Autosomal dominant mannose-binding lectin deficiency is associated with worse neurodevelopmental outcomes after cardiac surgery in infants

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ABSTRACT

Objectives: The *MBL2* gene is the major genetic determinant of mannose-binding lectin (MBL)—an acute phase reactant. Low MBL levels have been associated with adverse outcomes in preterm infants. The *MBL2*_{Gly54Asp} missense variant causes autosomal dominant MBL deficiency. We tested the hypothesis that *MBL2*_{Gly54Asp} is associated with worse neurodevelopmental outcomes after cardiac surgery in neonates.

Methods: This is an analysis of a previously described cohort of patients with nonsyndromic congenital heart disease who underwent cardiac surgery with cardiopulmonary bypass before age 6 months (n = 295). Four-year neurodevelopment was assessed in 3 domains: Full-Scale Intellectual Quotient, the Visual Motor Integration development test, and the Child Behavior Checklist to assess behavior problems. The Child Behavior Checklist measured total behavior problems, pervasive developmental problems, and internalizing/externalizing problems. A multivariable linear regression model, adjusting for confounders, was fit.

Results: *MBL2*_{Gly54Asp} was associated with a significantly increased covariate-adjusted pervasive developmental problem score ($\beta = 3.98$; $P = .0025$). Sensitivity analyses of the interaction between age at first surgery and MBL genotype suggested effect modification for the patients with *MBL2*_{Gly54Asp} ($P_{\text{interaction}} = .039$), with the poorest neurodevelopment outcomes occurring in children who had surgery earlier in life.

Conclusions: We report the novel finding that carriers of *MBL2*_{Gly54Asp} causing autosomal dominant MBL deficiency have increased childhood pervasive developmental problems after cardiac surgery, independent of other covariates. Sensitivity analyses suggest that this effect may be larger in children who underwent surgery at earlier ages. These data support the role of nonsyndromic genetic variation in determining postsurgical neurodevelopment-related outcomes in children with congenital heart disease. (J Thorac Cardiovasc Surg 2018;155:1139-47)

Mannose-binding lectin (MBL) deficiency and early surgery combined increase pervasive developmental problems.

Central Message

Autosomal dominant MBL deficiency is associated with worse neurodevelopmental outcomes after surgery for congenital heart disease. Neuroprotection is important as survival in patients increases.

Perspective

As survival for congenital heart surgery improves, the need for decreased morbidity has increased. We identify in this work a genetic variant that causes autosomal dominant mannose binding lectin deficiency and report its association with worse neurodevelopment. Its effects were exacerbated in younger patients. These findings could help in risk stratification to reduce morbidity.

See Editorial Commentary page 1148.

See Editorial page 1138.

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Abbreviations and Acronyms

APOE	= apolipoprotein E
CBCL 1.5-5	= Child Behavior Checklist for ages 1.5 to 5 years
CHD	= congenital heart disease
CHOP	= Children's Hospital of Philadelphia
CPB	= cardiopulmonary bypass
DHCA	= deep hypothermic circulatory arrest
FSIQ	= full-scale intelligence quotient
LOS	= length of stay
MBL	= mannose-binding lectin
PDP	= pervasive developmental problems
VMI	= visual-motor integration

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Congenital heart disease (CHD) is the most common human birth defect, frequently requiring surgical intervention with cardiopulmonary bypass (CPB) or circulatory arrest soon after birth. Although survival has improved, studies have identified a higher incidence of adverse neurologic and functional outcomes in survivors of neonatal surgery for CHD.^{1,2} Neurodevelopmental dysfunction is the most common adverse postsurgical outcome, with approximately 15% and 30% of survivors showing symptoms of pervasive developmental problems (PDPs) and attention-deficit hyperactivity disorder, respectively, by age 5 years.³

Mannose-binding lectin (MBL) is a hepatic-derived acute phase reactant that activates the complement system.⁴ Serum levels of MBL in humans are entirely determined by the *MBL2* gene (*MBL1* is a nonfunctional pseudogene).⁵ Prior work identified a glycine-to-aspartate missense variation at the 54th codon of the *MBL2* protein (*MBL2*_{Gly54Asp}, rs1800450, population minor allele frequency approximately 14%) as the cause of autosomal dominant MBL deficiency (Mendelian Inheritance in Man No. 614372). These patients manifest recurrent neonatal infections.^{6,7} A single copy of *MBL2*_{Gly54Asp} decreases serum MBL activity by approximately 90%—to the level of those with 2 copies of *MBL2*_{Gly54Asp},^{6,7} likely through a dominant-negative effect, whereby proteins with the aspartate protein change interfere with oligomerization of MBL into the mature, polymerized serum molecule that is then involved in carbohydrate recognition and initiation of the

complement cascade.⁸ In addition, a single copy of *MBL2*_{Gly54Asp} has been associated with increased prevalence of sepsis in neonates⁹ and adverse neurologic outcomes in preterm infants.¹⁰

Given the importance of MBL in immunity for neonates and its potential link to adverse neurologic events, we hypothesize that *MBL2*_{Gly54Asp} also affects neurodevelopment. Thus, in this study we sought to determine whether autosomal dominant MBL deficiency, as caused by *MBL2*_{Gly54Asp}, is associated with differential neurodevelopmental outcomes at 4-year follow-up in children with isolated CHD who required surgical palliation.

METHODS**Ethics Statement**

Participants were enrolled at the Children's Hospital of Philadelphia (CHOP) on a protocol approved by the institutional review boards of CHOP and the University of Washington (analysis) from October 1998 to April 2003. Informed, written consent was obtained from parents or guardians of all participants.

Patient Population

This is an analysis of a previously described prospective cohort of 550 participants enrolled in a prospective study at the CHOP to study neurodevelopmental dysfunction after surgical correction for CHD.¹¹⁻¹⁴ Patients aged 6 months or younger who underwent CPB with or without deep hypothermic circulatory arrest (DHCA) for repair of CHD were eligible for enrollment. Exclusion criteria included multiple distinct congenital anomalies, a likely genetic or phenotypic syndrome, and a language other than English spoken in the home. This study examined a subset of the cohort with both genetic and 4-year neurodevelopmental outcome data (n = 295).

Of the original 550 CHD cases, 381 returned for 4-year neurodevelopment assessment. An additional 53 participants were excluded due to presence of chromosomal/genetic abnormalities (eg, DiGeorge/22q11.2 deletion syndrome) that would be expected to bias the results due to high prevalence of poor neurodevelopmental outcomes compared with nonsyndromic CHD patients.^{3,15} Thirty-three patients were excluded due to a lack of high quality genotype data. There were 295 patients considered after these exclusions. Information on data collection (including further information on inclusion/exclusion criteria) operative management, and genotyping have been previously reported in detail.¹²

Genetic Evaluation to Exclude Syndromic CHD Participants

CHD participants were evaluated by a genetic dysmorphologist and genetic counselor at the 1-year and/or 4-year examinations. Patients were classified as having no indication of genetic syndrome or chromosomal abnormality (normal, isolated CHD), suspected genetic syndrome (suspect), or a definite genetic syndrome or chromosomal abnormality (genetic). Following this classification, genetics records for each patient with a CHD were individually reviewed by a second senior board-certified medical geneticist, blinded to the genetic data, to determine whether participants were to be included or excluded from the current analysis, which focuses on nonsyndromic participants. Due to this review, 53 CHD participants with known or suspected genetic syndromes were excluded from analysis due to the potential for genetic confounding effects on neurodevelopmental outcomes.^{3,15}

Four-Year Neurodevelopmental Examinations

Neurodevelopmental evaluations were performed between the fourth and fifth birthdays. Growth measurements (ie, weight, length, and head circumference) were recorded and a health history was obtained, focusing on the incidence of interim illnesses, hospitalizations, neurologic events or interim evaluations, current medication use, and parental concerns about health. Parents were asked specifically whether they had ever been told that their child had autism, Asperger syndrome, pervasive developmental disorder not otherwise specified, or attention-deficit hyperactivity disorder.

General child intelligence and visual-motor input integration were tested using the Wechsler Preschool and Primary Scale of Intelligence—Third Edition¹⁶ and the Beery-Buktenica Developmental Test of Visual-Motor Integration—Sixth Edition,¹⁷ respectively. Both the full-scale intelligence quotient (FSIQ) and visual-motor integration (VMI) score are standardized to a mean of 100 ± 15 .

Behavior skills were assessed through parental report by using the Child Behavior Checklist for ages 1.5 to 5 years (CBCL 1.5-5).¹⁸ The CBCL 1.5-5 is a questionnaire used to obtain parental reports of behavior problems demonstrated within the previous 6 months. The PDP score is among the clinically oriented scores resulting from the CBCL 1.5-5 and has been previously used to identify preschoolers at risk for autism.¹⁹ Other scores from the CBCL 1.5-5 include a total problem score and 2 broad behavioral indices (ie, internalizing and externalizing problems). The CBCL 1.5-5 total problems score consists of the sum of the scores for the 99 specific problem items on the form plus the highest scores for any written-in responses to item 100. The internalizing problems scores summarize specific scores regarding the behaviors of excessive emotional reactivity, anxious/depressed behavior, somatic complaints, and withdrawn behavior. The externalizing problems score summarizes the 2 specific behavior scores of attention problems and aggressive behavior. All raw scores (ie, PDP, total problems, externalizing problems, and internalizing problems) were transformed to *T* scores with mean = 50 and these *T* scores were directly used in analyses.

MBL^{Gly54Asp} (rs1800450) genotyping

Whole blood or buccal swab samples were obtained before surgery and were stored at 4°C. Genomic DNA was isolated from white blood cells and genotyping was performed using the Illumina HumanHap550-Quad+ BeadChip (Illumina Inc, San Diego, Calif) at the Center for Applied Genomics of CHOP. Patient genotypes at rs1800450, the DNA missense variant responsible for autosomal dominant MBL deficiency, were extracted from the larger, nonimputed genetic dataset using PLINK.²⁰

Apolipoprotein E genotyping

Genomic DNA was prepared and was used for determination of apolipoprotein E (*APOE*) genotypes as previously described.¹¹ *APOE* genotypes were classified into 3 groups as follows: $\epsilon 2$ ($\epsilon 2\epsilon 2$, $\epsilon 2\epsilon 3$, or $\epsilon 2\epsilon 4$) $\epsilon 3$ ($\epsilon 3\epsilon 3$), or $\epsilon 4$ ($\epsilon 4\epsilon 3$ or $\epsilon 4\epsilon 4$), and the *APOE* $\epsilon 2$ genotype was included as a covariate in the linear regression model for all phenotypes based on considerable prior published evidence that the *APOE* $\epsilon 2$ genotype is associated with markedly detrimental neurodevelopmental outcomes.^{3,11,21}

Statistical Analyses

All analyses and graphics were performed in R (R Foundation for Statistical Computing, Vienna, Austria) using standard regression packages.

Genetic ancestry was determined using previously described methods.²² Due to the mixed ancestry of the cohort, the first 3 principal component eigenvectors were used as covariates in the linear regression models for neurodevelopmental outcomes to adjust for potential population stratification.²³

Due to the autosomal dominant inheritance of MBL deficiency, we categorized patients with 1 or more copies of the *MBL^{Gly54Asp}* missense variant into a single group (hereafter termed MBL-deficient genotype).

The distribution of rs1800450 genotype among the cohort was 199 homozygous major, 84 heterozygous, and 12 homozygous minor (96 total MBL-deficient genotype patients).

Linear regression models were separately fit for the 6 considered neurodevelopmental outcomes (FSIQ, VMI score, and CBCL PDPs, total score, internalizing problems, and externalizing problems) to assess the association of MBL-deficient genotype with each outcome. A Bonferroni-adjusted threshold for significance was set at $\alpha = 0.0083$ to adjust for the 6 total neurodevelopmental outcomes tested (0.05 out of 6). Each linear regression model was adjusted for the previously reported confounding variables: the first 3 principal component eigenvectors for ancestry, gestational age, birth weight, birth head circumference, diagnostic class (coded as a dummy variable with diagnostic class 1 as the reference group),²⁴ preoperative intubation, preoperative length of stay (LOS), total minutes of CPB, total minutes of DHCA, delayed sternal closure, extracorporeal membrane oxygenation use, hematocrit at first surgery, postoperative LOS, mother's education at 4-year follow-up, and mother's socioeconomic status at 4-year follow-up. Diagnosis class was assigned based on preoperative diagnosis according to a previously proposed scheme²⁴: class I, 2-ventricle heart without arch obstruction; class II, 2-ventricle heart with arch obstruction; class III single-ventricle heart without arch obstruction; and class IV, single-ventricle heart with arch obstruction.

Plotting of Gene $\times$ Environment Interactions

A secondary analysis considering the possibility of effect modification of MBL deficiency by age at first surgery was fit using linear regression. Visualization of this gene $\times$ environment interaction was obtained through simplification of the linear regression coefficients: [Predicted PDP score = $\alpha + \beta_1$ (MBL deficient) + β_2 (age at first surgery) + γ (MBL deficient $\times$ age at first surgery)], such that the MBL deficient have coefficients equal to $\alpha + \beta_1 + \beta_2 + \gamma$, whereas those with normal MBL status have coefficients equal to $\alpha + \beta_2$.

RESULTS

Baseline characteristics of the studied cohort stratified by MBL status are presented in Table 1. A total of 199 patients had normal MBL status, as determined by their homozygous major genotype at rs1800450. An additional 96 participants were classified as having MBL-deficient genotype, as they carried 1 or 2 copies of the minor allele at rs1800450 (resulting in *MBL^{Gly54Asp}*, the missense variant causative for autosomal dominant MBL deficiency (minor allele frequency $F = 13\%$, see Mendelian Inheritance in Man No. 614372)). When comparing the 2 MBL groups and not adjusting for multiple contrasts, the MBL-deficient group had significant decreases in preoperative LOS (1.62 vs 2.37 days; $P = .002$) and postoperative LOS (8.85 vs 11.50 days; $P = .011$) as well as significant increases in average age at first surgery (57.7 vs 36.3 days; $P = .013$). We also report marginal differences in the distribution of maternal education at 4 years of follow-up ($P = .024$) and in self-reported race ($P = .060$) between the MBL-deficient genotype and normal MBL groups.

To determine whether MBL status was associated with 4-year neurodevelopmental outcomes, a multivariable linear regression model adjusting for numerous potential confounders (see Statistical Analysis) was applied. The differences in the other underlying risk factors between genotype

TABLE 1. Baseline and clinical characteristics of the 295 participants, stratified by mannose-binding lectin (MBL) status

	n	Normal MBL (n = 199)	MBL-deficient genotype (n = 96)	2-sided P value*
Male	295	110 (55)	56 (58)	.62
Gestational age (wk)	295	38.57 ± 1.87	38.45 ± 2.40	.82
Birth weight (kg)	295	3.17 ± 0.61	3.14 ± 0.71	.52
Birth head circumference (cm)	295	33.82 ± 1.49	33.85 ± 1.71	.90
Race	295			
American Indian/Alaska Native		4 (2)	3 (3)	.06
Asian		7 (4)	4 (4)	
Black		53 (27)	11 (12)	
Hispanic		8 (4)	5 (5)	
White		127 (64)	73 (76)	
APOE ε2 genotype	295	21 (10)	10 (11)	.99
Diagnostic class	295			
1		97 (49)	55 (57)	.59
2		23 (12)	9 (9)	
3		21 (11)	9 (9)	
4		58 (29)	23 (24)	
Preoperative intubation	295	62 (31)	24 (25)	.28
Preoperative LOS (d)	295	2.37 ± 3.01	1.62 ± 2.19	.002
Age at first surgery (d)	295	36.3 ± 51.6	57.7 ± 59.7	.013
Hematocrit at first surgery (%)	295	27.81 ± 3.78	27.67 ± 4.53	.94
Total CPB time (min)	295	64.5 ± 39.2	68.8 ± 37.0	.12
Total DHCA time (min)	295	24.4 ± 23.0	18.8 ± 22.9	.04
Delayed sternal closure	295	21 (11)	10 (10)	.97
ECMO use	295	5 (3)	3 (3)	.76
Postoperative LOS (d)	295	11.50 ± 11.08	8.85 ± 7.22	.011
Maternal education at 4 y	295			
Less than high school		11 (6)	6 (6)	.024
High school/some college		93 (46)	27 (29)	
College		65 (33)	44 (45)	
Graduate		30 (15)	19 (20)	
Maternal SES quintile at 4 y	295			
1		6 (3)	4 (4)	.85
2		17 (9)	7 (7)	
3		38 (19)	15 (15)	
4		64 (33)	31 (32)	
5		71 (36)	40 (42)	
FSIQ score	292	97.8 ± 17.7	99.3 ± 17.9	—
VMI score	294	94.9 ± 17.9	93.3 ± 16.6	—
CBCL 1.5-5 measures				
Total problems	295	47.4 ± 10.4	48.8 ± 12.9	—
Internalizing problems	295	48.1 ± 10.6	50.3 ± 12.9	—
Externalizing problems	295	46.4 ± 10.3	46.6 ± 11.8	—
PDPs score	295	54.08 ± 6.60	56.28 ± 8.81	—

Values are presented as n (%) or mean ± standard deviation. MBL, Mannose-binding lectin; ε2, genotype associated with poor neurodevelopmental outcomes; APOE ε2, apolipoprotein E gene genotype; LOS, length of stay; CPB, cardiopulmonary bypass; DHCA, deep hypothermic circulatory arrest; ECMO, extracorporeal membrane oxygenation; SES, socioeconomic status; FSIQ, full scale intellectual quotient; VMI, visual motor integration; CBCL 1.5-5, Child Behavior Checklist 1.5 to 5 years; PDPs, pervasive developmental problems. *Differences in proportions were assessed using χ^2 tests. Tests of differences in means were performed using the Wilcoxon rank-sum test.

groups are thus accounted for when testing the effect of genotype on each neurobehavioral outcome. Of the 6 tested neurodevelopmental outcomes, no association was noted for MBL-deficient genotype and the outcomes of FSIQ ($B = -2.78$; $P = .31$), VMI ($B = -2.89$; $P = .33$), or CBCL 1.5-5 externalizing problems ($B = 1.40$; $P = .46$) scores. Nominally significant associations were observed between the MBL-deficient genotype group and CBCL 1.5-5 total problems ($B = 3.23$; $P = .097$) and internalizing problems ($B = 3.33$; $P = .093$) scores. A statistically significant association, after Bonferroni correction for 6 tests ($\alpha = 0.0083$), was identified between the MBL-deficient genotype group and CBCL 1.5-5 PDP score ($B = 3.98$; $P = .0025$). These results are summarized in Table 2. Sensitivity analyses stratifying by self-reported race and diagnostic class did not demonstrate differences in the direction of effect for the MBL-deficient genotype.

We further sought to identify potential effect modification of the association between the MBL-deficient genotype and CBCL 1.5-5 PDP score by performing sensitivity analyses including an interaction term between MBL-deficient genotype with (separately) preoperative LOS, age at first surgery, total DHCA time, and postoperative LOS, all of which differed by MBL status (see Table 1). Of these, no interaction was noted between MBL-deficient genotype and preoperative LOS, total DHCA time, or postoperative LOS. However, an association was observed between age at first surgery and MBL-deficient genotype ($\gamma_{\text{interaction}} = -0.036$; $P_{\text{interaction}} = .039$) (Table 3). Plotting of this gene $\times$ environment interaction demonstrated a more deleterious neurodevelopmental effect (higher PDP scores) for neonates with the MBL-deficient genotype who underwent surgery at an early age (Figure 1). Patients who had surgery before age 30 days and were MBL deficient had a mean covariate-adjusted PDP score of 58, compared with 55 for the normal MBL group. In comparison, among patients who had surgery after age 90 days, both MBL-deficient and normal groups had a mean PDP score of 55.

To examine whether the interaction between age at first surgery and MBL-deficient genotype reflected differences in CHD severity, we performed a sensitivity analysis for the outcome of PDP score, stratified by CHD diagnostic class²⁴ (Table E1). Patients with CHD class 1 or 2 did not have significant associations for either MBL-deficient genotype or age at first surgery ($P > .05$). However, patients with CHD class 3 or 4 did have nominally significant associations between MBL deficiency and PDP scores ($P = .044$ and $P = .023$, respectively). Moreover, the interaction between MBL-deficient genotype and age at first surgery trended toward significance in both the class 3 ($P_{\text{interaction}} = .087$) and 4 ($P_{\text{interaction}} = .057$) groups. We also note that children in diagnostic class 3 and 4 tended to have an earlier age at first surgery (Figure E1). Thus,

TABLE 2. Association of autosomal dominant mannose-binding lectin (MBL) deficiency genotype with 4-year neurodevelopmental outcomes

Outcome*	β coefficient $\pm$ standard error	P value
FSIQ score [†]	-2.78 ± 2.75	.31
VMI score [†]	-2.89 ± 2.98	.33
CBCL total problems score [‡]	3.23 ± 1.94	.097
CBCL persistent developmental problems score [‡]	3.98 ± 1.30	.0025
CBCL internalizing problems score [‡]	3.33 ± 1.97	.093
CBCL externalizing problems score [‡]	1.40 ± 1.88	.46

FSIQ, Full Scale Intellectual Quotient; VMI, Visual Motor Integration; CBCL, Child Behavior Checklist 1.5 to 5 years. *Linear regression model for association of autosomal dominant MBL deficiency and 4-year neurodevelopmental outcomes adjusts for first 3 principal component eigenvectors (to adjust for genetic ancestry stratification), gestational age, birth weight, birth head circumference, *APOE* $\epsilon 2$ genotype, diagnostic class (class 1 as the reference group), preoperative intubation, preoperative length of stay, hematocrit at first surgery, total minutes of deep hypothermic circulatory arrest, total minutes of cardiopulmonary bypass, delayed sternal closure, use of extracorporeal membrane oxygenation, postoperative length of stay, mother's education at 4-year follow-up (lowest education group as the reference), mother's socioeconomic status at 4-year follow-up (lowest socioeconomic quintile as the reference), and age at first surgery. [†]For FSIQ and VMI scores, scores have a mean of 100, with lower scores and negative β coefficients corresponding to increased prevalence of problems in neurodevelopment. [‡]For CBCL outcomes, the *T* scores have a mean of 50, with higher scores and β coefficients corresponding to increased prevalence of problems in neurodevelopment.

although underpowered, we assessed a linear regression model on the outcome of PDP score using a 3-variable interaction term between CHD diagnostic class, age at first surgery, and MBL-deficient genotype ($P_{\text{interaction}} = .11$). We also note that CHD diagnostic class did not separately modify the association of MBL-deficient genotype ($P_{\text{interaction}} = 0.37$) or age at first surgery ($P_{\text{interaction}} = .19$) with the outcome of PDP scores.

To determine whether MBL-deficient genotype had an effect on long-term survival we performed an analysis on 422 previously described^{13,14} nonsyndromic CHD patients (see Table E2). From these analyses we conclude that MBL-deficient genotype is not associated with differential long-term survival (hazard ratio, 0.81; 95% confidence interval, 0.38-1.71; $P = .58$).

DISCUSSION

We report the novel finding of a deleterious neurodevelopmental effect in CHD surgery survivors, with carriers of 1 or more copy of the *MBL2*_{Gly54Asp} missense variant having significantly higher scores for covariate-adjusted PDPs at 3-year follow-up ($B = 3.98$; $P = .0025$). This association between MBL-deficient genotype and PDPs appeared more pronounced, with higher PDP scores in participants with both the MBL-deficient genotype and an earlier age at first surgery ($P_{\text{interaction}} = .039$). Although MBL-deficient genotype was not significantly associated with the other tested neurodevelopmental outcomes, the trend in the direction

TABLE 3. Full multivariable linear regression model coefficients for the outcome of pervasive developmental problems, including an interaction term between mannose-binding lectin (MBL) deficiency and age at first surgery

Variable	β coefficient $\pm$ standard error	% variation	P value
Intercept	55.72 $\pm$ 12.81	—	—
Male (%)	−1.04 $\pm$ 0.89	0.23	.24
Gestational age (wk)	−0.25 $\pm$ 0.26	0.47	.33
Birth weight (kg)	−0.0019 $\pm$ 0.0074	0.020	.80
Birth head circumference (cm)	0.15 $\pm$ 0.30	0.060	.63
PC1	−12.25 $\pm$ 11.29	—	—
PC2	12.52 $\pm$ 10.07	—	—
PC3	−6.26 $\pm$ 10.84	—	—
APOE ϵ 2 genotype (%)	3.49 $\pm$ 1.33	2.27	.0089
Diagnostic class (%)			
Diagnostic class 2	−0.62 $\pm$ 1.44	0.20	.67
Diagnostic class 3	1.80 $\pm$ 1.86	0.070	.33
Diagnostic class 4	2.49 $\pm$ 1.98	0.64	.21
Preoperative intubation (%)	0.79 $\pm$ 1.01	0.096	.43
Preoperative LOS (d)	0.11 $\pm$ 0.17	0.21	.50
Hematocrit at first surgery (%)	−0.23 $\pm$ 0.11	1.02	.043
Total DHCA time (min)	0.022 $\pm$ 0.038	0.049	.56
Total CPB time (min)	0.0038 $\pm$ 0.018	0.039	.83
Delayed sternal closure (%)	0.93 $\pm$ 1.53	0.16	.55
ECMO use (%)	2.74 $\pm$ 3.02	0.079	.37
Postoperative LOS (d)	0.12 $\pm$ 0.055	1.33	.024
Maternal education at 4 y			
High school/some college	−3.39 $\pm$ 2.00	0.47	.090
College	−3.55 $\pm$ 2.14	0.20	.098
Graduate	−4.35 $\pm$ 2.27	0.60	.057
Maternal SES quintile at 4 y			
2	2.81 $\pm$ 2.76	0.13	.31
3	1.89 $\pm$ 2.56	0.29	.46
4	0.91 $\pm$ 2.61	0.081	.73
5	1.87 $\pm$ 2.67	0.23	.48
Age at first surgery (d)	−0.0012 $\pm$ 0.011	0.11	.62
MBL deficiency (%)	3.98 $\pm$ 1.30	2.05	.0079
MBL deficiency $\times$ age at first surgery interaction	−0.036 $\pm$ 0.017	0.53	.039

Values are presented as mean $\pm$ standard deviation. PC, Principal component (eigenvectors to adjust for differing genetic ancestry); APOE ϵ 2, apolipoprotein E gene genotype associated with poor neurodevelopmental outcomes; LOS, length of stay; DHCA, deep hypothermic circulatory arrest; CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation; SES, socioeconomic status; MBL, mannose-binding lectin.

of effect for each association was consistent with a deleterious effect on neurodevelopment for the MBL-deficient genotype group. Overall, this novel association provides additional evidence that genetic variants are important modifiers of morbidity and disability after surgery for CHD.

Other investigators have reported a deleterious effect of MBL-deficient genotype on neurological outcomes in pre-term infants^{9,10} and that *Mbl* $-/-$ knockout mice were at increased risk of traumatic brain injury.²⁵ However,

evidence for a protective effect of MBL loss or insufficiency has also been reported. Orsini and colleagues²⁶ demonstrated a potential role of MBL in ischemic-reperfusion injury by illustrating that *Mbl* $-/-$ knockout mice were protected from focal cerebral ischemia injury compared with wild type animals.²⁶ Similar results were reported by the same group for the outcome of traumatic brain injury in a sample of 6 adult humans and 11 mice,²⁷ and by a separate group in 135 adult stroke patients.²⁸ Separately, in adult

FIGURE 1. Effect of mannose-binding lectin (MBL)-deficient genotype is modified by age at first surgery, with infants receiving surgery at an early age having significantly higher pervasive developmental problems (PDP) scores ($P_{\text{interaction}} = .039$). Predicted values of Child Behavior Checklist 1.5 to 5 years PDP score are $59.7 + 0.0372 \times (\text{age at first surgery, in days})$ for the MBL-deficient genotype group and $55.72 - 0.0012 \times (\text{age at first surgery, in days})$ for the normal MBL group.

cardiovascular disease, low MBL levels have been reported to be protective against multiorgan dysfunction²⁹ and postoperative myocardial infarction³⁰ after cardiac surgery.

Compared with adult and animal studies, investigations into the effect of MBL-deficient genotype in pediatric populations have more consistently reported deleterious effects. Notably, low MBL levels and MBL-deficient genotypes were noted in a higher proportion of premature neonates compared with term children.³¹ In addition, among premature neonates, those with low MBL levels at baseline had significantly increased risk of adverse neurologic outcomes.³² Further, MBL-deficient genotype has been associated with increased risk of respiratory distress syndrome,³³ sepsis,⁹ and adverse neurological outcomes in neonates.¹⁰ Within this context of pediatric disease, our results of a deleterious neurodevelopmental effect of MBL-deficient genotype are consistent with prior literature. We speculate that the potential mechanism through which MBL-deficient genotype exerts its adverse neurodevelopmental effects is indirect: through increased infection risk. This mechanism is strengthened through evidence of decreased complement (ie, C3/C4) levels in MBL-deficient genotype patients,³² and the consistent literature in pediatric populations that suggest an association between low MBL levels and increased rates of childhood infection,^{6,7} sepsis,⁹ and respiratory distress syndrome.³³ However, we also report from our data a decrease in both preoperative and postoperative LOS for the MBL-deficient genotype group, suggesting that, similar to the adult cardiovascular surgery literature,^{29,30} decreased MBL is protective against

immediate injury after cardiac surgery. Further research is necessary to clarify and further elucidate the role of MBL in the immediate setting after cardiac surgery, and in the longer-term with regard to neurologic and neurodevelopmental outcomes.

Some limitations of this study should be considered. First, statistical power was limited due to the size of the study and lack of comparable cohorts. We addressed this by limiting the number of primary tests to 6 neurodevelopmental outcomes that broadly assessed function and behavior. However, it is possible that other significant associations in untested neurodevelopmental outcomes were overlooked through this approach. Second, we were unable to measure MBL protein levels directly due to a lack of serum in this long-standing cohort. However, because the studied genetic variant (rs1800450 causing the *MBL2*^{Gly54Asp} missense variant and resulting autosomal dominant MBL deficiency) is expected to reduce MBL levels by 90%,^{6,7} we believe it is a suitable proxy for MBL levels in vivo. Finally, because these analyses were of 4-year neurodevelopmental outcomes requiring follow-up, there is the possibility of survivor bias. To identify if our results reflected survival bias and potentially worse neurodevelopmental outcomes in survivors with MBL deficiency, we performed a secondary analysis and determined that MBL-deficient genotype was not associated with differential survival.

In addition to the above limitations of our work, we note that our identified effect modification between MBL-deficient genotype and age at first surgery on the outcome

of PDP score could also be affected by the observation that those who undergo surgery before age 30 days tend to have more severe CHD³⁴ (Figure E1). Because patients with more severe CHD also tend to have poorer neurodevelopmental outcomes,¹² hypothetical survival of these children could have potentially demonstrated a more deleterious interaction between age at first surgery and MBL-deficient genotype on neurodevelopment. To assess this possibility, we performed a sensitivity analysis that demonstrated stronger associations for both MBL-deficient genotype and the interaction between age at first surgery and MBL deficiency with PDP scores in the class 3 and 4 children, who have 1 ventricle with (class 4) or without (class 3) aortic arch obstruction. While this sensitivity analysis was suggestive of further effect modification by CHD severity, modeling of 2-way (CHD class $\times$ age at first surgery or CHD class $\times$ MBL deficiency) and 3-way (CHD class $\times$ age at first surgery $\times$ MBL-deficient genotype) were not suggestive of significance ($P_{\text{interaction}} > .10$). Thus, although we conclude that CHD severity did not affect the deleterious association of MBL deficiency with PDP scores, CHD severity remains an important topic for future follow-up work in the study of neurodevelopment after surgical palliation.

CONCLUSIONS

We report the novel association of MBL-deficient genotype and poor neurodevelopmental outcomes in the setting of CHD. We further identified effect modification by age at first surgery, whereby children who underwent surgery at an early age and had the MBL-deficient genotype were at greater risk of deleterious neurodevelopmental outcomes. Given the importance of neurodevelopmental function in CHD survivors, validation of these results could lead to novel preventative and risk assessment strategies to decrease the long-term morbidity of CHD surgery.

Conflict of Interest Statement

Ms McDonald-McGinn has presented lectures on 22q11.2 deletion syndrome for Natera. All other authors have nothing to disclose with regard to commercial support.

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Key Words: congenital heart disease, neurodevelopment, genetics, MBL2, mannose-binding lectin, gene-by-environment interaction, gene-environment interactions

FIGURE E1. Distribution of age at first surgery and covariate-adjusted pervasive developmental problem (*PDP*) scores by congenital heart disease (CHD) diagnostic class.

TABLE E1. Sensitivity analysis by congenital heart disease diagnostic class on the association between age at first surgery and mannose-binding lectin (MBL) deficiency on the outcome of pervasive developmental problems

Variable	β coefficient $\pm$ standard error	<i>P</i> value ^{*,†}
Diagnostic class 1 (n = 152)		
Age at first surgery (d)	-0.00083 ± 0.014	.95
MBL deficiency (%)	2.66 ± 2.15	.76
MBL deficiency $\times$ age at first surgery interaction	0.0098 ± 0.022	.66
Diagnostic class 2 (n = 32)		
Age at first surgery (d)	-0.0042 ± 0.091	.65
MBL deficiency (%)	4.47 ± 4.90	.46
MBL deficiency $\times$ age at first surgery interaction	-0.028 ± 0.094	.77
Diagnostic class 3 (n = 30)		
Age at first surgery (d)	-0.018 ± 0.089	.085
MBL deficiency (%)	6.50 ± 2.77	.044
MBL deficiency $\times$ age at first surgery interaction	-0.025 ± 0.15	.087
Diagnostic class 4 (n = 81)		
Age at first surgery (d)	-0.036 ± 0.18	.045
MBL deficiency (%)	6.18 ± 2.66	.023
MBL deficiency $\times$ Age at first surgery interaction	-0.039 ± 0.20	.057

MBL, Mannose-binding lectin. *Linear regression model for association of autosomal dominant MBL deficiency and 4-year neurodevelopmental outcomes adjusts for first 3 principal component eigenvectors (to adjust for genetic ancestry stratification), gestational age, birth weight, birth head circumference, *APOE* $\epsilon 2$ genotype, preoperative intubation, preoperative length of stay, hematocrit at first surgery, total minutes of deep hypothermic circulatory arrest, total minutes of cardiopulmonary bypass, delayed sternal closure, use of extracorporeal membrane oxygenation, postoperative length of stay, mother's education at 4-year follow-up (lowest education group as the reference), mother's socioeconomic status at 4-year follow-up (lowest socioeconomic quintile as the reference), age at first surgery, and an interaction term between autosomal dominant MBL deficiency and age at first surgery. †Three-way interaction term between congenital heart disease diagnostic class, age at first surgery, and MBL deficiency on the outcome of pervasive developmental problem score did not demonstrate significant effect modification ($P = .11$). Separate regression models using a 2-way interaction term between congenital heart disease class and age at first surgery ($P = .19$) and congenital heart disease class and MBL-deficient genotype ($P = .37$) were not significant for effect modification.

TABLE E2. Multivariable Cox proportional hazards model coefficients for the association between mannose-binding lectin (MBL) genotype and long-term survival (N = 422; 47 total deaths during follow-up)

Variable	Hazard ratio (95% confidence interval)	P value
Male (%)	1.18 (0.61-2.27)	.62
Gestational age (wk)	1.07 (0.87-1.32)	.51
Birth weight (kg)	0.56 (0.28-1.13)	.11
PC1	—	—
PC2	—	—
PC3	—	—
Age at first surgery (d)	0.99 (0.99-1.01)	.90
<i>APOE</i> ε2 genotype (%)	1.55 (0.72-3.37)	.27
Diagnostic class (%)		
Diagnostic class 2	0.50 (0.056-4.51)	.54
Diagnostic class 3	5.79 (1.66-20.18)	.0058
Diagnostic class 4	6.57 (2.31-18.65)	.00041
Preoperative intubation (%)	0.65 (0.32-1.31)	.23
Preoperative LOS (d)	0.94 (0.79-1.13)	.51
Hematocrit at first surgery (%)	1.02 (0.94-1.09)	.69
Total DHCA time (min)	1.02 (1.01-1.04)	.0037
Total CPB time (min)	1.00 (0.99-1.01)	.73
Delayed sternal closure (%)	1.02 (0.45-2.30)	.97
ECMO use (%)	6.39 (2.76-14.80)	.000015
MBL deficiency (%)	0.81 (0.38-1.71)	.58

PC, Principal component (eigenvectors to adjust for differing genetic ancestry); *APOE* ε2, apolipoprotein E gene genotype; LOS, length of stay; DHCA, deep hypothermic circulatory arrest; CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation; MBL, mannose-binding lectin.