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The Mitochondrial Aspartate/Glutamate Carrier AGC1 and Calcium Homeostasis: Physiological Links and Abnormalities in Autism

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Abstract Autism spectrum disorder (ASD) is a severe, complex neurodevelopmental disorder characterized by impairments in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and behaviors. Recent evidence has unveiled an important role for calcium (Ca^{2+}) signaling in the pathogenesis of ASD. Postmortem studies of autistic brains have pointed toward abnormalities in mitochondrial function as possible downstream consequences of altered Ca^{2+} signaling, abnormal synapse formation, and dysreactive immunity. *SLC25A12*, an ASD susceptibility gene, encodes the Ca^{2+}-regulated mitochondrial aspartate–glutamate carrier, isoform 1 (AGC1). AGC1 is an important component of the malate/aspartate shuttle, a crucial system supporting oxidative phosphorylation and adenosine triphosphate (ATP) production. Here, we review the physiological roles of AGC1, its links to calcium homeostasis, and its involvement in autism pathogenesis.

Keywords Aspartate–glutamate carrier · Autism · Autistic disorder · Calcium signaling · Mitochondria · SLC25A12

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Introduction

Calcium ions represent one of the most versatile, ancient, and universal biological signaling systems known to regulate countless physiological phenomena, ranging from membrane potential to ion transporters, kinase activities, and transcription, among many others. In brain, calcium signals are essential not only for elementary forms of neuronal communication, such as synaptic transmission, but also for functions as complex as memory-related neuronal plasticity. Mitochondrial activity and Ca^{2+} signaling hold an intense cross-talk. On one hand, it has been firmly established that mitochondria participate in Ca^{2+} homeostasis by efficiently accumulating Ca^{2+} down the electrochemical gradient established across the inner mitochondrial membrane by the activity of the respiratory complexes. Mitochondrial Ca^{2+} accumulation is a tightly controlled process, in turn regulating functions as diverse as aerobic metabolism and induction of cell death [1]. On the other hand, intracellular Ca^{2+} levels modulate mitochondrial activity in two different ways: (a) *Ca^{2+} entry into mitochondria* via the Ca^{2+} uniporter, or rapid uptake mode mechanisms (by sequestering physiological-type pulses of Ca^{2+}) [2–4], is followed by (1) the Ca^{2+}-mediated activation of three dehydrogenases (NAD^+-isocitrate-, 2-oxoglutarate-, and pyruvate-dehydrogenase)

in the mitochondrial matrix [5]—this process causes an increase in mitochondrial NADH/NAD ratios, resulting in enhanced energy production; matrix Ca^{2+} has also been suggested (2) to activate ATP synthesis through a direct effect on F0F1-ATPase [6]; (b) even *without entering mitochondria*, Ca^{2+} modulates mitochondrial activity by regulating either (1) the mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase (FAD-GPDH) or (2) the activity of Ca^{2+}-dependent mitochondrial carriers (CaMCs), including the aspartate–glutamate carrier (AGC) and the ATP-Mg/Pi carrier. FAD-GPDH, together with cytoplasmic NAD-linked GPDH, acts as the glycerol-3-phosphate shuttle that transports reducing hydrogen equivalents into the mitochondria [7, 8]. FAD-GPDH is located at the outer face of the mitochondrial inner membrane and contains EF-hand motifs at the C-terminal end which are responsible for the activation by Ca^{2+} [9]. CaMCs are inserted into the internal mitochondrial membrane; their N-terminal end harbors several EF-hands (i.e., the archetypical calcium-binding motif) and leans into the intermembrane space, which separates the external and internal mitochondrial membranes. Ca^{2+} levels in the intermembrane space are in equilibrium with cytosolic Ca^{2+} and can activate CaMC-mediated transport across the inner mitochondrial membrane. Recently, the existence of a strong link between Ca^{2+} (dys)regulation, mitochondria, and cellular derangement in neurodegenerative disorders [10] has spurred further interest in the molecular mechanisms underlying the cross-talk between mitochondrial activity and Ca^{2+} homeostasis.

We shall now review the bidirectional links between mitochondria and Ca^{2+}, both in physiology and within the context of autism spectrum disorder (ASD), a severe neurodevelopmental disorder diagnosed in early childhood, where excessive intracellular Ca^{2+} signaling, abnormal mitochondrial activity, and enhanced oxidative stress appear to play important pathogenetic roles. Additional information specifically on excessive Ca^{2+} signaling in autism has been reviewed by Krey and Dolmetsch [11], whereas mitochondrial abnormalities in autistic patients have been reviewed in detail by Palmieri and Persico [12].

Insights into the Genomics and the Structure of the Mitochondrial Aspartate/Glutamate Carrier AGC1

The mitochondrial aspartate–glutamate carrier, isoform 1 (AGC1), encoded by the solute carrier family 25, member 12, gene (*SLC25A12*) located on chromosome 2q31 (Fig. 1), catalyzes an exchange between intra-mitochondrial aspartate and cytosolic glutamate, an important step in urea synthesis [13]. As a component of the malate–aspartate shuttle, AGC1 also has a role in the transfer of NADH-carried reducing equivalents from the cytosol into the mitochondrial matrix. In humans, there are two AGC isoforms: AGC1 and AGC2 (also named "aralar" and "citrin", respectively). AGC1 is expressed as 2.9- and 3.2-kb mRNAs predominantly in heart and skeletal muscle, with weaker expression in brain and kidney [14]. AGC1 is the main AGC isoform present in the adult brain, and it is expressed mainly in neurons. [14, 15]. The N-terminal portion of its predicted 678-amino acid sequence contains four EF-hand Ca^{2+}-binding domains, which were conclusively shown to bind Ca^{2+} in vitro and in vivo [16, 17]. Instead, the C-terminal half of AGC1, like the other members of the mitochondrial carrier family (MCF), contains six transmembrane domains and the characteristic MCF sequence motif [18]. Immunocytochemistry and cell fractionation studies showed that both exogenously expressed and endogenous AGC1 protein is localized within the mitochondria, inserted into the inner mitochondrial membrane with the N-terminal portion protruding in the intermembrane space [13].

Physiological Roles of AGC1

AGC is required for the transfer of mitochondrial aspartate to the cytosol in the malate–aspartate NADH shuttle (MAS) [19] (Fig. 2). MAS is also required during lactate metabolism and glycolysis to transfer reducing equivalents from the cytosol into the mitochondrial matrix. MAS consists of four enzymatic components: (1) malate dehydrogenase (cMDH, mMDH), located in the mitochondrial matrix and intermembrane space; (2) aspartate aminotransferase (cAAT and mAAT), also located in the mitochondrial matrix and

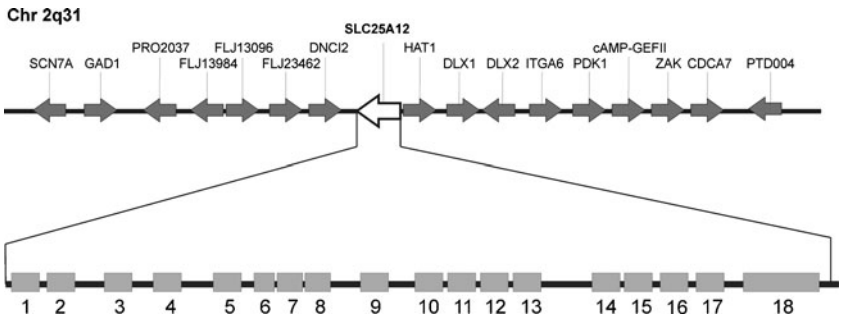

Fig. 1 Chromosomal position and genomic structure of the *SLC25A12* gene, which encodes the mitochondrial aspartate/glutamate transporter AGC1

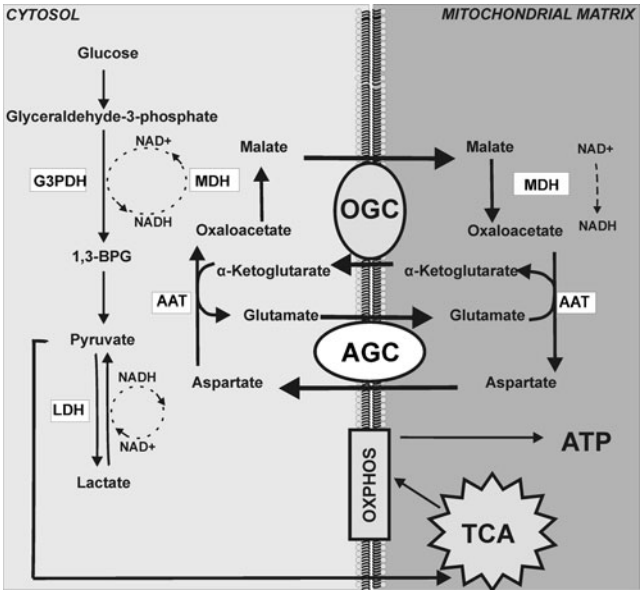

Fig. 2 The malate–aspartate shuttle (MAS) and ATP generation. MAS is the main pathway for the transfer of reducing equivalents in the form of NADH from the cytosol into the mitochondria. Cytoplasmic malate dehydrogenase reduces oxaloacetate to malate while oxidizing NADH to NAD^+. Malate then enters the mitochondrion where the reverse reaction is carried out by mitochondrial malate dehydrogenase. Movement of mitochondrial oxaloacetate to the cytoplasm is maintained by transamination to aspartate by aspartate aminotransferase with the amino group being donated by glutamate. The 2-oxoglutarate (α-ketoglutarate) generated leaves the mitochondrion for the cytoplasm. External mitochondrial membrane is not depicted since it is freely permeable to all indicated metabolites. *1,3-BPG* 1,3-bisphosphoglycerate, *AGC* aspartate/glutamate carrier, *AAT* aspartate aminotransferase, *G3PDH* glyceraldehyde-3-phosphate dehydrogenase, *LDH* lactate dehydrogenase, *MDH* malate dehydrogenase, *OGC* α-ketoglutarate/malate carrier, *TCA* tricarboxylic acid cycle

intermembrane space; (3) the malate-alpha-ketoglutarate antiporter (OGC); and (4) the glutamate–aspartate antiporter (AGC), both inserted into the inner mitochondrial membrane. MAS exerts its action in many tissues including liver and heart [20, 21]; in the latter, it is also the dominant NADH shuttle, with AGC1 expressed preferentially in atria [14].

Experimental evidence also points toward an important role for MAS in heart development during fetal and perinatal growth; MAS activity measured in mitochondria from neonatal porcine heart is about three times higher than MAS measured in adult heart [22, 23], where fatty acid metabolism bypasses the NADH shuttles. Strikingly, the activity of the heart mitochondrial Ca^{2+} uniporter undergoes a similar decrease along postnatal life in the rat [24]. However, despite these developmental changes in MAS activity, AGC transcripts and their protein products display higher levels in adult heart [23, 25].

MAS plays also a crucial role in the physiology of pancreatic β-cells by influencing glucose-induced activation of mitochondrial metabolism and insulin secretion [26–28], as shown in mice [29]. In particular, AGC1 is the only AGC isoform present in pancreatic islets and β-cells [21, 30], and its overexpression in INS-1E or pancreatic β-cell has been shown to significantly impact MAS function, leading to increased mitochondrial membrane potential and ATP levels with a concomitant reduction in mitochondrial NAD(P)H [30]. Conversely, *SLC25A12* knock-down in INS-1 β-cell lines resulted in undetectable levels of AGC1 protein and complete loss of MAS activity in isolated mitochondria and in significant decrease in glucose-stimulated insulin secretion [31]. When both shuttles were halted in mGPDH-deficient islets treated with aminooxyacetate, an inhibitor of the malate–aspartate shuttle, glucose-induced insulin secretion was almost completely abrogated [29].

MAS is also involved in early embryonic development. Pyruvate, but not lactate, can be used as an energy source in mouse zygotes (i.e., one-cell stage embryos), whereas after the first cleavage division, two-cell embryos can also use lactate [32]. Interestingly, zygotes practically lack MAS activity, which becomes quite active at the two-cell stage. This is not due to blunted gene expression, but rather to differences in levels of cofactors modulating enzymatic activities and resulting in the high K_m of the cytosolic aspartate aminotransferase (AAT in Fig. 2) [33]. In this scenario, aspartate becomes the rate-limiting factor: AGC may thus limit MAS function and the transfer of reducing equivalents at this particular developmental stage. In fact, raising cytosolic aspartate concentrations in zygotes by setting aspartate concentrations at 10 mM in their culture medium restores MAS activity. This, in turn, allows zygotes to use lactate as an energy substrate without rapidly developing NAD^+ depletion as occurs when MAS activity is lacking [33].

The relevance of AGC1 in the physiology of neurons has been elucidated by studies carried out in animal models. AGC1 is expressed in the adult CNS, particularly in neuron-rich areas and in the spinal cord, with no detectable expression in white matter [34]. The finding of high AGC1 levels and MAS activity in neurons agrees with earlier findings of high shuttle activity in synaptosomes [35] and its requirement for lactate utilization [36]. AGC1 knock-out mice display impaired motor coordination [37], a defect seemingly due to a marked decrease in myelin and in myelinated fibers across the central nervous system (CNS). This reduction in myelination is attributable to the role of AGC1 in the synthesis of brain aspartate and N-acetylaspartate (NAA). NAA is of paramount importance in myelin sheet formation by supplying acetyl groups [38–41]. Moreover, transaxonal transfer of NAA from neurons to oligodendrocytes has been postulated since neurons are the main producers of NAA in the adult brain, whereas the myelin sheet is obviously synthesized by oligodendrocytes.

AGC1 deficiency procures a large drop in NAA and aspartate levels both in the brain and in primary neuronal cultures derived from AGC1(−/−) mice, with a concomitant decrease in myelin lipid concentrations and in myelin-associated proteins (myelin basic protein and myelin-associated oligodendrocytic basic protein) [37]. Interestingly, the peripheral nervous system of AGC1(−/−) mice displays a significant decrease in myelin lipids and in myelin-associated proteins (MBP and OMGP), whereas it does not present alterations in the expression of galactocerebrosides, the basic component of myelin produced by oligodendrocytes. This dichotomy further confirms the selective role of neurons in providing oligodendrocytes with NAA, which will in turn provide acetyl groups for fatty acid synthesis and aspartate for protein synthesis. Thus, the reduction of brain aspartate levels and the lack of aspartate production in the brain mitochondria of AGC1(−/−) mice [37] suggest that a major route of aspartate production in the CNS is mitochondrial and that it depends on AGC1 for aspartate efflux into the cytosol. Accordingly, humans devoid of AGC1 display profound cerebral hypomyelination [42].

Calcium Regulation of AGC1 Activity

It is widely believed that increased cytosolic Ca^{2+} exerts a parallel activation of extramitochondrial ATPases and of oxidative phosphorylation (OXPHOS), thereby precisely balancing ATP consumption and production without major changes in ADP concentration [5, 43–47]. Ca^{2+} transport into the mitochondrial matrix and the subsequent activation of distinct intramitochondrial dehydrogenases [5, 43, 44, 48, 49] and of F0F1ATPase [46, 50] were assumed, until recently, to constitute the main regulatory mechanism of mitochondrial respiration and OXPHOS. Recently, Ca^{2+} has been found to regulate the glutamate-dependent state 3 respiration, by adjusting the supply of glutamate to mitochondria via AGC1 activity [51, 52]. As previously described, AGC1 has several Ca^{2+}-binding EF-hand motifs located on its long, hydrophilic amino-terminus which extends into the intermembrane space [13, 17, 53]. Further, the activity of AGC1 is strongly regulated by *extra*mitochondrial Ca^{2+} (which is in equilibrium with intermembrane Ca^{2+} levels), with an $S_{0,5}$ of 300 nM Ca_{free}^{2+} [53]. This value is in line with the $S_{0,5}$=260 nM measured for the *extra*mitochondrial Ca^{2+}-mediated activation of glutamate-dependent respiration of brain mitochondria [51, 52]. The electrogenic nature of AGC1 activity is realized by the co-transport of protons in addition to glutamate, making this carrier unidirectional [54]. Affinity is lower for glutamate than for aspartate (K_M=0.2 and 0.05 mM, respectively); the transport rate constant increases with membrane potential, while substrate affinities remain unchanged [13].

Calcium Homeostasis and AGC1 Activity in Autism Spectrum Disorder

A body of recent genetic evidence suggests that at least some ASD cases may result from abnormal Ca^{2+} homeostasis during neurodevelopment. Moreover, several genetic studies have identified autism-related genes encoding proteins either directly or indirectly controlling intracellular Ca^{2+} levels or regulated by cytosolic Ca^{2+} transients (Table 1). These molecules include ion channels, receptors, and Ca^{2+}-regulated signaling proteins, often times crucial to CNS development (Fig. 3). Gain-of-function mutations in the L-type voltage-gated Ca^{2+} channel $Ca_v1.2$ (*CACNA1C*) cause Timothy syndrome, a multisystem disorder including mental retardation and autism [55]. Similarly, mutations in the L-type voltage-gated Ca^{2+} channel $Ca_v1.4$ (*CACNA1F*) cause the incomplete form of X-linked congenital stationary night blindness (CSNB2): gain-of-function mutations cause CSNB2 frequently accompanied by cognitive impairment and either autism or epilepsy, whereas CSNB2 inactivation due to loss of-function mutations is not accompanied by neurological symptoms [56]. All of these gain-of-function mutations prevent voltage-dependent channel inactivation, leading to excessive Ca^{2+} influx. Also, mutations indirectly yielding increased cytosolic Ca^{2+} levels or amplifying intracellular Ca^{2+} signaling by hampering Ca^{2+}-activated negative feedback mechanisms have been found associated with autism [8, 57]. The bioelectrical instability resulting from these mutations nicely parallels the high prevalence of seizures and/or EEG abnormalities present among autistic individuals.

Apart from these rare high-penetrance mutations in genes encoding for ion channels, autism is a complex phenotype where common variants play a crucial role in defining the underlying genetic architecture and conferring vulnerability to the disease. Polygenic models allow for gene–gene and gene–environment interactions, able to explain the large inter-individual phenotypic variability routinely encountered by clinicians and present also in the general population, where autism is distributed as a continuous trait. Within this framework, we have recently assessed possible roles for the *SLC25A12* gene and for its protein product AGC1 in autism spectrum disorder [58]. We shall now briefly summarize our findings and examine how they can serve as a paradigm to disclose possible mechanisms underlying mitochondrial dysfunction in ASD. The involvement of AGC1 in neurodevelopmental disorders is not entirely surprising since the modulation of *SLC25A12* gene expression has prominent effects on neurodevelopment: an overexpression of *SLC25A12* boosting AGC1 transport activity yields enhanced neurite growth in vitro [59]; instead, *SLC25A12* disruption alters myelination and neurofilaments [60], further underscoring the critical role played by AGC1 in myelin formation. In our

Table 1 Calcium-related genes involved in autism spectrum disorder

Gene	Locus	Function	Ca^{2+} effect	Ref.
ATP13A4	3q29	Ca^{2+}-dependent P5-type ATPase, localized to the endoplasmic reticulum	Overexpression of ATP13A4 in COS-7 cells causes a significant increase in intracellular Ca^{2+} levels	[82]
ATP2B2	3p25.3	Plasma membrane calcium ATPase type 2 (PMCA2) expressed in excitable cells	P-type Ca^{2+} pump able to extrude cytosolic Ca^{2+} with fast activation and against large concentration gradients	[79]
CACNA1C	12p13.3	Alpha-1C subunit of L-type voltage-dependent Ca^{2+} channels	Ca^{2+} channel mediating a long-lasting influx of Ca^{2+} into the cell upon membrane depolarization to sustain the action potential	[55]
CACNA1F	Xp11.23	Alpha-1F subunit of L-type voltage-dependent Ca^{2+} channels		[56]
CACNA1H	16p13.3	Alpha-1H subunit of T-type voltage-dependent Ca^{2+} channels	Ca^{2+} channel mediating a transient influx of Ca^{2+} into the cell upon membrane depolarization to trigger the action potential	[83]
KCNMA1	10q22.3	Large-conductance voltage- and Ca^{2+}-activated K^+ channel	This channel causes faster repolarization, limiting voltage-dependent Ca^{2+} entry. Intracellular Ca^{2+} regulates the physical association between alpha and beta subunits	[57]
IL1RAPL1	Xp22.1-p21.3	IL1-receptor accessory protein-like 1, interacts with presynaptic *NCS-1* and with postsynaptic *PSD-95*	Postsynaptically *IL1RAPL1* and *PSD-95* stabilize dendritic spines and excitatory synapses. Presynaptically *IL1RAPL1* and *NCS-1* control Ca^{2+}-triggered exocytosis by silencing voltage-gated N-type Ca^{2+} channels	[84–86]
NCS1	9q34	Neuronal Ca^{2+}sensor-1, interacts with *IL1RAPL1*		[87]
CAPS2	12q21.1–q21.2	Ca^{2+}-binding protein with 2 EF-hand motifs	Regulation of Ca^{2+}-triggered exocytosis of dense-core vesicles containing BDNF and NT3	[88, 89]

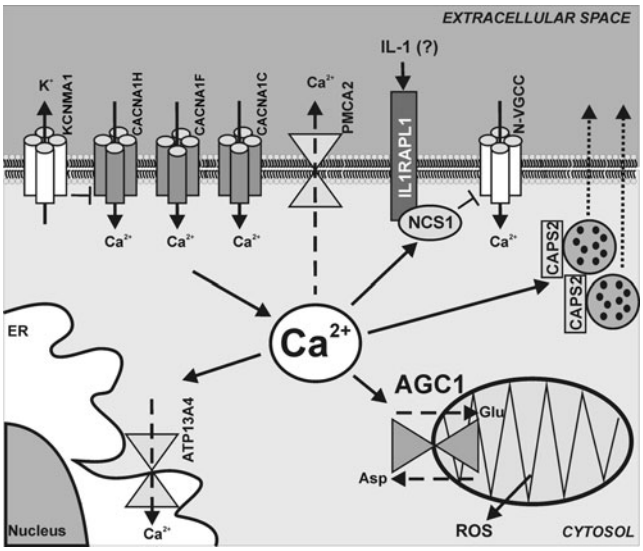

Fig. 3 Proteins encoded by calcium-related genes involved in autism spectrum disorder. A brief description of the function of each protein is provided in Table 1. *AGC1* mitochondrial aspartate/glutamate carrier, *ATP13A4* Ca^{2+}-dependent P5-type ATPase 4, *CACNA1C* alpha-1C subunit of L-type voltage-dependent Ca^{2+} channel, *CACNA1F* alpha-1F subunit of L-type voltage-dependent Ca^{2+} channel, *CACNA1H* alpha-1H subunit of T-type voltage-dependent Ca^{2+} channel, *CAPS2* Ca^{2+}-binding protein with two EF-hand motifs, *ER* endoplasmic reticulum, *IL1RAPL1* IL1-receptor accessory protein-like 1, *KCNMA1* large-conductance voltage- and Ca^{2+}-activated K^+ channel, *NCS1* neuronal Ca^{2+}sensor-1, *N-VGCC* neuronal voltage-gated calcium channel, *PMCA2* plasma membrane calcium ATPase type 2, encoded by the *ATP2B2* gene, *ROS* reactive oxygen species. ⊣ inhibition, *dashed line*—transport by a pump or by a transporter, *dotted line*—exocytosis

study, we have examined AGC transport rates, AGC1 expression levels, and *SLC25A12* genomic DNA and cDNA sequences in post-mortem temporocortical gray matter (Brodmann area 41/42 or 22) of six pairs of non-syndromic ASD patients and sex-, age-, and post-mortem interval (PMI)-matched controls [58]. Furthermore, we have performed a family-based genetic association study using genomic DNA extracted from 309 simplex and 17 multiplex families with a non-syndromic autistic proband [58]. The post-mortem study was performed on neocortical tissue from the superior temporal gyrus because this region hosts structural and functional abnormalities well documented in autistic individuals [61]. An approximately 3-fold increase in AGC transport rates was found in tissue homogenates from all six patients, compared to their matched controls. This increase was in no way correlated with a clinical history of seizures, EEG abnormalities, or with pharmacological treatment prior to death. Instead, excessive transport rates were consistently blunted by the Ca^{2+} chelator EGTA, and no difference in AGC transport rates was found in isolated mitochondria from patients and controls following removal of the Ca^{2+}-containing post-mitochondrial supernatant. Mitochondria from each control were then isolated and split into two aliquots, each exposed in parallel either to his/her own post-mitochondrial supernatant, or to the post-mitochondrial supernatant of his/her autistic match. AGC transport rates were activated 3.2- vs. 1.7-fold by the patient and control supernatants, respectively, and also this difference was blunted by EGTA. Finally, Ca^{2+} levels

measured directly in the post-mitochondrial supernatant using fura-2 were significantly higher in all six patients, compared to their matched controls [58]. A general activation of mitochondrial metabolism could be evinced by the increased expression of both AGC1 and cytochrome *c* oxidase activity in the brains of autistic patients. Furthermore, oxidized mitochondrial proteins were markedly increased in five of six patients vs. one of six controls [58].

In contrast with this wealth of biochemical data, sequencing of the genomic DNA and cDNA extracted from the same brain tissues did not reveal the existence of genetic polymorphisms in any way responsible for the biochemical abnormalities described above. In addition, we found no evidence of genetic association between AGC1 and autism in our sample. We can thus conclusively exclude that genetic variants, in the form of either non-synonymous coding mutations or common functional SNPs, represent the primary cause of the excessive AGC transport activity, mitochondrial metabolism, and, to a more variable degree, oxidative stress documented in each and every autistic brain tissue sample assessed in our study. Instead, the increased AGC1 transport activity, mitochondrial redox potential, and oxidative stress, possibly responsible for the downstream abnormalities in mitochondrial function [12], appear secondary to excessive Ca^{2+} levels in autistic brains [58]. On the other hand, unaffected brothers and sisters of autistic patients in our sample tend to inherit an *SLC25A12* haplotype which may mark a protective gene variant [58]. These results are not incompatible with genetic studies published to date, which have given conflicting results. Ramoz et al. [62] initially reported a significant association between autism and *SLC25A12* gene variants marked by the G allele at the two intronic SNPs *rs2056202* (I3-21A>G) and at *rs2292813* (I16+70A>G). This result was later confirmed by Segurado et al. [63], Turunen et al. [64], and in an extended sample recruited by Ramoz and colleagues [65], in addition to suggestive evidence of specific *SLC25A12* contributions to repetitive behaviors and rituals in autistic patients [66], which is not confirmed in our sample (Roberto Sacco and Antonio Persico, unpublished observation). On the other hand, no association was found by Blasi et al. [67], Rabionet et al. [68], Correia et al. [69], Chien et al. [70], and Palmieri et al. [58]. However, Palmieri et al. [58] is the only replication study incorporating also unaffected siblings in its experimental design and, with Rabionet et al. [68], not limiting the analysis to SNPs *rs2056202* and *rs2292813*, originally found associated with autism by Ramoz et al. [62].

Possible Roles for Other Calcium-Related Genes and for Environmental Factors

Our initial evidence, supporting abnormal Ca^{2+} homeostasis in the absence of pathogenic *SLC25A12* mutations in autism [58], spurred interest into epistatic interactions involving common variants in other Ca^{2+}-related genes. The *ATP2B2* gene, located on chromosome 3p25, appeared an especially good candidate to influence Ca^{2+} homeostasis and to interact with AGC1 in autism, as it encodes the plasma membrane calcium ATPase type 2 (PMCA2). This pump extrudes Ca^{2+} ions from the cytosol against very large concentration gradients and with faster kinetics compared to the more ubiquitous PMCA1 and PMCA4 [71] (Fig. 3). PMCA2 is thus mainly expressed in excitable cells, especially in the cerebral cortex and in cerebellar Purkinje cells [72]. Also, sensory hair cells in the human cochlea use PMCA2 to extrude Ca^{2+} flown into the cell through mechanosensitive transduction channels [73]. Interestingly, mutations in the *ATP2B2* gene have been shown to cause deafness in mice [74] and to carry in humans at least a modifier effect on hearing loss [75], which is highly prevalent among autistic individuals [76, 77]. Importantly, potential interactions between PMCA2 and AGC1 are bidirectional: on one hand, PMCA2 plays a pivotal role in determining intracellular Ca^{2+} levels, which regulate AGC1 activity; on the other hand, Ca^{2+} extrusion by PMCA2 occurs through the formation of an aspartyl phosphate intermediate [71], requiring AGC1 to provide the necessary aspartate.

Of note, recent studies clearly point toward a significant role for *ATP2B2* in autism. Gene-expression profiling of lymphoblastoid cell lines contrasting one sibling with autism and severe language impairment vs. one unaffected sibling in 21 discordant sib-pairs identified *ATP2B2* as the only differentially expressed gene among potential candidate genes located in the 3p25 chromosomal region [78]. Most importantly, our recent family-based association study involving an exploratory sample of 277 AGRE families and a replication sample of 406 families primarily recruited in Italy reports a significant association of *ATP2B2* gene variants with ASD in males [79]. Further evidence converging on the *ATP2B2* locus is also provided by a recent genome-wide association study performed by the Autism Genome Project [80].

Lastly, gene–gene interactions between *SLC25A12* and *ATP2B2* can also accommodate for environmental components involving agents able to affect Ca^{2+} homeostasis, such as polychlorinated biphenyls (PCBs). PCBs are important endocrine disruptors, known to promote Ca^{2+} entry and to enhance Ca^{2+} release from the endoplasmic reticulum by activating ryanodine receptors [81]. Prenatal PCB exposure may lead to the development of intracellular Ca^{2+} spikes of greater duration and/or amplitude in individuals carrying *ATP2B2* gene variants conferring reduced PMCA2 function and/or amounts. Excessive intracellular Ca^{2+} spikes would in turn modulate AGC1 activity, leading to abnormal energy metabolism and

enhanced oxidative stress [12]. This chain of perturbations would ultimately interfere with neuronal migration, dendritic spine formation, and synaptogenesis, especially in the parallel fiber-to-Purkinje cell synapse.

Conclusions and Perspectives

AGC1 plays a pivotal role in adjusting mitochondrial energy production to functional demands in excitable cells, through its responsiveness to cytosolic Ca^{2+} levels. AGC1 also exerts a profound influence on neurodevelopment not only through this mechanism but primarily by supplying some of the building blocks needed for myelin synthesis. Understanding these physiological functions and the underlying molecular links has been instrumental to begin addressing AGC1 contributions to disease states. *SLC25A12*, the gene encoding AGC1, is a putative autism susceptibility gene, and in numerous diseases, including autism, cellular Ca^{2+} homeostasis is dysregulated. Our recent progress in understanding abnormal Ca^{2+} homeostasis and mitochondrial functions in ASD will eventually allow investigators and clinicians to delineate whether and to what degree targeted therapeutic and preventive approaches can be designed on the basis of a strong pathophysiological foundation.

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Conflict of Interest We declare no conflict of interest.

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