Autism spectrum disorder-associated biomarkers for case evaluation and management by clinical geneticists

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David A Geier, BA
Institute of Chronic Illnesses, Inc., Silver Spring, MD, USA; and, CoMeD, Inc., Silver Spring, MD, USA

Mark R Geier, MD, PhD, FABMG, FACE
Author for correspondence
The Genetic Centers of America, 14 Redgate Court, Silver Spring, MD 20905, USA
Tel.: +1 301 989 0548
Fax: +1 301 989 1543
mgeier@comcast.net

"Depending on the etiology [of the autism spectrum disorder], associated medical risks may be identified, which may lead to screening and potential morbidity prevention in patients and other family members."

Autism spectrum disorders (ASDs) are neurodevelopmental disorders, usually diagnosed in early childhood and characterized by varying restrictions in communication and social interaction; as well as by atypical, repetitive behaviors including hyperactivity, poor attention, impulsivity, aggression, self-injury, tantrums and unusual responses to sensory stimuli [1]. Furthermore, common conditions often associated with an ASD diagnosis include gastrointestinal disease and dysbiosis [2], autoimmune disease [3] and mental retardation [4]. Based on a recent survey, ASDs occured in at least one in 150 children born in the USA during the early 1990s [5].

In April of 2008, the American College of Medical Genetics issued clinical practice guidelines recommending that clinical geneticists identify the etiology of ASDs and treat patients with the diagnosis. The work-up includes examining and evaluating the patient, the parents and the siblings, in order to establish a definitive etiology. Depending on the etiology, associated medical risks may be identified, which may lead to screening and potential morbidity prevention in patients and other family members. Specific recurrence risk counseling beyond general multifactorial information can be provided, and targeted testing of at-risk family members can be offered. In a number of cases (e.g., metabolic disorders), targeted therapies may be or become available, strongly justifying a medical genetics consultation for all ASD patients [6].

This article examines recognized, clinically available biomarker testing for the evaluation and treatment of ASDs. It will attempt to explain the clinical significance of the findings and, where possible, explore potential treatment options.

Porphyrin biomarkers

Porphyrins are derivatives of the heme synthesis pathway and afford a measure of xenobiotic exposures [7]. Recent studies, conducted in three separate continents, have examined urinary porphyrin profiles in ASDs [7–12]. In each of the studies, mercury-associated urinary porphyrin profiles were found to be significantly increased across the autism spectrum, from mild to severe ASD diagnoses [7–9]. Previous studies also demonstrated that chelation therapy in ASDs resulted in significant reductions in mercury-associated urinary porphyrin profiles [7–9]. Furthermore, using the Childhood Autism Rating Scale, a recognized test of ASD severity, researchers found a significant increasing correlation between mercury-associated urinary porphyrin profiles and Childhood Autism Rating Scale scores prior to blinded laboratory testing [12]. Providing further support for these correlations, other studies have shown that ASDs, relative to controls, had increased: brain mercury levels [13]; blood mercury levels [14]; mercury levels in baby teeth [15]; and mercury in the urine/fecal samples following chelation therapy [16]; as well as decreased excretion of mercury through first baby haircuts [17].

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Porphyrins can be examined in ASDs using Laboratory Corporation of America (LabCorp) random fractionated urinary porphyrin (Test#120980) and red blood cell fractionated porphyrin (Test#803445) testing. Additionally, 2,3-dimercapto-1-propanesulfonic acid and meso 2,3-dimercaptosuccinic acid, previously shown to significantly lower mercury body-burden (and hence lower urinary porphyrins) and help reduce mercury-associated neurodevelopmental toxicity [18], may improve clinical outcomes of ASDs [19]. The Autism Research Institute reported survey data collected from over 22,300 parents of ASDs. The survey includes a list of 45 medications, 23 nondrug supplements or biomedical treatments, and nine special diets used to treat ASDs. The parents rated the treatment on a six-point scale. Parents, assessing their children's condition before and after treatment, rated chelation therapy (or the removal of heavy metals) as the highest or best of these 77 choices. Interestingly, 76% of parents said that their child 'got better' on this treatment [20].

Trans-sulfuration biomarkers

The trans-sulfuration pathway starts with homocysteine. Homocysteine can either be remethylated to methionine or irreversibly removed from the methionine cycle by cystathionine β-synthase, which removes homocysteine from the methionine cycle and initiates the trans-sulfuration pathway for the synthesis of cysteine, glutathione, sulfate and taurine [21]. Recent ASD studies showed abnormal metabolites within this pathway [22–25], and a correlation between metabolite abnormalities and clinical severity [26]. In addition, low levels of the trans-sulfuration pathway metabolite, glutathione, may be associated with mercury-intoxication, as reflected by a significant correlation between levels of plasma oxidized glutathione and mercury-intoxication associated urinary porphyrins [12].

Trans-sulfuration biomarkers available from LabCorp include serum megaloblastic anemia profile (Test#706960), glutathione (Test#853002) and taurine (Test#910844). Supplementation with targeted nutritional interventions, such as methylcobalamin (vitamin B12), folinic acid, and pyroxidine (vitamin B6) in ASDs with trans-sulfuration abnormalities helped to improve clinical and laboratory findings [25,27].

Oxidative stress/inflammation biomarkers

Reactive oxygen species are a natural by-product of the normal metabolism of oxygen, which results in unstable atoms that cause disruption to other molecules and subsequent damage to cells. Recent studies revealed that biomarkers of oxidative stress were increased in ASDs [23–25,28–32]. Furthermore, investigators observed a significant correlation between decreased antioxidant proteins and the loss of previously acquired skills [29]. Investigators also found a correlation between Childhood Autism Rating Scale scores and advanced glycation end products known to promote neuro-inflammation, oxidative stress and neuronal degeneration [32]. In addition, investigators observed neuroglial activation and evidence of neuro-inflammation in ASDs [33].

Oxidative stress/inflammation biomarkers from LabCorp include an oxidative stress panel (Test#853047) and neopterin (Test#140335) testing. Aldactone® (spironolactone), a drug with demonstrated anti-inflammatory effects, was suggested as a therapeutic intervention for increased oxidative stress/inflammation in ASDs [34].

Hormonal biomarkers

The ratio of ASD cases between the sexes, with four boys diagnosed for every one girl, probably reflects a male vulnerability to developing ASDs; a hypothesis supported by multiple lines of evidence. Investigators reported that ASDs tend to display a hypermasculine profile on many cognitive tasks. Others have observed that ASDs also have lower-than-expected 2nd to 4th digit (2D:4D) ratios, which is correlated with higher ratios of fetal testosterone to fetal estrogen, as well as with lower verbal and higher numerical intelligence. Some neuroanatomical studies, comparing the brains of individuals with and without an ASD, reveal structural differences associated with high levels of fetal testosterone, including hemispheric asymmetries [35]. Interestingly, girls with abnormally high fetal testosterone levels, as a result of congenital adrenal hyperplasia, have a higher number of autistic traits than their unaffected sisters [36]. ASD girls were observed to have significantly delayed onset of menarche [37] (excess androgens have been linked to menstrual problems) and are more likely to display elevated rates of testosterone-related disorders in comparison with controls [38]. Other studies have shown elevated blood androgen metabolites in ASDs, in comparison with controls [22,39]. In addition, recent studies have helped to explain the biochemical basis for these increased androgen levels in ASDs [39].

Androgen biomarkers available from LabCorp include testicular function profile II (Test#035113), dehydroepiandrosterone (Test#004101), dehydroepiandrosterone-sulfate (Test#004697), androstenedione (Test#004705), androstane diol glucuronide (Test#140442), dihydrotestosterone (Test#500142), estradiol (Test#140244), estrone (Test#004564) and total estrogens (Test#004549) testing. Interventions for increased androgens in ASDs include drugs with known anti-androgen effects, such as Lupron® (leuprolide acetate), Androcur® (cyproterone acetate), and Aldactone® (spironolactone). These drugs were observed to result in significant clinical ameliorations in hyperactivity and/or impulsivity, stereotypy, aggression, self-injury, abnormal sexual behaviors and/or irritability behaviors that frequently occur in those with an ASD diagnosis [19,34,39]. Menstrual-aged females with ASDs may benefit from increased estrogen levels from Yaz® (drospirenone/ethinyl estradiol).

Mitochondrial dysfunction biomarkers

Recent research has supported a role for mild mitochondrial dysfunction among ASDs [40–42]. Investigators reported that levels of free and total carnitine and pyruvate were significantly reduced, while ammonia and alanine levels were considerably elevated, in ASDs. These are suggestive of mild mitochondrial dysfunction [40].

Mitochondrial dysfunction biomarkers available from LabCorp include carnitine (Test#706500), pyruvic acid (Test#004788), lactic acid (Test#004770) and ammonia (Test#007054) testing. Treatment of the mitochondrial dysfunction present in many

ASDs may include administration of the mitochondrial respiratory chain cofactors to enhance mitochondrial function, such as Carnitor® (L-carnitine).

Genetic biomarkers

Multiple lines of epidemiologic evidence support the strong role of genetics in the etiology of ASDs. Some of the most frequently reported chromosome regions with abnormalities associated with ASDs include 15q pericentromeric 11–13 region, 17p11, 22q11, 22q13, 16p11.2 and 2q37. Currently, array comparative genomic hybridization has emerged as a powerful new tool that promises further revolution of clinical genetic testing. In addition, Fragile X syndrome and mutations in the *MECP2* gene are reported in ASDs [6].

Other investigators have described the recently emerging importance of evaluating *MTHFR* gene mutations (i.e., polymorphisms) in ASDs [24,43]. The *MTHFR* gene codes for an essential enzyme in folate metabolism. MTHFR enzyme catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Methyltetrahydrofolate is essential in one-carbon-donor metabolism for the remethylation of homocysteine to methionine and the generation of metabolically active tetrahydrofolate in the methionine synthase reaction. Common SNPs of the C677T and the A1298C alleles in the *MTHFR* gene decrease the activity of the enzyme, and have been observed to occur at a significantly higher rate among ASDs in comparison with controls [24,43].

Genetic biomarkers are available from LabCorp, which offers blood chromosome (Test#052019), chromosome microarray (Test#510002), DNA Rett syndrome (Test#511180), Angelman/Prader Willi syndrome methylation assay (Test#511210), Fragile X syndrome (Test#510065) and *MTHFR* (Test#511238) testing. Genetic abnormalities are common among ASDs. Therefore, it is important that affected families seek genetic counseling to determine the family risk of transmission of ASD-associated genes, as well as to provide insights into behavior modification in order to help reduce the impact of genetic polymorphisms.

Conclusion

The present article provides a brief cataloging of the rapidly emerging, clinically available biomarkers that the clinician can use to evaluate ASDs, and can potentially help normalize with targeted treatments. The use of biomarkers will assist the clinical geneticist in making a differential diagnosis, and determine potential etiological factors contributing to ASDs. Finally, it is clear that, as additional research is performed, the clinical geneticist will need to give careful attention to the task of incorporating new testing and treatment options for ASDs.

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