HHS Public Access

Author manuscript

Am J Perinatol. Author manuscript; available in PMC 2024 June 06.

Published in final edited form as:

Am J Perinatol. 2024 May ; 41(Suppl 1): e2710–e2716. doi:10.1055/s-0043-1774312.

Genetic Predisposition to Adverse Neurodevelopmental Outcome of Extremely Low Birth Weight Infants

Michael W. Varner, MD[1], **Elizabeth A. Thom, PhD**[2,*], **C. Michael Cotten, MD, MHS**[3], **Susan R. Hintz, MD, MS**[4], **Grier P. Page, PhD**[5], **Dwight J. Rouse, MD**[6], **Brian M. Mercer, MD**[7,8], **Maged M. Costantine, MD**[9], **Yoram Sorokin, MD**[10], **John M. Thorp Jr., MD**[11], **Susan M. Ramin, MD**[12], **Marshall W. Carpenter, MD**[13], **Mary J. O'Sullivan, MD**[14], **Alan M. Peaceman, MD**[15], **George R. Saade, MD**[16], **Donald J. Dudley, MD**[17], **Steve N. Caritis, MD**[18], **Eunice Kennedy Shriver National Institute of Child Health Human Development Maternal-Fetal Medicine Units Network Neonatal Research Network**[#]

[1]Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, Utah

[2]Biostatistics Coordinating Center, George Washington University, Washington, District of Columbia

[3]Department of Pediatrics, Duke University, Durham, North Carolina

[4]Department of Pediatrics, Stanford University School of Medicine and Lucile Packard Children's Hospital, Palo Alto, California

[5]Social, Statistical and Environmental Sciences Unit, RTI International, Atlanta, Georgia

[6]Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, Alabama

[7]Case Western Reserve University-MetroHealth Medical Center, Cleveland, Ohio

[8]University of Tennessee, Memphis, Tennessee

[9]Department of Obstetrics and Gynecology, The Ohio State University, Columbus, Ohio

[10]Department of Obstetrics and Gynecology, Wayne State University, Detroit, Michigan

[11]Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, North Carolina

[12]Department of Obstetrics and Gynecology, University of Texas Health Science Center at Houston-Children's Memorial Hermann Hospital, Houston, Texas

[13]Department of Obstetrics and Gynecology, Brown University, Providence, Rhode Island

[14]Department of Obstetrics and Gynecology, University of Miami, Miami, Florida

Address for correspondence Michael W. Varner, MD, Department of Obstetrics and Gynecology, University of Utah Health Sciences Center, 30 North 1900 East, Room 2A226, Salt Lake City, UT 84132 (michael.varner@hsc.utah.edu).

[*]Deceased.

[#]A list of other members of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units Network and Neonatal Research Network are listed in the Supplemental Material (available in online version).

Conflict of Interest

None declared.

[15]Department of Obstetrics and Gynecology, Northwestern University, Chicago, Illinois

[16]Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas

[17]Department of Obstetrics and Gynecology, University of Texas Health Sciences Center at San Antonio, San Antonio, Texas

[18]Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract

Objective—This study aimed to evaluate whether there are genetic variants associated with adverse neurodevelopmental outcomes in extremely low birth weight (ELBW) infants.

Study Design—We conducted a candidate gene association study in two well-defined cohorts of ELBW infants (<1,000 g). One cohort was for discovery and the other for replication. The discovery case–control analysis utilized anonymized DNA samples and evaluated 1,614 single-nucleotide polymorphisms (SNPs) in 145 genes concentrated in inflammation, angiogenesis, brain development, and oxidation pathways. Cases were children who died by age one or who were diagnosed with cerebral palsy (CP) or neurodevelopmental delay (Bayley II mental developmental index [MDI] or psychomotor developmental index [PDI] < 70) by 18 to 22 months. Controls were survivors with normal neurodevelopment. We assessed significant epidemiological variables and SNPs associated with the combined outcome of CP or death, CP, mental delay (MDI < 70) and motor delay (PDI < 70). Multivariable analyses adjusted for gestational age at birth, small for gestational age, sex, antenatal corticosteroids, multiple gestation, racial admixture, and multiple comparisons. SNPs associated with adverse neurodevelopmental outcomes with $p < 0.01$ were selected for validation in the replication cohort. Successful replication was defined as $p < 0.05$ in the replication cohort.

Results—Of 1,013 infants analyzed (452 cases, 561 controls) in the discovery cohort, 917 were successfully genotyped for >90% of SNPs and passed quality metrics. After adjusting for covariates, 26 SNPs with $p < 0.01$ for one or more outcomes were selected for replication cohort validation, which included 362 infants (170 cases and 192 controls). A variant in SERPINE1, which encodes plasminogen activator inhibitor (PAI1), was associated with the combined outcome of CP or death in the discovery analysis ($p = 4.1 \times 10^{-4}$) and was significantly associated with CP or death in the replication cohort (adjusted odd ratio: 0.4; 95% confidence interval: 0.2–1.0; $p = 0.039$).

Conclusion—A genetic variant in SERPINE1, involved in inflammation and coagulation, is associated with CP or death among ELBW infants.

Keywords

candidate genes; extremely low birth weight; mental developmental delay; neurodevelopmental delay; preterm birth; polymorphisms; psychomotor delay; single-nucleotide polymorphisms

Preterm birth is a major risk factor for perinatal mortality and long-term neurodevelopmental disability.[1] Neurodevelopmental outcomes after preterm birth appear to be influenced by

complex interplay between genetic and environmental factors and have proven difficult to predict. Fetal gene polymorphisms in inflammation, angiogenesis, coagulation, brain development, and oxidation pathways, among others, have been associated with adverse neurodevelopmental outcomes after preterm birth, including cerebral palsy (CP) and developmental delay.[2–11] However, specific genetic risk loci have not been well defined, nor validated, and associations have been inconsistent among studies and populations. In addition, the contribution of genetic risk factors to neurodevelopmental outcomes in the highest risk infants, those born early preterm and at extremely low birth weight (ELBW), remains incompletely understood. However, early preterm and ELBW infants have dramatically increased risks of CP and developmental delay[1] and represent a population in whom genetic predispositions might reasonably be assumed to be more common. A better understanding of genetic risk factors for adverse neurodevelopmental outcomes after early preterm birth is important to gain the mechanistic insight necessary to develop effective prevention and treatment strategies.

Using a candidate gene approach and well-characterized discovery and validation cohorts, we hypothesized that candidate gene variants in the aforementioned pathways would be associated with adverse neurodevelopmental outcomes in a U.S. cohort of ELBW infants after controlling for significant epidemiological factors.

Materials and Methods

The discovery cohort was a multiracial population of 1,013 ELBW infants, born less than 1,000 g and enrolled between 1998 and 2001 in a study to assess associations between serial cytokine levels and neurodevelopmental delay.[12] Infant blood spot samples were processed for DNA, which was stored in the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Neonatal Research Network's anonymized DNA biorepository. Cases were defined as children that died by 1 year of life or were survivors with CP or neurodevelopmental delay at 18 to 22 months. Death was included because it is a competing outcome that precludes assessment of adverse neurodevelopmental outcomes. Neurodevelopmental testing was conducted by trained, certified examiners. CP was diagnosed according to prespecified standard criteria (gross motor delay, and abnormality in muscle tone, movement, and reflexes). Neurodevelopmental delay was defined by a score of <70 (equivalent to 2 standard deviations below the mean) on the Bayley Scales of Infant Development II in either the mental or psychomotor developmental indices (MDI and PDI, respectively). Infants with major congenital anomalies and suspected or known aneuploidy were excluded. Controls were survivors with normal neurodevelopment, defined as Bayley MDI and PDI ≥85 and no diagnosis of grade III/IV intraventricular hemorrhage, periventricular leukomalacia, or CP.

A pathway-based approach was used to identify candidate genes previously associated with neurodevelopmental outcomes after preterm birth as well as additional genes concentrated in inflammation, angiogenesis, brain development, and oxidation pathways. Tagging single-nucleotide polymorphisms (TagSNPs) were chosen for genotyping using HapMap (http://www.hapmap.org/) and the following criteria: (1) minor allele frequency greater than 10%, (2) r^2 value of at least 0.8, and (3) TagSNP tagged for at least six other SNPs.[13] To

represent the entire variation within a gene, additional SNPs approximately every 3,000 to 5,000 base pairs were included. DNA was extracted from stored blood spots on filter paper at −70°C. Genotyping was performed on whole-genome amplified DNA using the Illumina GoldenGate platform (Illumina, Inc., San Diego, CA) for 1,614 TagSNPs within the candidate genes. Data cleaning and analysis were performed using PLINK v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/). SNPs were removed with a low genotyping pass rate (greater than 10% of genotypes missing) or that were not in Hardy–Weinberg equilibrium (HWE) in infants with normal neurodevelopment. Individuals with more than 10% of genotypes missing were also removed. Separate association tests were performed for four different neurodevelopmental outcomes compared with infants with normal neurodevelopment: Analysis 1 (CP and/or death), Analysis 2 (CP), and Analysis 3 (mental delay), and Analysis 4 (motor delay). Infants that experienced more than one of the four outcomes evaluated were included as cases for each of those outcomes individually. None of the cases were included in any of the control group comparisons.

Epidemiological variables were tested for association with neurodevelopmental outcomes using stepwise regression. Because sex, gestational age at birth, small for gestational age, and use of antenatal corticosteroids has been shown in other studies to be associated with neurodevelopmental outcomes after preterm birth, these variables were included in planned analyses. Eigenvalues were included as covariables to adjust for racial admixture. For 800 infants in the discovery cohort, a previous genome wide scan (genome-wide association study [GWAS]) successfully genotyped markers to estimate a “dose effect” from various ancestries and determined eigenvalues that were assigned to these 800 infants. For infants without successful GWAS, markers among those tested on the GoldenGate platform were used to estimate eigenvalues. For SNP analyses, the minor, less frequent, allele for each SNP was tested for association with the defined neurodevelopmental outcomes in each analysis using QFAM-total in PLINK,[13] which is a total association test that uses between and within family components and performs a linear regression of phenotype on genotype. A permutation test with 10,000 iterations was then used to correct for family relatedness. False discovery rates are reported to adjust for multiple comparisons.

SNPs associated with one or more adverse neurodevelopmental outcomes with $p < 0.01$ were selected for validation in a replication cohort. Validation samples were derived from the *Eunice Kennedy Shriver* NICHD Maternal-Fetal Medicine Units Network randomized, placebo-controlled, double-masked multicenter clinical trial of magnesium sulfate for prevention of CP before anticipated preterm birth. Women with pregnancies between $24^{0/7}$ and $31^{6/7}$ weeks’ gestation and at risk of imminent preterm delivery were eligible for enrollment in this trial. The details of the trial, which was conducted between 1997 and 2004, have been previously reported.[14] Case/control definitions in the replication cohort were equivalent to the primary cohort, with the exception that neurodevelopmental outcomes were evaluated at or beyond 24 months of age. Neurodevelopmental testing was conducted by trained, certified examiners and infants with major congenital anomalies and suspected or known aneuploidy or syndromic causes of neurodevelopmental delay were excluded from the analysis. As in the primary cohort, four outcomes were evaluated: the combined outcome of CP or death, CP, mental delay, and motor delay. Cases and controls with available whole-genome amplified DNA were matched for race and sex. For multiple gestations, one

twin of each pair was randomly excluded to avoid inclusion of related individuals. SNP association analyses were performed using the minor allele defined in the discovery cohort after adjusting for gestational age at birth, preterm delivery < 28 weeks, small for gestational age, maternal education level, and treatment group. Significance in the validation cohort was defined as $p < 0.05$ and we required the genetic variant to be associated with the same defined outcome in both discovery and validation cohorts. All analyses in the replication cohort were performed using SAS statistical software (SAS Institute, Inc., Cary, NC).

The institutional review boards of the data coordinating center and the clinical sites where subjects were recruited approved the primary data collection for discovery and validation cohorts. This study was approved by the University of Utah Institutional Review Board.

Results

Discovery Cohort

Of 1,013 infants analyzed (452 cases, 561 controls) in the discovery cohort, a total of 1,494 SNPs were successfully genotyped in 966 infants. A total of 170 of the original 1,614 SNPs were excluded from the analysis, 105 based on failure to achieve HWE, and 93 for low genotyping. Twenty-eight SNPs overlapped in the missingness and HWE test failure. Forty-nine subjects with low genotyping rate were removed from analysis. A total of 917 subjects were successfully genotyped for >90% of SNPs and passed quality metrics and were included in the final analysis.

Characteristics of the discovery and replication cohorts are shown in Table 1. Compared with the discovery cohort, the validation cohort had a higher mean gestational age (25.9 vs. 30.4 weeks) and higher birth weight (763 vs. 1,536 g). Other characteristics were similar between cohorts.

Forty epidemiological variables were tested for association with each of the four neurodevelopmental analyses. Stepwise regression showed that in each analysis, there were no significant factors retained in the final model (data not shown). After controlling for clinically relevant covariables, including sex, gestational age at birth, small for gestational age, and use of antenatal corticosteroids, 26 SNPs with $p < 0.01$ are reported and shown in Table 2.

Replication Cohort

In the replication cohort, the previously identified 26 SNPs were successfully genotyped in 362 infants (170 cases and 192 controls). One of the original 26 SNPs was excluded due to failure of HWE at $p < 0.05$. All additional SNPs passed the requisite quality metrics and no subjects were removed from the analysis due to low genotyping rate.

A genetic variant in SERPINE1 (rs2227667) was associated with the combined outcome of CP or death in the discovery analysis ($p = 4.1 \times 10^{-4}$, FDR 0.46) and was significantly associated with CP or death in the adjusted analysis for the replication cohort ($p = 0.039$; adjusted odd ratio: 0.4; 95% confidence interval: 0.2–1.0). For this SERPINE1 SNP, the minor allele, G, was associated with reduced risk of CP/death in both discovery and

replication cohorts. However, this variant did not reach statistical significance for CP alone (p = 0.466; OR 0.7 [0.3–1.7]).

Discussion

This is a novel candidate gene study that analyzes adverse neurodevelopmental outcomes in two well-characterized U.S. cohorts of early preterm infants housed within the *Eunice Kennedy Shriver* NICHD Neonatal Research Network and Maternal-Fetal Medicine Units Network. After controlling for important epidemiological factors, a genetic variant in SERPINE1 was associated with the combined outcome of CP and death after early preterm birth in both discovery and validation cohorts.

The SERPINE1 gene on chromosome 7 encodes the serine protease inhibitor plasminogen activator inhibitor, or PAI1, a multifunctional protein and major inhibitor of fibrinolysis. Polymorphisms in SERPINE1 have been associated with PAI1 levels, but the specific effects of the reported polymorphism are not known. High levels of PAI1 have been associated with thrombosis, cardiovascular disease, and some cancers.[15] Gene mutations resulting in higher PAI1 transcriptional activity have also been associated with recurrent pregnancy loss, preeclampsia, and, most importantly, CP in early preterm infants.[7,16,17]

Strengths and Limitations

Our study has several strengths. Our discovery and validation cohorts were prospectively collected and key aspects of phenotyping and genotyping were similar, including the use of trained, certified examiners to prospectively ascertain key neurodevelopmental outcomes and use of consistent genetic methodology with careful consideration of potential confounders. Although children in these cohorts were not specifically sequenced for potential Mendelian causes of neurodevelopmental delay, all follow-up evaluations were conducted by trained, certified examiners and infants with major congenital anomalies and suspected or known aneuploidy or syndromic causes of neurodevelopmental delay were excluded from the analysis.

However, several weaknesses require acknowledgment. While time frames for enrollment and neurodevelopmental assessment were similar between discovery and replication cohorts, the discovery cohort enrolled infants born at a mean gestational age of 5 weeks earlier. This difference may have compromised our validation efforts, since genetic variants associated with adverse neurodevelopmental outcome may vary based on gestational age. In addition, the validation methodology we used cannot rule out false positive associations, nor can we be confident that true associations were not missed. We erred toward a conservative approach, restricting validation to SNPs that were associated with the same strict phenotype in both discovery and validation cohorts. Although the SERPINE1 was associated with the combined CP or death outcome in both discovery and validation cohorts, the false discovery rate in the discovery cohort is 0.46, and the statistical significance values in the adjusted analysis suggest that further replication analyses should be considered. Finally, SERPINE1 was not significantly associated with CP alone in the validation cohort, raising the possibility that it could be a variant associated with survival only.

Conclusion

The risk of central nervous system injury in ELBW infants is influenced by complex gene–environment interactions that are not well understood. This study supports the hypothesis that gene variants may influence the risk of death and adverse neurodevelopmental outcomes after preterm birth. Ultimately, identification of genetic susceptibility loci for poor neurodevelopmental outcomes after preterm birth improves our understanding of pathogenesis and may facilitate identification of new antenatal and postnatal neuroprotection strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Erin A.S. Clark, MD for assistance with concept design and manuscript development; Kathleen Jablonski, PhD, for statistical analysis; Allison Todd, MSN, RN for protocol development and coordination between clinical research centers; Steven J. Weiner, MS for protocol and data management; and Catherine Y. Spong, MD for protocol development and oversight.

Funding

This study is supported by U10 grants from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD; HD36790, HD21364, HD21373, HD21385, HD21397, HD21415, HD27851, HD27853, HD27856, HD27871, HD27880, HD27881, HD27904, HD34216, HD40461, HD40492, HD40498, HD40689, HD27869, HD34208, HD34116, HD40544, HD27915, HD34136, HD21414, HD27917, HD27860, HD40560, HD40545, HD40485, HD40500, HD27905, HD27861, HD34122, HD40512, HD53907, HD34210, HD21410, U01 HD36801, HD19897), MO1-RR-000080, and by the National Institute of Neurological Disorders and Stroke (NINDS). The National Center for Research Resources provided grant support for the Neonatal Research Network's Glutamine trial, which included the Genomic Study through cooperative agreements (General Clinical Research Center M01 grants RR30, RR32, RR39, RR70, RR80, RR633, RR750, RR997, RR6022, RR7122, RR8084, RR16587). Comments and views of the authors do not necessarily represent views of the NICHD, the National Institutes of Health, the Department of Health and Human Services, or the U.S. Government.

References

1. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet 2008;371(9608): 261–269 [PubMed: 18207020]
2. Gibson CS, Maclennan AH, Dekker GA, et al. Candidate genes and cerebral palsy: a population-based study. Pediatrics 2008;122 (05):1079–1085 [PubMed: 18977990]
3. Clark EA, Mele L, Wapner RJ, et al. ; *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Association of fetal inflammation and coagulation pathway gene polymorphisms with neurodevelopmental delay at age 2 years. Am J Obstet Gynecol 2010;203(01): 83.e1–83.e10
4. Harding D, Brull D, Humphries SE, Whitelaw A, Montgomery H, Marlow N. Variation in the interleukin-6 gene is associated with impaired cognitive development in children born prematurely: a preliminary study. Pediatr Res 2005;58(01):117–120 [PubMed: 15985687]
5. Harding DR, Dhamrait S, Whitelaw A, Humphries SE, Marlow N, Montgomery HE. Does interleukin-6 genotype influence cerebral injury or developmental progress after preterm birth? Pediatrics 2004;114(04):941–947 [PubMed: 15466088]
6. Costantine MM, Clark EA, Lai Y, et al. Association of polymorphisms in neuroprotection and oxidative stress genes and neurodevelopmental outcomes after preterm birth. Obstet Gynecol 2012;120(03):542–550 [PubMed: 22914463]

7. Nelson KB, Dambrosia JM, Iovannisci DM, Cheng S, Grether JK, Lammer E. Genetic polymorphisms and cerebral palsy in very preterm infants. Pediatr Res 2005;57(04):494–499 [PubMed: 15718364]

8. O'Callaghan ME, Maclennan AH, Gibson CS, et al. ; Australian Collaborative Cerebral Palsy Research Group. Genetic and clinical contributions to cerebral palsy: a multi-variable analysis. J Paediatr Child Health 2013;49(07):575–581 [PubMed: 23773706]

9. O'Callaghan ME, MacLennan AH, Haan EA, Dekker GSouth Australian Cerebral Palsy Research Group. The genomic basis of cerebral palsy: a HuGE systematic literature review. Hum Genet 2009;126(01):149–172 [PubMed: 19238444]

10. Gibson CS, MacLennan AH, Hague WM, et al. ; South Australian Cerebral Palsy Research Group. Associations between inherited thrombophilias, gestational age, and cerebral palsy. Am J Obstet Gynecol 2005;193(04):1437 [PubMed: 16202738]

11. Gibson CS, MacLennan AH, Goldwater PN, Haan EA, Priest K, Dekker GASouth Australian Cerebral Palsy Research Group. The association between inherited cytokine polymorphisms and cerebral palsy. Am J Obstet Gynecol 2006;194(03):674. e1–674.e11

12. Carlo WA, McDonald SA, Tyson JE, et al. ; *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Neonatal Research Network. Cytokines and neurodevelopmental outcomes in extremely low birth weight infants. J Pediatr 2011; 159(06):919–25.e3 [PubMed: 21798559]

13. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81(03):559–575 [PubMed: 17701901]

14. Rouse DJ, Hirtz DG, Thom E, et al. ; *Eunice Kennedy Shriver* NICHD Maternal-Fetal Medicine Units Network. A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy. N Engl J Med 2008;359(09):895–905 [PubMed: 18753646]

15. Milenkovic J, Milojkovic M, Jevtovic Stoimenov T, Djindjic B, Miljkovic E. Mechanisms of plasminogen activator inhibitor 1 action in stromal remodeling and related diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2017;161(04): 339–347 [PubMed: 29097819]

16. Dossenbach-Glaninger A, van Trotsenburg M, Dossenbach M, et al. Plasminogen activator inhibitor 1 4G/5G polymorphism and coagulation factor XIII Val34Leu polymorphism: impaired fibrinolysis and early pregnancy loss. Clin Chem 2003;49(07): 1081–1086 [PubMed: 12816904]

17. Buurma AJ, Turner RJ, Driessen JH, et al. Genetic variants in preeclampsia: a meta-analysis. Hum Reprod Update 2013;19(03): 289–303 [PubMed: 23300202]

Key Points

- Early preterm and ELBW infants have dramatically increased risks of CP and developmental delay.
- A genetic variant in SERPINE1 is associated with CP or death among ELBW infants.
- The SERPINE1 gene encodes the serine protease inhibitor plasminogen activator inhibitor.

Table 1

Subject characteristics[a]

	Discovery cohort					Replication cohort				
	Entire cohort (*n* = 917)	**CP/death (*n* = 253)**	**CP (*n* = 96)**	**MDI < 70 (*n* = 215)**	**PDI < 70 (*n* = 134)**	**Entire cohort (*n* = 362)**	**CP/death (*n* = 54)**	**CP (*n* = 21)**	**MDI < 70 (*n* = 93)**	**PDI < 70 (*n* = 80)**
Gestational age, wk (SD)	25.9 (1.9)	25 (1.8)	25.4 (1.9)	25.7 (2.0)	25.7 (2.1)	30.4 (3.0)	28.0 (2.8)	28.3 (2.7)	30.2 (3.3)	29.9 (3.3)
Birth weight, g (SD)	763 (140.9)	700 (141)	732 (136)	751 (140)	734 (140.9)	1,536 (570)	1,125 (460)	1,187 (466)	1,446 (533)	1,432 (561)
Small for gestational age, *n* (%)	143 (15)	29 (11.5)	13 (13.5)	33 (15.4)	27 (20.2)	30 (8.3)	4 (7.4)	2 (9.5)	14 (15.1)	8 (10.0)
Male, *n* (%)	474 (48.5)	140 (55.3)	53 (55.2)	125 (58.1)	78 (58.2)	227 (62.7)	33 (61.1)	15 (71.4)	61 (65.6)	51 (63.8)
Race, self-reported, *n* (%)										
White	488 (50)	105 (41.5)	39 (40.6)	84 (39.1)	60 (44.8)	236 (65.2)	34 (63.0)	11 (52.4)	70 (75.3)	44 (55.0)
Black	468 (47.9)	142 (56.1)	57 (59.4)	125 (58.1)	73 (54.5)	126 (34.8)	20 (37.0)	10 (47.6)	23 (24.7)	36 (45.0)
Other	21 (2.1)	6 (2.4)	0	6 (2.8)	1 (0.8)					
Ethnicity, self-reported, *n* (%)										
Hispanic	184 (18.8)	46 (18.2)	14 (14.6)	38 (18.7)	18 (13.4)	66 (18.2)	5 (9.3)	1 (4.8)	25 (26.9)	14 (17.5)
Non-Hispanic	793 (81.2)	207 (81.8)	82 (85.4)	177 (82.3)	116 (86.6)	296 (81.8)	49 (90.7)	20 (95.2)	68 (73.1)	66 (82.5)
Exposure to antenatal steroids, *n* (%)	749 (76.7)	184 (73)	73 (76.8)	164 (76.3)	104 (77.6)	353 (97.5)	54 (100.0)	21 (100.0)	91 (97.9)	78 (97.5)

Abbreviations: CP, cerebral palsy; MDI, mental developmental index; PDI, psychomotor developmental index; SD, standard deviation.

[a]Combined case number among categories totals greater than the entire cohort within discovery and replication cohorts since some individuals were analyzed for more than one outcome.

Table 2

Top candidates by neurodevelopmental outcome, adjusted for significant covariates

Gene	Function	SNP	MAF (cases)	MAF (controls)	*p*	FDR
Cerebral palsy						
TFAP2B	Brain transcription factor	rs2635727	0.24	0.34	9.0×10^{-4}	0.92
RELN	Neuronal migration in the developing brain	rs496535	0.33	0.39	3.3×10^{-3}	0.92
IGF1R	Tyrosine kinase receptor, IGF-1 and 2	rs8028620	0.34	0.41	3.4×10^{-3}	0.92
TFAP2B	Brain transcription factor	rs2817419	0.27	0.35	3.6×10^{-3}	0.92
RELN	Neuronal migration in the developing brain	rs2073559	0.44	0.53	5.0×10^{-3}	0.92
GRIN3A	Glutamate (NMDA) receptor subunit	rs17189632	0.40	0.30	5.5×10^{-3}	0.92
GRIN2B	Glutamate (NMDA) receptor subunit	rs2284424	0.18	0.18	5.9×10^{-3}	0.92
FZD4	7 transmembrane protein receptor	rs4144615	0.44	0.55	7.7×10^{-3}	0.99
Cerebral palsy/death						
SERPINE1[a]	Inhibits fibrinolysis	rs2227667	0.27	0.33	4.1×10^{-4}	0.46
IQGAP1	Scaffold protein involved in regulating various cellular processes	rs6496679	0.26	0.28	9.6×10^{-4}	0.54
GRIN2B	NMDA class of glutamate receptor	rs1161183	0.08	0.08	1.5×10^{-3}	0.55
IL1B	Proinflammatory cytokine	rs3136558	0.21	0.23	2.7×10^{-3}	0.77
LRP5	Transmembrane LDL receptor	rs491347	0.38	0.40	6.8×10^{-3}	0.90
LRP5	Transmembrane LDL receptor	rs599301	0.45	0.48	6.9×10^{-3}	0.90
EPAS1	Transcription factor involved in induction of genes via hypoxia	rs7594243	0.11	0.13	7.8×10^{-3}	0.90
IL1R1	Receptor for proinflammatory cytokine IL1	rs13020778	0.34	0.33	9.1×10^{-3}	0.90
Psychomotor delay (PDI < 70)						
ANGPT1	Angiogenesis	rs1283658	0.18	0.27	3.3×10^{-3}	0.99
IGF1R	Tyrosine kinase receptor, IGF-1 and 2	rs6598554	0.44	0.54	7.0×10^{-3}	0.99
CETP	Endoplasmic reticulum stress response to protein accumulation	rs4783962	0.16	0.18	9.0×10^{-3}	0.99
Mental delay (MDI < 70)						
GRIN2B	NMDA class of glutamate receptor	rs220549	0.51	0.46	1.2×10^{-3}	0.98
FLT1	VEGF (vascular endothelial growth factor) receptor	rs9508029	0.24	0.31	2.7×10^{-3}	0.98
RELN	Neuronal migration in the developing brain	rs144525	0.26	0.15	4.0×10^{-3}	0.98
KALRN	Nerve growth and axonal development	rs1708318	0.42	0.38	4.4×10^{-3}	0.98

Gene	Function	SNP	MAF (cases)	MAF (controls)	*p*	FDR
ABCA4	Craniofacial development	rs1931566	0.15	0.20	4.5×10^{-3}	0.98
IGFBP2	Ribosomal protein	rs1525608	0.38	0.46	7.7×10^{-3}	0.99
EPAS1	Transcription factor, induction via hypoxia	rs1868084	0.12	0.13	8.9×10^{-3}	0.99

Abbreviations: FDR, false discovery rate; MAF, minor allele frequency; MDI, mental developmental index; PDI, psychomotor developmental index; SNP, single-nucleotide polymorphism.

[a]Significant in validation analysis.