

# Autistic therapies focused by laboratory data.

## Part I: Organic acids

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### Introduction

*Autism is one of several currently recognized pervasive developmental disorders known collectively as Autistic Spectrum Disorders (ASD). Autism is characterised by delayed or impaired communication skills and social interaction, sensory hypo- or hyperactivity and repetitive behaviours. The broad pathophysiology of autism involves disruption of neural integration leading to failure of normal perception and learning functions.*

### Aetiology of Autism – the debate

There remains a divergence of scientific opinion about the aetiology of autism. Some maintain that multiple-loci genetic and epigenetic origins produce brain anatomical and functional alterations with little or no contribution from environmental factors <sup>1</sup>. This hypothesis has difficulty explaining data such as the rapid rise in incidence and positive patient responses to a variety of therapies <sup>2</sup> and the heterogeneity of the behavioural syndrome. There are strong arguments that genomic variations are only predisposing factors, upon which nutritional deficiencies, immunologic challenges and toxic chemical exposures act as triggering aetiologic elements <sup>3</sup>. The divergence of opinion arises from the attempt to define autism in purely behavioural or psychiatric terms, as opposed to thinking of it as a manifestation of neurotoxic, neuroimmune, and neurometabolic factors. There is inconclusive evidence implicating any single aetiologic factor.

Many clinicians have adopted the broad, multifactor aetiology where predisposing factors for autism include toxic chemicals, nutrient and antioxidant insufficiencies and genetic weaknesses, with particular emphasis on the metabolic fragility of methylation and glutathione biosynthetic pathways. Precipitating events include infections, food and airborne antigen exposures and inflammatory bowel responses.

### Pathophysiology

The pathophysiology is likely to progress from increased oxidative challenge, producing decreased glutathione status <sup>4</sup>, immune cell release of NMDA\* agonists disrupting regulation of glutamatergic neurons <sup>5,6</sup>, and decreased methylation capacity impacting dopamine DP4 receptors with consequences similar to those proposed in schizophrenia <sup>7</sup> and Parkinson's disease <sup>8,9</sup>. These changes can lead to critical impairment of neuronal membrane integrity, loss of calcium channel neurotransmitter regulation and defects in dopamine-mediated membrane phosphatide methylation. The net result is loss of neuronal integration necessary for normal perception and language ability and the onset of social withdrawal or self-stimulation

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\* N-methyl-D-aspartic acid, a water-soluble synthetic substance that mimics glutamate at NMDA receptors.

behaviours. Overall neuronal plasticity changes may result in brain morphology alterations as a response to the antecedent actions <sup>10</sup>.

### Focusing Therapeutic intervention

Such a complex, multifactor aetiology requires identification of individual weaknesses in order to focus therapeutic interventions for maximal effectiveness. The most pertinent weaknesses arising from the mix of specific gene alterations and environmental factors need to be identified in each individual. Evaluative tools must provide evidence from which individual therapies may be designed.

This review focuses on laboratory evaluation of organic acidurias for revealing metabolic weaknesses that may be responsive to aggressive nutritional, detoxification and gastrointestinal-immune balancing interventions.

### Organic Acid testing

Neonatal wards routinely perform organic acid testing to detect or confirm inborn errors of metabolism. Explosive growth in studies of inherited metabolic disorders continues to expand the list of abnormalities detected by urinary organic acid testing <sup>11</sup>. Modern instrumentation allows accurate measurement of levels in the normal physiological ranges for many organic acids. Abnormalities based on these ranges may be used to inspect for metabolic difficulties that may not express as obvious clinical conditions at birth. Sometimes the origin of the metabolic lesion is simple nutritional deficiency. For neonates, nutritional deficiencies may manifest due to inadequate maternal nutrient status. Single nucleotide polymorphisms can produce enzymes with lowered affinity for cofactors or altered metabolic regulatory proteins. A large number of these disorders are responsive to dietary intervention or nutrient supplementation at levels that shift the equilibrium towards enzyme saturation with cofactor <sup>12</sup>. Clinical laboratories are able to perform routine, remote assay for more than 40 organic acids on a simple overnight urine specimen.

One of the earliest applications of organic acid testing that became widely utilised was the detection of functional vitamin B12 deficiency in elderly populations by elevated methylmalonic acid in the presence of normal serum vitamin B12 concentrations <sup>13,14</sup>. The urinary methylmalonate: creatinine ratio test has been found to be more reliable than the test for serum methylmalonate <sup>15</sup>.

Interest in screening children for metabolic disorders has been accelerating in recent years. Abnormalities were found in 188 cases during screening recently completed in Asia, where methylmalonic aciduria (MMA) was the most common condition <sup>16</sup>. One third of these children were clinically responsive to vitamin B12 supplementation. Such early, pre-symptomatic intervention is an effective way to reduce morbidity due to nutrient insufficiency. Prevention of failure to thrive, developmental delay, dehydration, and coma due to vitamin B12 insufficiency has even been extended into prenatal diagnosis where systematic attempts to titrate maternally administered doses of vitamin B12 to foetal MMA response are being conducted <sup>17</sup>.

For routine metabolic screening, the analytical methods may be modified to allow lower detection limits and greater accuracy. Analytes are chosen for their potential to reveal specific nutrient insufficiencies, neurotransmitter turnover, detoxification function and intestinal microbial products <sup>18,19</sup>. A summary of analyte abnormalities and associated therapeutic strategies is shown in **Table 1**. Nutrient deficiencies produce analyte elevations because they effectively block the reaction required to clear the compound.

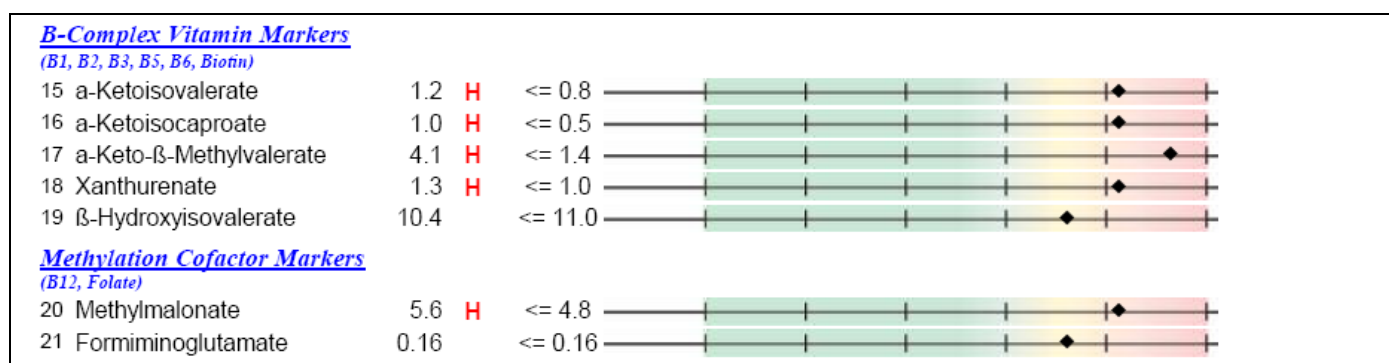
The scope of diagnostic capabilities from a single overnight urine specimen makes the profiling of organic acids in urine the single most powerful laboratory evaluation for finding individual weaknesses that can set the stage for neurotoxic consequences of autism. Whether folic acid

insufficiency is due to simple dietary deficiency or genetic polymorphism of the enzyme of the histidine catabolic pathway, an elevated formiminoglutamate is evidence that the patient is a candidate for aggressive folate supplementation. A 90-day follow up organic acid profile should show normalised concentrations if there was a simple dietary insufficiency. Genetic polymorphisms are generally more refractory to correction, and these patients may need much higher dosages for longer intervals.

**Table 1. Therapeutic strategies guided by organic acidurias**

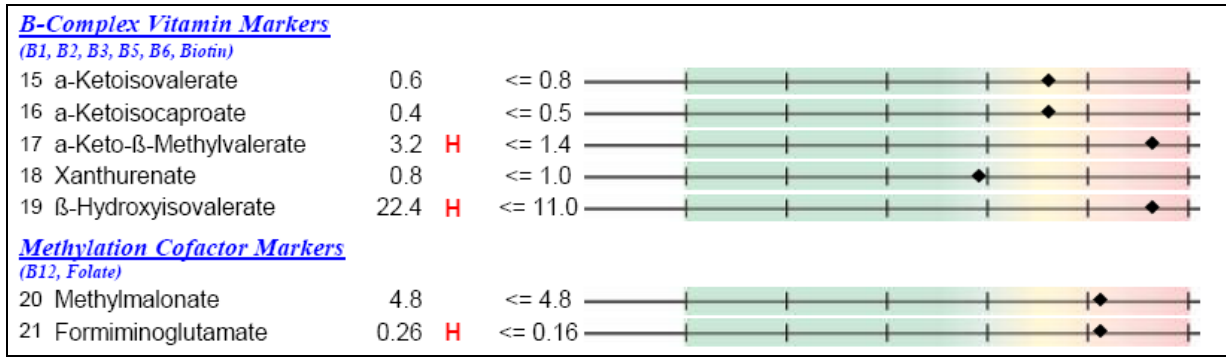
Abnormal acidurias	Indication	Ref.	Therapeutic strategy
<input type="checkbox"/> Adipate, suberate, ethylmalonate	Carnitine insufficiency	20	Improve mitochondrial efficiency with Carnitine & riboflavin
<input type="checkbox"/> Succinate, fumarate, malate, lactate	Coenzyme Q10 insufficiency	21	Coenzyme Q10
<input type="checkbox"/> Pyruvate & lactate	Low pyruvate dehydrogenase activity	22	Correct specific nutrient insufficiencies Lipoic acid
<input type="checkbox"/> Alpha-ketoisovalerate, <input type="checkbox"/> alpha-ketoisocaproate, <input type="checkbox"/> alpha-keto-beta-methylvalerate	Low branched-chain keto acid dehydrogenase activity	23	Vitamin B1, B3
<input type="checkbox"/> Xanthurenate & kynurenate	Vitamin B6 insufficiency	24	Vitamin B6
<input type="checkbox"/> Beta-hydroxyisovalerate	Biotin insufficiency	25	Biotin
<input type="checkbox"/> Methylmalonate	Vitamin B12 insufficiency	14	Vitamin B12
<input type="checkbox"/> Formiminoglutamate	Folic acid insufficiency	26	Folic acid
<input type="checkbox"/> p-hydroxyphenyllactate, <input type="checkbox"/> 8-hydroxy-2'-deoxyguanosine	Increased oxidative challenge	27 28	Improve water-soluble antioxidant status
<input type="checkbox"/> Quinolininate	Inflammatory response with potential neurotoxicity	29	Magnesium to antagonize glutamate neurotoxicity
<input type="checkbox"/> 2-Methylhippurate	Xylene exposure	30	Reduce xylene exposure
<input type="checkbox"/> Benzoate	Glycine conjugation	31	Supplemental glycine
<input type="checkbox"/> Sulphate	Low glutathione status	32	Improve glutathione status – NAC /
<input type="checkbox"/> Pyroglutamate	Glycine insufficiency	33	Methionine Taurine orally, GSH injection
<input type="checkbox"/> p-Hydroxyphenylacetate	Intestinal bacterial overgrowth	34	Antibiotics and probiotics to reduce
<input type="checkbox"/> Indican		35	inflammatory response potentially
<input type="checkbox"/> Arabinitol	Candidiasis	36	arising from gastrointestinal dysbiosis

The following figures illustrate various patterns of abnormalities found in autistic children. The unit of measurement in all figures is mcg of analyte per mg creatinine in overnight urine specimens. The charts display the positions of each laboratory result relative to the reference population. Hash marks represent reference population quintile divisions.



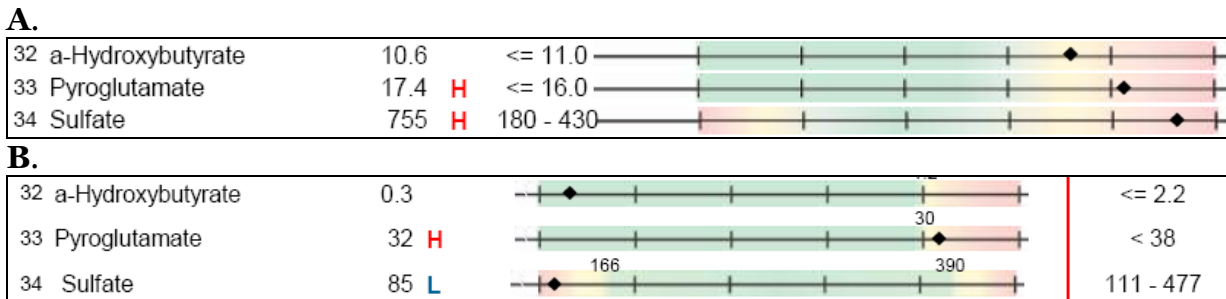
**Figure 1. Thiamine, pyridoxine, vitamin B12**

This 7 y/o female shows multiple keto acid elevations indicating potential to benefit from aggressive B-complex, especially B1, B5 and lipoic acid. In addition the high levels of xanthurenate and methylmalonate suggest that extra B6 and B12 are needed.



**Figure 2. Biotin, folic acid**

This 6 y/o male shows strong elevation of the biotin maker, beta-hydroxyisovalerate and of the folic acid marker, formiminoglutamate.



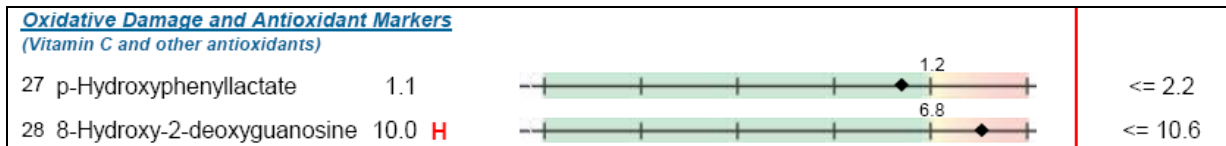
**Figure 3. Glutathione demand**

**A. Early stage glutathione demand**

The 7 y/o female reported in A shows the concurrent markers of glycine insufficiency (high pyroglutamate) and sulphate elevation that tend to appear in early stages of severe glutathione demand. Alpha-hydroxybutyrate is approaching the fifth quintile, indicating strong hepatic biosynthesis of glutathione.

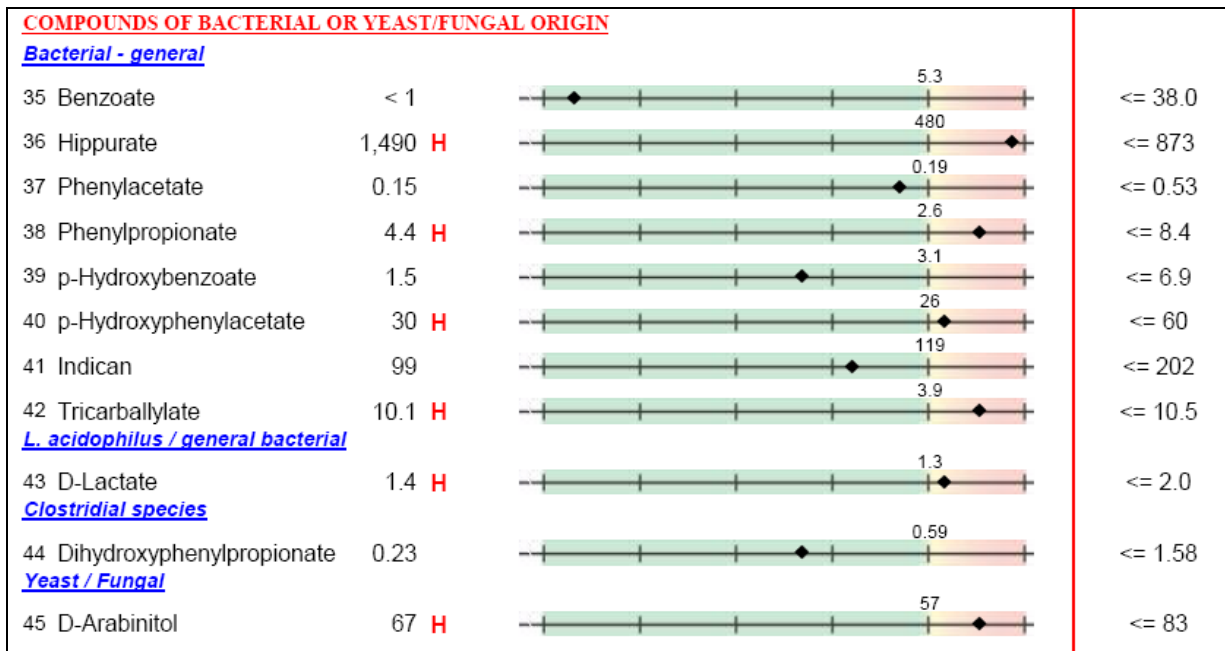
**B. Late stage glutathione demand**

The situation has progressed in B where sulphate has fallen to low levels seen in late stage total body depletion of glutathione while alpha-hydroxybutyrate output has fallen to first quintile levels, indicating difficulty sustaining hepatic glutathione synthesis from available sulphur amino acids.



**Figure 4. Insufficient antioxidant status**

Rates of DNA oxidative damage are reflected by levels of the oxidation product, 8-hydroxy-2’deoxyguanosine in urine. An elevated rate is found in this 7 y/o female. Such oxidative stress is the initiating event for depletion of glutathione reserves and various shifts of brain methylation found in autism.



**Figure 5. Compound bacterial and yeast overgrowth**

Multiple bacterial and yeast products are detected at elevated levels in this 2 y/o male. These products are absorbed from the transitional gut where the microbial population is most actively growing.

**Discussion**

The patterns shown in figures 1-5 demonstrate some of the great variety of potential sets of abnormalities that can arise due to the combination of genetic, nutritional and environmental toxicant exposure factors. Their correction by nutrient supplementation or intestinal microbial population adjustments have been shown to be highly efficacious. A follow up profile of organic acids in urine can demonstrate normalisation.

Nutrient insufficiencies, oxidative challenge and chronic inflammatory challenge from intestinal microbial overgrowth collectively constitute a significant part of the modifiable predisposing factors in autism. Their correction also may be critical for shortening the duration of autistic symptoms. Further study is needed to confirm the overall efficacy of such approaches. However, the large and rapidly growing number of anecdotal reports where the signs of autism were significantly relieved by correcting such factors is encouraging. Identifying nutrient and antioxidant insufficiencies from an organic acid profile on a single overnight urine specimen offers a significant advance to practitioners dealing with the multiple factors that can influence brain function in an autistic patient.

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