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Review

Mitochondrial dysfunction in autism spectrum disorders: Cause or effect?

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ABSTRACT

Autism Spectrum Disorders encompass severe developmental disorders characterized by variable degrees of impairment in language, communication and social skills, as well as by repetitive and stereotypic patterns of behaviour. Substantial percentages of autistic patients display peripheral markers of mitochondrial energy metabolism dysfunction, such as (a) elevated lactate, pyruvate, and alanine levels in blood, urine and/or cerebrospinal fluid, (b) serum carnitine deficiency, and/or (c) enhanced oxidative stress. These biochemical abnormalities are accompanied by highly heterogeneous clinical presentations, which generally (but by no means always) encompass neurological and systemic symptoms relatively unusual in idiopathic autistic disorder. In some patients, these abnormalities have been successfully explained by the presence of specific mutations or rearrangements in their mitochondrial or nuclear DNA. However, in the majority of cases, abnormal energy metabolism cannot be immediately linked to specific genetic or genomic defects. Recent evidence from post-mortem studies of autistic brains points toward abnormalities in mitochondrial function as possible downstream consequences of dysreactive immunity and altered calcium (Ca^{2+}) signalling.

1. Introduction

Autism Spectrum Disorders (ASDs), encompassing Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (PDDNOS), represent a group of severe neurodevelopmental disorders characterized by variable degrees of impairment in language, verbal and non-verbal communication, and social skills, as well as by repetitive behaviors and an excessive insistence on sameness, with onset prior to three years of age [1]. Collectively, the incidence of ASDs has dramatically risen during the last two decades from 2-5/10,000 to approximately 1-2/1000 children: broader diagnostic criteria and increased awareness in the medical community have certainly contributed to determine this trend, but a real increase in incidence possibly due to environmental factors cannot be excluded [2,3]. These disorders are often addressed as discrete clinical entities, but they should instead be viewed as a continuum, ranging from minimal autistic traits to severe autism [4].

No neuropsychiatric disorder displays genetic underpinnings as prominent as those documented by family and twin studies for autism, with heritability estimates above 90% and sibling recurrence risk as high as 3–6% for strictly defined autism [3]. However, only in approximately 10% of cases the disease is "syndromic", i.e. secondary to a known genetic disorder [3,5], whereas in the vast majority of patients, the origin of "non-syndromic", "primary" or "idiopathic" autism remains unknown. Genome-scans unveiled the existence of approximately 15–20 loci contributing to non-syndromic autism, albeit in complex fashion due to genetic heterogeneity, incomplete penetrance, phenocopies, gene–gene and gene–environment interactions [3].

The cytoarchitectonic abnormalities present in autistic brains are most compatible with reduced programmed cell death and/or increased cell proliferation, abnormal cell migration, and altered cell differentiation with reduced neuronal size, all pointing toward the first/second trimester of pregnancy as the critical time for deranged neurodevelopment in autism [6,7]. The detection of fine motor symptoms already on the day of birth or very early on in neonates later diagnosed with an ASD converges with neuropathological findings in dating prenatally the origin of the disease, although behavioral symptoms typically appear at 6–24 months [8–10]. Finally, large subgroups of ASD patients also display systemic signs and symptoms, including macrosomy [11], non-specific enterocolitis [12], immune dysreactivity [12,13] and renal oligopeptiduria [14]. Autism thus involves primarily, but not exclusively, the central nervous system (CNS) and should be viewed as a multi-

Abbreviations: AGC, aspartate/glutamate carrier; ASD, autism spectrum disorders; CK, creatine kinase; CNS, central nervous system; CNV, copy number variant; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malonyldialdehyde; OXPHOS, oxidative phosphorylation; ROS, reactive oxigen species; SAH, H-adenosylhomocysteine; SAM, S-adenosylmethionine

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organ systemic disorder encompassing several developmental components.

2. Primary mitochondrial disorders and autism

Interest into possible links between mitochondrial abnormalities and autism was initially spurred by the deleterious consequences of mitochondrial disorders on neurodevelopment. Indeed, mitochondrial disorders often result in CNS dysfunction, leading to developmental regression, learning disability, and various behavioural disturbances. Autism can indeed represent the main clinical presentation of a mitochondrial disease [15]. However, the clinical manifestations of mitochondrial disorders, even in the presence of well characterized mitochondrial DNA (mtDNA) mutations, are extremely heterogeneous, ranging from lesions affecting single tissues or organs, such as the optic nerve in Leber's hereditary optic neuropathy (LHON), or the cochlea in maternally inherited non-syndromic deafness, to myopathies, encephalomyopathies, cardiopathies, or complex multisystem syndromes with onset occurring anytime from neonatal to adult life [16]. Adult patients usually show signs of myopathy, associated with variable involvement of the CNS (ataxia, hearing loss, seizures, polyneuropathy, pigmentary retinopathy and, more rarely, movement disorders). Instead, children most frequently display severe psychomotor delay and generalized hypotonia, but symptoms can range from isolated myopathies, sometimes associated with cardiopathies, up to fatal multisystem syndromes [17,18]. The occurrence of muscle 'ragged red fibres' (RRFs), characterized by a segmental proliferation and accumulation of abnormal mitochondria under the sarcolemmal membrane, perhaps represents the best known morphological hallmark present in many, albeit not all, of these syndromes. Another common finding is the presence of muscle fibres that stain negative using cytochrome oxidase (COX, respiratory complex IV) histochemistry. However, these typical 'mitochondrial' clues may be absent in otherwise demonstrated mitochondrial disorders, such as LHON, and neuropathy ataxia and retinitis pigmentosa (NARP). The same also occurs in many paediatric cases: lactic acidosis and muscle tissue histology (even at the level of electron microscopy) will be negative in the majority of affected children, who rarely show "ragged red fibers" [15]. Finally, molecular investigations still fail to identify the responsible gene defect in 50% of adults affected by biochemically- and/or morphologically-defined mitochondrial disease. The percentage of undiagnosed cases increases to 80–90% for paediatric disorders [16].

Genetic mutations in mtDNA have been associated with myopathy, cardiomyopathy, neuropathy, seizures, optic atrophy, strokes, hearing loss, diabetes mellitus, and other clinical features [16]. In some cases, autism can directly stem from mutations in mtDNA, as documented in the following studies:

1) Pons et al. [19] reported two ASD patients carrying the 3243A>G mutation, located in the mtDNA $tRNA^{Leu(UUR)}$ gene. The same mutation was also present in the two mothers of two other autistic children, whose peripheral tissues available for investigation did not unveil this mutation. The 3243A>G mutation typically causes mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), but has also been associated with maternally inherited progressive external ophthalmoplegia, and with developmental delay and seizures [19]. The autistic phenotype in these mutation carriers appears highly heterogeneous. The behavioral triad of autistic disorder is often (but not always) associated with different combinations of neurological signs or symptoms, including developmental delay, clumsiness, attention deficit, neurologic deterioration during intercurrent illnesses especially in the presence of fever, microcephaly or macrocephaly. A fifth child presented with mitochondrial DNA depletion, which is typically associated with muscle hypotonia, seizures, myoclonus, and developmental delay. Notably, family history was generally positive for mitochondrial diseases in the maternal lineage, except in the presence of mitochondrial depletion.
2) Graf et al. [20] described one autistic patient carrying the 8363G>A mutation, affecting the mtDNA $tRNA^{Lys}$ gene. This patient was characterized by behavioral regression at age 2, extreme hyperactivity, lack of attention, mild fine and gross motor dyspraxia. His sister was severely affected with partial complex seizures, unsteady gait, myoclonus, swallowing dysfunction, and moderate cognitive impairment. Importantly, muscle biopsies unveiled 86% and 61% of mitochondrial DNA mutated in this girl and in the autistic proband, respectively, highlighting a probable dosage effect. One of the patient's two maternal half-sisters suffered from seizures, learning disabilities, fine tremors and mild motor dyspraxia. Another maternal half-sister and his mother were neurologically and psychiatrically healthy.
3) Weissman and Colleagues [21] reviewed the medical records of 25 autistic children with evidence of a definite or probable ($N=21$ and 4, respectively) mitochondrial disorder. In addition to the three core diagnostic features of autism (impaired communication, social interactions, and repetitive behaviours or restricted patterns of interest), these patients presented additional signs and symptoms in various combinations, most commonly including:

   (a) excessive fatigability and/or exercise intolerance ($N=19$, 76%);
   (b) gastrointestinal dysfunction ($N=16$, 64%), in the form of gastroesophageal reflux ($N=9$, 36%) and/or constipation ($N=8$, 32%);
   (c) structural or functional cardiovascular abnormalities ($N=7$, 28%);
   (d) facial dysmorphisms ($N=6$, 24%);
   (e) microcephaly ($N=4$, 16%) or macrocephaly ($N=4$, 16%);
   (f) marked developmental gross motor delays ($N=8$, 32%); and
   (g) growth retardation ($N=5$, 20%).

Clinical heterogeneity was paralleled by heterogeneity at the biochemical and genetic levels. Lactate levels were elevated in 19 (76%) patients; defects in complexes I, II, III, and IV, assessed in muscle and/or fibroblasts, were observed in 64%, 8%, 20% and 4% of patients, respectively. Magnetic resonance imaging (MRI) was frequently positive for brain structure abnormalities, detected in 10/21 (47.6%) patients, but no single abnormality appeared specifically associated. At the genetic level, three mutations of probable pathogenetic meaning were found: (a) 3397A>G in the ND1 subunit of complex I; (b) 4295A>G in mtDNA $tRNA^{Ileu}$, and (c) 11984 T>C in the ND4 subunit of complex I. These three missense mutations all cause aminoacid changes in highly conserved regions. Three other mutations (3394 T>C, 1039C>T, 11809 T>C) have unclear pathogenetic relevance.

In addition to mtDNA mutations, gene dosage abnormalities have been described by Fillano et al. [22] in five patients with autism carrying large deletions in their mtDNA. One recurrent deletion of 7.4 kb was present in three patients, whereas the remaining two patients unveiled at least three large deletions, including the 7.4 kb deletion. Approximately 5-15% of the mitochondrial genomes extracted from blood in four of these five patients carried these deletions. This leaves open the question of whether and to what extent mtDNA may undergo deletions more frequently in blood, as compared to muscle or to nervous tissue. Three of these patients, in addition to autism, also display ataxia and/or cardiomyopathy.

Several important conclusions can be drawn from these studies:

I) *At the clinical level*, autistic patients with an underlying mitochondrial disease can display highly heterogeneous clinical pictures. Some of their signs and symptoms are not unusual in idiopathic autism: for example, macrocephaly and macrosomy are present in approximately 20% of autistic patients [11]; gastrointestinal dysmotility is relatively frequent