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Genetic Variation, Magnesium Sulfate Exposure, and Adverse Neurodevelopmental Outcomes Following Preterm Birth

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Abstract

OBJECTIVE: To evaluate the association of magnesium sulfate (MgSO₄) exposure and candidate gene polymorphisms with adverse neurodevelopmental outcomes following preterm birth.

METHODS: We performed a nested case-control analysis of a randomized trial of maternal MgSO₄ before anticipated preterm birth for prevention of cerebral palsy (CP). Cases were children who died by 1 year of life or were survivors with abnormal neurodevelopment at age 2 years. Controls were race- and sex-matched survivors with normal neurodevelopment. We analyzed 45 candidate gene polymorphisms in inflammation, coagulation and vascular regulation pathways and their association with 1) psychomotor delay, 2) mental delay, 3) CP and 4) combined outcome of death/CP. Logistic regression analyses, conditional on maternal race and child sex, and adjusted for treatment group, gestational age at birth and maternal education, were performed.

RESULTS: Four hundred and six subjects, 211 cases and 195 controls, were analyzed.

Psychomotor delay: The strongest association was for IL6R (rs 4601580) in which each additional copy of the minor allele was associated with an increased risk of psychomotor delay (aOR 3.3; 95%CI 1.7-6.5). *Mental delay:* Three SNPs in IL6R were associated with mental developmental delay. Additional SNPs in IL6, MBL2, and F7 showed MgSO₄ treatment interaction. *CP:* TLR4 (rs4986790) was associated with CP (aOR 5.5; 95%CI, 1.1-26.9). SNPs in IL1 β and PAI1 showed MgSO₄ treatment interaction. *Death/CP:* SNPs in F7 and NOS3 were associated with the combined outcome of death/CP.

CONCLUSION: Candidate gene polymorphisms are associated with death and adverse neurodevelopmental outcomes following preterm birth. MgSO₄ may abrogate this genotype association for some loci.

PRECIS

Candidate gene polymorphisms are associated with death and adverse neurodevelopmental outcomes in children born preterm; MgSO₄ may abrogate this association for some genetic loci.

Keywords

Candidate genes; magnesium sulfate; mental developmental delay; neurodevelopmental delay; preterm birth; polymorphisms; psychomotor delay; single-nucleotide polymorphisms (SNPs)

INTRODUCTION

Increasing evidence suggests that neurodevelopmental outcomes after preterm birth are influenced by both genetic and environmental factors. Exposure to magnesium sulfate (MgSO₄) before anticipated early preterm birth has been shown to reduce the risk of gross

motor dysfunction and cerebral palsy (CP) in surviving children in several clinical trials and in meta-analyses.¹⁻⁵

Although the neuroprotective role of MgSO₄ is well established, the mechanism of fetal neuroprotection remains uncertain. MgSO₄ may work via one or more mechanisms including 1) promotion of vascular stability, 2) prevention of hypoxic-ischemic reperfusion injury, 3) reduction in excitatory amino acid damage by acting as a noncompetitive N-methyl-D-aspartic acid (NMDA) receptor antagonist, and/or 4) mitigation of cytokine-mediated inflammatory injury.^{6,7} Intrauterine inflammation and the fetal inflammatory response syndrome (FIRS) have been associated with preterm birth, cerebral white-matter damage (periventricular leukomalacia) and CP.⁸⁻¹² A protective mechanism involving mitigation of cytokine-mediated injury, either directly or indirectly, is therefore particularly intriguing.

In exploratory studies, fetal gene polymorphisms in inflammation, coagulation and vascular regulation pathways have been associated with adverse neurodevelopmental outcomes after preterm birth, including CP and neurodevelopmental delay.¹³⁻¹⁹ We sought to confirm these previous observations and also hypothesized that MgSO₄ may influence these genotype associations in a unique gene-environment interaction. Our objective was to evaluate the associations between candidate gene polymorphisms and MgSO₄ exposure with adverse outcomes, including death, CP and neurodevelopmental delay, in a cohort of early preterm births.

MATERIALS AND METHODS

Subjects

Children enrolled in the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network randomized, placebo-controlled, double-masked multicenter clinical trial of MgSO₄ for prevention of CP before anticipated preterm birth were studied. Women with singleton or twin gestations between 24 0/7 and 31 6/7 weeks' gestation and at risk of imminent preterm delivery were eligible for enrollment. The primary outcome was a composite of stillbirth or infant death by 1 year of life, and moderate or severe CP at or beyond 2 years of age. Neurodevelopmental delay was included as a secondary outcome, assessed using the Bayley Scales of Infant Development II administered at 2 years of age. The details of the trial, which was conducted between 1997 and 2004, have been previously reported.¹

Our nested case-control analysis aimed to evaluate the association of MgSO₄ exposure and candidate gene polymorphisms with death and abnormal neurodevelopment by age 2 years following preterm birth. Inclusion criteria were 1) enrollment in the original MgSO₄ randomized controlled trial, 2) neurological outcome data at age 2 in survivors, and 3) available DNA for genotyping. Cases either died by 1 year of life or were survivors with abnormal neurodevelopment, defined by CP or neurodevelopmental delay. 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), which were

administered by centrally certified psychologists or psychometrists. CP was assessed by centrally-certified pediatricians or pediatric neurologists according to pre-specified criteria (gross motor delay, and abnormality in muscle tone, movement, and reflexes).¹ Controls survived with normal neurodevelopment, defined as Bayley MDI and PDI ≥ 85 and no diagnosis of grade III/IV intraventricular hemorrhage, periventricular leukomalacia, or CP.

The study group for this analysis is illustrated in Figure 1. In the primary study, 2,241 women (2,444 fetuses) participated. We excluded fetal deaths and infants with major congenital malformations. We also excluded children lost to follow-up and children with no available DNA sample. Randomly selected control-group children were matched to case-group children for self-reported maternal race/ethnicity and child sex to minimize the chance of misidentifying a race-based or sex-based genotype association. We excluded samples that either failed to genotype and/or had $>30\%$ of genotypes missing, and then excluded the corresponding case or control group subjects, in order to maintain the matching design and ensure valid and accurate genotyping results. One twin from each pair was randomly excluded to avoid the issue of including related individuals in the analysis. This resulted in 406 subjects, 211 cases and 195 controls.

Selection of Polymorphisms for Genotyping

We used a candidate gene approach to evaluate 45 polymorphisms in 19 candidate genes within inflammation, coagulation and vascular regulation pathways (Table 1).

Polymorphisms were selected based on previously described associations with CP and/or neurodevelopmental delay or involvement in hypothesized causal pathways.

DNA Extraction, Amplification and Genotyping

DNA was extracted from fetal cord serum using the PureGene® DNA Purification System (Qiagen) per the manufacturer's protocols. To increase the available DNA for testing, whole-genome amplification (WGA) was completed on each sample using the GenomePlex® Whole Genome Amplification Kit (Sigma-Aldrich). Genotyping of single nucleotide polymorphisms (SNPs) was accomplished using TaqMan® assays (Applied Biosystems). Genotyping for the 1L1RN variable number tandem repeat polymorphism (VNTR) was performed using a fluorescently labeled primer during PCR followed by size determination by capillary electrophoresis against an internal size standard on a 3130xL Genetic Analyzer (Applied Biosystems).

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.

Separate analyses compared the association of candidate gene polymorphisms with 1) CP, 2) the combined outcome of CP/death, 3) psychomotor delay, and 4) mental delay. Death and CP were analyzed as a combined outcome, as was done in the primary randomized clinical trial, since death and CP are competing outcomes.¹ Because this secondary analysis was performed as part of the MgSO₄ trial, in which the treatment affected the risk of adverse

neurodevelopmental outcomes, analyses first determined if MgSO₄ modified the relationship between each polymorphism and the adverse neurodevelopmental outcomes described above (treatment interaction). If this treatment interaction was not present, then polymorphisms were analyzed for cases and controls with both treatment groups combined. If this interaction was present, polymorphisms were analyzed according to treatment group. Conditional logistic regression analysis, stratified by maternal race/ethnicity and child sex, was used to account for the matching of cases with controls. Additionally, covariates known *a priori* to be associated with neurodevelopmental outcomes following preterm birth (gestational age at birth, maternal education level) were included in the models. Initial analyses assumed an additive genetic model, in which each copy of the minor allele confers additional risk. Significant genetic associations were further explored using dominant and recessive genetic models.

Exact tests for Hardy-Weinberg equilibrium were performed on control subjects for each polymorphism.²⁰ As this was an exploratory study, no adjustments were made for multiple comparisons and all comparisons are reported. A p-value <0.05 was considered to be statistically significant. All calculations were performed using SAS software version 9.3 (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. After review by the University of Utah IRB, this secondary analysis was determined to be exempt from IRB approval procedures secondary to de-identification of data and study samples prior to analysis.

RESULTS

Four hundred and six subjects, 211 cases and 195 controls, were analyzed. Among the cases, there were 44 infant deaths, 25 children with CP, 95 children with psychomotor delay and 113 children with mental delay. Some children had more than one outcome and were included in more than one analysis.

Children identified from the parent cohort who satisfied the case inclusion criteria, but had no available DNA, were previously compared with the children with DNA samples available for analysis.²² Children with DNA available for analysis were more likely to be born at a later gestational age and more likely to be singleton.

The demographic and clinical characteristics of study subjects are shown in Table 2. Cases delivered significantly earlier than controls, with a mean gestational age of 29.3 vs. 30.9 weeks (P<0.001). The majority of preterm births among cases and controls occurred following preterm premature rupture of membranes. Cases and controls were similar with regard to sex distribution, exposure to antenatal steroids and MgSO₄, chorioamnionitis, and maternal race/ethnicity. Maternal education was significantly less in cases vs. controls (P<0.001). Both maternal education and gestational age at delivery were included as covariates in the logistic regression analyses.

In the control group, each polymorphism was in Hardy-Weinberg equilibrium, with the exception of IL6 SNP rs1800871 (p<0.001) which was subsequently excluded from analysis.

Significant genotype results ($p < 0.05$), stratified by outcome, are presented in Table 3. For SNPs with evidence of MgSO_4 treatment interaction, results are stratified by outcome and treatment group and presented in Table 4.

Psychomotor Delay Analyses

One hundred and ninety subjects, 95 cases and 95 controls, were analyzed for the psychomotor delay outcome. Four SNPs in the interleukin 6 receptor (IL6R), one SNP in mannose binding lectin 2 (MBL2), and one SNP in plasminogen activator inhibitor 1 (PAI1) were associated with psychomotor delay (Table 3). There was no evidence of MgSO_4 treatment interaction at these loci. The strongest association with psychomotor delay was for a SNP in IL6R (rs 4601580) where each additional copy of the minor allele, T, was associated with an increased risk of psychomotor delay with an odds ratio (OR) of 3.3 (95% confidence interval (CI), 1.7-6.5) using an additive model.

Mental Delay Analyses

Two hundred and twenty-six subjects, 113 cases and 113 controls, were analyzed for the mental delay outcome. Three SNPs in IL6R were associated with mental developmental delay without evidence of MgSO_4 treatment interaction (Table 3). For example, for rs6687726, the minor allele, A, was associated with mental delay with an OR of 2.5 (95%CI, 1.1-5.4) using a dominant genetic model. Additional SNPs in interleukin 6 (IL6), MBL2, and factor VII (F7) showed evidence of MgSO_4 treatment interaction (Table 4). The minor allele at the IL6 locus (rs1554606) was associated with reduced risk of mental delay in the magnesium treatment group (OR 0.3; 95%CI, 0.1-0.7); a genotype association was not observed in the placebo group (OR 1.0; 95%CI, 0.6-1.8).

Cerebral Palsy Analyses

One hundred and twenty-five subjects, 25 cases and 100 controls, were analyzed for CP. One SNP in toll-like receptor 4 (TLR4) was associated with CP with an OR of 5.5 (95%CI, 1.1-26.9) for each copy of the minor allele (Table 3). SNPs in interleukin 1 beta (IL1 β) and PAI1 showed evidence of MgSO_4 treatment interaction. Minor alleles at these loci were associated with increased risk of CP in the placebo group; exposure to MgSO_4 appeared to abrogate these genotype associations.

Death or Cerebral Palsy Analyses

Two SNPs, one in F7 and one in nitric oxide synthase 3 (NOS3) were associated with the combined outcome of death or CP (Table 3). There was no evidence of MgSO_4 treatment interaction for this outcome.

DISCUSSION

We used a candidate gene approach to investigate the role of genetic variation and MgSO_4 in neurodevelopment after preterm birth. In the parent trial, treatment with MgSO_4 in women at imminent risk for delivery between 24 and 31 weeks of gestation resulted in a significant decrease in the risk of moderate to severe CP in surviving children. Our nested case-control analysis suggests that genetic variants within inflammation, coagulation, and vascular

regulation genes may modify neurodevelopmental outcomes after preterm birth. In addition, treatment with MgSO₄ appears to modify these genotype associations at loci within IL6, MBL2, F7, IL1 β , and PAI1 genes. These results may help to identify genetic targets for strategies aimed at improving neurodevelopmental outcomes after preterm birth and may also help us to understand the neuroprotective effect of MgSO₄.

Polymorphisms Related to Inflammation

In this analysis, polymorphisms in the inflammatory cytokine gene IL6 and its receptor (IL6R) were associated with both psychomotor and mental delay. IL6 polymorphisms have previously been associated with impaired cognitive and motor development following preterm birth.^{18,23} Genetic variation within IL6 has also been linked to CP in children born at term and near-term.^{16,24,25} Evidence of MgSO₄ treatment interaction at an IL6 locus (rs1554606) lends further support to the hypothesis that MgSO₄ treatment effect may, directly or indirectly, involve mitigation of cytokine damage. Considerably less is known about genetic variation in IL6R and the impact on neurodevelopmental outcomes. Amniotic fluid levels of IL6 are influenced by both IL6 and IL6R polymorphisms and there is evidence to suggest that the IL6R genotype may be more important.²⁶ Our most significant finding is an increased risk of psychomotor delay associated with carriage of an IL6R polymorphism (rs4601580). The functional consequence of this IL6R polymorphism is unknown. The potential contribution of genetic variation in the IL6R to neurodevelopmental outcomes after preterm birth deserves further attention.

Polymorphisms in mannose binding lectin 2, MBL2, gene that result in reduced levels of circulating MBL were associated with reduced risk of psychomotor delay and showed evidence of magnesium treatment interaction for mental delay in this study. MBL plays a role in innate immunity, including activation of the complement cascade. MBL gene polymorphisms have previously been associated with increased risk of cerebral palsy in children born preterm after perinatal viral exposure²⁷ and with adverse neurological outcomes in a small population of children born at or before 32 weeks gestation.²⁸ These studies suggest that decreased serum MBL levels may be associated with increased risk of adverse neurodevelopmental outcome after preterm birth, possibly due to increased risk of infection-mediated injury. Conversely, adult literature and animal models suggest that loss-of-function MBL gene polymorphisms may be associated with improved outcomes in ischemia reperfusion brain injury (e.g. traumatic brain injury and acute stroke).²⁹⁻³¹ While MBL gene polymorphisms have been associated with neurologic outcomes in adult and pediatric populations, the relative neuroprotective or harmful characteristics of MBL remain to be elucidated. MBL may have both neuroprotective and injurious properties, depending on clinical circumstances.

A polymorphism in the toll-like receptor 4 gene, TLR4, was associated with increased risk of CP in this analysis. TLR4 is an antigen-binding receptor important in the activation of the innate immune system. Several studies have associated the rs4986790 polymorphism with reduced host immune response in the presence of Gram-negative bacterial infections.^{32,33} This polymorphism has been associated reduced risk of CP in a prior analysis of a mixed gestational age cohort.³⁴

A polymorphism in the cytokine interleukin 1 beta, IL1 β , gene showed evidence of magnesium treatment interaction for the outcome of cerebral palsy in this analysis. Previous studies have reported the association between genetic variation in the IL1 β gene with cerebral palsy and adverse neurodevelopmental outcomes.^{23,35}

Polymorphisms Related to Thrombosis or Thrombolysis

Plasminogen activator inhibitor 1, PAI1, gene polymorphisms were associated with reduced risk of psychomotor delay and showed evidence of magnesium treatment interaction for CP in this analysis. Encoded by the SERPINE1 gene, PAI1 is an inhibitor of fibrinolysis. PAI1 polymorphisms have previously been associated with CP in children born preterm.^{13,15} A meta-analysis did not find an association between PAI1 and CP.²⁴

A F7 polymorphism was associated with the combined outcome of cerebral palsy/death and showed evidence of magnesium treatment interaction for the mental delay outcome. Genetic variation in the Factor VII, F7, gene has been associated with CP in a previous study of children born very prematurely.¹⁵

Polymorphisms Related to Vascular Regulation

An endothelial nitric oxide synthase 3, NOS3, gene polymorphism was associated a reduced risk of the combined outcome of CP or death in this analysis. Nitric oxide synthases are a family of enzymes catalyzing the production of nitric oxide, which is an important cellular signaling molecule that modulates vascular tone and is involved in angiogenesis and neural development, among other roles. Polymorphisms in endothelial NOS, eNOS, have been previously associated with CP.¹⁵ Polymorphisms of the inducible isoform, iNOS, which is involved in the immune response, have also been associated with risk of CP in preterm children in previous analyses.^{13,36}

The strengths of this study include the evaluation of a large cohort of preterm children with appropriate controls and well-characterized obstetrical and neurodevelopmental outcomes. However, neurodevelopmental testing at age 2 may be of limited predictive value for longer-term outcomes. Whether the genetic polymorphisms we report have any longer-term neurocognitive associations of clinical significance is uncertain, and cannot be extrapolated from this data. In addition, our results may not be generalizable to other preterm birth populations and our findings need to be validated in other cohorts.

We acknowledge that some of 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, previous studies have demonstrated associations between inflammation, coagulation, and vascular regulation gene variants and fetal/neonatal central nervous system injury. These studies lend support and biologic plausibility to our findings. In order to evaluate the possible effect of multiple comparisons, we applied the Holm-Bonferroni method post hoc, where a p-value of $< 1.3 \times 10^{-3}$ would be considered to be statistically significant. Applying this correction, the SNP in IL6R (rs 4601580) that was associated with an increased risk of psychomotor delay (OR 3.3; 95%CI, 1.7-6.5) remained statistically significant at $p < 0.001$, withstanding the possible effect of multiple comparisons. No other SNPs met this threshold. In this exploratory analysis,

reporting and discussing all associations with a p-value <0.05 is important in order to inform selection of polymorphisms for evaluation and validation in other cohorts.

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, particularly for the CP analysis, may result in true genotype-phenotype associations being missed. Further studies in other populations are needed to confirm or refute the genetic associations described. The sample size and lack of placental pathology also prohibited analysis of the interaction of genotype with maternal/intrauterine infection and neonatal sepsis, gene-environment interactions of obvious interest.

Multiple polymorphisms in IL6 and IL6R were selected based on previously described associations with neurodevelopmental outcomes and involvement in hypothesized causal pathways. The candidate SNPs selected for this analysis do not fully capture the genetic variation within each gene. Formal haplotype analyses of these genes, via inclusion of additional loci, should be the focus of future studies.

The risk of central nervous system injury in preterm children 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, and may also influence the response to MgSO₄ neuroprophylaxis. Ultimately, a better understanding of genetic factors that predispose to these outcomes may lend mechanistic insight and may inform future clinical trials focused on prevention and treatment.

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Figure 1.
Selection of cases and controls from the magnesium sulfate randomized trial.¹

Table 1.**Genetic Variants Included in the Analysis**

Gene	Symbol	Chr:Position	RS Number
C-reactive protein	CRP	1:159682233	rs1205
Interleukin 1 β	IL1 β	2:113594867 2:113595829 2:113598107	rs16944 rs1143623 rs4848306
Interleukin 1 receptor antagonist	IL1RN	2	VNTR *
Interleukin 6	IL6	7:22768707 7:22766645 7:22766246 7:22766221 7:22759655 7:22768572 7:22775663 7:22763009	rs1554606 rs1800795 rs1800796 rs1800797 rs1880243 rs2069840 rs11766273 rs12700386
Interleukin 6 receptor	IL6R	1:154368928 1:154389196 1:154418879 1:154394417 1:154426947 1:154400015 1:154422067 1:154400320 1:154404380	rs952146 rs4075015 rs4537545 rs4601580 rs4845374 rs4845618 rs4845625 rs6687726 rs7549338
Interleukin 10	IL10	1:206946634 1:206946407 1:206946897	rs1800871 rs1800872 rs1800896
Interleukin 13	IL13	5:131995964	rs20541
Mannose binding lectin 2	MBL2	10:54531226 10:54531685 10:54532014	rs1800451 rs7096206 rs11003125
Tumor necrosis factor α	TNFA	6:31543031	rs1800629
Toll-like receptor 1	TLR1	4:38807654	rs5743551
Toll-like receptor 2	TLR2	4:154607126 4:154626317	rs4696480 rs5743708
Toll-like receptor 4	TLR4	9:120475302 9:120475602	rs4986790 rs4986791
Factor II	F2	11:46761055	rs1799963
Factor V	F5	1:169519049	rs6025
Factor VII	F7	13:113773159	rs6046
Plasminogen activator inhibitor 1	PAI1	7:100781445 7:100769706	rs7242 rs1799768
Plasminogen activator inhibitor 2	PAI2	18:61564394 18:61570529	rs6098 rs6104
Methyltetrahydrofolate reductase	MTHFR	1:11854476 1:11856378	rs1801131 rs1801133
Nitric oxide synthase 3	eNOS3	7:150689943 7:150690176	rs1800779 rs3918226

Chr, Chromosome; RS, reference sequence

* Variable number tandem repeat, 86–base pair, intron 2

Table 2.

Maternal and Neonatal Characteristics of Cases and Controls *

Characteristic	CP (n=25)	Controls (n=100)	P	CP/ Death (n=69)	Controls (n=138)	P	Psycho-motor delay (n=95)	Controls (n=95)	P	Mental delay (n=113)	Controls (n=113)	P
Ethnicity	12 (48.0)	48 (48.0)	>0.99	37 (53.6)	74 (53.6)	>0.99	37 (39.0)	37 (39.0)	>0.99	52 (46.0)	52 (46.0)	>0.99
African American	12 (48.0)	48 (48.0)		25 (36.2)	50 (36.2)		41 (43.2)	41 (43.2)		27 (23.9)	27 (23.9)	
Caucasian	1 (4.0)	4 (4.0)		7 (10.1)	14 (10.1)		17 (17.9)	17 (17.9)		34 (30.1)	34 (30.1)	
Hispanic												
Maternal education (y)	12 [11–14]	12 [12–14.5]	0.21	12 [11–13]	12 [12–14]	0.03	12 [10–13]	12 [11–14]	0.04	11 [10–12]	12 [10–14]	0.007
Allocated to magnesium sulfate	13 (52.0)	47 (47.0)	0.65	36 (52.2)	69 (50.0)	0.77	44 (46.3)	45 (47.4)	0.88	53 (46.9)	51 (45.1)	0.79
Chorioamnionitis, clinical	3 (12.0)	15 (15.0)	>0.99	12 (17.4)	16 (11.6)	0.25	12 (12.6)	12 (12.6)	>0.99	14 (12.4)	13 (11.5)	0.84
Gestational age, birth (wk)	27.9 [26.6–29.9]	31.1 [29.1–32.1]	<0.001	27.1 [25.7–29.1]	31.1 [29.1–32.3]	<0.001	29.7 [26.7–31.6]	31.6 [29.6–32.4]	<0.001	30.1 [27.7–31.7]	31.3 [29.7–32.4]	<0.001
Preterm delivery, < 37 weeks	25 (100.0)	99 (99.0)	>0.99	68 (98.6)	135 (97.8)	>0.99	93 (97.9)	91 (95.8)	0.68	109 (96.5)	110 (97.4)	>0.99
Preterm delivery, < 28 weeks	13 (52.0)	11 (11.0)	<0.001	42 (60.9)	16 (11.6)	<0.001	32 (33.7)	12 (12.6)	<0.001	33 (29.2)	8 (7.1)	<0.001
Cesarean delivery	13 (52.0)	35 (35.0)	0.12	34 (49.3)	45 (32.6)	0.02	38 (40.0)	39 (41.1)	0.88	36 (31.9)	43 (38.1)	0.33
Singleton	20 (80.0)	94 (94.0)	0.04	59 (85.5)	132 (95.7)	0.01	86 (90.5)	87 (91.6)	0.80	106 (93.8)	108 (95.6)	0.55
Male gender	18 (72.0)	72 (72.0)	>0.99	44 (63.8)	88 (63.8)	>0.99	60 (63.2)	60 (63.2)	>0.99	76 (67.3)	76 (67.3)	>0.99

CP, cerebral palsy

Data are median [interquartile range] or n (%).

* Combined case number among categories totals greater than 211 since some individuals were analyzed for more than one outcome.

Table 3.
Genotype Frequencies and Odds Ratios for Cases and Controls for Neurodevelopmental Outcomes

Gene	rs	Allele	Cases [*]			Controls [*]			Adjusted OR		Model ^{††}		
			n	MM	Mm	mm	n	MM	Mm	mm		(95% CI) [†]	P [†]
Psychomotor Delay													
IL6R	4601580	T/A	91	23.1	49.5	27.5	86	37.2	45.4	17.4	3.3 (1.7–6.5)	<0.001	Additive
IL6R	7549338	C/G	88	33.0	50.0	17.1	88	48.9	38.6	12.5	3.7 (1.4–9.7)	0.008	Dominant
IL6R	4845625	T/C	85	31.8	55.3	12.9	90	51.1	33.3	15.6	4.4 (1.5–12.4)	0.006	Dominant
IL6R	4845618	G/T	91	19.8	58.2	22.0	89	38.2	46.1	15.7	1.7 (1.0–2.9)	0.04	Additive
MBL2	7096206	G/C	89	74.2	24.7	1.1	89	68.5	25.8	5.6	0.3 (0.1–0.8)	0.02	Additive
PAI1	7242	G/T	89	37.1	43.8	19.1	85	25.9	48.2	25.9	0.4 (0.2–0.8)	0.01	Additive
Mental Delay													
IL6R	4537545	C/T	95	24.2	47.4	28.4	98	36.7	40.8	22.5	2.2 (1.2–3.9)	0.01	Additive
IL6R	6687726	A/G	109	18.4	51.4	30.3	109	33.9	41.3	24.8	2.5 (1.1–5.4)	0.02	Dominant
IL6R	4845625	T/C	98	32.7	50.0	17.4	107	44.9	43.0	12.2	1.7 (1.0–2.9)	0.04	Additive
Cerebral Palsy													
TLR4	4986790	G/A	24	75.0	25.0	0	97	89.7	10.3	0	5.5 (1.1–26.9)	0.03	Additive
Cerebral Palsy or Death													
F7	6046	A/G	56	85.7	14.3	0	115	74.8	22.6	2.6	0.1 (0.03–0.6)	0.006	Additive
NOS3	3918226	T/C	67	97.0	3.0	0	131	90.8	8.4	0.8	0.1 (0.01–0.8)	0.03	Additive

OR, odds ratio; CI, confidence interval; m, minor allele; M, major allele; IL6R, interleukin 6 receptor; MBL2, mannose binding lectin 2; PAI1, plasminogen activator inhibitor 1; TLR4, toll-like receptor 4; F7, factor VII; NOS3, nitric oxide synthase 3

Only tests with $P < 0.05$ after adjustment are shown

^{*} Overall number of cases or controls, and the percent of genotype for each polymorphism.

[†] All regression models adjusted for gestational age at birth, maternal education level, and exposure to magnesium sulfate. Maternal race/ethnicity and child sex were controlled through matching.

^{††} Type of genetic model (additive, dominant, or recessive)

Table 4. Genotype Frequencies and Odds Ratios for Cases and Controls, by Magnesium Sulfate Exposure

		Magnesium Sulfate Exposure										No Magnesium Sulfate Exposure															
Gene	rs	Allele	Cases*			Controls*			Adjusted OR (95% CI) [†]				P [‡]	Cases*			Controls*			Adjusted OR (95% CI) [†]				P [‡]	Hetero-geneity ^{††}	Model [§]	
			n	MM	Mm	mm	n	MM	Mm	mm	n	MM		Mm	mm	n	MM	Mm	mm	n	MM	Mm	mm				
Mental Delay																											
IL6	154606	T/G	50	52.0	42.0	6.0	48	37.5	43.8	18.8	0.3 (0.1–0.7)	0.007	57	47.4	42.1	10.5	59	52.5	35.6	11.9	1.0 (0.6–1.8)	0.97	0.02			Additive	
F7	6046	A/G	41	82.9	17.1	0	44	72.7	22.7	4.6	0.4 (0.1–1.4)	0.15	49	79.6	18.4	2.0	51	82.4	17.7	0	4.1 (0.9–20.0)	0.08	0.03			Additive	
MBL2	1880451	T/C	52	78.9	19.2	1.9	45	66.7	26.7	6.7	0.5 (0.2–1.1)	0.08	58	74.1	24.1	1.7	60	78.3	18.3	3.3	1.7 (0.7–4.4)	0.26	0.04			Additive	
Cerebral Palsy																											
IL1β	1183623	G/C	13	76.9	23.1	0	46	65.2	34.8	0	0.2 (0.03–1.5)	0.12	12	41.7	41.7	16.7	52	76.9	21.2	1.9	5.0 (1.1–22.2)	0.03	0.01			Additive	
PAI1	1789768	A/G	13	53.9	30.8	15.4	46	39.1	39.1	21.7	0.6 (0.2–1.5)	0.26	11	9.1	36.4	54.6	53	47.2	35.9	17.0	3.5 (1.1–11.0)	0.03	0.02			Additive	

OR, odds ratio; CI, confidence interval; m, minor allele; M, major allele; IL6, interleukin 6; F7, factor VII; MBL2, mannose binding lectin 2; IL1β, Interleukin 1β; PAI1, plasminogen activator inhibitor 1

Only tests with P for heterogeneity < 0.05 after adjustment are shown

* Overall number of cases or controls, and the percent of genotype for each polymorphism.

[†] All regression models adjusted for gestational age at birth and maternal education level. Maternal race/ethnicity and child sex were controlled through matching.

^{††} Tests the heterogeneity of effect by exposure to magnesium sulfate.

[§] Type of genetic model (additive, dominant, or recessive)