Correspondence

Reply to: Lack of Diagnostic Utility of "Amino Acid Dysregulation Metabotypes"

To the Editor:

Our article, entitled "Amino Acid Dysregulation Metabotypes: Potential Biomarkers for Diagnosis and Individualized Treatment for Subtypes of Autism Spectrum Disorder," provides an important step toward establishing a reliable biological marker of increased risk for a diagnosis of autism spectrum disorder (ASD) (1). This publication is based on data from the Children's Autism Metabolome Project (CAMP) (NCT02548442), which has enrolled 1100 children with ASD, developmental delay, and typical development. This study had the goal of determining whether a subset of children with ASD had altered branch chain amino acid metabolism. The conclusion is that approximately 17% of children with ASD demonstrate such an imbalance of these amino acids. The article has been criticized by Sainani and Goodman (2). We believe that these criticisms are based on a lack of understanding of the state of biomarker research in ASD and specifically on the iterative, multistep process that research on biomarkers must take to establish a diagnostic test. We address each of their concerns.

As Goodman and Sainani correctly point out, positive predictive value (PPV) is typically driven by prevalence of the disorder in the likely test population. We have defined our use of PPV in the article with statements such as the following: "We wish to note that our use of PPV was not adjusted for prevalence of ASD in the general population or for populations at greater risk of ASD"; "The PPV reported is based on the CAMP study population and was used as one facet of the criteria to define metabotypes"; and "We do not imply that a general population screen using the AADMs [amino acid dysregulation metabotypes] would achieve a similar specificity or PPV." Diagnostic values were based on the CAMP study population to compare biomarker performance between a training set and a test set. The focus of our study was to demonstrate the existence of specific metabotypes in the CAMP study population.

Sainani and Goodman speculate that the specificity of the AADMs would be lower in populations with other developmental disorders. We acknowledged this possibility in the limitations section of the discussion that "the specificity of AADMs for ASD relative to other neurodevelopmental disorders is currently unclear." Only through further clinical research can we accurately estimate the specificity of the AADMs for ASD.

Sainani and Goodman (2) view the fact that the mean concentrations of the tested values were similar between cases and control subjects as a problem. This criticism does not acknowledge the enormous biological heterogeneity observed within ASD (3–7) and the fact that mean differences are rarely observed in any biological feature. Our metabotyping approach identifies subpopulations of ASD subjects with values not observed in the majority of typically developing subjects without regard to mean values. We confirmed the reproducibility of these metabotype populations in a test set of 342 subjects. Our underlying hypothesis is that subpopulations of ASD exist owing to the heterogeneous underlying biology of ASD. There is a wide range of genetic and environmental factors that contribute to the biological underpinnings of ASD. Associating these factors with metabotypes within the ASD population provides important clues to developing targeted interventions.

Sainani and Goodman (2) suggest that the age of the subject could lead to differences in metabolism. However, we did not identify differences in means within 6-month age bins of the AADM population—evidence that age is not a contributing factor [see Supplemental Table S11 in Smith *et al.* (1)]. Research is currently underway to determine if the identified metabotypes are stable over time; defining and characterizing a biomarker is a long-term, multistep interactive process, and not all facets of this process can be reported in a single publication.

The fourth point made by Sainani and Goodman (2), that a biomarker-based test would be useful only if it could detect cases at earlier ages than behavioral tests, demonstrates a lack of understanding of the current state of ASD diagnosis and treatment. Currently, behavioral testing can reliably diagnose children beginning at 24 months of age (8), but the median age of diagnosis is 4.3 years (9). The CAMP study offers the opportunity to assess children as young as 18 months of age. These metabotypes hold the promise of more personalized treatment by providing a tool for a physician to seek earlier assessment by a neurodevelopmental specialist.

The point that the test does not satisfy the first criterion for a decision tool because it does not affect subsequent actions ignores the usefulness of earlier referral and treatment. It also does not acknowledge that biochemical information gained from the test can help guide testing, disease stratification, and treatment decisions. The metabotype-based test panels are not intended to replace the standard of care, but rather to identify children at risk for ASD who can be referred to a specialist for further evaluation. Earlier intervention is well known to improve outcome, so the suggestion that a diagnostic test is worthless because it does not replace the next step in the standard of care is without merit.

The final comment made by Sainani and Goodman demeans the authors of the letter. They imply that ethical, unbiased research cannot be done through public–private collaborations. Yet, the National Institutes of Health National Center for Advancing Translational Sciences begins its description of such relationships by stating, "Public-private partnerships are an important way to accelerate translational science. Each party brings its unique expertise and assets to the table to solve a common challenge" (10). Conflicts were reported appropriately to the *Journal* and are reflected in the manuscript. Partnerships have resulted in important advances in health care, such as GE Medical's research collaboration with many universities—partnerships responsible for innovations in medical imaging that are on the market today, improving and saving lives (11,12).

Our study will be the first in a series of reports based on analyses of the metabolism of CAMP subjects using multiple

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metabolite profiling techniques developed at Stemina Biomarker Discovery, Inc. (Madison, WI), in collaboration with the expertise of scientists at the MIND Institute (University of California, Davis). We hope that this information will improve the basic understanding of ASD and facilitate earlier diagnosis and more precise and complete treatment of this complex disorder.

Alan M. Smith
Elizabeth L.R. Donley
Robert E. Burrier
Joseph J. King
David G. Amaral

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AMS is an employee of Stemina Biomarker Discovery, Inc., and is an inventor on provisional patent application 62/623,153 entitled "Amino Acid Analysis and Autism Subsets" filed on January 29, 2018. ELRD is an equity owner in Stemina Biomarker Discovery, Inc., and is an inventor on provisional patent application 62/623,153 entitled "Amino Acid Analysis and Autism Subsets" filed on January 29, 2018. REB is an employee of Stemina Biomarker Discovery, Inc., and is an inventor on provisional patent application 62/623,153 entitled "Amino Acid Analysis and Autism Subsets" filed on January 29, 2018. JJK was an employee of Stemina Biomarker Discovery, Inc., and is an inventor on provisional patent application 62/623,153 entitled "Amino Acid Analysis and Autism Subsets" filed on January 29, 2018. DGA receives research funding from the National Institutes of Health, the Simons Foundation, and Stemina Biomarker Discovery, Inc.; and is on the Scientific Advisory Boards of Stemina Biomarker Discovery, Inc., and Axial Therapeutics.

Article Information

From the Stemina Biomarker Discovery, Inc. (AMS, ELRD, REB, JJK), Madison, Wisconsin; and the Medical Investigation of Neurodevelopmental Disorders (MIND) Institute (DGA) and Department of Psychiatry and Behavioral Sciences (DGA), University of California, Davis, Davis, California.

Address correspondence to David G. Amaral, Ph.D., The MIND Institute, University of California, Davis, 2825 50th Street, Sacramento, CA 95817; E-mail: dgamaral@ucdavis.edu.

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