FAILURE TO VALIDATE ASSOCIATION OF MANNOSE- BINDING LECTIN DEFICIENCY WITH ADVERSE

NEURODEVELOPMENTAL OUTCOMES AFTER CARDIAC SURGERY IN INFANTS

To the Editor:

Mannose-binding lectin (MBL) is an acute-phase reactant that activates the complement system. A genetic variant of *MBL2*, rs1800450, with a minor allele frequency of approximately 14%, is associated with the protein change *MBL2*^{Gly54Asp} and causes autosomal dominant MBL deficiency. We recently reported in this *Journal* that rs1800450 was associated with increased pervasive developmental problems after cardiac surgery at 4-year follow-up.¹ Most initial reports of genotype-phenotype associations are not subsequently validated. Ideally, validation should be performed in a similar population. The phenotype assessed in the validation study should be the same as in the initial report, and similar covariates should be measured. We were previously able to validate our findings of deleterious neurodevelopmental outcomes associated with the *APOE* ϵ 2 allele² with data from the Pediatric Heart Network (PHN),³ and sought to similarly validate our findings related to *MBL2*^{Gly54Asp}.

We used data from 231 subjects from the combined Infant Single Ventricle (ISV) and Single Ventricle Reconstruction (SVR) trials and performed separate linear regressions on the outcomes of Psychomotor Developmental Index and Mental Developmental Index, with adjustment for other known risk factors (see Table 1 for a full list of variables). We found a marginally significant association ($\alpha = 0.01$ or $0.05/5$, adjusting for 5 total statistical tests) between *MBL2*^{Gly54Asp} and Psychomotor Developmental Index ($P = .076$) at 14 months of follow-up after cardiac surgery. The direction of effect for *MBL2*^{Gly54Asp} ($\beta = 5.11$) was opposite that of *APOE* ϵ 2 ($\beta = -9.23$; $P = .014$), however, indicating a protective effect for *MBL2*^{Gly54Asp} that conflicts with our previous findings. For our analyses with Mental Developmental Index, we found no evidence of

TABLE 1. Summary of associations for *APOE* ϵ 2 and *MBL2*^{Gly54Asp} with neurodevelopmental outcomes in the Pediatric Heart Network data

Outcome*	N	<i>APOE</i> ϵ 2		<i>MBL2</i> ^{Gly54Asp}	
		β	P value	β	P value
MDI	231	-3.49	.31	1.69	.52
PDI	231	-9.23	.014	5.11	.076
FSII-R General	189	4.05	.18	-3.91	.037
FSII-R Total	189	2.92	.35	-3.41	.074
FSII-R Activity	189	2.93	.39	-3.11	.14

MDI, Mental Developmental Index at 14 months of follow-up; PDI, Psychomotor Developmental Index at 14 months of follow-up; FSII-R, Functional Status II-R at 2 to 3 years of age. *Analyses adjusted for clinical site, sex, ethnicity (as a factor variable), birth weight, gestational age, presence of genetic anomaly, type of congenital heart disease (as a factor variable), age at first surgery, total cardiopulmonary bypass time, total aortic crossclamp time, total deep hypothermic circulatory arrest time, medical insurance, socioeconomic status z score, poverty z score, total number of severe adverse events, and postsurgical stay in days.

association for *MBL2*^{Gly54Asp} ($\beta = 1.68$; $P = .52$) in multivariable regression analyses.

We also performed an analysis of a subset of the Single Ventricle Reconstruction trial (N = 189) for the outcome of Functional Status II-R at 2 to 3 years of age (FSII-R). We found marginally significant associations for *MBL2*^{Gly54Asp} with decreased FSII-R General ($\beta = -3.91$; $P = .037$) and FSII-R Total ($\beta = -3.41$; $P = .074$) scores in multivariable analysis. We found no association between *MBL2*^{Gly54Asp} and FSII-R Activity score ($\beta = -3.11$; $P = .14$). We again note that the direction of effect is opposite that of *APOE* ϵ 2 (Table 1) and reflects a consistent neuroprotective effect for *MBL2*^{Gly54Asp} across all FSII-R domains tested.

In summary, we could not validate our previous finding that *MBL2*^{Gly54Asp} is associated with poorer neurodevelopmental outcomes.¹ In the PHN data, we do not find significant associations after correction for multiple comparisons. The findings do, however, suggest a neuroprotective effect across tested domains. These results could reflect underlying differences between cohorts, because data from the Child Behavior Checklist, from which the variable of pervasive developmental problems (the primary outcome from our previous study) was defined, was not available in the PHN cohorts. In addition, these results may reflect differential effects of MBL deficiency at varying stages of neurodevelopment.⁴ Alternatively, this *MBL2* variant may reflect the effects of another, closely correlated, causal variant in this region. Regardless of the underlying reason for our lack of validation, further investigation is needed into the molecular mechanism of effect for MBL deficiency on neurodevelopment the better to understand and prevent sequelae of cardiac surgery in infancy.

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BACK TO THE DRAWING BOARD Reply to the Editor:

Neonates and infants with complex congenital heart disease are at increased risk for adverse neurodevelopmental outcomes relative to children without heart disease. If risk factors for such adverse outcomes could be identified, alternative treatment approaches tailored to different phenotypes might be developed. Further, insights about the mechanistic explanation for adverse outcomes associated with certain risk factors may suggest strategies to mitigate the potential deleterious impact of the risk factor. In this context, a recent report describing worse neurodevelopmental outcomes after congenital heart surgery in patients with mannose-binding lectin (MBL) deficiency was particularly intriguing.¹ Interestingly, there was also a suggestion in the report that this association may be age related, in that the association was greatest in younger patients. As with any novel observation, the findings must be validated and confirmed; unfortunately, however, the same investigators were unable to do so when they studied mannose-binding lectin deficiency in 2 similar populations.² Indeed, mannose-binding lectin deficiency actually appeared to be neuroprotective in certain subpopulations.

Although the original observation was not confirmed in the validation study, we believe that the follow-up report by Kim and colleagues² is nonetheless very valuable. Most fundamentally, investigators interested in neurodevelopment can be confident that they have the most current information available. Perhaps even more importantly, the community must acknowledge the intellectual rigor of Kim and colleagues² in “correcting” the record. All too often, a promising initial report appears in the literature, but no confirmatory (or contradictory) sequel is available. Is this because no validation study was done? Or is it because the attempt at replication was unsuccessful, and a negative study was less attractive to both investigators and editors? In an era in which trust in the scientific community can no longer be taken for granted (if it ever was), Kim and colleagues² have provided both first-class science and an example of how to “do it right.” They are to be applauded for providing what the noted sage Paul Harvey might have called “the rest of the story.”