achieve statistical significance, largely because only 19 children developed hemiparetic cerebral palsy. High SNAPPE-II predicted a positive screen for an autism spectrum disorder and a small head circumference. Thus high SNAPS might be indicators of immaturity and vulnerability that supplement risk information conveyed by gestational age variables.(37,38)

6. Bronchopulmonary dysplasia

In an attempt to gain a better understanding of why children who developed chronic lung disease are more likely than others to have later developmental impairments (63,64), we sought a better understanding of neonatal lung disease. Within nearly all strata of antenatal, placental and neonatal variables, growth restricted infants were at increased bronchopulmonary dysplasia risk compared with infants who were not growth restricted. (60) Fetal growth restriction was the only maternal or antenatal characteristic that predicted bronchopulmonary dysplasia risk after adjustment for antenatal and neonatal characteristics. Neither chorioamnionitis nor umbilical vasculitis was associated with increased risk.

We identified two forms of lung dysfunction during the first two postnatal weeks that predicted chronic lung disease. (49) The most severe is early and persistent pulmonary dysfunction (an $\text{FiO}_2 \geq 0.23$ on all days between 3 and 7 days of life and receiving $\text{FiO}_2 > 0.25$ on Day 14) (N=576). The other form first begins during the second postnatal week and is labeled pulmonary deterioration (an $\text{FiO}_2 < 0.23$ at any time between 3 and 7 days and receiving $\text{FiO}_2 > 0.25$ on day 14) (N=484).

We also identified two forms of chronic lung disease. Both are defined by oxygen dependency at 36 weeks post-menstrual age. The more severe form, however, also requires the child be ventilator dependent (48).

7. Indicators of inflammation predict neonatal brain damage

Prior to planning the ELGAN Study, we postulated that the phenomena that lead to preterm birth contribute to cerebral white matter damage and cerebral palsy. (65) In the ELGAN cohort, we identified six disorders leading to very preterm birth. (50) Based on histological changes and microbiological findings, these disorders were categorized into two broad groups. One group, characterized by the recovery of organisms from the placenta and histological

inflammation of the placenta, is associated with preterm labor, preterm premature rupture of the fetal membranes, placental abruption, and cervical insufficiency. The second group, characterized by the absence of organisms and the presence of histological features associated with disorders at the maternal-fetal interface, is associated with preeclampsia and delivery for fetal indications. We infer that disorders causing preterm delivery can be grouped broadly into disorders associated with aberrations of placental function and those associated with intrauterine inflammation.

This classification has helped us better understand the association between pregnancy disorders and brain outcomes. Compared to infants delivered for preeclampsia, those delivered for preterm labor, preterm premature rupture of membranes, and cervical insufficiency were at increased risk of ventriculomegaly, while those delivered for preterm labor, and intrauterine growth restriction were at increased risk of an echolucent lesion. (51) Children born to women who presented with preterm labor or preterm premature rupture of membranes were at increased risk of diparetic cerebral palsy, while children whose mother presented with cervical insufficiency were at increased risk of both diparetic and quadriparetic cerebral palsy. These associations did not achieve statistical significance, in part because of the relatively small numbers of children with each of these forms of cerebral palsy. Nevertheless, they are in keeping with the hypothesis that processes related to the disorders that lead to delivery much before term might also influence the risk of white matter damage and later clinical correlates.

To identify microorganisms associated with maternal and fetal inflammation, we collected samples of placenta parenchyma under sterile conditions and cultured these samples for bacteria and *Mycoplasmas*. Placentas delivered following preterm labor had the highest rates, which decreased with increasing gestational age from 79% at 23 weeks to 43% at 27 weeks. The lowest rate of organism recovery was from placentas delivered by Cesarean section for severe preeclampsia (25%).(54)

The presence of neutrophils in the umbilical cord is a biomarker of fetal inflammation.(66) In the ELGAN cohort, funisitis was associated with the recovery of specific microorganisms from the placenta parenchyma, including *Actinomyces*, *Prevotella bivia*, *Corynebacterium* species, *E. coli*, *Peptostreptococcus magnus*, multiple species of *Streptococci*, and *Mycoplasma* species, including *Ureaplasma urealyticum*.(35) These observations, and the previously described association of fetal vasculitis and brain damage in preterm infants,(31) suggests that specific microorganisms in the placenta can lead to fetal inflammation, which in turn can lead to brain damage. Consistent with this possibility, is the finding in the ELGAN cohort that recovery of *Ureaplasmas* or bacteria from the placenta was associated with a higher risk of an echolucent lesion, and the recovery of bacteria was associated with a higher risk of ventriculomegaly.(55)

Recovery of microorganisms from placenta parenchyma, including skin microflora, predicted both ventriculomegaly and an echolucent lesion. (67) Polymicrobial infections predicted quadriparetic cerebral palsy, while recovery of skin flora predicted diparetic cerebral palsy. Ventriculomegaly is the only brain lesion and diparesis the only cerebral palsy diagnosis predicted by histologic inflammation of the placenta. Microorganism recovery need not be accompanied by placenta inflammation to predict ventriculomegaly or an echolucent lesion of the brain during the first few postnatal months or motor impairments at age 24 months.

Evidence that postnatal inflammation adversely affects the developing brain comes from our finding that late bacteremia and necrotizing enterocolitis were each associated with an increased risk of a Bayley Scales of Infant Development Mental Development Index more than 3 standard deviations below the mean (i.e., < 55).(48)

8. Brain structure-function relationships

We have found that ultrasound markers of white matter damage, defined as either ventriculomegaly or a parenchymal echolucent lesion, are predictive of all three forms of cerebral palsy that we studied (41). Forty-five percent of children with ventriculomegaly and 52% of those with an echolucent lesion developed cerebral palsy. One of these findings was present in 61% of children with spastic quadriplegia, 58% of those with hemiparesis, and 30% of those with spastic diparesis. Consistent with other studies,(68) only 6% of children who had an entirely normal ultrasound scan when in the neonatal intensive care unit developed cerebral palsy. Nonetheless, these children represented half of all cohort members with cerebral palsy.

Ultrasound-detected white matter damage predicted delayed mental development, but not as well as it predicted cerebral palsy. (52) While moderate/severe ventriculomegaly was associated with an almost 14-fold increase in the odds of cerebral palsy, this ultrasound lesion is associated with only a 3-fold increase in the odds of Mental Development Index < 70. An echolucent lesion is associated with an 18-fold increase in the odds of cerebral palsy, but only a 2.7-fold increase in the odds of Mental Developmental Index < 70. Fully 23% of infants who had no ultrasound lesion had a Bayley Scales of Infant Development Mental Development Index more than 2 standard deviations below the mean of the reference sample (i.e., < 70).

Ultrasound abnormalities were only slightly more predictive of delayed psychomotor development than of delayed mental development. Moderate/severe ventriculomegaly was associated with a 3.5-fold increase in the odds of Psychomotor Development Index < 70, and echolucent lesions with a 4.5-fold increase.

In the ELGAN cohort, microcephaly (i.e., head circumference more than 2 standard deviations below the mean) at 2 years, but not at birth, is associated with severe motor and cognitive impairments at 2 years. (45) Seventy-one percent of children with congenital microcephaly had a normal head circumference at 2 years and had neurodevelopmental outcomes comparable to children whose head circumference was normal at birth and 2 years. Microcephaly at 2 years was associated with an increased risk of an Mental Developmental Index <70, a cerebral palsy diagnosis, and screening positive for an autism spectrum disorder. The risks were further increased if the child also had cerebral white matter damage on an early ultrasound scan.

9. Screening for autism spectrum disorder in ELGANS

In the past decade increasing attention has been directed to autism spectrum disorders and routine screening during preschool years is now recommended.(69) For this reason we screened ELGAN study participants at 2 years of age using the M-CHAT. (44) Fully 21% of ELGAN subjects screened positive for an autism spectrum disorder. Among the children who appeared to be at increased risk of screening positive were those who had major motor, vision or hearing impairments. After eliminating those who had such impairments that might have interfered with M-CHAT screening, 16% of children, or nearly triple the expected rate, still screened positive. Even after eliminating children whose Mental Developmental Index was <70, 10% still screened positive.

Four of the six “critical” M-CHAT items (Does not point to indicate interest. Does not bring objects to show you. Does not imitate you. Does not look at toy when you point to it) were among the items most commonly failed by children who had motor, cognitive, visual or hearing limitations.(70) The children who have these limitations are much more likely than others to fail these and other items, including those that do not require the functional ability they lack.

Summary and future directions

The ELGAN Study's primary goal has been to identify the antecedents of brain damage, as detected with ultrasound, as well as with clinical examinations, among the most vulnerable preterm infants, those born before 28 weeks of gestation. We are seeking to identify not only initiators and damage promoters that increase the risk of brain damage, but also damage modulators, growth factors, and other molecules and processes that decrease the risk. With this information, clinical trials can be designed to evaluate how effectively a reduction or blockade of initiators or damage promoters prevents white matter damage, and to evaluate how effectively inflammation modulators and protectors (such as neurotrophins) prevent/minimize white matter damage.

An ongoing objective of the ELGAN investigators is to contribute to the theoretical foundations in the field of developmental epidemiology, resulting in a number of papers that attempt to integrate research findings into a conceptual framework.(3,5,^{8,10-12,17,18,71-77})

The ELGAN Study offers a number of methodological strengths. First, the study included a large number of infants, making it unlikely that we will miss important associations due to lack of statistical power. Second, we selected infants based on gestational age, not birth weight, in order to minimize confounding due to factors related to fetal growth restriction.(20) Third, we collected all of our data prospectively. Fourth, we minimized observer variability as best we could in the interpretation of ultrasound scans (1) and assessments of neurodevelopmental functions(4), as well as assessments of placenta and retinal pathology. Fifth, examiners were not aware of the medical histories of the children they examined, thereby minimizing "diagnostic suspicion bias".(78) Sixth, attrition in the first two years was modest, with information about the two-year assessment available from 83% to 91% of survivors, depending on the outcome of interest.

The weaknesses of our study are those of all observational studies. We are unable to distinguish between causation and association as explanations for what we found. In addition, the sickest infants were more likely to be treated aggressively than others who were not quite so sick, making our study prone to confounding by indication(79,80).

As described above, we have completed only some of the papers we plan. Analyses of blood levels of cytokines, chemokines, adhesion molecules, hormones, and growth factors are underway. Once these data are available we will analyze associations between these biomarkers and the outcomes we have assessed.

We have created a tissue bank containing specimens of placenta, umbilical cord and blood spots from all infants in this study, so that future analyses can be carried out when justified by new knowledge and technology.

Because assessments of function at 2 years are imperfect predictors of school-age clinical expressions of brain damage, we are applying for additional funding for evaluations of the ELGAN Study participants at 9 years of age, when we can assess cognitive capacity, language, reading, and computational abilities, impairments in attention and executive function, autism, epilepsy, as well as structural brain abnormalities on magnetic resonance imaging.

Acknowledgments

Financial Disclosures: This study was supported by a cooperative agreement with The National Institute of Neurological Disorders and Stroke (NINDS) (SU01NS040069-05). The authors gratefully acknowledge the contributions of our subjects and their families, as well as those of our colleagues.

Abbreviations

ELGAN	extremely low gestational age newborn
M-CHAT	Modified Checklist for Autism in Toddlers
SNAP	Score for Acute Neonatal Physiology

Reference List

1. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. *Seminars in Reproductive Medicine* 2007;25:21–39. [PubMed: 17205421]
2. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. *Pediatr Res* 1997;42:1–8. [PubMed: 9212029]
3. Dammann O, O'Shea TM. Cytokines and Perinatal Brain Damage. *Clinics in Perinatology* 2008;35:643–663. [PubMed: 19026332]
4. Dammann O, Leviton A. The role of the fetus in perinatal infection and neonatal brain injury. *Curr Opin Pediatr* 2000;12:99–104. [PubMed: 10763757]
5. Dammann O, Leviton A. Inflammatory brain damage in preterm newborns - dry numbers, wet lab, and causal inferences. *Early Human Development* 2004;79:1–15. [PubMed: 15282118]
6. Leviton A, Dammann O. Coagulation, inflammation, and the risk of neonatal white matter damage. *Pediatric Research* 2004;55:541–545. [PubMed: 14973176]
7. Hagberg H, Dammann O, Mallard C, Leviton A. Preconditioning and the developing brain. *Seminars in Perinatology* 2004;28:389–395. [PubMed: 15693395]
8. Leviton A, Dammann O, Durum SK. The adaptive immune response in neonatal cerebral white matter damage. *Annals of Neurology* 2005;58:821–828. [PubMed: 16250014]
9. Dammann O, Leviton A, Gappa M, Dammann CEL. Lung and brain damage in preterm newborns, and their association with gestational age, prematurity subgroup, infection/inflammation and long term outcome. *Bjog-An International Journal of Obstetrics and Gynaecology* 2005;112:4–9. [PubMed: 15715586]
10. Dammann O, Leviton A. Perinatal brain damage causation. *Developmental Neuroscience* 2007;29:280–288. [PubMed: 17762196]
11. Dammann O. Persistent neuro-inflammation in cerebral palsy: a therapeutic window of opportunity? *Acta Paediatrica* 2007;96:6–7. [PubMed: 17187594]
12. Bueter W, Dammann O, Leviton A. Endoplasmic reticulum stress, inflammation, and perinatal brain damage. *Pediatr Res*. 2009 (in press).
13. Back SA, Riddle A, McClure MM. Maturation-dependent vulnerability of perinatal white matter in premature birth. *Stroke* 2007;38:724–730. [PubMed: 17261726]
14. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurology* 2009;8:110–124. [PubMed: 19081519]
15. Leviton A, Gressens P. Neuronal damage accompanies perinatal white-matter damage. *Trends Neurosci* 2007;30:473–478. [PubMed: 17765331]
16. Johnston MV, Ishida A, Ishida WN, Matsushita HB, Nishimura A, Tsuji M. Plasticity and injury in the developing brain. *Brain & Development* 2009;31:1–10. [PubMed: 18490122]
17. Dammann O, Bueter W, Leviton A, Gressens P, Dammann CEL. Neuregulin-1: A potential endogenous protector in perinatal brain white matter damage. *Neonatology* 2008;93:182–187. [PubMed: 17993737]
18. Dammann O, Leviton A. Brain damage in preterm newborns: might enhancement of developmentally-regulated endogenous protection open a door for prevention? *Pediatrics*. 1999
19. Drummond PM, Colver AF. Analysis by gestational age of cerebral palsy in singleton births in north-east England 1970-94. *Paediatric and Perinatal Epidemiology* 2002;16:172–180. [PubMed: 12060314]

20. Arnold CC, Kramer MS, Hobbs CA, McLean FH, Usher RH. Very low birth weight: a problematic cohort for epidemiologic studies of very small or immature neonates. *Am J Epidemiol* 1991;134:604–613. [PubMed: 1951265]
21. O'Shea TM, Klinepeter KL, Dillard RG. Prenatal events and the risk of cerebral palsy in very low birth weight infants. *American Journal of Epidemiology* 1998;147:362–369. [PubMed: 9508103]
22. Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. *JAMA* 1997;278:207–211. [PubMed: 9218666]
23. Savitz, D. *Interpreting Epidemiologic Evidence*. Oxford University Press; New York: 2003. New York
24. O'Shea TM. Definition and classification of cerebral palsy - an epidemiologist perspective. *Developmental Medicine and Child Neurology* 2007;49:29–30.
25. Leviton A, Dammann O, Allred EN, Kuban K, Pagano M, Van Marter L, Paneth N, Reuss ML, Susser M. Antenatal corticosteroids and cranial ultrasonographic abnormalities. *American Journal of Obstetrics and Gynecology* 1999;181:1007–1017. [PubMed: 10521769]
26. Dammann O, Allred EN, Van Marter LJ, Dammann CEL, Leviton A. Bronchopulmonary dysplasia is not associated with ultrasound-defined cerebral white matter damage in preterm newborns. *Pediatric Research* 2004;55:319–325. [PubMed: 14605242]
27. Hansen AR, Collins MH, Genest D, Heller D, Schwarz S, Banagon P, Allred EN, Leviton A. Very low birthweight infant's placenta and its relation to pregnancy and fetal characteristics. *Pediatric and Developmental Pathology* 2000;3:419–430. [PubMed: 10890926]
28. Kuban K, Allred EN, Dammann O, Pagano M, Leviton A, Share J, Abiri M, DiSalvo D, Doubilet P, Kairam R, Kazam E, Kirpekar M, Rosenfeld DL, Sanocka U, Schonfeld S, for the Developmental Epidemiology Network. Topography of cerebral white matter disease of prematurity studied prospectively in 1607 very-low-birthweight infants. *J Child Neurol* 2001;16:401–408. [PubMed: 11417604]
29. Leviton A, Paneth N, Susser M, Reuss ML, Allred EN, Kuban K, Sanocka U, Hegyi T, Hiatt M, Shahrivar F, Van Marter LJ. Maternal receipt of magnesium sulfate does not seem to reduce the risk of neonatal white matter damage. *Pediatrics* 1997;99:E2. [PubMed: 9099777]
30. Leviton A, Paneth N, Reuss ML, Susser M, Allred EN, Dammann O, Kuban K, Van Marter LJ, Pagano M. Hypothyroxinemia of prematurity and the risk of cerebral white matter damage. *Journal of Pediatrics* 1999;134:706–711. [PubMed: 10356138]
31. Leviton A, Paneth N, Reuss ML, Susser M, Allred EN, Dammann O, Kuban K, Van Marter LJ, Pagano M. Maternal infection, fetal inflammatory response, and brain damage in very low birth weight infants. *Pediatric Research* 1999;46:566–575. [PubMed: 10541320]
32. Dammann O, Allred EN, Kuban ECK, Van Marter LJ, Pagano M, Sanocka U, Leviton A. Systemic hypotension and white-matter damage in preterm infants. *Developmental Medicine and Child Neurology* 2002;44:82–90. [PubMed: 11852927]
33. Kuban K, Sanocka U, Leviton A, Allred EN, Pagano M, Dammann O, Share J, Rosenfeld R, Abiri M, DiSalvo D, Doubilet P, Kairam R, Kazam E, Kirpekar M, Schonfeld S, Members of the Development Epidemiology Network. White matter disorders of prematurity: Association with intraventricular hemorrhage and ventriculomegaly. *J Pediatr* 1999;134:546.
34. Hecht JL, Allred EN, Kliman HJ, Zambrano E, Doss BJ, Husain A, Pflueger SMV, Chang CH, Livasy CA, Roberts D, Bhan I, Ross DW, Senagore PK, Leviton A. Histological characteristics of singleton placentas delivered before the 28th week of gestation. *Pathology* 2008;40:372–376. [PubMed: 18446627]
35. Hecht JL, Onderdonk A, Delaney M, Allred EN, Kliman HJ, Zambrano E, Pflueger SM, Livasy CA, Bhan I, Leviton A. Characterization of chorioamnionitis in 2nd-trimester C-section placentas and correlation with microorganism recovery from subamniotic tissues. *Pediatric and Developmental Pathology* 2008;11:15–22. [PubMed: 18237241]
36. Hecht JL, Kliman HJ, Allred EN, Pflueger SM, Chang CH, Doss BJ, Roberts D, Livasy CA, Bhan I, Zambrano E, Ross DW, Senagore P, Husain AN, Leviton A. Reference weights for placentas delivered before the 28th week of gestation. *Placenta* 2007;28:987–990. [PubMed: 17573110]
37. Dammann O, Naples M, Bednarek F, Shah B, Kuban KCK, O'Shea TM, Paneth N, Allred EN, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. SNAP-II and

- SNAPPE-II and the risk of structural and functional brain disorders in infants born before the 28th week of gestation. *Neonatology* 2010;97:71–82. [PubMed: 19672122]
38. Dammann O, Shah B, Naples M, Bednarek F, Zupancic J, Allred EN, Leviton, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. SNAP-II and SNAPPE-II as predictors of death among infants born before the 28th post-menstrual week. Inter-institutional variation. *Neonatology*. 2009 (in press).
 39. Kuban JD, Allred EN, Leviton A. Thymus involution and cerebral white matter damage in extremely low gestational age neonates. *Biology of the Neonate* 2006;90:252–257. [PubMed: 16790992]
 40. Kuban K, Adler I, Allred E, Batton D, Bezinque S, Betz BW, Cavenagh E, Durfee S, Ecklund K, Feinstein K, Fordham LA, Hampf F, Junewick J, Lorenzo R, McCauley R, Miller C, Seibert J, Specter B, Wellam J, Westra S, Leviton A. Observer variability assessing US scans of the preterm brain: the ELGAN study. *Pediatr Radiol* 2007;37:1201–1208. [PubMed: 17901950]
 41. Kuban K, Allred E, O'Shea TM, Paneth N, Pagano M, Dammann O, Leviton A, Duplessis A, Westra S, Bassan H, Krishnamoorthy K, Junewick J, Olomu N, Romano E, Seibert J, Engelke S, Karna P, Batton D, O'Connor S, Keller C, Miller C, the ELGAN Investigators. Cranial ultrasound lesions in the NICU predict cerebral palsy at age 2 years in children who were born at extremely low gestational age. *J Child Neurol* 2008;24:63–72. [PubMed: 19168819]
 42. Kuban K, Allred E, O'Shea TM, Paneth N, Pagano M, Leviton A, ELGAN Study Cerebral Palsy Algorithm Group. An algorithm for identifying and classifying cerebral palsy in young children. *Journal of Pediatrics* 2008;153:466–472. [PubMed: 18534210]
 43. Kuban KCK, O'Shea M, Allred E, Leviton A, Gilmore H, DuPlessis A, Krishnamoorthy K, Hahn C, Soul J, O'Connor SE, Miller K, Church PT, Keller C, Bream R, Adair R, Miller A, Romano E, Bassan H, Kerkerling K, Engelke S, Marshall D, Milowic K, Wereszczak J, Hubbard C, Washburn L, Dillard R, Heller C, Burdo-Hartman W, Fagerman L, Sutton D, Karna P, Olomu N, Caldarelli L, Oca M, Lohr K, Scheiner A. Video and CDROM as a training tool for performing neurologic examinations of 1-year-old children in a multicenter epidemiologic study. *Journal of Child Neurology* 2005;20:829–831. [PubMed: 16417880]
 44. Kuban KCK, O'Shea TM, Allred EN, Tager-Flusberg H, Goldstein DJ, Leviton A. Positive Screening on the Modified Checklist for Autism in Toddlers (M-CHAT) in Extremely Low Gestational Age Newborns. *Journal of Pediatrics* 2009;154:535–540. [PubMed: 19185317]
 45. Kuban KCK, Allred EN, O'Shea TM, Paneth N, Westra S, Miller C, Rosman NP, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. Developmental correlates of head circumference at birth and at two years in a cohort of extremely low gestational age newborns (ELGANs). *J Pediatr*. 2009 (in press).
 46. Bose CL, Van Marter L, Laughon M, O'Shea TM, Allred EN, Karna P, Ehrenkranz R, Boggess K, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. Fetal growth restriction is followed by an increased risk of chronic lung disease among infants born before the 28th week of gestation. *Pediatrics*. 2009 published online August 17, 2009.
 47. Laughon M, Bose C, Allred E, O'Shea M, Van Marter LJ, Bednarek F, Leviton A. Factors associated with treatment for hypotension in extremely low gestational age newborns during the first postnatal week. *Pediatrics* 2007;119:273–280. [PubMed: 17272616]
 48. Laughon M, O'Shea TM, Allred EN, Bose C, Kuban K, Van Marter L, Ehrenkranz R, Leviton A, ELGAN Study Investigators. Chronic lung disease and the risk of developmental delay at two years of age in children born before 28 weeks postmenstrual age. *Pediatrics* 2009;124:637–648. [PubMed: 19620203]
 49. Laughon M, Allred EN, Bose CL, O'Shea TM, Van Marter L, Ehrenkranz R, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. Patterns of respiratory disease during the first two weeks of life in extremely preterm infants. *Pediatrics* 2009;123:1131.
 50. McElrath TF, Hecht JL, Dammann O, Boggess K, Onderdonk A, Markenson G, Harper M, Delpapa E, Leviton A, ELGAN Study Investigators. Pregnancy disorders that lead to delivery before the 28th week of gestation: an epidemiologic approach to classification. *Am J Epidemiol* 2008;168:980–989. [PubMed: 18756014]
 51. McElrath TF, Allred EN, Kuban KCK, O'Shea TM, Paneth N, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. Maternal antenatal characteristics predict

- ultrasound abnormalities of the preterm birth, and likely later cerebral palsy. *Am J Epidemiol.* 2009 (in press).
52. O'Shea TM, Kuban KCK, Allred EN, Paneth N, Pagano M, Dammann O, Bostic L, Brooklier K, Butler S, Goldstein DJ, Hounshell G, Keller C, McQuiston S, Miller A, Pasternak S, Plesha-Troyke S, Price J, Romano E, Solomon KM, Jacobson A, Westra S, Leviton A. Neonatal cranial ultrasound lesions and developmental delays at 2 years of age among extremely low gestational age children. *Pediatrics* 2008;122:E662–E669. [PubMed: 18762501]
 53. Onderdonk AB, Hecht JL, McElrath TF, Delaney ML, Allred EN, Leviton A. Colonization of second-trimester placenta parenchyma. *American Journal of Obstetrics and Gynecology* 2008;199:52.e1–52.e10. [PubMed: 18313635]
 54. Onderdonk AB, Delaney ML, Dubois AM, Allred EN, Leviton A. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. *American Journal of Obstetrics and Gynecology* 2008;198:e1–e7. [PubMed: 18166321]
 55. Olomu IN, Hecht JL, Onderdonk AO, Allred EN, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. Perinatal correlates of Ureaplasma urealyticum in placenta parenchyma of singleton pregnancies that end before 28 weeks of gestation. *Pediatrics* 2009;123:1329–1336. [PubMed: 19403499]
 56. Martin CR, Brown YF, Ehrenkranz R, O'Shea TM, Allred EN, Belfort MB, McCormick MC, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. Nutritional practices and growth velocity in the first month of life in extremely low gestational age newborns. *Pediatrics* 2009;124:649–657. [PubMed: 19651583]
 57. Begg MD, Parides MK. Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. *Statistics in Medicine* 2003;22:2591–2602. [PubMed: 12898546]
 58. Leviton A, Blair E, Dammann O, Allred E. The wealth of information conveyed by gestational age. *Journal of Pediatrics* 2005;146:123–127. [PubMed: 15644836]
 59. Dammann O, Naples M, Bednarek F, Shah B, Kuban KCK, O'Shea TM, Paneth N, Allred EN, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. SNAP-II and SNAPPE-II and the risk of structural and functional brain disorders in infants born before the 28th week of gestation. *Neonatology.* 2009 (in press).
 60. Bose CL, Van Marter L, Laughon M, O'Shea TM, Allred EN, Karna P, Ehrenkranz R, Boggess K, Leviton A, for the Extremely Low Gestational Age Newborn (ELGAN) Study Investigators. Fetal growth restriction is followed by an increased risk of chronic lung disease among infants born before the 28th week of gestation. *Pediatrics.* 2009 (in press).
 61. Richardson DK, Gray JE, McCormick MC, Workman K, Goldmann DA. Score for Neonatal Acute Physiology: a physiologic severity index for neonatal intensive care. *Pediatrics* 1993;91:617–623. [PubMed: 8441569]
 62. Richardson DK, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: Simplified newborn illness severity and mortality risk scores. *Journal of Pediatrics* 2001;138:92–100. [PubMed: 11148519]
 63. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, Wrage LA, Poole K. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. *Pediatrics* 2005;116:1353–1360. [PubMed: 16322158]
 64. Schmidt B, Asztalos EV, Roberts RS, Robertson CMT, Sauve RS, Whitefield MF, for the Trial of Indomethacin Prophylaxis in Preterms (TIPP) Investigators. Impact of bronchopulmonary dysplasia, brain injury, and severe retinopathy on the outcome of extremely low-birth-weight infants at 18 months. *JAMA* 2003;289:1124–1129. [PubMed: 12622582]
 65. Leviton A. Preterm Birth and Cerebral Palsy - Is Tumor-Necrosis-Factor the Missing Link. *Developmental Medicine and Child Neurology* 1993;35:553–556. [PubMed: 8504899]
 66. Park CW, Lee SM, Park JS, Jun JK, Romero R, Yoon BH. The antenatal identification of funisitis (fetal inflammation) with a rapid MMP-8 bedside test. *American Journal of Obstetrics and Gynecology* 2007;197:S21.
 67. Leviton A, Hecht JL, Onderdonk AO, Kuban K, O'Shea TM, Allred EN, Paneth N, for the ELGAN Study Investigators. Microbiological and histologic characteristics of the extremely preterm infant's

- placenta predict white matter damage and later cerebral palsy. The ELGAN Study. *Pediatric Research*. 2009 (in press).
68. Lupton AR, O'Shea TM, Shankaran S, Bhaskar B, NICHD Neonatal Network. Adverse neurodevelopmental outcomes among extremely low birth weight infants with normal head ultrasound: Prevalence and antecedents. *Pediatrics* 2005;115:673–680. [PubMed: 15741371]
 69. Johnson CP, Myers SM. Identification and evaluation of children with autism spectrum disorders. *Pediatrics* 2007;120:1183–1215. [PubMed: 17967920]
 70. Luyster RJ, Kuban KCK, O'Shea TM, Paneth N, Allred EN, Leviton A, for the ELGAN Study Investigators. The Modified Checklist for Autism in Toddlers (M-CHAT) in extremely low gestational age newborns: Individual items associated with cognitive, motor, vision, and hearing limitations. *Journal of Developmental and Behavioral Pediatrics*. 2009 (provisional acceptance).
 71. Dammann O, Durum SK, Leviton A. Modification of the infection-associated risks of preterm birth and white matter damage in the preterm newborn by polymorphisms in the tumor necrosis factor-locus? *Pathogenesis* 1999;1:171–177.
 72. Dammann O, Leviton A. Infection remote from the brain, neonatal white matter damage, and cerebral palsy in the preterm infant. *Semin Pediatr Neurol* 1998;5:190–201. [PubMed: 9777677]
 73. Dammann O, Durum S, Leviton A. Do white cells matter in white matter damage? *Trends in Neurosciences* 2001;24:320–324. [PubMed: 11356502]
 74. Leviton A, Dammann O, O'Shea TM, Paneth N. Adult stroke and perinatal brain damage: Like grandparent, like grandchild? *Neuropediatrics* 2002;33:281–287. [PubMed: 12571781]
 75. Dammann O, Kuban KCK, Leviton A. Perinatal infection, fetal inflammatory response, white matter damage, and cognitive limitations in children born preterm. *Mental Retardation and Developmental Disabilities Research Reviews* 2002;8:46–50. [PubMed: 11921386]
 76. Dammann O, Leviton A. Inflammation, brain damage and visual dysfunction in preterm infants. *Seminars in Fetal & Neonatal Medicine* 2006;11:363–368. [PubMed: 16581321]
 77. Dammann O. Risk, predictability and biomedical neo-pragmatism. *Acta Paediatrica* 2009;98:1093–1095. [PubMed: 19638012]
 78. Sackett DL. Bias in analytic research. *J Chron Dis* 1979;32:51–63. [PubMed: 447779]
 79. Walker AM. Confounding by indication. *Epidemiology* 1996;7:335–336. [PubMed: 8793355]
 80. Signorello LB, McLaughlin JK, Lipworth L, Friis S, Sørensen HT, Blot WJ. Confounding by indication in epidemiologic studies of commonly used analgesics. *Am J Ther* 2002;9:199–205. [PubMed: 11941379]

Table 1

Maternal and pregnancy characteristics that are correlates of inclusion or exclusion at each stage of the study. These are column percents.

Maternal and pregnancy characteristics		Sample					
		Cranial ultrasound		Survived to 24 months		Evaluated at 24 months	
		Yes	No	Yes	No	Yes	No
Racial identity	White	58	69	60	50	61	50
	Black	29	22	27	37	27	34
	Other	13	10	13	13	12	16
Maternal age	<21	14	16	14	16	13	28
	21-35	68	67	67	71	67	61
	>35	18	16	19	14	20	10
Years of education	<12	17	15	17	21	16	27
	12 (High School)	28	27	27	31	27	26
	>12 to <16	24	27	23	28	23	27
	16 (College)	18	21	18	13	19	16
	>16	13	9	14	6	15	3
Married	Yes	56	69	57	50	59	39
Public insurance	Yes	42	34	40	52	39	51
Primigravida	Yes	40	40	41	35	41	45
Conception assistance	Yes	20	34	21	13	22	15
Antenatal steroids	Complete	62	61	64	56	64	58
	Partial	27	25	26	29	25	34
	None	11	14	10	14	10	8
Pregnancy complication	PTL	44	45	45	42	45	38
	pPROM	21	39	22	18	22	23
	PE	14	0	13	17	13	9
	Abruption	10	2	11	9	11	10
	Cerv Insufficiency	6	6	6	6	5	15
Cesarean delivery	Fetal Indication	5	8	4	8	4	5
	Yes	65	63	66	58	67	61

Maternal and pregnancy characteristics	Sample					
	Cranial ultrasound		Survived to 24 months		Evaluated at 24 months	
	Yes	No	Yes	No	Yes	No
Maximum number of infants	1455	51	1198	257	1102	96
Total	1506		1455		1198	