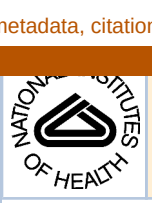

Published in final edited form as:

Am J Obstet Gynecol. 2010 July ; 203(1): 83.e1–83.e10. doi:10.1016/j.ajog.2010.01.047.

Association of Fetal Inflammation and Coagulation Pathway Gene Polymorphisms with Neurodevelopmental Delay at Age 2

Erin A. S. CLARK, M.D.,
Departments of Obstetrics and Gynecology at the University of Utah, Salt Lake City, UT

Lisa MELE, M.S.,
The George Washington University Biostatistics Center, Washington, DC

Ronald J. WAPNER, M.D.,
Drexel University, Philadelphia, PA

Catherine Y. SPONG, M.D.,
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD

Yoram SOROKIN, M.D.,
Wayne State University, Detroit, MI

Alan PEACEMAN, M.D.,
Northwestern University, Chicago, IL

Jay D. IAMS, M.D.,
The Ohio State University, Columbus, OH

Kenneth J. LEVENO, M.D.,
University of Texas Southwestern Medical Center, Dallas, TX

Margaret HARPER, M.D.,
Wake Forest University Health Sciences, Winston-Salem, NC

Steve N. CARITIS, M.D.,
University of Pittsburgh, Pittsburgh, PA

Menachem MIODOVNIK, M.D.,
Columbia University, New York, NY

Brian M. MERCER, M.D.,
Case Western Reserve University, Cleveland, OH

John M. THORP, M.D.,
University of North Carolina at Chapel Hill, Chapel Hill, NC

Susan M. RAMIN, M.D.,
The University of Texas Health Science Center at Houston, Houston, TX

Corresponding Author: Erin A. S. Clark, MD, University of Utah School of Medicine, Suite 2B200, 30 N 1900 E, Salt Lake City, UT 84132, business phone: 801-585-5156, fax: 801-585-2594, erin.clark@hsc.utah.edu.

*The other members of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units Network are listed in the Appendix.

Presented at the 29th annual meeting of the Society for Maternal-Fetal Medicine, San Diego, CA, January 29–31, 2009

Marshall CARPENTER, M.D., and
Brown University, Providence, RI

Dwight J. ROUSE, M.D.
University of Alabama at Birmingham, Birmingham, AL

for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network*

Abstract

OBJECTIVE—Evaluate the association between fetal inflammation and coagulation gene single-nucleotide polymorphisms (SNPs) and neurodevelopmental delay at age 2.

METHODS—Case-control secondary analysis of a randomized trial of single- versus multiple-course corticosteroids. Multiplex assay assessed 46 SNPs. Cases had mental developmental and/or psychomotor delay at age 2. Controls had normal neurodevelopment.

RESULTS—125 cases and 147 controls were analyzed. Allele frequencies were different between cases and controls for IL1-β –511 (p=0.009), IL4R 148 (p=0.03), IL6 -174 (p=0.02), and IL6 -176 (p=0.007). Genotype frequencies were different for IL1-β-511 (p=0.03) and IL6 -174 (p=0.04). Results for IL1-β –511, IL4R 148, and IL6 -176 remained significant after logistic regression analysis. IL1-β-511 and IL6 -176 minor alleles were associated with increased risk of neurodevelopmental delay, OR 3.1 (1.2–8.2) and 2.2 (1.2–3.9), respectively. IL4R 148 minor allele was protective, OR 0.6 (0.4–0.9).

CONCLUSION—Fetal SNPs in IL1-β, IL4R and IL6 may be associated with neurodevelopmental delay at age 2.

KEY WORDS/PHRASES

Coagulation; inflammation; neurodevelopmental delay; single-nucleotide polymorphisms (SNPs)

INTRODUCTION

There is accumulating evidence to support the hypothesis that neurodevelopmental outcomes after preterm birth are influenced by both genetic and environmental factors. The fetal inflammatory response syndrome (FIRS) is a multisystem disorder that has been associated with preterm birth, cerebral white-matter damage (periventricular leukomalacia), and cerebral palsy (CP).[1–3] Fetal proinflammatory cytokines IL6, IL1-β and tumor necrosis factor (TNF)-α have been associated with fetal/neonatal central nervous system (CNS) injury.[3] Further, polymorphisms in genes related to cytokine function (TNF-α, mannose-binding lectin, IL8 and lymphotoxin-α) have been associated with an increased risk of CP in recent studies.[4, 5]

Perinatal thromboembolic events are also increasingly recognized as a cause of neurologic disabilities such as CP, epilepsy, and other cognitive abnormalities.[6] Polymorphisms in genes related to thrombosis or thrombolysis (factor V Leiden, prothrombin gene mutation G20210A, Factor VII, plasminogen activator inhibitor I and methylenetetrahydrofolate reductase) have been associated with an increased risk of CP.[7, 8] The interaction between the inflammation and coagulation pathways may be important to the mechanism of fetal CNS injury. Perinatal arterial ischemic stroke (a common cause of hemiplegic CP) has been linked to several independent maternal risk factors, including chorioamnionitis.[9] There is compelling evidence that activated coagulation factors contribute to neonatal white matter damage through enhanced inflammation, rather than occlusion of cerebral blood vessels.[10]

While previous studies suggest that certain single nucleotide polymorphisms (SNPs) in inflammation and coagulation pathway genes increase vulnerability to cerebral palsy, it is uncertain whether SNPs in one or both of these pathways are associated with other forms of neurodevelopmental delay. We hypothesized that children with neurodevelopmental delay at age 2, when compared to children with normal neurodevelopment, have differences in inflammation and/or coagulation pathways gene SNPs.

MATERIALS AND METHODS

Subjects

The subjects evaluated were children enrolled in the NICHD Maternal Fetal Medicine Units (MFMU) Network randomized, double-masked, placebo-controlled, multicenter clinical trial of single- versus multiple-course antenatal corticosteroids. The primary aim of this trial was to assess the clinical efficacy and safety of repeated doses of antenatal corticosteroids in pregnancies at risk for preterm birth. The details of the trial, which was conducted between 2000 and 2003, have been previously reported. [11, 12]

A secondary aim of the MFMU Network antenatal corticosteroid trial was the correlation of steroid regimen with neurodevelopmental outcomes as assessed by the Bayley Scales of Infant Development, second edition, at age 2 years.[12] The Bayley Scales consist of mental and psychomotor developmental indices (MDI and PDI). A Bayley score of 85 is 1 SD below the mean and consistent with mild neurodevelopmental delay. A Bayley score of 70 is 2 SD below the mean and consistent with more significant delay. Placental samples and fetal cord serum were collected in a subset of subjects enrolled in the MFMU Network antenatal corticosteroid trial per study protocol.

Our secondary analysis aimed to evaluate the association between SNPs in fetal inflammation and coagulation pathway genes and neurodevelopmental delay at age 2 years. Inclusion criteria consisted of 1) maternal enrollment in the MFMU Network antenatal corticosteroid trial, 2) Bayley scores at age 2 years and, 3) DNA extracted from placental samples or fetal cord serum. Cases were children with mental and/or psychomotor delay, defined by a Bayley MDI and/or PDI <85 at age 2 years. Cerebral palsy cases (as defined in the primary trial)[11] were excluded from the analysis in order to test our hypothesis of an association between inflammation and coagulation gene SNPs and neurodevelopmental delay at age 2 in the absence of cerebral palsy. Controls were children with normal neurodevelopment, defined by Bayley MDI and PDI ≥85.

The study group is illustrated in Figure 1. From the original steroid trial, 583 children were discharged alive. Of these, 4 died between discharge and follow-up, and 23 were not available because they were born at a center that discontinued participation in the MFMU Network in 2001. Of the remaining 556 children who were available for follow-up, 459 (83%) had Bayley scores at age 2–3 years. After exclusion of CP cases (n=7), 452 children were eligible for participation in our analysis. There were 40 twin pairs in the original steroid trial. For this study, one twin from each pair (n=40) was randomly excluded to avoid the issue of including related individuals in the analysis. Of the remaining 412 infants, DNA from placental tissues and/or fetal serum was available for 272 infants, 125 cases and 147 controls.

After IRB review, this study was determined to be exempt from IRB approval procedures secondary to de-identification of data and study samples prior to this analysis.

DNA Extraction

DNA was extracted from placental samples and/or umbilical cord serum collected at the time of delivery. Placental samples were either fresh (n=145) or formalin-fixed and paraffin-embedded (n=92). DNA was extracted from approximately 0.7 gm of fresh placental tissue

using the PureGene DNA Purification System (Qiagen, Valencia, CA) as per the manufacturer's protocols. DNA from the formalin-fixed paraffin-embedded samples was extracted from 3–6, five micron sections obtained from the paraffin block and isolated using the PureGene DNA Purification System as per the manufacturer's protocols. DNA was also extracted from umbilical cord serum samples (n=190). Briefly, 250–1000 μL of cord serum was centrifuged at 10,000 × g for 10 minutes to pellet any cells in the sera. The supernatant was discarded and DNA was extracted with the same procedure used for placental samples.

Selection of SNPs for Genotyping

We selected 46 SNPs in 27 inflammation and coagulation genes based on the available literature and hypothesized causal pathways. We also included SNPs that have been reported to be associated with cerebral palsy. [4, 5, 8, 13] The SNPs included in our custom multiplex assay are shown in Table 1.

Genotyping

The 48-Plex GenomeLab™ SNPStream® Genotyping System and accompanying automated genotype calling software (Beckman Coulter, Fullerton, CA) was used for genotyping. Polymerase-chain-reaction (PCR) assays and extension primers for these SNPs were designed with the use of Beckman Coulter's Autoprimer multiplex primer design engine (www.Autoprimer.com). PCR and extension reactions were performed according to the manufacturer's instructions. Controls were appropriately included in each 384-well plate. Researchers and laboratory personnel were blinded to the case/control status of the biologic samples.

When DNA was available from both placenta and serum, the placental samples were used preferentially secondary to higher quality DNA. In subjects with a placental tissue genotyping success rate of <85%, the serum samples were also genotyped with a goal of increasing the number of SNP results. In 25 subjects with both placental tissue and sera genotyped, manufacturer's automated allele calling resulted in at least one discordant SNP between the two samples. Comparison of data quality allowed a correct genotype to be easily identified in most cases. If the subject's correct genotype could not be easily identified, no genotype was reported for that SNP. In all but one case, it was the serum data that was questionable secondary to the low intensity of the genotype signal due to poor quality and low concentration of the extracted DNA. In 3 subjects, the data for both serum and placental samples was strong, but many SNPs were discordant. These 3 subjects were removed from the analysis. Because the serum samples tended to have lower DNA quality, the automated genotype results were individually reviewed. If data quality did not allow for a clear genotype determination for a given serum sample, no genotype was reported.

Statistics

Demographic and clinical characteristics of cases and controls were compared using the Chi-square or Fisher's Exact test for categorical variables and the Wilcoxon Rank Sum test for continuous variables. Differences in allele and genotype frequencies between cases and controls were tested for each SNP with the use of the Chi-square or Fisher's exact tests, as appropriate. SNPs found to be significantly related to MDI and/or PDI <85 in the univariate analyses ($p<0.05$) were further analyzed using multiple logistic regression modeling. Genotypes were included as covariates in a series of regression models assuming either an additive, dominant, or recessive genetic pattern. The additive model assumes that having 2 copies of minor allele has twice the effect of having 1 copy, the dominant model assumes that having at least one copy of the minor allele is sufficient for disease, and the recessive model assumes that two copies of the minor allele are needed for disease. Other covariates included gestational age at delivery, small for gestational age (SGA) status (based on a birth weight less

than the 10th percentile of published standards)[14], gender, steroid treatment group, race, smoking, and maternal level of education. Of the three ways of examining genotype, the regression model that had the highest likelihood score was considered to be the best-fitting model for the respective SNP. The sample size and the rarity of some genotypes and phenotypes limited further modeling of interactions.

Tests for Hardy-Weinberg equilibrium (chi-square or Fisher's exact) were performed on control subjects for each SNP significant in the univariate analysis. As this was an exploratory study, no adjustments were made for multiple comparisons and all comparisons are reported. A p-value of less than 0.05 was considered to be statistically significant. All calculations were performed using SAS software (SAS Institute, Inc, Cary, NC).

RESULTS

The demographic and clinical characteristics of those included vs. excluded from our study based on availability of biologic samples for DNA analysis are shown in Table 2. Subjects excluded from our study were born at an earlier gestational age (p=0.01), had a lower birthweight (p=0.01) and were more likely to have received multiple courses of antenatal corticosteroids (p=0.007). The demographic and clinical characteristics of our study subjects are shown in Table 3. Significant differences between cases and controls were observed for race/ethnicity (p<0.001) and maternal education (p=0.01).

We achieved DNA PCR amplification for 35 of the 46 SNPs included on the custom multiplex assay (76%). Primer interaction, a common problem in multiplex assays, likely accounted for the PCR failures that were observed. The average genotyping call rate per subject sample was 63%. This genotyping call rate is reflective of the DNA degradation that was observed among the formalin-fixed paraffin-embedded placental samples.

Table 4 lists allele and genotype frequencies for the 35 SNPs among case and control subjects. Allele frequencies were significantly different between cases and controls for IL1-β –511 (p=0.009), IL4R 148 (p=0.03), IL6 -174 (p=0.02), and IL6 -176 (p=0.007). Genotype frequencies were significantly different between cases and controls for IL1-β-511(p=0.03) and IL6 -174 (p=0.04).

SNPs found to be significantly related (p<0.05) to MDI and/or PDI <85 in the univariate analyses (IL1-β –511, IL4R 148, IL6 -174 and -176) were further analyzed using multiple logistic regression modeling. Results of the logistic regression analysis for association of genotype with neurodevelopmental delay at age 2 years are presented in Table 5. Carriers of the IL1-β-511 and IL6 -176 minor alleles were more likely (OR 3.1, 95% CI 1.2–8.2, and 2.2, 95% CI 1.2–3.9, respectively) to have neurodevelopmental delay than control subjects, after adjustment for gestational age at delivery, SGA, gender, steroid treatment group, race, smoking, and maternal level of education. Carriers of the IL4R 148 minor allele were less likely to have neurodevelopmental delay (OR 0.6, 95% CI 0.4–0.9). The IL6 -174 genotype was not significantly associated with neurodevelopmental delay in the logistic regression analysis. When various genotype models were tested, the best-fitting model was recessive for IL1-β –511, dominant for IL6 -176, and additive for IL4R 148.

In the control group, each SNP that was significant in the univariate analysis was in Hardy-Weinberg equilibrium, with the exception of IL6 -176 (p<0.0001).

COMMENT

We used a candidate gene association approach to investigate the role of inflammation and coagulation pathway gene SNPs in cognitive development in pregnancies at-risk for preterm

birth. Our results suggest an association between SNPs in IL-1β, IL6, and IL4R and neurodevelopmental delay at age 2. Biologically this is plausible, as inflammation and/or coagulation pathway-mediated fetal/neonatal brain injury could occur to a milder extent than necessary for a diagnosis of cerebral palsy and could also occur outside the vulnerable motor pathways of the developing brain.

Perinatal concentrations of the proinflammatory cytokines IL1-β and IL6 have been shown to correlate directly with the development of periventricular white matter injury and subsequent cerebral palsy.[3] The IL1-β-511 SNP is in the enhancer-promoter region of the gene and the A allele has been associated with higher levels of IL1-β.[15, 16] The IL6 -174 and -176 SNPs are also in the enhancer-promotor region of the gene, and although functional assays have been somewhat conflicting, the G allele at position -174 has been associated with a stronger IL6 response.[17, 18] SNPs that are capable of altering circulating cytokine levels have been associated with CP, either directly through cytokine-mediated brain damage or indirectly through altered immune response to infection.[4, 5, 8] Our study suggests that proinflammatory cytokine gene SNPs may also increase the risk of subsequent neurodevelopmental delay.

The IL4R 148 (ile50val) polymorphism is in the extracellular domain of the alpha chain of the IL4R complex. The IL4R plays a critical role in IL4 binding and signal transduction, and therefore IL4-mediated IgE production and Th2 inflammatory reactions. Variants in IL4Rα have been reported to be associated with atopic phenotypes, leading to the hypothesis that the IL4R alpha chain is a major atopy locus. [19–22] Our study suggests that genetic alterations to the extracellular domain of IL4R may contribute to neurodevelopmental outcomes in pregnancies at high risk for preterm birth.

Although polymorphisms in genes related to thrombosis or thrombolysis have been associated with an increased risk of CP,[7, 8] coagulation pathway gene polymorphisms were not associated with neurodevelopmental delay in this study. Since the interaction between the inflammation and coagulation pathways may be critical to the mechanism of fetal CNS injury, studies with sufficient power to evaluate gene-gene interactions should be pursued.

It should be noted that neurodevelopmental testing at age 2 is of limited predictive value, particularly for subtle deficits. Whether inflammation gene variants have any longer-term neurocognitive effects of clinical significance is uncertain, and cannot be extrapolated from this data.

Genes or SNPs in linkage disequilibrium with those we have identified, rather than the genes and SNPs that we report, may be the actual causative variants associated with neurodevelopmental outcomes. In addition, missing genotyping data and sample size may result in true genotype-phenotype associations being missed. Further studies in other populations are needed to confirm or refute the genetic associations described.

We acknowledge that the observed associations between genotype and neurodevelopmental delay may be due to chance as multiple analyses increase the likelihood of identifying chance statistical associations. However, multiple previous studies have demonstrated a strong association between proinflammatory cytokines and fetal/neonatal central nervous system injury. In addition, variation in the IL6 gene has been shown to be associated with impaired cognitive development in children born prematurely. [23] These studies lend support and biologic plausibility to our findings.

Sample size did not permit analysis with stratification by race/ethnicity, and we acknowledge this as a study limitation. This may have been addressed by genotyping of Ancestry Informative Markers (AIMS), which would have allowed us to control and adjust for racial admixture.[24] However, this was cost-prohibitive for this exploratory analysis. The sample size and lack of

placental pathology also prohibited analysis of the interaction of genotype with maternal/intrauterine infection and neonatal sepsis, a gene-environment interaction of obvious interest.

The demographic and clinical characteristics of subjects included vs. excluded in this analysis (Table 2) suggest that our subjects are not entirely representative of the original steroid trial. We speculate that the differences between subjects included versus excluded may reflect more difficult sample collection in subjects born at an earlier gestational age (and lower birth weight). In these babies, delivery circumstances and resuscitation efforts may have negatively influenced the ability to collect adequate biologic specimens. We are uncertain as to why subjects that were excluded were more likely to have received antenatal multiple-course steroids. These subject characteristics were included in our regression analysis, and are unlikely to be a significant source of bias.

The risk of central nervous system injury in preterm infants is influenced by complex gene-environment interactions that are not yet well understood. The strengths of this study include the evaluation of a large cohort of infants at risk for preterm birth with appropriate controls and well-characterized obstetrical and neurodevelopmental outcomes. This study adds to the current evidence that certain inflammation gene variants may contribute to adverse neurodevelopmental outcomes and suggests that further investigation of genetic polymorphisms as contributors to susceptibility to neurodevelopmental delay in preterm infants is warranted. Ultimately, a better understanding of genetic factors that predispose to these outcomes may lead to identification of new prevention strategies and future prevention/intervention trials.

Acknowledgments

Supported by grants from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (HD21410, HD27869, HD27917, HD27860, HD27915, HD34116, HD34208, HD34136, HD40500, HD40485, HD40544, HD40545, HD40560, HD40512, HD40485, HD36801) and M01-RR-000080 from the National Center for Research Resources

The following subcommittee members participated in protocol development and coordination between clinical research centers (Michelle Di Vito, R.N. and Francee Johnson, R.N., B.S.N.), protocol/data management and statistical analysis (Elizabeth Thom, Ph.D.), and protocol development and oversight (Donald J. Dudley, M.D.). We also thank Dr. Michael Varner for his invaluable assistance with protocol development, manuscript preparation, and mentorship to Dr. Erin Clark.

References

1. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol 1998;179:194–202. [PubMed: 9704787]
2. Romero R, Gomez R, Ghezzi F, et al. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. Am J Obstet Gynecol 1998;179:186–93. [PubMed: 9704786]
3. Yoon BH, Jun JK, Romero R, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. Am J Obstet Gynecol 1997;177:19–26. [PubMed: 9240577]
4. Gibson CS, MacLennan AH, Goldwater PN, Haan EA, Priest K, Dekker GA. The association between inherited cytokine polymorphisms and cerebral palsy. Am J Obstet Gynecol 2006;194:674, e1–11. [PubMed: 16522396]
5. Gibson CS, Maclennan AH, Dekker GA, et al. Candidate genes and cerebral palsy: a population-based study. Pediatrics 2008;122:1079–85. [PubMed: 18977990]
6. deVeber GA, MacGregor D, Curtis R, Mayank S. Neurologic outcome in survivors of childhood arterial ischemic stroke and sinovenous thrombosis. J Child Neurol 2000;15:316–24. [PubMed: 10830198]
7. Gibson CS, MacLennan AH, Hague WM, et al. Associations between inherited thrombophilias, gestational age, and cerebral palsy. Am J Obstet Gynecol 2005;193:1437. [PubMed: 16202738]

8. Nelson KB, Dambrosia JM, Iovannisci DM, Cheng S, Grether JK, Lammer E. Genetic polymorphisms and cerebral palsy in very preterm infants. Pediatr Res 2005;57:494–9. [PubMed: 15718364]

9. Lee J, Croen LA, Backstrand KH, et al. Maternal and infant characteristics associated with perinatal arterial stroke in the infant. JAMA 2005;293:723–9. [PubMed: 15701914]

10. Leviton A, Dammann O. Coagulation, inflammation, and the risk of neonatal white matter damage. Pediatr Res 2004;55:541–5. [PubMed: 14973176]

11. Wapner RJ, Sorokin Y, Thom EA, et al. Single versus weekly courses of antenatal corticosteroids: evaluation of safety and efficacy. Am J Obstet Gynecol 2006;195:633–42. [PubMed: 16846587]

12. Wapner RJ, Sorokin Y, Mele L, et al. Long-term outcomes after repeat doses of antenatal corticosteroids. N Engl J Med 2007;357:1190–8. [PubMed: 17881751]

13. Gibson CS, MacLennan AH, Goldwater PN, Haan EA, Priest K, Dekker GA. Mannose-binding lectin haplotypes may be associated with cerebral palsy only after perinatal viral exposure. Am J Obstet Gynecol 2008;198:509, e1–8. [PubMed: 18455525]

14. Alexander GR, Kogan MD, Himes JH. 1994–1996 U.S. singleton birth weight percentiles for gestational age by race, Hispanic origin, and gender. Matern Child Health J 1999;3:225–31. [PubMed: 10791363]

15. Chen H, Wilkins LM, Aziz N, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum Mol Genet 2006;15:519–29. [PubMed: 16399797]

16. Rogus J, Beck JD, Offenbacher S, et al. IL1B gene promoter haplotype pairs predict clinical levels of interleukin-1beta and C-reactive protein. Hum Genet 2008;123:387–98. [PubMed: 18369665]

17. Reiman M, Kujari H, Ekholm E, Lapinleimu H, Lehtonen L, Haataja L. Interleukin-6 polymorphism is associated with chorioamnionitis and neonatal infections in preterm infants. J Pediatr 2008;153:19–24. [PubMed: 18571528]

18. Rivera-Chavez FA, Peters-Hybki DL, Barber RC, O'Keefe GE. Interleukin-6 promoter haplotypes and interleukin-6 cytokine responses. Shock 2003;20:218–23. [PubMed: 12923492]

19. Kruse S, Japha T, Tedner M, et al. The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. Immunology 1999;96:365–71. [PubMed: 10233717]

20. Mitsuyasu H, Izuhara K, Mao XQ, et al. Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma. Nat Genet 1998;19:119–20. [PubMed: 9620765]

21. Ober C, Leavitt SA, Tsalenko A, et al. Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. Am J Hum Genet 2000;66:517–26. [PubMed: 10677312]

22. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Engl J Med 1997;337:1720–5. [PubMed: 9392697]

23. 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;58:117–20. [PubMed: 15985687]

24. Kosoy R, Nassir R, Tian C, et al. Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. Hum Mutat 2008;30:69–78. [PubMed: 18683858]

APPENDIX

In addition to the authors, other members of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network are as follows:

University of Utah — D. Dudley, M. Varner, K. Anderson, A. Guzman, A. Crowley, M. Fuller

Drexel University — M. DiVito, A. Sciscione, V. Berghella, M. Pollock, M. Talucci

Wayne State University — M. Dombrowski, G. Norman, A. Millinder, C. Sudz, D. Driscoll

The Ohio State University — F. Johnson, M. Landon, S. Meadows, P. Shubert

Northwestern University — G. Mallett

University of Texas Southwestern Medical Center — D. Weightman, L. Fay-Randall, P. Mesa

Wake Forest University Health Sciences — P. Meis, M. Swain, C. Moorefield

University of Pittsburgh — T. Kamon, K. Lain, M. Cotroneo

Columbia University — F. Malone, V. Pemberton, S. Bousleiman

Case Western Reserve University — P. Catalano, C. Milluzzi, C. Santori

University of North Carolina at Chapel Hill — K. Moise, K. Dorman

The University of Texas Health Science Center at Houston — L. Gilstrap, M. Day

Brown University — D. Allard, J. Tillinghast

University of Alabama at Birmingham — A. Northern, K. Bailey

The George Washington University Biostatistics Center — E. Thom, F. Galbis-Reig, L. Leuchtenburg

Eunice Kennedy Shriver National Institute of Child Health and Human Development — D. McNellis, K. Howell, S. Pagliaro

MFMU Network Steering Committee Chair *(Vanderbilt University Medical Center)* — S. Gabbe

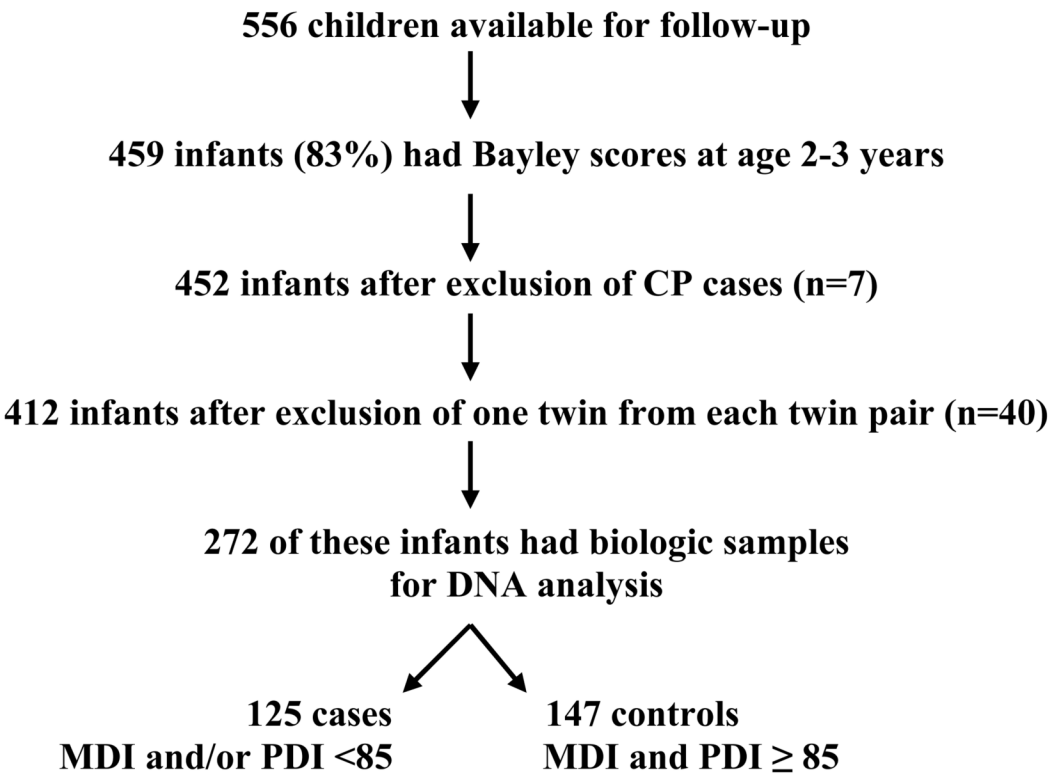

Figure 1. Identification of Cases and Controls

From the original steroid trial, 583 children were discharged alive. Of these, 4 died between discharge and follow-up and 23 were not available because they were born at a center that discontinued participation in the MFMU Network in 2001; cases and controls were selected from the remaining 556 children who were available for follow-up.

Table 1

Single Nucleotide Polymorphisms Studied

Gene	Symbol	Polymorphism	RS Number
Lymphotoxin-α (Tumor Necrosis Factor-β)	LTA	thr26asn(C/A)	rs1041981*
Mannose-Binding Lectin	MBL2	−550(G/C)	rs11003125
		gly54asp(A/G)	rs1800450
		gly57glu(A/G)	rs1800451*
		arg52cys(T/C)	rs5030737*
		−221(G/C)	rs7096206
Tumor Necrosis Factor-α	TNF	−308(G/A)	rs1800629
		−376(G/A)	rs1800750*
		−238(G/A)	rs361525
Tumor Necrosis Factor Receptor Superfamily Member 6	TNFRSF6	−670(G/A)	rs1800682
Interleukin 1-α	IL1A	−889(C/T)	rs1800587
Interleukin 1-β	IL1B	+3962(C/T)	rs12621220
		−511(A/G)	rs16944
Interleukin 1 Type 1 Receptor	IL1RN	+970(C/T)	rs3917365
Interleukin 4	IL4	−590(C/T)	rs2234648
		582(A/G)	rs35648164
Interleukin 4 Receptor	IL4R	gln576arg(A/G)	rs1801275
		ile50val(A/G)	rs1805010
Interleukin 5 Receptor-α	IL5RA	−80(G/A)	rs2290608
Interleukin 6	IL6	−174(G/C)	rs1800795
		−176(G/C)	rs2234683
Interleukin 9	IL9	553(A/C)	rs2066760
		thr113met(T/C)	rs2069885*
Interleukin 10	IL10	−819(C/T)	rs1800871*
		−1082(G/A)	rs1800896
Interleukin 13	IL13	arg130gln(T/C)	rs20541
Factor II	F2	20210(G/A)	rs1799963
Factor V Leiden	F5	arg506gln(A/G)	rs6025
		arg353gln(T/C)	rs6046*
Factor XIII	F13	val34leu(T/G)	rs5985
Methylenetetrahydrofolate Reductase	MTHFR	1298(A/C)	rs1801131
		677(C/T)	rs1801133
Thrombomodulin	THBD	1418(C/T)	rs1042579*
Plasminogen Activator Inhibitor 1	PAI1	+11053(G/T)	rs7242*
Fibrinogen-β Polypeptide Chain	FGB	−455(G/A)	rs1800790*
Thrombin Activatable Fibrinolysis Inhib.	TAFI	1040(C/T)	rs1926447
Platelet Glycoprotein Ia (Integrin, α2)	ITGA2	873(G/A)	rs1062535*
β-Adrenergic Receptor	ADRB2	arg16gly(A/G)	rs1042713

Gene	Symbol	Polymorphism	RS Number
		gln27glu(C/G)	rs1042714
Nitric Oxide Synthase 3, Endothelial	NOS3	−690(C/T)	rs3918226
		glu298asp(T/G)	rs1799983
		−922(A/G)	rs1800779
Selectin P	SELP	val640leu(T/G)	rs6133
		thr756pro(C/A)	rs6136
Transforming Growth Factor-β1	TGFB1	−800(G/A)	rs1800468
		−509(C/T)	rs1800469

* PCR amplification was not successful

Table 2

Demographic and Clinical Characteristics of Children Included vs. Excluded

Characteristic	Included	Excluded*	P
Subjects, n	272	180	
Gender, n (%)			
Male	143 (52.6)	89 (49.4)	0.51
Female	129 (47.4)	91 (50.6)	
Race/ethnicity†, n (%)			
White	92 (33.8)	37 (35.2)	0.59
Black	104 (38.2)	44 (41.9)	
Other	76 (27.9)	24 (22.9)	
GA at delivery†, mean ± SD, wk	35.2 ± 3.8	34.2 ± 3.7	**0.01**
GA at delivery†, n (%)			
≥37 wks	108 (39.7)	27 (25.7)	**0.03**
32–36 wks	111 (40.8)	49 (46.7)	
<32 wks	53 (19.5)	29 (27.6)	
Birth weight, mean ± SD, gm	2396.3 ± 778.7	2059.4 ± 721.4	**0.01**
Neurodevelopment, n (%)			
MDI and/or PDI < 85	125 (46.0)	86 (47.8)	0.94
MDI and/or PDI < 70	50 (18.4)	37 (20.6)	0.86
Chorioamnionitis†, n (%)	8 (2.9)	3 (2.9)	1.00
Maternal tobacco use†, n (%)	55 (20.2)	25 (23.8)	0.44
Antenatal multiple-course steroids†, n (%)	126 (46.3)	65 (61.9)	**0.007**
Maternal education†, mean ± SD, y	12.3 ± 2.7	12.3 ± 2.2	0.55

*One twin from each pair (n=40) and subjects lacking DNA for analysis (n=140) were excluded

†272 pregnancies included vs. 105 pregnancies excluded

Table 3

Demographic and Clinical Characteristics of Children with Neurodevelopmental Delay (Cases) vs. Controls

Characteristic	Cases	Controls	P
Study sample, n	125	147	
Gender, n (%)			
Male	69 (55.2)	74 (50.3)	0.42
Female	56 (44.8)	73 (49.7)	
Race/ethnicity, n (%)			
White	25 (20.0)	67 (45.6)	**<0.001**
Black	55 (44.0)	49 (33.3)	
Other	45 (36.0)	31 (21.1)	
GA at delivery, mean ± SD, wk	35.4 ± 3.7	35.0 ± 3.9	0.36
GA at delivery, n (%)			
≥ 37 wks	53 (42.4)	55 (37.4)	0.53
32–36 wks	51 (40.8)	60 (40.8)	
<32 wks	21 (16.8)	32 (21.8)	
Birth weight, mean ± SD, kg	2383.4 ± 719.2	2407.2 ± 828.2	0.83
Neurodevelopment, n (%)			
MDI and PDI ≥ 85	0	147 (100)	
MDI and/or PDI <85	125 (100)	0	
MDI and/or PDI <70	50 (40.0)	0	
Chorioamnionitis, n (%)	3 (2.4)	5 (3.4)	0.73
Maternal tobacco use, n (%)	22 (17.6)	33 (22.4)	0.32
Antenatal multiple-course steroids, n (%)	54 (43.2)	72 (49.0)	0.34
Maternal education, y	11.8 ± 2.7	12.6 ± 2.6	**0.01**

Table 4

Genotypic Distributions in Control Children and Children with Neurodevelopmental Delay (Bayley MDI and/or PDI <85)*

Gene Symbol	Polymorphism	Allele	Controls n(%)	Cases n(%)	P	Genotype	Controls n(%)	Cases n (%)	P
LTA	thr26asn(C/A)	C	92(40)	61(39)	0.84	CC	14(12)	11(14)	0.73
		A	140(60)	97(61)		CA	64(55)	39(49)	
						AA	38(33)	29(37)	
MBL2	−550(G/C)	G	105(67)	49(56)	0.07	GG	35(45)	13(30)	0.19
		C	51(33)	39(44)		GC	35(45)	23(52)	
						CC	8(10)	8(18)	
	gly54asp(A/G)	A	28(13)	13(8)	0.10	AA	2(2)	2(2)	0.10
		G	182(87)	151(92)		AG	24(23)	9(11)	
						GG	79(75)	71(87)	
	−221(G/C)	G	156(72)	100(68)	0.53	GG	63(58)	38(52)	0.71
		C	62(28)	46(32)		GC	30(28)	24(33)	
						CC	16(15)	11(15)	
TNF-α	−308(G/A)	G	178(86)	116(85)	0.94	GG	76(73)	48(71)	0.56
		A	30(14)	20(15)		GA	26(25)	20(29)	
						AA	2(2)	0	
	−238(G/A)	G	149(71)	135(78)	0.14	GG	47(45)	49(56)	0.26
		A	61(29)	39(22)		GA	55(52)	37(43)	
						AA	3(3)	1(1)	
TNFRSF6	−670(C/T)	C	131(63)	112(71)	0.11	CC	37(36)	41(52)	0.06
		T	77(37)	46(29)		CT	57(55)	30(38)	
						TT	10(10)	8(10)	
IL1-α	−889(C/T)	C	120(70)	67(68)	0.81	CC	39(45)	20(41)	0.75
		T	52(30)	31(32)		CT	42(49)	27(55)	
						TT	5(6)	2(4)	
IL1-β	+3962(C/T)	C	102(65)	65(69)	0.54	CC	30(38)	20(43)	0.72
		T	54(35)	29(31)		CT	42(54)	25(53)	
						TT	6(8)	2(4)	
	−511(A/G)†‡	A	57(29)	65(42)	**0.009**	AA	8(8)	16(21)	**0.03**
		G	141(71)	89(58)		AG	41(41)	33(43)	

Gene Symbol	Polymorphism	Allele	Controls n(%)	Cases n(%)	P	Genotype	Controls n(%)	Cases n (%)	P
						GG	50(51)	28(36)	
IL1RN	+970(C/T)	C	191(83)	141(79)	0.32	CC	79(69)	54(61)	0.44
		T	39(17)	37(21)		CT	33(29)	33(37)	
						TT	3(3)	2(2)	
IL4	−590(C/T)	C	217(94)	163(96)	0.49	CC	103(90)	79(93)	0.72
		T	13(6)	7(4)		CT	11(10)	5(6)	
						TT	1(1)	1(1)	
	582(A/G)	A	50(28)	30(31)	0.62	AA	4(5)	3(6)	0.83
		G	126(72)	66(69)		AG	42(48)	24(50)	
						GG	42(48)	21(44)	
IL4R	gln576arg(A/G)	A	127(61)	91(57)	0.42	AA	44(42)	26(33)	0.28
		G	81(39)	69(43)		AG	39(38)	39(49)	
						GG	21(20)	15(19)	
	ile50val,	A	104(44)	67 (34)	**0.03**	AA	23(19)	12(12)	0.10
	148 (A/G)†	G	132(56)	131(66)		AG	58(49)	43(43)	
						GG	37(31)	44(44)	
IL5RA	−80(G/A)	G	147(60)	110(57)	0.60	GG	61(50)	49(51)	0.27
		A	99(40)	82(43)		GA	25(20)	12(13)	
						AA	37(30)	35(36)	
IL6	−174(G/C)†‡	G	125(72)	81(84)	**0.02**	GG	44(51)	35(73)	**0.04**
		C	49(28)	15(16)		GC	37(43)	11(23)	
						CC	6(7)	2(4)	
	−176(G/C)†	G	159(63)	101(50)	**0.007**	GG	69(55)	40(40)	0.09
		C	93(37)	99(50)		GC	21(17)	21(21)	
						CC	36(29)	39(39)	
IL9	553(A/C)	A	7(4)	6(6)	0.46	AA	0	0	0.55
		C	181(96)	102(94)		AC	7(7)	6(11)	
						CC	87(93)	48(89)	
IL10	−1082(G/A)	G	71(36)	42(28)	0.11	GG	12(12)	4(5)	0.21
		A	125(64)	108(72)		GA	47(48)	34(45)	
						AA	39(40)	37(49)	

Gene Symbol	Polymorphism	Allele	Controls n(%)	Cases n(%)	P	Genotype	Controls n(%)	Cases n (%)	P
IL13	arg130gln(T/C)	T	57(26)	43(23)	0.59	TT	7(6)	5(5)	0.86
		C	165(74)	141(77)		TC	43(39)	33(36)	
						CC	61(55)	54(59)	
F5	arg506gln(A/G)	A	57(33)	26(27)	0.26	AA	12(14)	6(12)	0.42
		G	115(67)	72(74)		AG	33(38)	14(29)	
						GG	41(48)	29(59)	
F13	val34leu(T/G)	T	99(39)	65(33)	0.17	TT	17(13)	15(15)	0.05
		G	157(61)	135(67)		TG	65(51)	35(35)	
						GG	46(36)	50(50)	
MTHFR	1298(A/C)	A	9(4)	6(4)	0.88	AA	1(1)	1(1)	0.88
		C	207(96)	150(96)		AC	7(6)	4(5)	
						CC	100(93)	73(94)	
	677(C/T)	C	131(68)	113(74)	0.22	CC	47(49)	40(53)	0.14
		T	61(32)	39(26)		CT	37(39)	33(43)	
						TT	12(13)	3(4)	
TAFI	1040(C/T)	C	192(80)	147(77)	0.39	CC	76(63)	55(57)	0.70
		T	48(20)	45(23)		CT	40(33)	37(39)	
						TT	4(3)	4(4)	
ADRB2	arg16gly(A/G)	A	100(47)	63(41)	0.28	AA	23(22)	13(17)	0.55
		G	112(53)	89(59)		AG	54(51)	37(49)	
						GG	29(27)	26(34)	
	gln27glu(C/G)	C	58(27)	32(22)	0.22	CC	6(6)	3(4)	0.42
		G	154(73)	116(78)		CG	46(43)	26(35)	
						GG	54(51)	45(61)	
NOS3	−690(C/T)	C	149(80)	87(81)	0.93	CC	63(68)	37(69)	1.00
		T	37(20)	21(19)		CT	23(25)	13(24)	
						TT	7(8)	4(7)	
	glu298asp(T/G)	T	45(26)	17(16)	0.05	TT	6(7)	1(2)	0.17
		G	125(74)	87(84)		TG	33(39)	15(29)	
						GG	46(54)	36(69)	
	−922(A/G)	A	128(58)	101(62)	0.41	AA	43(39)	38(47)	0.50
		G	92(42)	61(38)		AG	42(38)	25(31)	

Gene Symbol	Polymorphism	Allele	Controls n(%)	Cases n(%)	P	Genotype	Controls n(%)	Cases n (%)	P
						GG	25(23)	18(22)	
SELP	val640leu(T/G)	T	73(37)	43(28)	0.10	TT	15(15)	7(9)	0.29
		G	127(63)	109(72)		TG	43(43)	29(38)	
						GG	42(42)	40(53)	
	thr756pro(C/A)	C	34(15)	24(15)	0.85	CC	9(8)	4(5)	0.47
		A	188(85)	140(85)		CA	16(14)	16(20)	
						AA	86(77)	62(76)	
TGFB1	−800(G/A)	G	105(65)	54(57)	0.24	GG	29(36)	16(34)	0.07
		A	57(35)	40(43)		GA	47(58)	22(47)	
						AA	5(6)	9(19)	
	−509(C/T)	C	35(19)	16(15)	0.39	CC	1(1)	0	0.66
		T	145(81)	88(85)		CT	33(37)	16(31)	
						TT	56(62)	36(69)	

*Because of missing genotype results, the number of cases and controls for each genotype do not necessarily total the 125 cases and 147 controls included in the study.

†p-value <0.05 for allele distribution difference between cases and controls

‡p-value <0.05 for genotype distribution difference between cases and controls

Table 5

Logistic Regression Analysis for Association of Genotype with Neurodevelopmental Delay at Age 2 Years*

Gene	Polymorphism	RS Number	OR (95% CI)†	Best Model
IL1-β	−511A/G	16944	3.1 (1.2–8.2)	Recessive
IL4-R	Ile50Val, 148 (A/G)	1805010	0.6 (0.4–0.9)	Additive
IL6	−176G/C	2234683	2.2 (1.2–3.9)	Dominant
IL6	−174G/C	1800795	0.5 (0.2–1.2)	Dominant

*Covariates included gestational age at delivery, small for gestational age (SGA) status, gender, steroid treatment group, race, smoking, and maternal level of education.

†OR, odds ratio; CI, confidence interval.