AGC1 Deficiency Associated with Global Cerebral Hypomyelination

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SUMMARY
The mitochondrial aspartate–glutamate carrier isoform 1 (AGC1), specific to neurons and muscle, supplies aspartate to the cytosol and, as a component of the malate–aspartate shuttle, enables mitochondrial oxidation of cytosolic NADH, thought to be important in providing energy for neurons in the central nervous system. We describe AGC1 deficiency, a novel syndrome characterized by arrested psychomotor development, hypotonia, and seizures in a child with a homozygous missense mutation in the solute carrier family 25, member 12, gene SLC25A12, which encodes the AGC1 protein. Functional analysis of the mutant AGC1 protein showed abolished activity. The child had global hypomyelination in the cerebral hemispheres, suggesting that impaired efflux of aspartate from neuronal mitochondria prevents normal myelin formation.

CASE REPORT
Our patient was a 3-year-old girl, the firstborn child of distantly related Swedish parents, delivered after an uncomplicated pregnancy. Development was normal during the first months of life. Delayed psychomotor development was noted at approximately 5 months of age. At the age of 7 months, the girl began having seizures. She presented with episodes of apnea and later, sporadic tonic seizures developed, with focal clonic jerks. Physical examination revealed severe muscular hypotonia and
Figure 1. MRI Scans in the Patient.
Panel A shows T₂-weighted MRI scans obtained from our patient when she was 8 months of age and 1 year 4 months of age. The supratentorial brain volume is reduced, with prominent cortical sulci, enlarged ventricles, and a lack of development of low signal in hemispheric white matter. At 2 years 9 months of age (Panel B), persistent high signal in the white matter reveals that the age-related low signal, typical of myelination, did not develop normally. There is some myelination in the internal capsule, but in the corona radiata it does not reach the normally lower signal as compared with that from the cerebral cortex. There is thus a substantial lack of myelination in the cerebral hemispheres, whereas normal myelination is noted in the brainstem and cerebellum. The globus pallidus and putamen show some loss of volume. Panel C shows a representative 1H spectrum from the left frontal white matter of the patient (bottom), with a normal spectrum shown (top) for reference. The N-acetyl aspartate (NAA) peak is severely reduced in comparison with the myo-inositol (mIns) and creatine (Cr) peaks. There is no substantial peak around 1.3 to 1.4 ppm, corresponding to the position of lactate and lipids, respectively. Similar findings were seen for the deep and cortical gray matter (not shown). Cho denotes choline, Cr₂ creatine (a second peak), and Glx glutamate and glutamine.

Psychomotor retardation affecting mainly motor skills. She had poor head control and could not roll over or grasp objects. Eye contact and smiling response could be elicited only with difficulty. The plasma lactate level was increased, at 6 mmol per liter, and there was one episode of a slightly elevated lactate level in the cerebrospinal fluid, at 2.6 mmol per liter (normal range, 0.5 to 2.3), in relation to an episode of apnea, but levels were otherwise within the normal range. Plasma amino acid levels were normal; the glutamate level was 70 µmol per liter (1.0 mg per deciliter) (normal range, 0 to 200 [0 to 2.9]), and the aspartate level was 5 µmol per liter (0.7 mg per deciliter) (normal range, 0 to 25 [0 to 0.3]). Repeated electroencephalography, beginning at 9 months of age, showed generalized slowing of background activity but no interictal epileptiform discharges. As of the last examination, at 3 years 8 months of age, there has been essentially no further progress in psychomotor development. The patient cannot sit without support, crawl, or be pulled to a standing position. Severe spasticity has developed, with generalized hyperreflexia. The epilepsy is treated with carbamazepine and levetiracetam. Her height, weight, and head circumference are within the normal ranges.
The Regional Ethics Committee at Karolinska Institutet approved this study. We obtained written informed consent from both parents.

**METHODS AND RESULTS**

**MAGNETIC RESONANCE INVESTIGATIONS**

We carried out magnetic resonance imaging (MRI) of the brain when the patient was 8 months of age, 1 year 4 months of age, and 2 years 9 months of age (Fig. 1A and 1B). We observed a global lack of myelination in the cerebral hemispheres, with reduced supratentorial cerebral volume. This was obvious at 8 months of age and more pronounced at 1 year 4 months of age. There was a slight improvement in myelination in the centrum semiovale and pyramidal tracts at 2 years 9 months of age, but there was no corresponding low signal in the white matter (as compared with the cortex), typical of normal development, on T₂-weighted imaging. Myelination remained lacking, over time, in the periphery of the frontal, occipital, and temporal lobes. In contrast, the cerebellum, brainstem, and thalami were essentially normal with regard to the MRI signal, configuration, and volume.
We sequenced all 18 exons and exon–intron junctions of SLC25A12 (National Center for Biotechnology Information GeneID 8604) from amplified genomic DNA, using a kit (Big Dye Terminator v3.1 Cycle Sequencing kit, Applied Biosystems) and a DNA analyzer (model 3730, Applied Biosystems). We found a homozygous c.1769A→G transition, causing a Q590R missense mutation, in exon 17 (Fig. 3A). Both parents were heterozygous for the mutation, which was absent in 100 unaffected Swedish controls, each of whom had four Swedish grandparents.

**FUNCTION AND EXPRESSION OF MUTANT AGC1**

Wild-type and mutant (Q590R) AGC1 were produced in *Escherichia coli* CO214(DE3), a mutant strain of *E.coli* BL21(DE3), and liposomes were reconstituted with recombinant proteins in the presence of aspartate or glutamate, as described previously.1,7 We removed external substrate from the proteoliposomes by means of extrusion chromatography, initiated transport at 25°C by adding [14C]aspartate or [14C]glutamate to proteoliposomes, and terminated transport by adding pyridoxal 5'-phosphate for a final concentration of 15 mM and bathophenanthroline for a concentration of 10 mM.1 The amount of wild-type and

**Figure 3 (facing page). The Q590R Mutation in the SLC25A12 Gene Encoding Mitochondrial Aspartate–Glutamate Carrier Isoform 1 (AGC1).**

In Panel A, direct sequencing of the mutant SLC25A12 gene from the patient and the wild-type gene from a control shows a homozygous A→G transition at nucleotide 1769, resulting in a Q590R amino acid substitution in the AGC1 protein. In Panel B, amino acid alignment shows that Q590 is highly conserved in the aspartate–glutamate carriers AGC, AGC1, and AGC2 but not in other mitochondrial carriers, including the related glutamate carriers GC1 and GC2. Blocks of the same color show groups of the same or similar amino acids. The human AGC1 has the National Center for Biotechnology Information accession number NP_003696.2. Af denotes Aspergillus fumigatus, Ag Anopheles gambiae, Bt Bos taurus, Dd Dictyostelium discoideum, Dm Drosophila melanogaster, Dr Danio rerio, Hs Homo sapiens, Gg Gallus gallus, Mm Mus musculus, Nc Neurospora crassa, Nf Neosartorya fischeri, Nv Nasonia vitripennis, Rn Rattus norvegicus, Sc Saccharomyces cerevisiae, and Xt Xenopus tropicalis. Panels C and D show structural-homology models of AGC1, illustrating the consequences of the Q590R mutation. Docking of aspartate (shown in the van der Waals representation, colored in yellow) in the Q590 wild-type AGC1 (Panel C) and the Q590R mutant AGC1 (Panel D) is shown from the lateral side. Stick representations highlight some residues that are located within 4 Å of the substrate. Portions of helices I and II (in Panels C and D) and portions of the salt-bridge network (in Panel C only) are rendered transparent to facilitate viewing of the substrate and side chains of some amino acids. The transmembrane α-helices H1 through H6 are shown, and purple surfaces highlight the salt-bridge network between residues D348 and K451, E448 and K543, and D540 and K351.
mutant proteins incorporated into liposomes was about 20% of that added to the reconstitution mixture. The mutant form of AGC1 was unable to transport aspartate or glutamate, even after 60 minutes of incubation (Fig. 2B and 2C).

We observed that AGC1 colocalized with mitochondria in fibroblasts from both the patient and controls, and we found normal levels of AGC1 and porin in total and mitochondrial fractions of the patient’s lymphocytes. Moreover, mutant AGC1 coprecipitated with mitochondrial membranes to the same extent as did wild-type AGC1, indicating its ability to integrate in the inner mitochondrial membrane (see the Supplementary Material).
brainstem nuclei, sometimes together with symmetrical focal lesions in the basal ganglia and impaired oxidative phosphorylation.

Resonance spectroscopy is a metabolic marker of lactate accumulation detected with the use of magnetic resonance imaging (MRI). Furthermore, MRI showed global cerebral hypomyelination with relatively spared gray matter and without obvious signal changes in the basal ganglia and brainstem, despite the fact that AGC1 expression is restricted to neurons.

**DISCUSSION**

This study sheds light on the role of AGC1 in the central nervous system. AGC1 expression in the central nervous system is restricted to neurons and is absent in white matter. AGC1 is a component of the malate–aspartate shuttle, which is thought to be required for mitochondrial oxidation of cytosolic NADH in the brain (Fig. 2D). Magnetic resonance findings are heterogeneous in patients with mitochondrial encephalopathy due to impaired oxidative phosphorylation, but there are some commonalities, including symmetrical focal lesions in the basal ganglia and brainstem nuclei, sometimes together with cortical infarct-like lesions. In addition, lactate accumulation detected with the use of magnetic resonance spectroscopy is a metabolic marker of impaired oxidative phosphorylation. However, our patient had no substantial accumulation of lactate detected on magnetic resonance spectroscopy. Furthermore, MRI showed global cerebral hypomyelination with relatively spared gray matter and without obvious signal changes in the basal ganglia and brainstem, despite the fact that AGC1 expression is restricted to neurons.

These findings do not support compromised neuronal energy production by the respiratory chain as a major pathogenetic mechanism in AGC1 deficiency, suggesting that the malate–aspartate shuttle may not be bioenergetically essential in the central nervous system. The glycerol-3-phosphate cycle, known to be active in the brain, is an alternative pathway of transfer for reducing equivalents into the mitochondria. It could not compensate for inhibition of the malate–aspartate shuttle in isolated synaptosomes, but it may compensate for a compromised malate–aspartate shuttle in vivo.

Neuronal aspartate is a substrate for the formation of N-acetyl aspartate by aspartate-N-acetyltransferase in mitochondria and microsomes. N-acetyl aspartate produced in neurons undergoes transaxonal transfer to oligodendrocytes, where it supplies acetyl groups for the synthesis of myelin lipids (Fig. 2D). The phenotype of Slc25a12-knockout mice suggests that myelin formation depends on aspartate transport from mitochondria to the cytosol. Motor-coordination deficits developed in these mice, from day 12 onward, along with impaired myelination in the central nervous system. In parallel, there was a striking deficit in levels of aspartate and N-acetyl aspartate in the brain and in cultured neurons, suggesting that the major site of aspartate production in the central nervous system is the mitochondrion, that AGC1 affects aspartate efflux into the cytosol, and that this efflux is essential for N-acetyl aspartate formation. The knockout mice had normal numbers of neurons, suggesting that the malate–aspartate shuttle is not essential for the survival of neurons in these animals. MRI revealed a global lack of myelination in the cerebral hemispheres of our patient, and N-acetyl aspartate levels were drastically reduced, as determined by proton magnetic resonance spectroscopy. These findings are concordant with the phenotype of the Slc25a12-knockout mouse. Although N-acetyl aspartate reduction is considered a marker of neuronal viability and number, the patient had relatively conserved gray matter, supporting our hypothesis that the major pathogenetic mechanism of her syndrome is impaired neuronal aspartate transport, which prevents myelination by failing to provide N-acetyl aspartate to oligodendrocytes. The Slc25a12-knockout mouse thus seems to represent a model of the human disease.

The hypomyelination in our patient was confined to the cerebral hemispheres, with findings...
essentially normal in the cerebellum and brainstem. In the mouse, AGC1 and AGC2 have overlapping expression patterns during embryonic development, and full tissue-specific expression is attained postnatally.\(^2,10\) The ontogeny of tissue-specific expression of AGC1 in the human central nervous system is unknown, and residual expression of AGC2 may explain the regional differences seen in our patient.

The difference in phenotypes associated with AGC1 deficiency and AGC2 deficiency is consistent with the different tissue-expression patterns of these two isoforms, resulting in metabolic disturbances in the central nervous system and the liver, respectively. In both cases, the major pathogenic mechanism is reduced mitochondrial aspartate efflux. In the liver, aspartate is needed for the urea cycle, whereas in the brain, it is required for N-acetyl aspartate and myelin formation. In conclusion, we describe a phenotype associated with AGC1 deficiency, with a presentation of severe, early postnatal psychomotor developmental arrest, as well as hypotonia and seizures. A dominating feature of the syndrome is global cerebral hypomyelination.

Supported by grants from the Swedish Research Council (12198), the Karolinska Institutet, and the Stockholm County Council (to Dr. Wedell) and from Ministero dell’Università e della Ricerca, the Italian Human ProteomeNet (project RBRN07BMCT_009), and Apulia Region Neurobiotech (Progetto Strategico 124) (to Dr. Palmieri).

No potential conflict of interest relevant to this article was reported.

**REFERENCES**


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