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Among Children Born Extremely Preterm a Higher Level of Circulating Neurotrophins Is Associated with Lower Risk of Cognitive Impairment at School Age

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Abstract

Objectives—To test the hypothesis that higher blood levels of neurotrophic proteins (proteins that support neuronal survival and function) in the first 2 weeks of life are associated with a lower risk of cognitive impairment at 10 years.

Study design—We evaluated 812 10-year-old children with neonatal blood specimens enrolled in the multicenter prospective Extremely Low Gestational Age Newborn Study, assessing 22 blood proteins collected on 3 days over the first 2 weeks of life. Using latent profile analysis, we derived a cognitive function level based on standardized cognitive and executive function tests. We defined high exposure as the top quartile neurotrophic protein blood level on ≥2 days either for ≥4 proteins

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List of additional members of the ELGAN Study is available at www.jpeds.com(Appendix).

or for a specific cluster of neurotrophic proteins (defined by latent class analysis). Multinomial logistic regression analyzed associations between high exposures and cognitive impairment.

Results—Controlling for the effects of inflammatory proteins, persistently elevated blood levels of ≥4 neurotrophic proteins were associated with reduced risk of moderate (OR, 0.35; 95% CI, 0.18–0.67) and severe cognitive impairment (OR, 0.22; 95% CI, 0.09–0.53). Children with a cluster of elevated proteins including angiopoietin 1, brain-derived neurotrophic factor, and regulated upon activation, normal T-cell expressed, and secreted had a reduced risk of adverse cognitive outcomes (OR range, 0.31–0.6). The risk for moderate to severe cognitive impairment was least with 0–1 inflammatory and >4 neurotrophic proteins.

Conclusions—Persisting elevations of circulating neurotrophic proteins during the first 2 weeks of life are associated with lowered risk of impaired cognition at 10 years of age, controlling for increases in inflammatory proteins.

Advances in neonatal intensive care have increased the survival of extremely preterm children born at <28 weeks of gestation.[1] Increased survival rates have not been accompanied by similar improvements in neurodevelopmental outcomes, and one-quarter of survivors have cognitive impairment.[2,3] Reduction of the risk of cognitive impairment depends on an improved understanding of its etiology.

The Extremely Low Gestational Age Newborn (ELGAN) Study was designed to test the hypothesis that perinatal inflammation is associated with persisting brain structural and functional disorders. In the ELGAN cohort of about 1000 children born at <28 weeks of gestation, neonatal elevations of specific inflammation-associated protein biomarkers in blood robustly predicted cognitive impairment at 2 years of age.[4,5] These indicators of neonatal systemic inflammation also were associated with impaired cognition at 10 years of age.[6]

In the ELGAN Study, blood samples were taken in the first 2 weeks of life and we measured levels of neurotrophic proteins, including growth factors, neurotrophins, and angiotrophins that might influence developmental outcomes.[7,8] These proteins support the growth, survival, and differentiation of developing neurons. Lower levels of these proteins may signal less resilience against inflammation-associated injury and higher levels may prevent damage.[9] Inflammation-related proteins are associated with cognitive impairment, and neurotrophic proteins may be associated with better cognitive outcomes, but elevations within these 2 protein families that operate in opposite directions frequently occur simultaneously. We, therefore, tested the hypothesis that higher blood levels of neurotrophic proteins measured in the first 2 weeks of life would be associated with a lesser risk of cognitive impairment at 10 years of age, controlling for the presence of inflammation-associated proteins.

Methods

The ELGAN Study is a multicenter, observational study of the risk of structural and functional neurologic disorders in extremely preterm infants. From 2002 to 2004, women delivering at <28 weeks of gestation were asked for consent to enroll their child into the

study. This analysis includes 812 children (a subset of the surviving 1200 study participants) who had ≥2 sets of neonatal blood samples for proteins and were evaluated at 10 years of age (Figure 1; available at www.jpeds.com). This study was approved by the institutional review boards of all participating institutions and informed consent was obtained from all participants.

Cognitive Assessment and Derivation of Levels of Function

Child assessments included the full-scale IQ from the School-Age Differential Ability Scales–II Verbal and Nonverbal Reasoning scales[10] and executive function (EF) (from the School-Age Differential Ability Scales–II Verbal and Nonverbal Reasoning scales and the Developmental NEeuroPSYchological Assessment, 2nd Edition[11]).

Even though the development and maturation of neural circuits underlying aspects of IQ and EF differ from each other,[12] both can be reliably measured by 10 years of age.[13] As previously reported, we evaluated cognitive outcomes using latent class analysis (LCA) classifications, which represented both IQ and EF abilities, to provide a better predictor of adaptive outcomes, such as academic success, than IQ alone.[2,14] Children were classified into their most likely latent class for analysis. With LCA, we identified 4 subgroups of children in our cohort corresponding with overall cognitive functioning that was normal (34% of cohort; normal mean IQ and EF scores), low-normal (41%; mean IQ and EF scores ranging from 0.5 to 1.5 SDs below norm), moderately impaired (17%; mean IQ and EF measures from 1.5 to 2.5 SDs below norm), and severely impaired (8%; mean IQ and EF measures from 2.5 to 4.0 SDs below norm).[2]

Assessment of Inflammation and Neutrophic Proteins

Blood Protein Measurements.—Drops of whole blood were collected on postnatal days 1 (range, 1–3 days), 7 (range, 5–8 days), and 14 (range, 12–15 days).[15,16] Protein concentration quartiles were normalized for gestational age and day of collection.[17] Because single day elevations of proteins are not as strongly associated with cognitive outcomes as are persistent elevations,[6] we defined protein concentration elevation as being in the highest quartile on ≥2 of 3 measures obtained.

Identification of Clusters of Neurotrophic and Inflammation-Associated Proteins with LCA.—The specific neurotrophic proteins evaluated are listed in Table I (available at www.jpeds.com). Given that the blood levels of neurotrophic proteins under study might correlate with one another, we conducted separate LCA analyses on each postnatal day, fitting models with 2–5 classes and choosing an appropriate model based on fit statistics, entropy, interpretability, and consistency of results across days (Table II; available at www.jpeds.com). Analyses consistently identified 3 subgroups of children with similar patterns of neurotrophin elevations. Based on these analyses, we categorized children into 3 distinct subgroups (Table III; available at www.jpeds.com). The neurotrophic-related protein group (NRG) 1 had ≤2 elevated proteins; NRG2 had elevations of ≥3 proteins including ≥2 of the following 3 neurotrophic proteins: regulated upon activation, normal T-cell expressed, and secreted (RANTES), brain-derived neurotrophic factor (BDNF), and

angiopoietin 1 (Ang-1); and NRG3 had low levels on ≥2 of 3 NRG2 proteins, RANTES, BDNF, and Ang-1, but elevated levels of ≥3 of the other neurotrophic proteins (Table III).

The analyses also included values for 8 inflammation-related proteins obtained at the same 3 time points as the neurotrophic proteins. These inflammatory-related proteins have been associated with structural and functional neurological outcomes in previous ELGAN Study analyses Table I.[4,18,19] As with the neurotrophic proteins, we conducted LCA on postnatal days 1, 7, and 14 and found that a 3-class solution was most consistent across all 3 days (Table II). Based on these analyses, we identified 3 distinct subgroups of children: inflammatory group (IRG) 2 had ≥3 elevated proteins that included elevation of either C-reactive protein (CRP) or serum amyloid A (SAA). IRG3 had normal CRP and SAA but had elevations of ≥3 other inflammatory proteins (Table III).

We a priori operationally defined neurotrophic protein exposure in 2 ways. First, we considered the number of sustained elevated inflammatory and sustained elevated neurotrophic proteins as measures of the breadth of inflammatory or neurotrophic exposure (0–1 proteins [referent group], 2–3 proteins, >4 proteins). Second, we considered the at-risk subgroups of children based on a pattern of elevated proteins derived from LCA.

Statistical Analyses

We tested the hypothesis that elevation of neurotrophic proteins in the first 2 weeks of life is associated with a decreased risk of cognitive impairment. Because high concentrations of inflammation-related proteins can prompt high concentrations of putative neurotrophic proteins,[20–29] in our analyses we controlled for elevations of inflammatory proteins when evaluating risks associated with neurotrophic proteins.

Maternal and infant characteristics of the study sample were compared across neurotrophin risk groups with a χ^2 test; characteristics associated with risk groups at $P < .05$ were considered potential confounders. Multivariable multinomial logistic regression models were used to examine adjusted associations of elevated inflammatory and neurotrophic proteins with cognitive impairment at 10 years of age. All analyses controlled child sex and maternal education.[3,30] Secondary analyses also controlled for additional potential confounding by variables found to be associated with either neurotrophic or inflammatory protein exposure in preliminary analyses. Adjusted associations based on these logistic regression models were described with ORs and 95% CIs. In secondary analyses, variables found to be associated with either neurotrophic or inflammatory protein exposure were examined for confounding and controlled for if the adjusted ORs for neurotrophic or inflammatory proteins changed by ≥10%. Secondary analyses also examined the association between number of neurotrophic and inflammatory proteins and IQ alone, categorized as <70, 70–85, and >85.

Results

Demographic Characteristics and Protein Elevations

Maternal and child characteristics, including maternal body mass index and maternal smoking status, did not significantly differ across neurotrophic protein risk groups of

children ($P > .10$), with the exception of birth weight z-score ($P < .05$) (Table IV). Children with a greater number of elevated neurotrophic proteins were more likely to have elevated inflammatory proteins ($P < .001$). For example, 32% of children with >4 elevated neurotrophic proteins had >4 elevated inflammatory proteins, but only 4% of children with 0–1 elevated neurotrophic proteins had >4 elevated inflammatory proteins. Elevated inflammatory proteins were associated with maternal race, marital status, smoking during pregnancy, and birth weight z-score category (data not shown). Among these risk factors, only maternal race was found to confound the association between elevated proteins and cognitive impairment in multivariable analyses described elsewhere in this article.

Patterns of Inflammatory and Neurotrophic Protein Elevations

In each of 3 strata defined in terms of the number of elevations of inflammatory proteins (columns), the risk of cognitive impairment decreased as the number of elevations of neurotrophic proteins increased (Figure 2). For example, for children with >4 elevated inflammatory proteins, the percent with cognitive impairment decreased from 56.3% to 22.0% as the number of neurotrophic proteins increased from 0 to 1 to >4 (OR, 0.22; 95% CI, 0.07–0.70). Similarly, for children with 0–1 elevated inflammatory proteins, the percent with cognitive impairment decreased from 22.2% to 10.0% (OR, 0.39; 95% CI, 0.17–0.89). In the 3 strata defined by the number of elevations of neurotrophic proteins, the risk of cognitive impairment increased with elevated number of inflammatory proteins. Multiple logistic regression analysis of these data (Table IV) show significant effects of both neurotrophic ($P = .004$) and inflammatory proteins ($P < .001$), with no significant multiplicative interaction ($P = .670$). The risk for moderate or severe cognitive impairment was greatest in the presence of >4 inflammatory and 0–1 neurotrophic proteins (56%) and least when there were 0–1 inflammatory and >4 neurotrophic proteins (10%).

Associations between Protein Elevations and Cognitive Impairment

Adjusting for the number of elevations of inflammatory proteins, elevation of >4 neurotrophic proteins was associated with a reduced risk of moderate and severe cognitive impairment (Table V). Elevation of 2–3 neurotrophic proteins also was associated with decreased risk of cognitive impairment in the severely impaired and low normal groups of children. Secondary analyses examining confounding by other maternal and infant characteristics identified race as a confounder; associations of >4 elevated inflammatory and neurotrophic proteins with severe and moderate cognitive impairment were somewhat attenuated, but remained significant after additionally controlling for race. Secondary analyses using IQ alone as the outcome showed similar associations with elevated proteins. For IQ categories, both 2–3 and >4 elevated inflammatory proteins significantly increased the odds of an IQ of <70, but not an IQ of 70–85, whereas both 2–3 and >4 elevated neurotrophic proteins were associated with lower odds of an IQ of <70, but not an IQ of 70–85 (data not shown).

The latent class category resulting from the analysis of neurotrophic proteins that was characterized by elevated proteins, including 2 of 3 proteins (RANTES, BDNF, or Ang-1), was associated with a lesser risk of cognitive impairment for the severely impaired, moderately impaired, and low-normal categories (Table VI). The latent class with elevated

levels of other neurotrophic proteins, but not RANTES, BDNF, and Ang-1, was not associated with cognitive outcome (Table VI). In LCA-defined inflammation subgroups of children, IRG2 (pre-dominantly SAA and CRP elevations) was significantly associated with all 3 adverse cognitive outcome groups relative to the reference group, but IRG3 (≥3 proteins other than SAA and CRP) was significantly associated with increased risk in the moderately impaired children only.

There were no significant interactions ($P > .65$) between neurotrophic and inflammatory proteins related to cognitive outcomes. Adjustments for birth weight z-score, the only demographic or birth characteristic associated with the neurotrophic protein LCA groups of children, did not substantively change the association between neurotrophic protein elevations and cognitive outcome.

Discussion

Whereas the sustained presence of inflammation-related proteins in the blood during the first 2 weeks after birth was associated with adverse cognitive outcomes at 10 years of age, increased levels of circulating neurotrophic proteins were associated with a lesser risk of cognitive impairment. The lower risk for cognitive impairment associated with elevation of neurotrophic proteins was evident whether indexed by the number of elevated proteins or by a cluster of neurotrophic proteins derived from LCA that included RANTES, BDNF, and Ang-1. With both approaches, the association was stronger with severe compared with moderate cognitive impairment.

We examined the association between elevated neurotrophic proteins in the first weeks of life with cognitive abilities, adjusting for inflammation. This approach was undertaken based on the notion that inflammation-related and neurotrophic proteins correlate with each other but seem to influence the risk of cognitive impairment in opposite directions. That inflammation-related and neurotrophic proteins are both elevated more often than expected by chance, suggests ≥2 possible underlying biological models. In 1 model, inflammatory and neurotrophic protein elevations have a common initiator, prompting an inflammatory cascade as well as enhancing production of neurotrophic proteins. A number of in vitro neonatal models suggest that BDNF and other neurotrophins are decreased rather than increased when exposed to a lipopolysaccharide stimulus, suggesting that this mechanism is less likely.[24] A second, more likely model invokes neurotrophic protein elevations as a consequence of inflammation, possibly as a nonspecific upregulation of many proteins or an attempt of the body to”downregulate” inflammation.

As part of our evaluation of 12 proteins thought to have neurotrophic properties, we sought to understand whether these neurotrophic proteins fluctuated independently or in concert. LCA identified 3 patterns of elevated neurotrophic protein values. One group of children, NRG2, with elevations of RANTES, BDNF, and Ang-1, were least at risk for adverse cognitive outcomes. Support for a neuroprotective role for RANTES, BDNF, and Ang-1 includes the association of RANTES with a decreased risk of attention deficit hyperactivity disorder in our cohort.[31] Also, in preterm infants, higher BDNF values are associated with lower odds of failing developmental milestones[32] and developing retinopathy of prematurity.

[33] In rodent brain ischemia and head trauma models, BDNF also is associated with neuroprotective effects.[34,35] Ang-1 in experimental models seems to ameliorate the impact of cerebral ischemia and stroke in rats.[36,37]

We found no interaction between elevated neurotrophic and inflammatory proteins with respect to cognitive risk. This finding implies that elevated neurotrophic proteins are protective against cognitive impairment regardless of the level of inflammatory proteins, rather than only providing protection in the presence of elevated inflammatory proteins. However, statistical tests for interaction have lower power, and this null result should be interpreted cautiously.

We conceptualized our outcome as cognitive impairment and chose to summarize IQ and EF variables using LCA, which identified subgroups of children by degree of cognitive impairment. Other approaches to identifying impairment categories, such as categorizing impairment based on IQ and EF z-scores or a factor analysis approach, would also be valid. Similarly, because severity and breadth of inflammatory exposure seems to be a critical predictor of outcome,[6] we conceptualized inflammatory and neurotrophic protein exposure as representing exposure categories and used LCA to define the exposure categories.

Brain development in the extremely preterm infant involves dynamic and critical processes that are distinguishable from those occurring in the more mature brain, and many of these developmental processes seem to be adversely affected in several ways. First, to a greater extent than in the term-born infant's brain, the immature brain demonstrates vigorous dendritic and axonal growth (particularly growth cone proliferation), as well as myelinogenesis and angiogenesis.[38] Second, developing preoligodendrocytes and subplate neurons at 24–32 weeks of gestation are extremely vulnerable to physiological perturbations.[39] Third, most programmed cell death (apoptosis) in neuronal populations occurs prenatally, whereas cell death in glia populations as well as production and pruning of connections are largely postnatal events.[40] Fourth, to a greater extent than infants born at term, preterm infants encounter a host of potentially prenatal, perinatal, and postnatal harmful exposures, including inflammatory processes that frequently precipitate early delivery or occur in the context of postnatal illnesses, such as chronic lung disease, necrotizing enterocolitis, and sepsis.[38,41] Fifth, preterm infants may have reduced capacities to synthesize proteins that promote cell growth or survival in the amounts needed for normal development,[42] particularly when exposed to adverse stresses, such as inflammation and infection, which occur commonly in extremely preterm infants.[9,43]

Our finding that the presence of proteins with neurotrophic properties reduces risks for adverse cognitive impairment support the notion that such proteins can stimulate oligodendrocyte progenitor cell proliferation,[44] induce oligodendrocyte progenitors "to undergo continuous self-renewal,"[45] minimize oligodendrocyte progenitor and neuronal apoptosis,[46,47] and rescue motor neurons from axotomy.[48–50]

These results suggest that there are complex determinants of adverse outcomes involving an interplay between imposed risks and host resistance and resilience. Both risk and protective factors likely are under the control of genetic and local environmental or epigenetic

influences, which may either enhance or dampen inflammatory or neurotrophic and neuroprotective mechanisms.[51] For example, inflammatory-promoting clinical conditions associated with low gestational age (such as chorioamnionitis, chronic lung disease [or protracted intubation],[52] necrotizing enterocolitis,[53] and sepsis[52]) may contribute to heightened adverse outcome risk. In contrast, antenatal exposure to magnesium,[54] socioeconomic advantage,[30] or exposures that enhance neurotrophic concentrations may positively influence the balance between risk and neuroprotection. Calabrese et al and Dhobale reviewed the effects of neurotrophic proteins in the perinatal period and noted that elevation of certain neurotrophins, including BDNF, are associated with maternal preeclampsia and fetal growth restriction, whereas neurotrophin-3 is diminished in the presence of placental inflammation.[24,55] Further complicating our understanding of determinants of outcome is the observation that many of the proteins we characterize as either inflammatory or having neurotrophic properties may be pleiotrophic, functioning to enhance risk in some contexts and to protect brain function in others. For example, Ang-1 seems to have neurotrophic properties in these analyses, yet under other circumstances has proinflammatory characteristics.[56,57] Similar data exist for BDNF,[58–60] basic fibroblast growth factor,[61] insulin-like growth factor-1,[62] and erythropoietin.[63,64]

Our findings complement the notion that exogenously administered neurotrophic proteins might be therapeutic,[65–69] lending support to growth factor clinical trials aimed at benefitting cognitive outcome,[70–72] including one involving extremely preterm infants.[73] The next steps should include analyses that evaluate the role that antecedent prepregnancy, prenatal, and postnatal characteristics have in modulating inflammation-associated and neurotrophic proteins, and to explore epigenetic mechanisms that likely play a role in such modulations.

Our study has several strengths. We included a large number of infants, collected our data prospectively, and had only modest attrition across 10 years of follow-up. Examiners at 2 and 10 years were not aware of the medical histories of the children they examined, and our analyses of protein data are of high quality, with high content validity.[16,74,75]

Although we sampled a wide range of inflammation-associated proteins known to be associated with neurologic damage, and a number of neurotrophic proteins, we did not evaluate all known inflammation-associated or neurotrophic proteins. We selected proteins on the basis of likely involvement in the fetal or neonatal inflammatory response or brain-protective properties, and the accuracy with which they could be measured reliably in whole blood spots using the Meso Scale Discovery and the Luminex multiplex platforms. Finally, rather than reporting absolute protein concentration values, we used a distribution-based definition of protein elevation based on gestational age, postnatal day, and the interval between processing blood samples, because normal values are not known, and values appear to be influenced by these factors.

We conclude that elevated levels of circulating neurotrophic proteins in the first 2 weeks after birth seem to decrease the risk of cognitive impairment at 10 of age years in children born extremely preterm. ■

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Appendix

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Glossary

ANG	Angiopoietin
BDNF	Brain-derived neurotrophic factor
CRP	C-reactive protein
EF	Executive function
ELGAN	Extremely Low Gestational Age Newborn
IRG	Inflammatory risk group
LCA	Latent class analysis
NRG	Neurotrophin group

RANTES	Regulated upon activation, normal T-cell expressed, and secreted
SAA	Serum amyloid A

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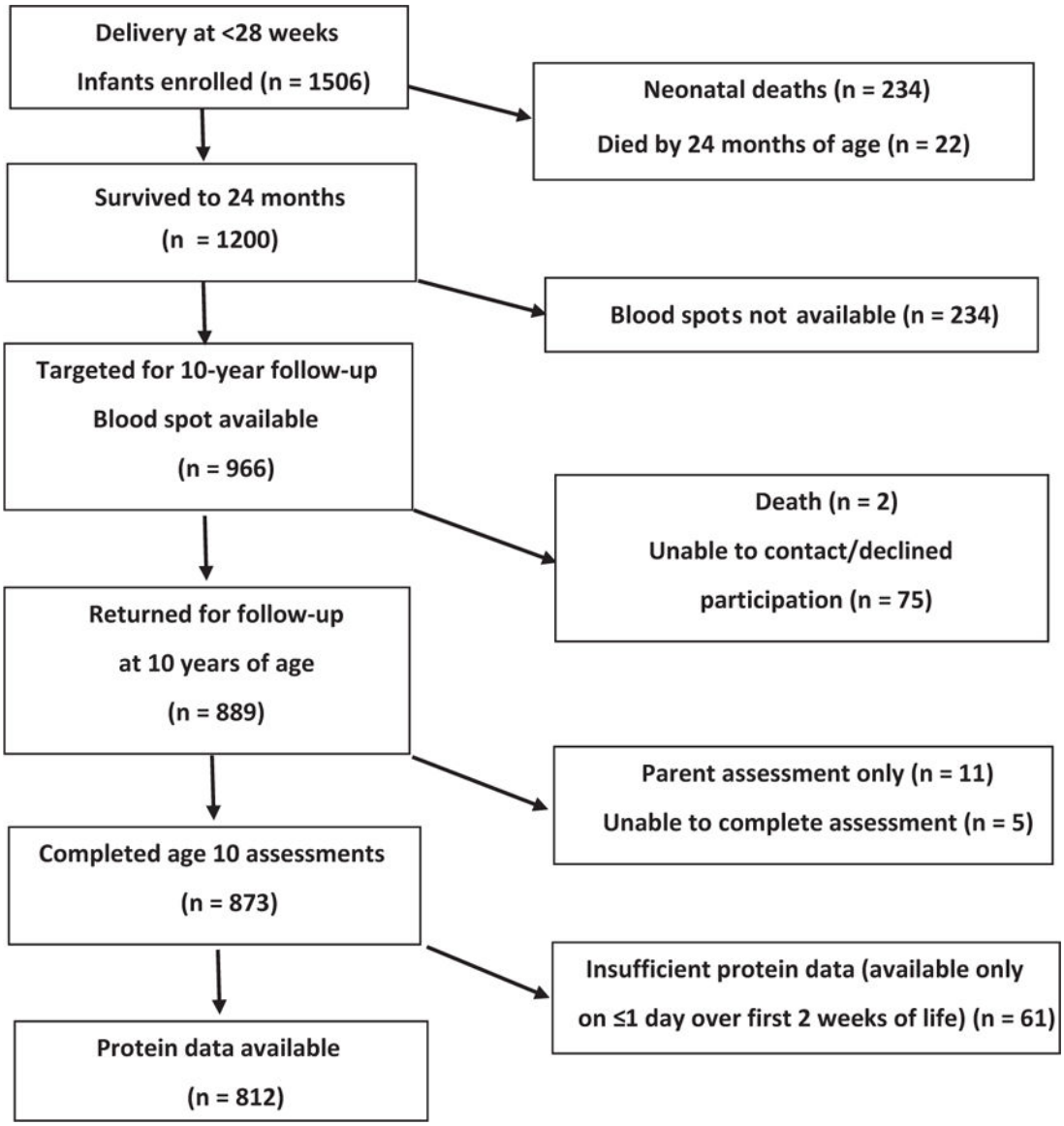

Figure 1.
Study enrollment flow chart.

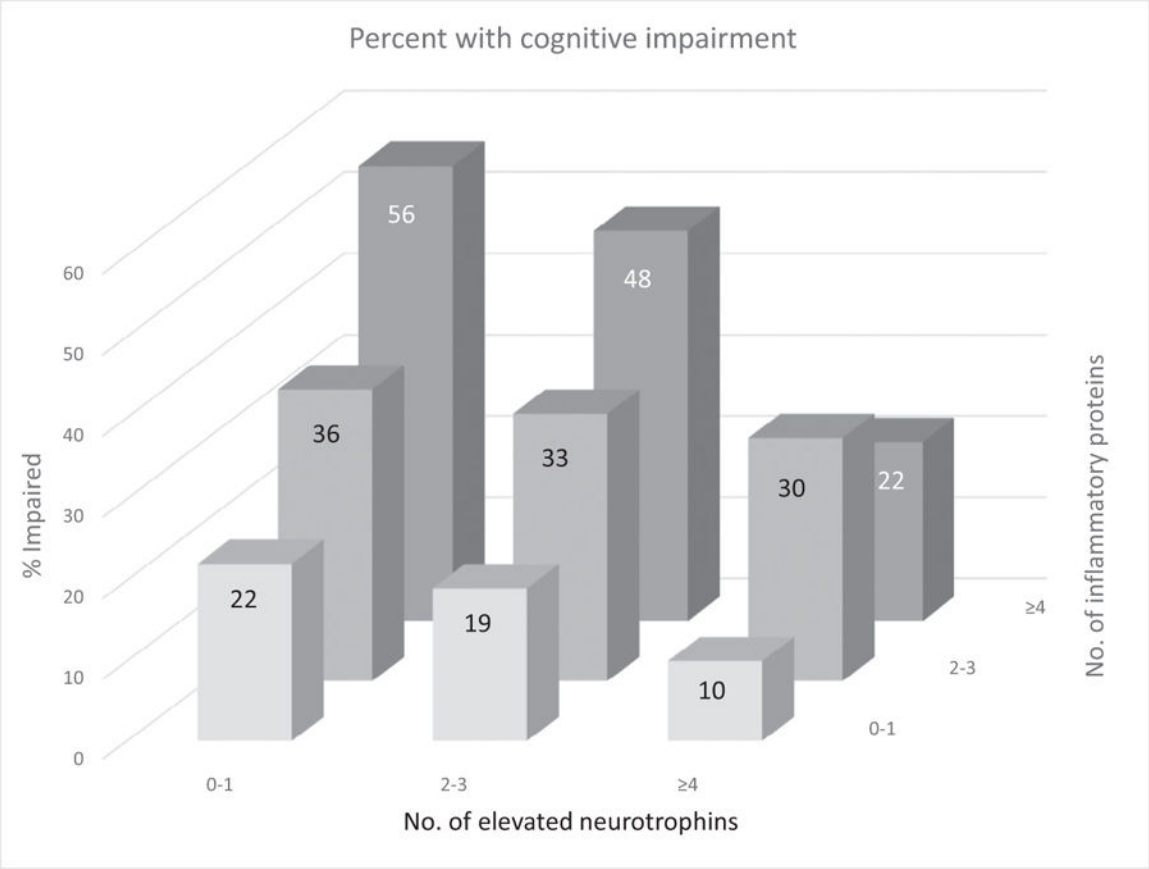

Figure 2.

Percent of children with moderate or severe cognitive impairment as a function of the number of elevated neurotrophic and inflammatory proteins.

Table I.

Inflammation-related and neurotrophic proteins considered in analyses

Inflammation-related proteins	Proteins with neurotrophic properties
Interleukin-1b (IL-1β)	Interleukin-6 receptor (IL-6R)
Interleukin-6 (IL-6)	Regulated upon activation, and normal T-cell expressed, and (presumably) secreted (RANTES)
Tumor necrosis factor-alpha (TNF-α)	Brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (BFGF)
Intercellular adhesion molecule-1 (ICAM-1)	Insulin-like growth factor-1 (IGF-1)
Interleukin-8 (IL-8)	Vascular endothelial growth factor (VEGF)
Matrix metallopeptidase 9 (MMP-9)	Vascular endothelial growth factor receptor-1 (VEGF-R1)
C-reactive protein (CRP)	Vascular endothelial growth factor receptor-2 (VEGF-R2)
Serum amyloid A (SAA)	Placental growth factor (PIGF)
	Angiopoietin 1 (ANG-1)
	Angiopoietin 2 (ANG-2)
	Thyroid-stimulating hormone (TSH)

Table II.

Fit indices for LCA on neurotrophic and inflammatory proteins*

Day of life	No. of classes	AIC	BIC	SSABIC	Lo-Mendell-Rubin *P* value†	Entropy
Fit indices for LCA on neurotrophic proteins						
1	2	9491.2	9608.6	9529.2	<.001	0.85
	3	9301.1	9479.5	9358.8	<.001	0.75
	4	9248.2	9487.6	9325.6	.327	0.72
	5	9197.2	9497.6	9294.4	.073	0.73
7	2	9626.6	9744.3	9664.9	<.001	0.79
	3	9443.8	9622.7	9502.1	.001	0.77
	4	9378.9	9619.0	9457.1	.135	0.78
	5	9312.4	9613.7	9410.5	.089	0.77
14	2	8947.0	9062.2	8982.8	<.001	0.83
	3	8780.0	8955.2	8834.6	<.001	0.71
	4	8679.8	8914.9	8753.0	.005	0.78
	5	8589.6	8884.7	8681.5	.142	0.78
Fit indices for LCA on inflammatory proteins						
1	2	6217.5	6297.2	6243.3	<.001	0.83
	3	6034.2	6156.2	6073.7	<.001	0.86
	4	5896.0	6060.3	5949.2	<.001	0.85
	5	5878.5	6085.0	5945.3	.074	0.86
7	2	6596.8	6676.9	6622.9	<.001	0.77
	3	6379.6	6502.0	6419.4	<.001	0.82
	4	6269.6	6434.4	6323.2	<.001	0.84
	5	6252.0	6459.2	6319.5	.013	0.85
14	2	5810.8	5889.2	5835.2	<.001	0.82
	3	5680.0	5799.9	5717.3	<.001	0.88
	4	5575.9	5737.3	5626.2	<.001	0.84
	5	5572.3	5775.1	5635.4	.152	0.84

AIC, Akaike information criteria; *BIC*, Bayesian information criteria; *SSABIC*, sample size adjusted Bayesian information criteria.

* Separate analyses were conducted for proteins measured at days 1, 7, and 14.

† Lo-Mendell-Rubin adjusted likelihood ratio test of k vs k-1 classes.

Table III.

Inflammatory and neurotrophic proteins through LCA*

	IRG 1 Low elevation (n = 634)	IRG 2† ≥3 Elevated proteins including CRP or SAA (n = 130)	IRG 3‡ ≥3 Elevated proteins Not CRP or SAA (n = 48)
IRG			
CRP	6.8	86.9	.0
SAA	6.9	68.5	.0
IL-1β	7.1	43.8	62.5
IL-6	6.1	51.5	52.1
TNF-alpha	7.4	54.6	75.0
IL-8	4.3	58.5	68.7
ICAM-1	8.4	62.3	39.6
MMP-9	8.5	33.1	56.2
	NRG 1 Low Elevation (n = 552)	**NRG 2§ 2 of RANTES, BDNF, Ang-1 Elevated (n = 133)**	**NRG 3¶ 3 + Other Proteins Elevated (n = 127)**
NRG			
RANTES	5.8	77.4	12.6
BDNF	5.8	85.7	5.7
Ang-1	2.1	87.2	12.3
IL-6R	7.2	43.6	51.2
BFGF	6.2	28.6	48.4
IGF-1	13.6	17.3	41.0
VEGF	10.1	42.9	40.9
VEGF-R1	10.2	30.1	40.9
VEGF-R2	7.4	51.1	40.9
PIGF	8.2	19.5	41.0
Ang-2	5.6	42.9	36.9
TSH	14.1	26.3	40.9

BFGF, Basic fibroblast growth factor; *ICAM-1*, intercellular adhesion molecule-1; *IGF*, insulin-like growth factor; *IL*, interleukin; *MMP*, matrix metallopeptidase; *PIGF*, placental growth factor; *TNF*, tumor necrosis factor; *TSH*, thyroid-stimulating hormone; *VEGF*, vascular endothelial growth factor.

*The columns represent the percentage for each protein listed on the left that have sustained highest quartile value in each of the 3 inflammatory or neurotrophic risk groups of children.

[†] IRG2 has a preponderance of high values for CRP and SAA and lesser elevations of the other proteins.

[‡] IRG3 has a reduced likelihood of having high values for CRP or SAA while having elevated values of the other inflammatory proteins.

[§] NRG2 has a preponderance of high values for RANTES, BDNF, and Ang-1 and lesser elevations of the other proteins.

[¶] NRG3 has reduced likelihood of having high values for RANTES, BDNF, and Ang-1, while having elevated values of the other neurotrophic proteins.

Table IV.

Description of the sample of children with measured proteins (n = 812)

		Neurotrophic risk group* (proteins included in top quartile)			No. of elevated neurotrophic proteins†		
	Overall n	1: None (n = 552)	2: RANTES, BDNF, ANG-1 (n = 133)	3: >3—not RANTES, BDNF, ANG-1 (n = 127)	0–1 (n = 395)	2–3 (n = 232)	≥4 (n = 185)
Maternal characteristics							
Racial identity							
White	505	62	68	62	63	59	68
Black	208	27	19	27	26	31	20
Other	90	11	13	10	11	10	12
Hispanic							
Yes	80	10	14	7	9	9	11
No	731	90	86	93	91	91	89
Body mass index							
Under	64	8	7	11	9	6	10
Normal	390	49	52	50	49	50	51
Over	152	20	22	13	22	16	19
Obese	179	23	20	26	21	29	20
Education, years							
≤12	322	40	43	40	41	38	45
>12, <16	185	24	22	24	22	27	22
≥16	283	36	35	36	38	35	33
Marital status, single							
Yes	329	40	37	47	38	43	42
No	483	60	63	53	62	57	58
Public insurance							
Yes	286	36	31	37	37	33	34
No	526	64	69	63	63	67	66
Cigarette exposure in pregnancy							
None	567	73	68	68	74	70	67
Passive	120	14	16	20	14	15	18
Smoker	108	13	16	12	12	15	14

		Neurotrophic risk group* (proteins included in top quartile)			No. of elevated neurotrophic proteins†		
	Overall n	**1: None (n = 552)**	**2: RANTES, BDNF, ANG-1 (n = 133)**	**3: >3—not RANTES, BDNF, ANG-1 (n = 127)**	**0–1 (n = 395)**	**2–3 (n = 232)**	**≥4 (n = 185)**
Newborn characteristics							
Sex							
Male	413	51	47	55	51	53	48
Female	399	49	53	45	49	47	52
Gestational age, weeks							
23–24	173	21	20	24	21	24	20
25–26	366	44	46	48	45	44	46
27	273	35	35	28	35	32	34
Birth weight z-score							
<−2	49	7	2	8	6	6	6
≥−2, <−1	106	12	11	19	11	16	14
≥−1	657	81	87	73	82	79	81
Inflammatory proteins							
IRG1	634	87	65	51			
IRG2	130	11	23	32			
IRG3	48	2	12	17			
Inflammatory proteins							
0–1	540				85	59	38
2–3	164				11	27	30
≥4	108				4	14	32

*No significant differences in characteristics by neurotrophic risk group except for birth weight z-score ($P = .043$) and inflammatory proteins ($P < .001$).

†No significant differences in characteristics by number of neurotrophic proteins except for inflammatory proteins ($P < .001$).

Table V.

Impairment on LCA measure*

	LCA-based cognitive impairment level (n = 812)			
	Severe n = 64	**Moderate n = 135**	**Low Normal n = 333**	**Normal n = 280**
Elevated inflammatory proteins				
0–1	Ref	Ref	Ref	Ref
2–3	1.67 (0.75–3.73)	**3.47 (1.98–6.09)**	1.34 (0.84–2.14)	Ref
≥4	**5.68 (2.30–14.00)**	**5.89 (2.83–12.24)**	**2.72 (1.49–4.99)**	Ref
Elevated neurotrophic proteins				
0–1	Ref	Ref	Ref	Ref
2–3	**0.48 (0.24–0.97)**	0.70 (0.41–1.19)	**0.66 (0.44–0.99)**	Ref
≥4	**0.22 (0.09–0.53)**	**0.35 (0.18–0.67)**	**0.63 (0.39–1.00)**	Ref

Values are adjusted OR (95% CI).

Bold values represent ORs for which the 95% confidence limits do not include 1; ie, significantly different from 1 at $P < .05$.

*At 10 years of age for those with elevated inflammatory and neurotrophic proteins on 2 of the first 3 postnatal measurements (controlling for infant sex, maternal education, and the other set of proteins).

Table VI.

Cognitive impairment *

	LCA-based cognitive impairment level (n = 812)			
	Severe n = 64	**Moderate n = 135**	**Low Normal n = 333**	**Normal n = 280**
Elevated inflammatory proteins				
IRG1	Ref	Ref	Ref	Ref
IRG2	**3.79 (1.72–8.36)**	**3.93 (2.08–7.41)**	**2.54 (1.48–4.34)**	Ref
IRG3	2.18 (0.61–7.78)	**3.18 (1.29–7.83)**	1.53 (0.69–3.38)	Ref
Elevated neurotrophic proteins				
NRG1	Ref	Ref	Ref	Ref
NRG2	**0.31 (0.12–0.79)**	**0.46 (0.24–0.88)**	**0.62 (0.39–0.98)**	Ref
NRG3	0.85 (0.37–1.95)	1.03 (0.55–1.95)	1.00 (0.60–1.67)	Ref

IRG2 defined by elevation of CRP or SAA with other proteins having little or modest elevations.

IRG3 defined by ≥3 other protein elevations without CRP and SAA elevations.

NRG2 defined by elevation of 2 of RANTES, BDNF, or ANG-1 with other proteins having little or modest elevations.

NRG3 defined by ≥3 other proteins elevated protein elevations without CRP and SAA elevations.

Values are adjusted OR (95% CI).

Bold values represent ORs for which the 95% confidence limits do not include 1; ie, significantly different from 1 at $P < .05$.

*At 10 years of age for those with sustained elevated inflammatory and neurotrophic proteins (controlling for infant sex and maternal education).