Apolipoprotein E genotype and neurodevelopmental sequelae of infant cardiac surgery

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Background: There has been increasing recognition of adverse neurodevelopmental sequelae in some children after repair of congenital heart defects. Even among children with the same cardiac defect, significant interindividual variation exists in developmental outcome. Polymorphisms of apolipoprotein E have been identified as a risk factor for worse neurologic recovery after central nervous system injury.

Methods: A single-institution prospective study of patients ≤ 6 months of age undergoing cardiopulmonary bypass for repair of congenital heart defects was undertaken to evaluate the association between apolipoprotein E genotype and postoperative neurodevelopmental dysfunction. Developmental outcomes were evaluated at 1 year of age by using the Bayley Scales of Infant Development.

Results: One-year evaluation was performed in 244 patients. After adjustment for preoperative and postoperative covariates—including gestational age, age at operation, sex, race, socioeconomic status, cardiac defect, and use of deep hypothermic circulatory arrest—the apolipoprotein E $\epsilon 2$ allele was associated with a worse neurologic outcome as assessed by the Psychomotor Developmental Index of the Bayley Scales of Infant Development ($P = .036$). Patients with the apolipoprotein E $\epsilon 2$ allele had approximately a 7-point decrease in the Psychomotor Developmental Index.

Conclusions: Apolipoprotein E $\epsilon 2$ allele carriers had significantly lower Psychomotor Development Index scores at 1 year of age after infant cardiac surgery. The effect was independent of ethnicity, socioeconomic status, cardiac defect, and use of deep hypothermic circulatory arrest. An effect of the apolipoprotein E $\epsilon 4$ allele was not detected. Genetic polymorphisms that decrease neuroresiliency and impair neuronal repair after central nervous system injury are important risk factors for neurodevelopmental dysfunction after infant cardiac surgery.

A dramatic reduction in mortality after surgical correction of congenital heart defects (CHD) in recent years has been accompanied by increasing recognition of adverse neurodevelopmental sequelae in some survivors.¹⁻³ Evaluation of children after neonatal repair of CHD demonstrates a pattern of neurodevelopmental sequelae characterized by cognitive impairment, speech and language abnormalities, impaired visuospatial and visuomotor skills, attention deficit hyperactivity disorder, motor delays, and learning disabilities.⁴⁻⁶ Cerebral ischemia in the perioperative period has been proposed to be a primary mechanism of central nervous system (CNS) injury. Management strategies during the operation—including type of support (deep hypothermic circulatory arrest [DHCA] or continuous cardiopulmonary bypass [CPB]), hemodilution, degree of cooling, and blood gas management—have been implicated as factors in postoperative neurodevelopmental dysfunction.¹⁻⁸ These potential risk factors do not fully explain the frequency or pattern of neurodevelopmental dysfunction. There is significant interindividual variation in developmental outcome, even among children with the same cardiac defect, suggesting that other patient-specific factors may be important determinants of CNS injury.

The role of genetic polymorphisms that increase susceptibility to neurologic injury has not been explored in children with CHD. Apolipoprotein E (APOE) is an important regulator of cholesterol metabolism.⁹⁻¹² APOE-containing lipoproteins are the primary lipid transport vehicles in the CNS. There is increasing evidence that APOE is important for neuronal repair.^{9,11-15} There are 3 common isoforms of APOE (E2, E3, and E4), which are encoded by 3 alleles (ϵ 2, ϵ 3, and ϵ 4, respectively) and vary by single amino acid substitutions. A strong association has been validated between the APOE ϵ 4 allele and Alzheimer's disease.¹⁰ The APOE genotype has been shown to have an important role as a determinant of neurologic recovery after CNS ischemia, intracerebral hemorrhage, and traumatic brain injury.¹³⁻¹⁷ There is evidence of an association of APOE genotype with neurocognitive decline after cardiac surgery in adults.¹⁸⁻²⁰ We evaluated the association between postoperative neurologic dysfunction and APOE genotype in infants undergoing cardiac surgery.

Methods

Patient Population

The Institutional Review Board at The Children's Hospital of Philadelphia approved the study. Patients 6 months of age or less undergoing CPB, with or without DHCA, for repair of CHD were eligible. Exclusion criteria included (1) multiple congenital anomalies, (2) recognizable genetic or phenotypic syndrome other than chromosome 22q11 microdeletions, and (3) language other than English spoken in the home.

Informed consent was obtained from the parent or guardian. Operations were performed by 4 cardiac surgeons with a dedicated team of cardiac anesthesiologists. Alpha-stat blood gas management was used. DHCA was used at the surgeon's discretion. The mean hematocrit after institution of CPB and hemodilution was $26\% \pm 3.5\%$. Before DHCA, patients underwent core cooling with topical hypothermia to a nasopharyngeal temperature of 18°C . Modified ultrafiltration was performed in all patients.

APOE Genotype Determination

Whole blood was obtained before the operation and stored at 4°C . Genomic DNA was prepared and used to determine APOE genotypes by using a previously published method.^{18,21}

One-Year Neurodevelopmental Examination

Children were evaluated at 12 months of age, corrected for prematurity, plus or minus 2 weeks. All study personnel were blinded to the genotype. Socioeconomic status (SES) and ethnicity were determined by parental reporting. Developmental assessment included the Bayley Scales of Infant Development, which yielded 2 scores: the Psychomotor Development Index (PDI) and the Mental Developmental Index (MDI). The MDI assesses memory, problem solving, early number concepts, generalization, vocalizations, and language and social skills. The PDI assesses control of gross muscle function, including crawling and walking, as well as fine muscle skills necessary for prehension, use of writing instruments, and imitation of hand movements. Both the MDI and PDI are normalized to a mean of 100 with an SD of 15. They yield an accurate assessment of the child's developmental status at 1 year but are not predictors of later outcome, such as IQ in adolescence. The evaluation also included medical history, growth measurements, and detailed neurologic examination assessing active and passive tone, reflexes, gross motor skills, and visual and auditory responses. Patients were evaluated by a genetic dysmorphologist. Because recognition of genetic syndromes in neonates may be difficult, some children with genetic abnormalities had been enrolled as infants. Chromosome analysis and testing for microdeletions of chromosome 22q11 were performed as indicated.

Data Analysis

Patients were grouped according to cardiac diagnosis: group 1 consisted of patients with hypoplastic left heart syndrome (HLHS); group 2 consisted of patients with conotruncal defects (tetralogy of Fallot, transposition of the great arteries, truncus arteriosus, and so on); and all other defects were coded as group 3. Patients were additionally coded according to a previously described classification incorporating anatomy and perioperative physiology that has been shown to be predictive of perioperative mortality.²² Class I is 2 ventricles with no aortic arch obstruction, class II is 2 ventricles with aortic arch obstruction, class III is single ventricle with no arch obstruction, and class IV is single ventricle with arch obstruction. Patients with tetralogy of Fallot and transposition of the great arteries are in class I, whereas patients with HLHS or variants are in class IV. Results of the genetic evaluations were classified as *normal* if no genetic or chromosomal abnormality was demonstrated, *abnormal* if a specific diagnosis was confirmed, and *suspect* if there was evidence of a genetic syndrome that could not be confirmed. Results of the neurologic examinations were classified

as *normal* if no abnormalities or only mild abnormalities that did not affect motor skills were found, *suspect* when there was a moderate degree of abnormality (ie, moderate hypotonia with generally normal reflexes and mildly abnormal motor skills), and *abnormal* whenever significant abnormalities of tone, reflexes, or motor skills were present.

Statistical Analysis

The primary outcome was the PDI, chosen on the basis of the Boston Circulatory Arrest Study finding that use of DHCA was associated with a significant decrease in the PDI.² Secondary measures of neurologic outcome included the MDI, head circumference at 1 year, and neuromuscular examination. The primary predictor of interest was *APOE* genotype. Subjects were grouped into the $\epsilon 2$ group ($\epsilon 2\epsilon 2$ and $\epsilon 3\epsilon 2$), the $\epsilon 3\epsilon 3$ group, and the $\epsilon 4$ group ($\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$). There were no $\epsilon 2\epsilon 2$ patients in the study population. Five $\epsilon 2\epsilon 4$ subjects were excluded from the analyses because the effects of the $\epsilon 2$ and $\epsilon 4$ alleles are opposing in Alzheimer's disease. Twelve subjects with a missing *APOE* genotype were also excluded. Because PDI was selected as the primary outcome, we did not make multiple comparison adjustments for analyses of the additional correlated outcomes. Baseline evaluation of *APOE* genotype effects used analysis of variance with 2 degrees of freedom (*df*), and further investigation of the *APOE* effects after covariate adjustment focused on separate comparisons of the $\epsilon 2$ group versus the $\epsilon 3\epsilon 3$ group and the $\epsilon 4$ group versus the $\epsilon 3\epsilon 3$ group.

Linear regression was used to characterize the crude and adjusted association between the 1-year outcomes PDI, MDI, and head circumference. Logistic regression was used for the categorical neuromuscular examination outcome. Suspect and abnormal neuromuscular examination groups were pooled to form a "not normal" group and thus derive a binary response variable. Three regressions were performed for each outcome. First, the marginal association between genotype and outcome was considered without adjustment for any covariates. Next, the preoperative adjusted regressions included preoperative characteristics of race (white, black, or other), sex, SES, gestational age, birth weight, head circumference percentiles, diagnostic group, and suspected or definite genetic disorder. Apgar scores were not significantly associated with outcome, and estimates of genotype effect did not substantially change with or without adjustment for Apgar score. Because 11 children were missing Apgar data, Apgar scores were dropped from the regression model rather than imputing the scores or dropping those cases. Missing percentiles of birth weight ($n = 2$) and head circumferences at birth ($n = 24$) were imputed by using the percentile at the 1-year follow-up examination. Subjects missing birth head circumference measurements were dropped from the analysis of 1-year head circumference. Finally, the preoperative and postoperative adjusted regression included these variables plus variables related to the cardiac repair. Operative variables included class (I-IV), operation, age and weight at operation, hematocrit on CPB, total support time (duration of CPB plus DHCA), number of bypass runs, and use of DHCA. The relationship between *APOE* genotype group and all covariates was explored by analysis of variance for quantitative traits and by χ^2 analysis for categorical traits.

Interaction between genotype and major preoperative or postoperative covariates was tested by including interaction terms in the preoperative and postoperative adjusted regression analysis. Because the power to detect genotype by covariate interactions was expected to be low, fitted genotype effects for patient subsets were examined to determine whether the same general trends were manifested. Specifically, the signs of coefficients for the *APOE* effects were examined in subgroups on the basis of presence of a suspected or definite genetic disorder, males versus females, Caucasian children, SES, diagnostic group, support time, and use of DHCA.

Results

Between October 1, 1998, and September 20, 2001, 467 eligible patients underwent cardiac surgery (Table 1). Twenty patients died before enrollment. A total of 368 patients (82%) were enrolled. Thirteen patients (3.5%) died during the initial hospitalization, and 15 additional patients (4.1%) died after hospital discharge before 1 year of age. There were 340 potential patients for the 1-year evaluation, and 244 (72%) returned. The study population consisted of 244 patients in whom the 1-year evaluation was completed by September 20, 2002. Two additional premature infants born before September 20, 2001, underwent evaluation in October 2002 and are not included in the study population. There was a trend toward a lower return rate among black infants and those with more complex heart defects (class IV). However, the primary reason for not returning was geographic location, because no travel funds were available. There was no difference in *APOE* genotype between returning and nonreturning patients.

On the Bayley scales, the mean PDI score was 77.0 ± 18.0 , and the mean MDI score was 88.6 ± 15.5 . The MDI was ≤ 70 in 28 patients (11%), and the PDI was ≤ 70 in 80 patients (33%). The neuromuscular examination had abnormal or suspect results in 96 patients (39%), with hypotonia in 85; hypertonia in 5; mixed tonal abnormalities in 3; and asymmetry, spastic quadriplegia, and hyperreflexia in 1 each. Head circumference at 1 year was at or below the fifth percentile in 60 patients (25%). Genetic examination results were normal in 175 patients, a definite genetic abnormality was present in 29 (12%; including 13 with 22q11 microdeletions), and 40 patients were classified as having suspect results.

No demographic or preoperative characteristics were significantly related to *APOE* genotype (Table 2). Although the test for differences of *APOE* distribution between male and female infants was not significant ($P = .130$), there was a trend for a deficit of $\epsilon 4$ -containing genotypes in males. Allele frequencies in Caucasian males in this sample were $\epsilon 2$, 0.081; $\epsilon 3$, 0.812; and $\epsilon 4$, 0.108; for Caucasian females, they were $\epsilon 2$, 0.075; $\epsilon 3$, 0.769; and $\epsilon 4$, 0.157. Allele frequencies in Caucasian subjects are expected to be 0.085 for $\epsilon 2$, 0.768 for $\epsilon 3$, and 0.147 for $\epsilon 4$. The *APOE* genotype

TABLE 1. Characteristics of the patient population

Variable	Returned (n = 244)*		Did not return (n = 94)		Hospital death (n = 13)		Late death (n = 15)	
	No.	%	No.	%	No.	%	No.	%
Race								
Asian/Pacific Islander, Hispanic, Native American, other	42	17	21	22	4	31	1	7
Black, not Hispanic	31	13	29	31	3	23	3	20
White, not hispanic	171	70	44	47	6	46	11	73
Sex								
Female	145	59	58	62	8	62	8	53
Male	99	41	36	38	5	38	7	47
APOE genotype								
ε2ε2	0		2	2.1	0		0	
ε2ε3	28	11.5	11	11.7	2	15.4	1	6.7
ε2ε4	5	2.0	4	4.3	1	7.6	0	
ε3ε3	147	60.2	53	56.4	6	46.2	8	53.3
ε3ε4	48	19.7	16	17.0	2	15.4	4	46.7
ε4ε4	4	1.7	1	1.1	0		0	
Not done	12	4.9	7	7.4	2	15.4	2	13.3
Gestational age (wk)								
Mean ± SD	38.5 ± 2.1		38.5 ± 1.8		36.8 ± 2.6		37.1 ± 4.4	
n	242		80		9		14	
Birth weight (g)								
Mean ± SD	3172 ± 634		3124 ± 573		2752 ± 459		2680 ± 793	
n	243		83		11		14	
Birth head circumference (%)								
Mean ± SD	43 ± 31		38 ± 29		37 ± 31		42 ± 36	
n	231		77		7		14	
Apgar at 1 min								
Mean ± SD	7.8 ± 1.6		7.9 ± 1.3		7.0 ± 1.7		7.51 ± 1.5	
n	233		81		10		14	
Apgar at 5 min								
Mean ± SD	8.6 ± 0.9		8.8 ± 0.6		8.4 ± 1.0		8.4 ± 0.8	
n	233		81		10		14	
Diagnostic group								
HLHS	35	14.3	18	19.1	6	46.2	7	46.6
Conotruncal defects	135	55.3	54	57.4	2	15.4	4	26.7
Other defects	74	30.4	22	23.5	5	35.4	4	26.7
Operative class								
Two ventricles: no arch obstruction	143	58.6	43	45.7	3	23.1	3	20.0
Two ventricles: arch obstruction	21	8.6	14	14.9	1	7.7	1	6.7
Single ventricle: no arch obstruction	29	11.9	5	5.3	1	7.7	3	20.0
Single ventricle: arch obstruction	51	20.9	32	34.1	8	61.5	8	53.3
Age at first operation (d)								
Mean ± SD	43 ± 5.4		53 ± 6.3		29 ± 4.7		39 ± 5.9	
Median (range)	7 (1-186)		14 (1-188)		6 (1-126)		6 (1-178)	
Weight at first operation, kg (mean ± SD)	3.96 ± 1.32		3.93 ± 1.26		2.99 ± 0.93		3.35 ± 1.02	

*Two premature infants born before September 20, 2001, were scheduled for evaluation in October 2002.

distribution did not significantly differ from Hardy-Weinberg proportions.²¹ APOE genotype frequencies are expected to vary by race.²²⁻²⁵ Although this variation was not statistically significant, the expected deficit of ε4 alleles in Asians was seen. The distribution of male and female subjects did not differ significantly by race ($P = .17$; data not shown). Operative variables for the first operation and for the entire first year of life are shown in Tables 3 and 4,

respectively. None was related to APOE genotype (all $P > .2$).

The neurologic outcomes, stratified by APOE genotype, are shown in Table 5 and are shown for the Caucasian subgroup in Table 6. All other analyses use all races. Regression analyses unadjusted for any covariates showed a significant APOE effect for PDI ($P = .016$; 2 *df*) and neuromuscular examination ($P = .032$; 2 *df*; Figure 1). In

TABLE 2. Preoperative variables stratified by *APOE* genotype

Variable	Missing <i>APOE</i> and $\epsilon 2\epsilon 4$		$\epsilon 2\epsilon 2$, $\epsilon 2\epsilon 3$		$\epsilon 3\epsilon 3$		$\epsilon 3\epsilon 4$, $\epsilon 4\epsilon 4$		All		<i>P</i> value*
	No.	%	No.	%	No.	%	No.	%	No.	%	
Race											
Asian/Pacific Islander, Hispanic, Native American, other	2	11.7	3	10.7	31	21.1	7	13.5	43	17.6	.308
Black, not hispanic	5	29.4	3	10.7	16	10.9	10	19.2	34	13.9	
White, not hispanic	10	58.9	22	78.6	100	68.0	35	67.3	167	68.5	
Sex											
Female	8	47.1	10	35.7	57	38.8	28	53.8	103	42.2	.130
Male	9	52.9	18	64.3	90	61.2	24	46.2	141	57.8	
SES											
Groups 1 through 3	4	23.5	13	46.4	41	28.1	19	36.5	77	31.7	.124
Groups 4 and 5	13	76.5	15	53.6	105	71.9	33	63.5	166	68.3	
Genetic exclusion											
Normal	10	58.9	19	67.9	110	74.8	36	69.2	175	71.7	.759
Abnormal	2	11.7	5	17.9	16	10.9	6	11.6	29	11.9	
Unknown	5	29.4	4	14.2	21	14.3	10	19.2	40	16.4	
Gestational age (wk)											
Mean $\pm$ SD	38.3 $\pm$ 2.3		38.5 $\pm$ 2.8		38.4 $\pm$ 2.1		39.1 $\pm$ 1.8		38.6* $\pm$ 2.1		.151
n	17		28		147		52		244		
Birth weight (%)											
Mean $\pm$ SD	48.8 $\pm$ 31.0		55.5 $\pm$ 31.9		49.2 $\pm$ 29.5		59.4 $\pm$ 29.4		52.*3 $\pm$ 30.0		.091
n	17		28		147		51		243		
Birth head circumference (%)											
Mean $\pm$ SD	45.7 $\pm$ 30.0		43.1 $\pm$ 34.9		41.6 $\pm$ 30.9		43.6 $\pm$ 29.7		42.2* $\pm$ 31.0		.920
n	15		26		141		49		231		
Apgar at 1 min											
Mean $\pm$ SD	8.2 $\pm$ 0.7		7.5 $\pm$ 1.6		7.7 $\pm$ 1.7		7.8 $\pm$ 1.7		7.7* $\pm$ 1.6		.665
n	17		27		140		49		233		
Apgar at 5 min											
Mean $\pm$ SD	8.9 $\pm$ 0.3		8.4 $\pm$ 1.0		8.7 $\pm$ 1.0		8.7 $\pm$ 0.7		8.6* $\pm$ 0.9		.319
n	17		27		140		49		233		

*Does not include $\epsilon 2\epsilon 4$ and missing genotypes.

each case, infants in the *APOE* $\epsilon 2$ group performed less well than those with the reference $\epsilon 3\epsilon 3$ genotype. Comparing the $\epsilon 2$ group with the other groups pooled gave *P* values of .006, .041, .041, and .27 for PDI, MDI, neuromuscular examination, and head circumference, respectively (Table 7).

The *APOE* $\epsilon 2$ effect on PDI remained significant after adjusting for the preoperative (*P* = .023) or preoperative and postoperative (*P* = .036) covariates (Table 7). After adjustment for covariates, the *APOE* $\epsilon 2$ group had an approximately 7-point decrease in PDI score. The smaller $\epsilon 2$ effect on MDI was not significant after preoperative (*P* = .09) or preoperative plus postoperative (*P* = .13) covariate adjustment, although the trend of poorer outcome among the $\epsilon 2$ subjects persisted. The effect of *APOE* $\epsilon 2$ on neuromuscular examination was no longer significant after preoperative (*P* = .18) or preoperative plus postoperative (*P* = .26) covariate adjustment. Head circumference at 1 year did not have a significant *APOE* genotype effect with or without covariate adjustment.

An *APOE* $\epsilon 4$ effect was not seen for any outcome. The regression coefficients for the covariate-adjusted regression were near 0 for PDI and MDI. After covariate adjustment, nonsignificantly negative coefficients were seen for the $\epsilon 4$ effect on PDI, MDI, and head circumference.

There was no statistically significant interaction between the pooled *APOE* $\epsilon 2$ group and presence of a genetic syndrome, sex, race, SES, diagnostic group, operative class, support time, or use of DHCA in the analysis of PDI or MDI. Power to detect interactions was expected to be low in this sample size; therefore, the estimated *APOE* coefficients specific to covariate strata were examined for consistency. For prediction of PDI, the coefficients of the pooled group compared with the $\epsilon 3\epsilon 3$ reference group were negative for all strata evaluated, except for diagnostic group 1 (HLHS; 1.2; SD, 7.3) and Hispanic race (13.1; SD, 17.0). The effect of the $\epsilon 2$ group on PDI was -4.1 (SD, 3.9) in subjects with normal genetic evaluation results and -6.9 (SD, 8.0) in subjects who were thought to have a genetic disorder. The $\epsilon 2$ coefficient for PDI was -1.7 (SD, 5.3) in females and

TABLE 3. Perioperative variables stratified by *APOE* genotype: first operation

Variable	Missing <i>APOE</i> and $\epsilon 2\epsilon 4$		$\epsilon 2\epsilon 2$, $\epsilon 2\epsilon 3$		$\epsilon 3\epsilon 3$		$\epsilon 3\epsilon 4$, $\epsilon 4\epsilon 4$		All		<i>P</i> value*
	No.	%	No.	%	No.	%	No.	%	No.	%	
Operative class											
Two ventricles: no arch obstruction	12	70.6	14	50.0	88	59.9	29	55.8	143	58.6	.554
Two ventricles: arch obstruction	2	11.8	2	7.1	10	6.8	7	13.4	21	8.6	
Single ventricle: no arch obstruction			5	17.9	16	10.9	8	15.4	29	11.9	
Single ventricle: arch obstruction	3	17.6	7	25.0	33	22.4	8	15.4	51	20.9	
Diagnostic group											
HLHS	2	11.8	6	21.4	22	15.0	5	9.6	35	14.3	
Conotruncal defects	10	58.8	14	50.0	80	54.4	31	59.6	135	55.3	
Other defects	5	29.4	8	28.6	45	30.6	16	30.8	74	30.4	.704
Surgeon											
1	5	29.4	3	10.7	36	24.5	12	23.1	56	23.0	.488
2	10	58.8	16	57.2	75	51.0	22	42.3	123	50.4	
3	1	5.9	3	10.7	15	10.2	9	17.3	28	11.5	
4	1	5.9	6	21.4	21	14.3	9	17.3	37	15.1	
Age at surgery (d)											
Mean $\pm$ SD		39 $\pm$ 50		49 $\pm$ 58		42 $\pm$ 55		44 $\pm$ 52		43* $\pm$ 54	.852
n		17		28		147		52		244	
Weight (kg)											
Mean $\pm$ SD		3.63 $\pm$ 1.09		3.77 $\pm$ 1.37		3.98 $\pm$ 1.32		4.10 $\pm$ 1.36		3.98* $\pm$ 1.33	.584
n		17		28		147		52		244	
Hematocrit on CPB											
Mean $\pm$ SD		27 $\pm$ 4		26 $\pm$ 3		26 $\pm$ 3		26 $\pm$ 4		26* $\pm$ 3	.625
n		17		28		147		52		244	
Total support time (min)											
Mean $\pm$ SD		90 $\pm$ 44		89 $\pm$ 47		92 $\pm$ 41		99 $\pm$ 47		93* $\pm$ 43	.467
n		17		28		147		52		244	
Total CPB time (min)											
Mean $\pm$ SD		66 $\pm$ 44		65 $\pm$ 40		70 $\pm$ 44		81 $\pm$ 47		72* $\pm$ 44	.217
n		17		28		147		52		244	
Total DHCA time (min)											
Mean $\pm$ SD		24 $\pm$ 19		24 $\pm$ 26		22 $\pm$ 23		19 $\pm$ 22		21* $\pm$ 23	.580
n		17		28		147		52		244	

*Does not include $\epsilon 2\epsilon 4$ and missing genotypes.

−10.4 (SD, 4.2) in males. The $\epsilon 2$ coefficient for MDI analysis was negative in all strata except diagnostic group 1 (HLHS; 3.2; SD, 6.6) and class IV patients (1.7; SD, 6.0).

For the analysis of PDI and MDI, there was no significant interaction between the *APOE* $\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$ genotype groups and presence of a genetic syndrome, sex, race, SES, diagnostic group, operative class, or DHCA. For neuromuscular examination, the $\epsilon 2$ group generally had worse examination results across the strata evaluated. A marginally significant *APOE* genotype $\times$ sex interaction ($P = .0581$) was detected in the analysis of neurologic examination. Only the $\epsilon 2$ group males had worse examination results than the $\epsilon 3\epsilon 3$ genotype group (−1.8; SD, 0.8); the $\epsilon 2$ group coefficient for females was 1.0 (SD, 0.9).

For the prediction of head circumference, a marginal *APOE* genotype $\times$ genetic diagnosis interaction was de-

tected ($P = .0503$). The coefficient for the $\epsilon 2$ group without evidence of a genetic disorder was −7.4 (7.2), whereas that for the $\epsilon 2$ group with evidence of a genetic disorder was 20.0 (SD, 14.3). The $\epsilon 4$ coefficients without and with evidence of a genetic disorder were 0.05 (SD, 5.5) and 22.9 (SD, 13.6), respectively.

Discussion

There is a significant effect of the *APOE* $\epsilon 2$ allele to predict lower PDI at 1 year of age after infant cardiac surgery; this is independent of race, SES, cardiac defect, and use of DHCA. This finding is consistent with the hypothesis that *APOE* genotypes other than the common $\epsilon 3\epsilon 3$ predict decreased neuroresiliency after CNS injury. An *APOE* $\epsilon 4$ effect is not detectable.

TABLE 4. Operative data: first year of life

Variable	Missing APOE and ε2ε4		ε2ε2, ε2ε3		ε3ε3		ε3ε4, ε4ε4		All		P value*
	No.	%	No.	%	No.	%	No.	%	No.	%	
Total number of separate operations with CPB											
1	15	88.2	18	64.3	100	68.0	39	75.0	172	70.5	.700
2	2	11.8	10	35.7	42	28.6	13	25.0	67	27.5	
3					4	2.7			4	1.6	
4					1	0.7			1	0.4	
Total number of episodes of CPB											
1	12	70.6	17	60.7	95	64.6	37	71.2	161	66.0	.377
2	4	23.5	10	35.7	43	29.3	9	17.3	66	27.0	
3	1	5.9			5	3.4	4	7.7	10	4.1	
4			1	3.6	4	2.7	2	3.8	7	2.9	
Cumulative duration of DHCA (min)											
Mean ± SD	27 ± 23		31 ± 33		28 ± 33		22 ± 26		27* ± 31		.366
n	17		28		147		52		244		
Single longest episode of DHCA (min)											
Mean ± SD	23 ± 18		23 ± 23		22 ± 23		19 ± 21		21* ± 23		.646
n	17		28		147		52		244		
Cumulative duration of CPB (min)											
Mean ± SD	71 ± 44		81 ± 44		92 ± 62		100 ± 59		93* ± 59		.383
n	17		28		147		52		244		

*Does not include ε2ε4 and missing genotypes.

TABLE 5. Neurodevelopmental outcomes at 1 year stratified by APOE genotype

Variable	ε2ε2, ε2ε3		ε3ε3		ε3ε4, ε4ε4		All		P value*
	No.	%	No.	%	No.	%	No.	%	
Psychomotor Developmental Index									
Mean ± SD	67.89 ± 18.28		77.86 ± 17.74		78.17 ± 15.04		76.70* ± 17.47		.016
n	28		147		52		244		
Mental Developmental Index									
Mean ± SD	83.32 ± 17.43		89.65 ± 14.66		89.58 ± 14.09		88.85* ± 14.97		.144
n	28		147		52		244		
Results of neuromuscular examination									
Normal	11	39.3	89	60.5	36	69.2	148	60.7	.032
Abnormal or suspect	17	60.7	58	39.5	16	30.8	96	39.3	
Head circumference at testing (%)									
Mean ± SD	29.93 ± 26.63		36.81 ± 30.83		38.80 ± 28.69		36.41* ± 29.85		.434
n	28		146		52		243		

*Does not include ε2ε4 and missing genotypes.

The gene for the human *APOE* is located on the long arm of chromosome 19 and codes for a 299–amino acid protein.¹¹ The most common allele in humans is ε3, and there are 2 other common alleles, ε2 and ε4, whose protein products differ from that of ε3 by single amino acid substitutions. There are 3 homozygous genotypes (ε2ε2, ε3ε3, and ε4ε4) and 3 heterozygous genotypes (ε2ε3, ε2ε4, and ε3ε4). Allele frequency varies according to ethnic background.^{21–27}

The APOE protein is the receptor ligand for low-density lipoprotein cholesterol. APOE-containing lipoproteins are the primary lipid transport vehicles in the CNS. APOE

modulates lipoprotein levels by effects on clearance rate, lipolytic conversion, and very-low-density lipoprotein (VLDL) triglyceride production.¹¹ In addition, APOE has an important role in mobilization and redistribution of cholesterol and phospholipids during remodeling of neuronal membranes. The normal function of APOE within neurons is thought to be maintenance of microtubular integrity and stabilization of the neuronal cytoskeleton.¹¹ APOE synthesis is upregulated by astrocytes and oligodendrocytes after CNS injury or cerebral ischemia. After an injury, APOE immunoreactivity increases first in astrocytes and later in neurons. The temporal changes in localization and levels of

Figure 1. Mental Developmental Index (MDI) and Psychomotor Development Index (PDI) stratified by apolipoprotein E (APOE) genotype. **P* = .006 versus other groups pooled, unadjusted for covariates. #*P* = .041 versus other groups pooled, unadjusted for covariates. The dashed line represents the expected population mean.

TABLE 6. Outcomes for the white subgroup

Variable	ε2ε2, ε2ε3		ε3ε3		ε3ε4, ε4ε4		All		<i>P</i> value*
	No.	%	No.	%	No.	%	No.	%	
Psychomotor Developmental Index									
Mean ± SD	65.91 ± 19.13		75.31 ± 16.89		75.89 ± 14.21		74.12* ± 16.89		.047
n	22		100		35		167		
Mental Developmental Index									
Mean ± SD	82.45 ± 18.96		90.31 ± 14.75		88.91 ± 15.85		88.90* ± 15.76		.149
n	22		100		35		167		
Results of neuromuscular examination									
Normal	7	31.8	55	55.0	22	62.9	91	54.5	.065
Not normal	15	68.2	45	45.0	13	37.1	76	45.5	
Head circumference at testing (%)									
Mean ± SD	27.89 ± 26.68		40.54 ± 32.11		38.80 ± 28.43		38.37* ± 30.73		.217
n	22		99		35		166		

*Does not include ε2ε4 and missing genotypes.

APOE are consistent with an upregulation of APOE by astrocytes in response to injury, release into the extracellular space, and uptake by neurons. APOE immunoreactivity after global ischemia is localized to vulnerable regions such as the caudate and CA1 region of the hippocampus. Studies in *APOE* knockout mice have shown that APOE deficiency worsens neuronal injury after cerebral ischemia and compromises the blood-brain barrier after CNS injury.^{9,11,28} Mechanisms by which APOE may modify CNS injury include protection against oxidative stress, modulation of the glial response to inflammation, and a direct neurotropic effect on injured neurons.^{7,9,29,30}

Infants undergoing cardiac surgery are at risk for CNS injury and adverse neurodevelopmental sequelae that significantly diminish the quality of life for these children and their parents.¹⁻⁶ The need for early intervention, special

education, and rehabilitative services results in considerable cost to society. Significant interindividual variation in outcome exists that cannot be completely explained by previously reported risk factors.^{3,5,6} Recent studies suggest that *APOE* ε4 may be a risk factor for neurocognitive decline after heart surgery in adults; however, the evidence is not conclusive.¹⁸⁻²⁰ The mechanisms of CNS injury during infant cardiac surgery are poorly understood and are likely different from those in adults.⁷ Children with CHD often have congenital CNS anomalies; in addition, many are hypoxic before and after surgery. Use of DHCA results in a period of global cerebral ischemia. Mechanisms of CNS injury in infants undergoing cardiac surgery include hypoxia/ischemia, emboli, reactive oxygen species, and inflammatory microvasculopathy.⁷ *APOE* genotype has been shown to modify the inflammatory

TABLE 7. Logistic regression coefficients for *APOE* genotype group effects

Variable	<i>APOE</i>	Unadjusted	Preoperative adjusted regression	Preoperative and postoperative adjusted regression
PDI	ε2	−9.971 (3.553), <i>P</i> = .006*	−7.221 (3.150), <i>P</i> = .023*	−6.979 (3.305), <i>P</i> = .036*
	ε4	0.309 (2.780), <i>P</i> = .91	−0.569 (2.743), <i>P</i> = .82	−0.362 (2.601), <i>P</i> = .89
MDI	ε2	−6.325 (3.071), <i>P</i> = .041*	−4.893 (2.866), <i>P</i> = .089	−4.661 (3.047), <i>P</i> = .128
	ε4	−0.069 (2.403), <i>P</i> = .98	−0.899 (2.300), <i>P</i> = .70	−1.094 (2.403), <i>P</i> = .65
Head circumference	ε2	−6.880 (6.162), <i>P</i> = .27	−8.206 (5.880), <i>P</i> = .16	−6.580 (6.215), <i>P</i> = .29
	ε4	1.990 (4.823), <i>P</i> = .68	−0.951 (4.986), <i>P</i> = .84	−1.086 (4.890), <i>P</i> = .83
Neuromuscular examination result†	ε2	−0.864 (0.422), <i>P</i> = .041	−0.675 (0.508), <i>P</i> = .184	−0.605 (0.540), <i>P</i> = .26
	ε4	0.382 (0.343), <i>P</i> = .26	0.580 (0.428), <i>P</i> = .176	0.722 (0.455), <i>P</i> = .113

The coefficients are relative to the common ε3ε3 genotype group. The SDs are given in parentheses.

**P* < .05.

†(0, Suspect or abnormal; 1, normal; coefficients are the log odds ratios).

response to CPB and is a risk factor for postoperative renal dysfunction.^{29,31,32}

Previous studies have implicated *APOE* ε4 as a risk factor for recovery after brain injury and for Alzheimer's disease.⁹⁻¹³ Few studies have focused on the ε2 allele; most classified patients as ε4 positive or ε4 negative and did not assess the ε2 allele because of its rarity. *APOE* ε2 has been shown to adversely influence recovery after ischemic stroke and cardiac arrest and to increase the risk of intracerebral hemorrhage.^{12,14-17} The frequency of all 3 *APOE* alleles suggests some balancing selection; certain alleles are better under some conditions, and other alleles are better under other conditions.

Although this study provides strong evidence of an adverse effect of the *APOE* ε2 allele on neurodevelopmental outcome, there are several limitations. Children with CHD are heterogeneous, and many factors besides the genetic profile affect outcome. However, the sequential analysis performed in this study adjusted for both patient and procedural factors known to affect outcome. Furthermore, no interaction of *APOE* genotype with these factors could be identified. In addition, the *APOE* ε2 allele is rare, and the possibility that the *APOE* genotype is a surrogate for some unidentified factor cannot be excluded. There is evidence that the *APOE* alleles are heterogeneous within themselves, and some variants may be more strongly related to outcome than others. Full sequencing of the *APOE* region of 72 African American and European individuals detected many single nucleotide polymorphisms. These studies suggest that there is a substructure within the common ε2, ε3, and ε4 alleles.³³ In other words, each of these alleles is heterogeneous, although primarily in the noncoding regions. Although it is possible that an unidentified subset of the ε2 alleles is associated with poor outcome, rather than the entire ε2 group, a direct effect of the coding change is the most likely cause of an association. The lack of any trend

toward a performance difference among carriers of the ε4 allele suggests that a true ε4 effect was not missed. Finally, neurodevelopmental assessment at 1 year of age is not predictive of long-term outcome. Only further evaluation of these children at an older age can determine whether *APOE* genotype is a risk factor for worse long-term outcome.

In summary, the *APOE* ε2 allele predicts worse neurodevelopmental outcome at 1 year of age after infant repair of CHD. This *APOE* genotype-environment interaction demonstrates that genetic polymorphisms that impair neuroresiliency and CNS recovery may explain some of the interindividual variation in developmental outcome after surgery for CHD. Further studies, with more reliable and thorough testing, are necessary as the children reach school age to determine whether *APOE* genotype predicts a worse long-term neurodevelopmental outcome.

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