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Emerging Clues and Altered Metabolic Findings in Autism: Breakthroughs and Prospects from Omics Studies

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Abstract

Autism spectrum disorder (ASD) is a pervasive broad-spectrum disorder that involves multifaceted delays in the development of many basic, social, and communication skills. The etiology of ASD is multifactorial and involves interplay among genetic, neurologic, hormonal, metabolic, immune-inflammatory, and environmental factors; which further complicate both the diagnosis and management in affected individuals. This review delineates the underpinnings of clinical challenges posed by ASD, like clinical heterogeneity of patient presentation, unresolved ASD genomic map and deficient drug therapy. Besides, it addresses the emerging pathogenic, etiologic, and diagnostic clues of diverse origins, encompassing genetic-, neurotransmitter-, androgenic- and immunoinflammatory-anomalies in ASD patients, as availed by advances in Omics technologies (like genomics and proteomics). Moreover, as unravelled by metabolomics studies, metabolic flaws that pertain to amino acids, fatty acids, mitochondrial anomalies, and oxidative-stress are highlighted and further correlated with the extent of clinical picture and malbehaviors coherent with ASD patients. Lastly, future directions are underlined to promote and rationalize ASD research so as to help translate its findings into remedy.

Keywords: Autism (ASD); Emerging clues; Etiology; Biomarkers; Altered metabolism; Hypertestosteronemia; Management

Introduction and Challenges Posed by Autism

Autism (autism spectrum disorder, ASD), is a pervasive broad-spectrum developmental disorder that appears in the first 3 years of life. It involves variable, multifaceted delays in the development of many basic, social, and communication skills. Although ASD is more frequently encountered (four times) in boys than in girls, it remains without known racial, ethnic, or social boundaries. The exact cause of autism has been largely elusive; but appears to be multifactorial and involves interplay among diverse triggers including genetic, neurologic, hormonal, metabolic, immune-inflammatory, and environmental factors. Besides, etiology studies also implicated certain types of infections, and maternal problems during pregnancy or at birth. Accordingly, a plethora of studies have agreed that there is no single definitive cause for autism [1,2].

Challenges with diagnosis and management of autism

Clinical heterogeneity among affected individuals: ASD represents a broad-range disease with a wide margin of diversity and variability among its patients, which makes our understanding of autism far from complete. Indeed, the widely variable set of risk factors, favour diversity and inconsistence of the clinical picture among ASD patients.

ASD genome is still largely unresolved (the complete spectrum of genetic component): This is further aggravated by the complexity of the Central Nervous System (CNS) and scarcity of knowledge on brain development within the first 3 years of a child age.

Gender bias in the prevalence of ASD (4 folds in favor of men) mandates extensive specialized research to better resolve and clarify the underlying basis of such sex differences.

Most such patients have glitches in communicating, expressing or complaining about their problems and suffer.

Medical treatment with drugs against ASD has been, thus far, limited, which inevitably makes other social and behavioral treatments predominantly involved as mainstay modalities [1-4]. Likewise, a diagnostic biomarker/s that can early and unequivocally detect ASD has not been yet identified.

Emerging Etiological and Risk-Factors in ASD

Thanks to recent advances in Omics technologies and the epigenetic research area, mechanisms such as genomic imprinting, epimutations and methylation have been introduced to help unravel some of the ASD mysteries [5].

Genetic anomalies: Lessons from recent genomics studies

Recently, studies employing large-scale "whole exome sequencing" and genomic screening have spotted new candidate genes with wide implications in the pathogenesis of ASD. Intriguingly, however, these findings indicated that one gene alone is not enough to pose a significant risk for ASD; but alternatively, several risk variant genes are likely involved. These genetic advents, likewise, concluded that the diagnosis and prognosis of ASD are further complicated by the notions that many of the discovered genes can also associate with other neurological disorders than ASD [6].

Chromosomal aberrations at many sites have been documented in ASD by conventional cytogenetic techniques that were subsequently substantiated by large-scale genomics [7]. Analyses of plenty of cases

unequivocally concluded the association of definitive chromosomal regions with autism. These include 1q21, 2q37, 7q11.23, 15q11-13, 16p11.2, 17p11.2, 22q11.2 and 22q13. Besides, whole-genome array-based analyses have shown genomic imbalances in about 10% of the ASD cases [8]. In particular, the most frequent site of autosomal anomalies in ASD is chromosome 15, with the duplication of 15q11-q13 being the most encountered alteration. Interestingly, this region contains about 30 genes, most of which are highly associated with ASD, neurobehavioral disorders, cognitive deficits, hypotonia and seizures [9,10]. The second most common abnormality in ASD is a phenotypic microdeletions and microduplications in the 16p11.2, which entails incomplete symptoms of variable expressivity and neurodevelopmental impairments including intellectual disability and epilepsy on top of autism. This phenotype occurs in about 0.5% of autism patients [11,12].

Epigenetic modifications and interplay with environmental factors: Evidence from genomics

Epigenetic mechanisms involve modifications to chromatin and genomes without altering the DNA sequence (genotype), as exemplified by methylation and acetylation of the genetic materials or its histone protein. Accordingly, epigenetic modifications can switch genes on and off by altering their availability for transcription. Paradoxically, the latter epigenetic modifications can further become programmed or (imprinted), thereby disrupting the usual patterns of epigenomes and creating "epimutations" which, if not corrected, can be transmitted to subsequent generations [13]. Accordingly, the relevance / association of imprinted genes to ASD is that they are highly expressed in the human brain, and that human neurons are known to undergo massive methyl-modifications all over the developmental and postnatal life.

Recently, epigenetic mechanisms were found to be recruited by a variety of stress-based conditions, including cellular exposure to environmental factors, oxidative stress (OS), drugs, and toxins. Interestingly, epigenetic abnormalities have wide implications that directly relate to neurodevelopmental diseases. The association between ASD and epigenetics came from the detection in ASD patients of genes that control epigenesis and genetic mutations in imprinted regions therein. For instance, among the most common chromosomal anomalies in ASD are duplications of the imprinted 15q11–13 region, which was also proven to be inheritable [14]. The DNA methylation process is induced by methionine synthase, an enzyme whose activity is known to be downregulated by OS and environmental insults. When the activity of this enzyme is impaired, affected individuals can display attention deficits or typical autistic behaviors (in response to altered expression of genes that are controlled by methylation) [15,16]. Consequently, environmental factors can, conceivably, alter intracellular pathways during embryonic development, causing epigenetic alterations in neurons, thereby linking the impact of environmental insults with individual behavior differences [17]. It, therefore, follows that the etiology of ASD is not merely via congenital genetic defects but can besides be evoked by environmental factor-dependent epigenetic mechanisms. Environmental toxicants that have evidently been positively associated with ASD encompass toxic wastes, pesticides, polychlorinated biphenyls, and heavy metals such as lead, arsenic and mercury [18,19].

Lately, numerous studies have examined the relationship between perinatal exposure to polluted air, ozone, or traffic pollution and the risk of (ASD). These studies revealed a consistent and compelling evidence to suggest a causal association between air pollution and the development of ASD [20]. Diet and nutrition have been of an appealing concern in investigating the etiology of ASD. It has been found that a majority of children with ASD display unusual gastrointestinal symptoms, along with an enhanced intestinal permeability. Moreover, disparate microbiotic content between ASD patients and controls has been established. Therefore, nutrition-related factors were suggested to play a notable role in the etiology of ASD and its manifestations. Likewise, a plethora of observations indicated that nutrition, among other environmental elements, might trigger an unstable base of genetic or epigenetic predisposition that puts forward to autism [21].

Nutritional status of individuals with autism spectrum disorders has not yet received enough attention. Research examining anthropometric measurements reveals an abnormally accelerated rate of growth among children with autism. Further, inadequate micronutrient- but adequate macronutrient-intakes are increasingly reported. Thus, several lines of evidence implied that patients with ASD disorders are likely to display dysregulated amino acid metabolism, increased concentrations of homocysteine, and/or decreased levels of the vitamins folate, B-6, B-12 and vitamin-D, thereby suggesting possible biomarkers for an early diagnosis of ASDs [22]. Other studies have reported that supplemental nutrients such as omega-3 fatty acids, zinc, magnesium, and some phytochemicals may evoke moderate benefits, while avoidance of food allergens, chemicals, and chelation therapy can afford some aid to autism patients. However, research investigating their association with disease severity and other comorbid psychiatric/non-psychiatric factors has been incomplete [23].

The relevance of OS to etiology and severity of ASD was substantiated by many experimental and clinical observations. Thus, a lower level of mitochondrial reduced-glutathione (GSH, a natural antioxidant defense mechanism) existed in individuals with ASD, as compared to unaffected ones. Moreover, ASD severity has been inversely associated with GSH levels and other biological markers of cellular OS [24-26]. Furthermore, postmortem brain samples from ASD patients demonstrated low levels of GSH and altered activity of antioxidant-enzymes, along with clues of massive oxidative damage to proteins, enzymes and DNA, with mounting levels of lipid peroxides [26-28].

Neurotransmitter signalling and implications in ASD

Neurotransmitter system dysfunction has been widely implicated in ASD by modulating neuronal cell migration, differentiation, and ultimately the development of the brain [29,30]. Numerous neurotransmitter systems have been assessed and found widely implicated in the pathogenesis of ASD. Pertinent such neurotransmitters include the GABAergic, glutamatergic and serotonergic systems [31].

Synaptic vesicles store neurotransmitters such as gamma-aminobutyric acid (GABA) and glutamate [32]. Alterations in neurotransmission and/or synaptic storage of neurotransmitters can trigger serious neurologic disorders, including ASD [33]. The structural composition of synapses may be altered by mutations or deletions in other scaffold proteins, which may ultimately alter the number or composition of available neuro-receptors, as demonstrated with mGluR, NMDA and AMPA receptors. Disrupted neurologic signaling, firing or communication induced by various genetic mutations, can stand as a primary antecedent in the development of

autism [34]. Gene screening and associated neurologic studies showed that genetic alterations towards the excitatory neurotransmitter glutamate are evidently involved in ASD pathogenesis during the early development of age (1-3 years), by affecting neuronal plasticity and cognitive functions [35,36].

A main inhibitory neurotransmitter in the brain is **gamma-aminobutyric acid (GABA)** [37]. GABA is biosynthesized from the aminoacid glutamate via the enzyme glutamic acid decarboxylase (GAD) [38]. GAD exists in two isoforms, GAD67 and GAD65, which are encoded by the GAD1 and GAD2 genes [39]. GABA is crucially needed in early developments, neuronal differentiation and in phases of maturation as well [40]. Reduced production or signaling of GABA can cause hyperexcitability state along with cognitive dysfunction [41]. The high incidence of epilepsy in patients with autism has notably motivated research on the GABA neurotransmitter system in individuals afflicted with ASD [42].

Neurochemical abnormalities that associate with pathophysiology of ASD involve lower expression of GAD65 and GAD67, which entails subsequent downregulation of GABAergic transmission [43]. Fatemi and coworkers [44] have shown marked decline in expression of the two GAD isoforms. Furthermore, the evidently diminished platelet-GABA levels in ASD patients [45] and the appreciable reduction in GABAA and GABAB receptors in brains of post-mortem ASD patients [46,47] attest to a deficient GABAergic system in ASD patients. Collectively, both animal model and clinical studies on ASD are in favor of "blunted GABAergic transmission", which should motivate and direct future research to promote the upregulation of already-deficient GABA neuroinhibitory system in ASD patients.

On the other hand, a prominently and massively reported neurotransmitter in ASD has been **serotonin (5-Hydroxytryptamine, 5-HT)**. 5-HT is synthesized from tryptophan aminoacid initially by the enzyme tryptophan-hydroxylase which converts tryptpohan to 5-hydroxy-tryptophan. A subsequent “decraboxylation” reaction governs the mature 5-HT neurotransmitter [48,49]. Serotonin is a neuromodulator that orchestrates critical steps in neuronal development, such as synaptogenesis, cell proliferation, differentiation, and glial growth [50,51]. Serotonin also regulates many aspects of cognitive functions in the brain cortex by targeting GABAergic inhibition [52]. Such concerted actions for 5-HT further emphasize its utility as a therapeutic target in ASD.

Numerous observations have revealed that the pathophysiology of ASD involves hyperserotonemia in up to 50% of individuals with ASD (with a concomitantly lower brain serotonin levels), thereby indicating that ASD associates with a distorted serotonergic distribution [49,53-55]. Likewise, a circumstantial evidence in support of 5-HT involvement in autism is that defective brain serotonin levels are known to associate with many repetitive and aggressive behaviors like spinning, stepping, and self-hitting, that are common in ASD patients [56]. This view is further substantiated by studies on 5-HT with first-degree relatives of ASD patients, who have interestingly also exhibited serotonin-related psychiatric disorders, such as depression and obsessive-compulsive disorders [54,57]. In line, severity of some typical behavioral problems in ASD was linked with signaling status of the serotonin 5HT1D receptor and availability of the 5-HT precursor L-tryptophan [58]. Thus, mutations in genes encoding the rate-limiting enzymes in catabolism of L-tryptophan, like 2,3 dioxygenase gene, are believed to also underlie the alterations in serotonin levels in ASD [59]. Several studies in individuals with ASD have shown lower serotonin transporter binding capacity in various brain regions [60,61]. In another study, using an animal model of food allergy to cow's milk protein, increased levels of serotonin were detected peripherally in the intestine, while lower levels of serotonin occurred in the brain. Meanwhile, reduced dopamine levels were concurrently observed in the prefrontal cortex. Such neurotransmitter changes were associated with reduced social behavior, cognition, and increased repetitive attitude in mice, thereby reproducing ASD-coherent social malbehaviors [62]. Accordingly, the highest level of certainty for ASD relationship with monoamines was evident with the serotonergic system. Not surprisingly, thence, many clinical trials have targeted the use of serotonergic drugs to palliate symptoms of ASD. These included selective serotonin reuptake inhibitors (SSRI), 5-HT-2A receptor antagonists, tricyclic antidepressants and mixed ligands for dopamine / 5-HT receptors (like Aripiprazole). The only two FDA-approved medications against ASD, namely Risperidone and Aripiprazole, are serotonergic antipsychotics, at least in part. These medications are intended to alleviate the ASD core symptoms of irritability, disrupted sociability, and aggressive/self-injurious malbehaviors [3-5].

As said, neurotransmitter levels and effects can likewise influence neuronal developments, events that are evidently altered in many ASD patients, as documented with the disruption in pruning and fine-tuning of neurons during development. Evidence that substantiates this hypothesis came from imaging of the brains of children with autism. Thus, pathologic anomalies were identified in multiple brain regions [63], but pathologies of the cortical, limbic and basal ganglia were the most prominent. Comparing children with autism with typically-developing ones revealed that anomalies also included volumetric and metabolic differences in both of the frontal cortex and caudate nucleus [64,65]. Moreover, some reports also described abnormally diffuse and unfocused connections between the frontal cortex and caudate [66].

Hypertestosteronemia: Pathologic implications and therapeutic prospects

Autism spectrum disorder (ASD) is a well-recognized, life-long, neurodevelopmental disorder that affects 1.5% of children in average, with also a strong gender basis towards males relative to females (4.5 fold higher incidence) [67]. Investigators outlined that some individuals diagnosed with an ASD show considerable worsening in symptoms by the time of puberty [68]. Among the symptoms that deteriorate were disruptive behavior, destructiveness, restlessness, and impairment of essential social and academic proficiency. Therefore, many studies have envisaged that elevated male hormones (androgens) may play a clinically important role in the condition of ASD-affected patients, and that reduction of androgens in individuals diagnosed with ASD would significantly ameliorate such ASD-linked clinical symptoms [69]. Studies revealed that gonadotropin-releasing hormone (GnRH) analogues, like leuprolide acetate, evoked gonadal suppression (lower testosterone levels) in ASD patients that associated with milder symptoms of anxiety, sexual desire, aggressive behaviors, agitation, restlessness, and obsessive manners [70].

Immune-inflammatory rage in ASD: Evidence from proteomics

Immune dysfunction may well translate to defective neurodevelopment, altered cognitive function and distorted behavior, events that are typical of ASD. Furthermore, ASD patients have consistently displayed both immune dysregulation and inflammation at diverse cellular, molecular and genetic levels that included brain

tissues from ASD patients. The cytokines IL-2 and IL-4 are known to trigger repetitive and cognitive behaviors. Thus, mice injected with IL-2 underwent a "repetitive" climbing behavior, consonant with features of ASD [71,72].

Proteomics, an analogous term with genomics, is the large-scale study/screening of proteins and their functions. Thus, the "proteome" is the entire set of proteins produced by an organism or system. **Proteomics analyses revealed** escalation of several immune proteins and pro-inflammatory cytokines in plasma of ASD patients, [71,73,74]. Likewise, rises of some pro-inflammatory cytokines were found in postmortem frontal cortex brain samples of ASD patients [75]. Compelling evidence, as well, indicated that alterations in immune mediators that arise at early stage of growth can modify aspartate (NMDA) receptor-mediated excitatory synaptic transmission, a scenario that was described in ASD [76]. Microglias, the first and main line of active immune defense of the CNS, may also release proinflammatory cytokines and free radicals if chronically activated [77]. In autism, microglial activation, along with changes in microglial morphology and their gene expression were evident in numerous brain areas linked to behavior and cognition [78,79]. Taken together, while there is a compelling evidence indicating a pivotal role for the immune system in ASD, future studies are requested to dissect out its exact contribution, delineate its temporal involvements, and advance such findings into medical interventions.

Metabolic Flaws in ASD

Differential diagnostic and pathogenic impacts; and the role of metabolomics therein. Background

Because ASD is a heterogeneous syndrome that involves a plethora of neurological, developmental, and organ abnormalities, it seems to have no unique and well-defined metabolic disruptive pattern. Alternatively, in ASD-affected individuals, there can be some possibilities of metabolic and physiological aberrations that could contribute to symptoms, cellular injury, and vulnerability to stress and noxious environmental factors. The most prominent and influential anomalies in ASD include OS, impaired mitochondrial metabolism, defective fatty acid metabolism/distribution pattern; and, accordingly, immune-inflammatory flares [80,81]. However, interestingly, for the currently reported forthcoming metabolic alterations in ASD, the data obtained from the periphery (blood or tissues) may not necessarily be replicated in the "CNS" of children with ASD, thereby reflecting the complexity and diversity of the disrupted regulatory mechanisms in ASD.

Undoubtedly, the recent advent of Omics studies to ASD has largely availed prompt, massive high-throughput screening of multiple metabolites wherein flaws can be spotted and comparatively interpreted to come up with novel candidates for subsequent scrutiny as a clinical target.

Metabolomics studies: Pathobiologic, diagnostic and therapeutic utilities

The "metabolome" is the compilation of all metabolites (metabolic products) in a biological system (cell, organ or organism). Thence, metabolomics is the scientific assessment of cellular metabolic changes in response to a particular challenge like a disease, stress or a chemical. Recent advances in DNA sequencing and mass spectrometry technologies, as per metagenomics and metabolomics, have allowed us to integrate knowledge from different systems, thereby permitting the unfolding of many health-related mysteries. Moreover, these breakthroughs availed prompt holistic approaches to study the inter-relationship, interaction and causality among many diverse metabolites; as well as their correlations with definitive physiologic or pathologic condition (disease). This has particularly been of prime application to idiopathic or complicated/heterogeneous disorders such as diabetes, multiple sclerosis, and autism (ASD) [81,82]. Although several biochemical markers have been associated with ASDs, there is still no specific laboratory test for these conditions. Metabolomics studies are committed to extensive, prompt and precise screening to identify likely altered metabolites in ASD that could be of diagnostic and/or therapeutic promise [83,84].

Using metabolomics, a representative study [82] aimed to identify a fingerprint pattern of metabolic perturbance in ASD, using urinary specimens from ASD children against age-matched controls. Empowering a combination of liquid- and gas-chromatography-based mass spectrometry, 53 metabolites were found to be significantly different in the ASD group. Of which, the levels of crucial biologically-active aminoacids such as glycine, serine, threonine, alanine, histidine, glutamate and the organic acid taurine, were appreciably lower in ASD children. Similarly, the level of antioxidant carnosine was also lower in ASD children. Taurine is an inhibitory aminoacid whose plasma level is increased in autism [85]. It was suggested that its higher level is a compensatory phenomenon for the raised (excitatory glutamate) levels in ASD [86]. An also relevant value for taurine is its metabolic cross-talk with the ASD-involved antioxidant "glutathione" [87]. Furthermore, numerous gut bacterial marker metabolites (microbiomes) were notably lower in ASD children, who also concurrently showed gastrointestinal dysfunction. Thus, collectively, this metabolomics study managed to detect abnormal metabolites in ASD at three different aspects, namely; aminoacid metabolism, oxidative stress, and gut-microbiomes.

Another study had targeted the aminoacid tryptophan, an essential nutrient and a precursor to many crucial biological metabolites such as serotonin, quinolinic acid, and kynurenic acid, which direct neurodevelopment and synaptogenesis [88]. In addition, quinolinic acid gives rise to the coenzyme NAD+, a vital energy carrier in mitochondria, while tryptophan metabolic pathways afford the coenzyme NADH. Notably, likewise, the levels of quinolinic and kynurenic acid are strongly influenced by the activity of the immune system. Therefore, tryptophan metabolism entails profound biologic implications that culminate into brain development, neuroimmune activity and mitochondrial function.

In this study, metabolic profiling of lymphoblastoid cells from several ASD patients (against controls) showed a reduced generation of NADH, when tryptophan was the sole energy source of these cultured cells. These findings largely concurred with the behavioral traits and clinical picture of the ASD cell-donors [88]. Furthermore, the low level of NADH in the presence of tryptophan was not observed in cell lines from non-ASD patients with other intellectual disability, schizophrenia or even ASD conditions that lack the behavioral traits. The authors further indicated that analysis of a previous gene expression study showed abnormal levels of genes involved in tryptophan metabolic pathways in 10 patients. Thus, diminished tryptophan metabolism virtually provides a robust biochemical basis and possible trigger towards a diagnostic approach for ASD. Altogether, these metabolomics studies open new horizons in planning, approaching, undertaking and delineating aspects of metabolic derangements in

ASD, and, therefore spur further specialized studies to substantiate the therapeutic and clinical values of these findings.

Oxidative stress: impact on ASD manifestations, and diagnostic biomarkers

Oxidative stress (OS), excessive availability of oxidative (destructive) free-radicals and reactive-oxygen/nitrogen species, interferes with cellular/protein integrity and functions, thereby exacerbating tissue damage and inactivation of biologically-important proteins like enzymes, hormones, neurotransmitters, receptors and transporters. Unequivocally, OS has been documented with most of ASD children, and also correlated well with the severity of autistic abnormalities. Accordingly, in ASD, aspects and biomarkers of OS like lipid peroxidation, risen rate of DNA damage and deficient-antioxidant enzymes (Catalase, Superoxide-dismutase, glutathione peroxidase) or -reduced-glutathione (a cellular protector) in blood/urine have been widely reported. Levels of these OS markers correlated well with vulnerability to oxidative hemolysis and ASD symptoms, albeit via diverse mechanisms and manifestations [89,90].

General biomarkers of OS, not only confined to ASD, include "**reduced glutathione**", **lipid-peroxides**" and **glutathione peroxidase**, which can be measured in the blood and/or the urine. Although not-specific enough as diagnostic markers of ASD, they reflect a serious oxidation condition (OS) that mandates supplementation of antioxidants to patient to block further clinical deteriorations [79,91]. A more reliable and sensitive biomarker of OS that showed high credibility in ASD, has been *F2t-Isoprostanes (F2-IsoPs),* which can be determined either in blood or in urine, and has been found indicative of impaired cognitive function, development, and disrupted behavior for ASD subjects [92].

Plasma *3-chlortyrosine (3CT)*, reflects both reactive nitrogen species and myeloperoxidase activity (a proinflammatory enzyme), and appears to escalate in ASD patients primarily with mitochondrial dysfunction [93], thereby indicating a mitochondrial-driven OS and inflammation. Another, also mitochondrial OS marker, is **3-Nitrotyrosine (3NT)**, which rises in plasma of ASD patients in response to oxidative protein damage and neuronal death, thereby also concurring with the extent of behavioral anomalies and aggression observed with ASD patients [94]. Lately, in ASD patient blood, increases were detected in levels of **thioredoxin (TRX)**, a redox-regulating protein with antioxidant activity, indicating the utility of this protein as an oxidative-stress marker in autism [95].

Mitochondrial dysfunction: Contribution to ASD, and biomarkers

Because mitochondria are the most important cellular organelles that drive "aerobic metabolism", "energy production and storage" and "respiration", their dysfunction can entail a plethora of physical and functional disorders including neurodegeneration, as typically observed in ASD. Not surprisingly, many children with ASD demonstrated a variety of mitochondrial dysfunction consonant with the severity of ASD, thereby attesting to causality among the two events [96,97]. Interestingly, the underpinnings (molecular basis) of mitochondrial dysfunctions have been likewise verified in ASD. Many such studies implied that in patients with ASD mitochondrial dysfunctions are predominantly acquired rather than genetic [96]. Accordingly, subsequent investigations revealed a crucial role for environmental factors, OS, pesticides, medications like valproate, and heavy metals as impulsive upstream triggers to mitochondrial disruption [98-103]. Laboratory markers to diagnose mitochondrial dysfunction are based on detecting a decline of aerobic metabolism and fatty-acid oxidation; and include measurements of lactate, pyruvate and lactate-to-pyruvate ratio, carnitine, creatine kinase (CK), and the transaminase enzymes AST and ALT [97].

Fatty acid (FA) composition in ASD: a biomarker of membrane stability and function.

Initial experimental research with animal models of neurological diseases indicated altered brain lipid and FA profiles, which also prompted clinical research in this direction [104]. Consequently, it turned out that patients with ASD showed reduced proportions of poly-unsaturated fatty acids (PUFA) in brain tissues and RBC-membranes, thereby reducing fluidity of membranes in these cells [105,106]. Likewise, in ASD, there has been a rise in ω6/ω3 FA ratio, indicative of erroneous FA synthesis, elongation and metabolism [107]. Such FA anomalies correlated positively with the incidence and extent of ASD mal-behavior and aggression, and were found mostly of non-genetic origin. It is noteworthy also that FA-evoked glitches with ASD patients can turn more serious when the mitochondrial membranes are affected [108]. To assess altered FA composition and membrane fluidity, analysis of RBC FA-composition and unsaturation (double bond) sites has been a useful tool and a diagnostic marker to judge the integrity and stability of cellular membranes in vital tissues, including the brain [105-108].

Conclusion and Future Directions

The past decade has witnessed unprecedented advances in the understanding of ASD's etiology and pathophysiology that was availed by empowering a set of extensive, automated, high throughput Omics-based sciences [109-111]. Thus far, genomics, epigenetic, proteomics and metabolomics studies on ASD have demonstrated the complexity of etiology and heterogeneity of clinical picture among ASD patients. Moreover, the recently reported rises in ASD prevalence, along with deficient or narrow-scoped drug therapy, pose an urgent challenge to revolutionize ASD research and translate findings into remedy. The only two FDA-approved drug-therapies, thus far, belong to conventional psychotropics, and have shown feeble success in rectifying all of the ASD-pertaining malbehaviors, on top of their considerable adverse reactions. While antiandrogen therapy has conferred some promise, it remains of confined scope to cases with hypertestosteronemia, and is still under assessments. Therefore, future efforts in the area of experimental and clinical therapeutics are warranted. Another challenge and urgent need is to come up with a specific diagnostic biomarker/s to enable early diagnosis and rational, evidence-based interventions, thereby reducing the burden of ASD on patients and their families.

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