Correspondence

Lack of Diagnostic Utility of "Amino Acid Dysregulation Metabotypes"

To the Editor:

We read with interest the article by Smith *et al.* (1) on amino acid dysregulation metabotypes (AADMs). The hypothesis that there may be subtypes of autism spectrum disorder (ASD) marked by measurable metabolic changes is interesting. However, we believe that the data presented in the article demonstrate that the test described has minimal or no diagnostic utility. Here, we describe five serious problems that led to this conclusion.

The authors compared blood metabolites in 516 children with ASD (cases) and 164 normal control subjects. Specifically, they measured concentrations of individual amino acids as well as ratios of amino acids—glutamine, glycine, and ornithine to leucine, isoleucine, and valine. The authors classified high values of these measurements as having a glutamine, glycine, or ornithine AADM.

The first problem is with the reported diagnostic accuracy values. The authors report that testing positive for any of the three AADMs yields a sensitivity of 16.7%, specificity of 96.3%, and positive predictive value (PPV) of 93.5%. However, PPV depends on disease prevalence and cannot be calculated from case-control samples like this, in which the percentage of cases is artificially set by the investigators (2). The prevalence of ASD in the sample was 76%, so even a weak test, which this is, can raise this to a PPV of 93.5%. If applied to the general population, which has a 1.7% ASD prevalence (1), the test would have a PPV of just 7% (Figure 1). Even if applied to a high-risk population—younger male siblings of affected sisters—the PPV would still be just 48%, with a wide 95% confidence interval—28% to 68% (Figure 1).

A second problem is that the actual specificity is certain to be lower than reported, with a profound impact on the clinical utility. As noted by the authors, the control group—normally developing children—are not those to whom the test will or should be applied. Rather, the relevant control group for a proper specificity value would be those with behavioral or developmental problems needing differentiation from autism, or perhaps high-risk children without the disease. Specificity will surely be lower in this group, and even a small specificity decrease will devastate test performance. With the reported numbers, the negative likelihood ratio (LR) is 0.86 (95% confidence interval, 0.82–0.91), virtually useless in ruling out autism, and the positive LR is 4.6 (95% confidence interval, 2.0–10.2), a weak rule-in test. If the specificity in a proper control group decreased just from 96% to 90%, which is probably conservative, the positive LR would decrease from a weak 4.6 to an essentially useless 1.7. If the specificity dropped to 83.3%, it would literally be useless: positive LR = negative LR = 1.0.

A third problem is that the mean concentrations and ratios of the tested values were similar between cases and control subjects. While cases were more likely to fall in the upper tails of the distributions, they were also overrepresented in the lower tails; cases simply had more variability than control subjects [see Figure 2 in Smith *et al.* (1)]. This is noted by the authors, but weakens both biologic plausibility and generalizability. Many factors besides metabolic function could affect this variation. For example, children with ASD have more erratic eating behaviors than normal children, which could lead to wider swings in nutrient levels (3,4). The control group was also about 3 months younger than the ASD group, and younger toddlers may have more homogeneous diets.

Fourth, a biomarker test for autism would be useful only if it could detect cases at earlier ages than behavioral tests. This study provides no evidence that the AADM test can pick up autism sooner than behavioral testing— all cases in the study were diagnosed behaviorally. To show that this biomarker test has clinical utility, the researchers will need to evaluate the test prospectively on young, disease-free or prediagnosis children or retrospectively on blood samples that were taken before diagnosis. Though the authors found no relationship between age and the mean values of the metabolic ratios, this does not mean that the test is reliable at the youngest ages, as this result was likely dominated by the large majority of the cases, who were older than 24 months of age, with a mean age of 34.5 $\pm$ 7.9 months.

The last problem is that this test does not satisfy the first criterion for a decision tool, that it affects subsequent actions. The reference standard is the behavioral and cognitive assessments applied in this study. If this test is negative, the reference standard still must be done, because a negative test is almost informationless. If the test is positive, even in a high-suspicion population, it does not raise the chance of ASD high enough to avoid using the reference standard to decide on subsequent management. Neither test result removes the need to apply the reference standard, the definition of a clinically useless test.

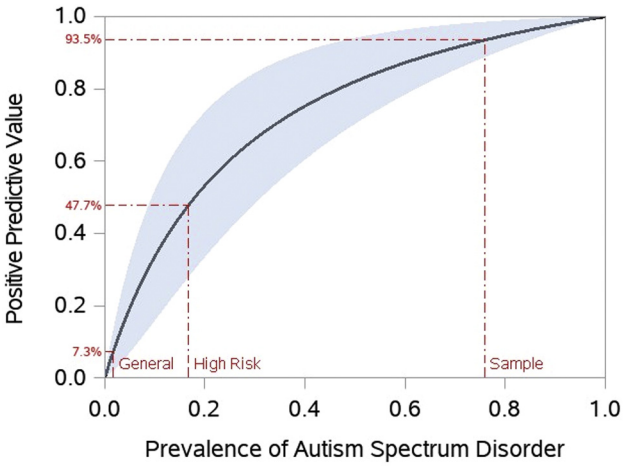

Figure 1. The positive predictive value depends on the prevalence of autism spectrum disorder in the test population. The 95% confidence bands were calculated according to the formula in Mercaldo *et al.* (7). Dashed red lines indicate prevalence in the general population (1.7%) (1), the study sample (75.9%), and a specific high-risk population—younger male siblings of affected sisters (16.7%) (8).

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Finally, we note that all the authors of this article report a financial conflict of interest with a company, Stemina Biomarker Discovery, Inc. (Madison, WI), that is commercializing this test. The webpages of NeuroPointDX (a division of Stemina Biomarker Discovery, Inc.) cite this publication as the scientific basis for the "NPDX AA test for autism." They state that it "can identify about 30% of children with autism," differing from the 16.7% sensitivity reported in the paper (5). A news article (6) about the study reported, "The test, to be drawn first-thing-in-the-morning, did not need approval from the U.S. Food and Drug Administration but instead will be handled by state-regulated clinical laboratories. It's already been approved for use in 45 states, including Wisconsin."

For the reasons noted, we believe that the evidence reported in this study does not support the use of this test for either diagnosing ASD or guiding patient care.

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The authors report no biomedical financial interests or potential conflicts of interest.

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