BRIEF COMMUNICATION

The ketogenic diet compensates for AGC1 deficiency and improves myelination

*†Maria Dahlin, ‡Daniel A. Martin, §Zandra Hedlund, ¶Monica Jonsson, **††Ulrika von Döbeln, and †††‡‡Anna Wedell

Epilepsia, 56(11):e176–e181, 2015
doi: 10.1111/epi.13193

Dr. Maria Dahlin is a senior consultant in neuropediatrics and epileptology at Karolinska Hospital.

Summary

The brain aspartate-glutamate carrier (AGC1) is specifically expressed in neurons, where it transports aspartate from the mitochondria to the cytosol, and plays a role in transfer of nicotinamide adenine dinucleotide (NADH)-reducing equivalents into the mitochondria as a part of the malate-aspartate shuttle. Deficient function of AGC1 underlies an inborn error of metabolism that presents with severe hypotonia, arrested psychomotor development, and seizures from a few months of age. In AGC1 deficiency, there is secondary hypomyelination due to lack of N-acetylaspartate (NAA), which is normally generated by acetylation of aspartate in the neuron and required for fatty acid synthesis by the adjacent oligodendrocyte. Based on experiences from AGC2 deficiency, we predicted that reduced glycolysis should compensate for the metabolic defect and allow resumed myelination in AGC1 deficiency. Carbohydrate restriction was therefore initiated in a patient with AGC1 deficiency at 6 years of age by introducing a ketogenic diet. The response was dramatic, clinically as well as radiologically. Psychomotor development showed clear improvement, and magnetic resonance imaging (MRI) indicated resumed myelination. This is the first successful treatment of secondary hypomyelination reported. Because AGC1 is driven by the proton gradient generated by the neuronal mitochondrial respiratory chain, the results have potential relevance for secondary hypomyelination in general.

KEY WORDS: Aspartate-glutamate carrier, Inborn error of metabolism, Ketogenic diet, Malate-aspartate shuttle, Redox status, Secondary hypomyelination.

Deficiency of the mitochondrial aspartate-glutamate carrier isoform 1 (AGC1) encoded by *SLC25A12* causes an inborn error of metabolism (IEM) that presents with severe hypotonia, arrested psychomotor development, and seizures from a few months of age (OMIM number 612949). Only two unrelated cases have been described.[1,2] AGC transports aspartate from the mitochondria to the cytosol in exchange for glutamate plus a proton, and plays a role in the transfer of nicotinamide adenine dinucleotide (NADH)-reducing equivalents from the cytosol into the mitochondria as a component of the malate-aspartate shuttle (MAS) (Fig. 1). AGC1 is the only isoform expressed in the adult central nervous system (CNS) and skeletal muscle,[3] whereas in liver, AGC2 is specifically expressed. In the CNS, AGC1 expression is restricted to neurons.[4] Impaired transfer of reducing equivalents into mitochondria attenuates oxidative phosphorylation. In addition, NADH generated by glycolysis accumulates in the cytoplasm, increasing the $NADH/NAD^+$

Accepted August 14, 2015; Early View publication September 24, 2015.
*Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden; †Department of Neuropediatrics, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden; ‡Department of Neuroradiology, Karolinska University Hospital, Stockholm, Sweden; §Department of Nutrition, Karolinska University Hospital, Stockholm, Sweden; ¶Center of Child Neurology and Habilitation, Östersund Hospital, Östersund, Sweden; **Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; ††Center for Inherited Metabolic Diseases (CMMS), Karolinska University Hospital, Stockholm, Sweden; and ‡‡Department of Molecular Medicine and Surgery, Science for Life Laboratory, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

Address correspondence to: Anna Wedell, Center for Inherited Metabolic Diseases (CMMS), Karolinska Institutet, Karolinska University Hospital, L7:05, S-17176 Stockholm, Sweden. E-mail: Anna.Wedell@ki.se

ratio. Disrupted AGC1 function also prevents efflux of aspartate from the mitochondria to the cytosol. Neuronal aspartate is a substrate for N-acetylaspartate (NAA) formation by aspartate-N-acetyltransferase (Fig. 1A).[5] NAA produced in neurons undergoes transaxonal transfer to oligodendrocytes, where it supplies acetyl groups for synthesis of myelin lipids.[6,7] The severe secondary hypomyelination seen in AGC1 deficiency[1] thus fits well as a result of disruption of this pathway.

An *Agc1* knockout mouse model has been generated.[8] These mice developed motor-coordination deficits from day 12 onward, along with prominent hypomyelination throughout the CNS and striking deficits in levels of aspartate and NAA in the brain and in cultured neurons. The phenotype of the *Agc1* knockout mouse thus corresponds well with the clinical picture of AGC1 deficiency, and lends further support to the proposed pathogenetic mechanism.

Mutations in *SLC25A13* encoding AGC2 cause type 2 citrullinemia (CTLN2, OMIM number 603471), characterized by episodes of hyperammonemic encephalopathy.[9] In CTLN2, the lack of aspartate in the cytosol of the hepatocyte causes disruption of the urea cycle (Fig. 1B). CTLN2 patients have a preference for protein- and fat-rich food and an aversion toward carbohydrates. Carbohydrates aggravate the disease by causing significant increases in plasma ammonia and citrulline, and a low-carbohydrate diet is recommended.[10] The beneficial effect of carbohydrate restriction can be understood in light of the biochemical defect (Fig. 1B). Glycolysis generates NADH; carbohydrate restriction thus reduces the NADH/NAD^+ ratio. This favors generation of aspartate by the cytosolic part of the MAS, since the bidirectional enzyme malate dehydrogenase (MDH) is regulated by this ratio. Increased cytosolic aspartate compensates for AGC2 deficiency, and function of the urea cycle is resumed.

The successful compensation of the biochemical defect in CTNL2 by carbohydrate restriction has implications for AGC1 deficiency. Furthermore, when cerebellar slice cultures from *Agc1* knockout mice were incubated with pyruvate, a positive effect on myelination was observed.[11] This indicates that cytosolic aspartate production with subsequent NAA and myelin lipid formation can be achieved by lowering the NADH/NAD^+ ratio in the neuron. A treatment that theoretically could accomplish this is the ketogenic diet (KD). We therefore initiated treatment with the KD in our patient with AGC1 deficiency, at the age of 6 years. Her response to the treatment was dramatic.

Methods

Case report

The patient is the first child to distantly related Swedish parents.[1] Delayed psychomotor development was noted from around 5 months. She did not acquire head control, roll over, or grasp objects. Physical examination showed severe muscular hypotonia and psychomotor retardation. At 7 months, seizures started and soon became daily (Table S1). From this age until the start of diet treatment at 6 years of age, there was essentially no further psychomotor development.

Dietary treatment

A modified version of the Johns Hopkins Hospital protocol including supplements was used.[12,13] The patient started the KD without fasting on a ketogenic ratio of 1:1 of fat to protein and carbohydrates. The ratio was successively increased to 3:1 at 10 days, to 3.5:1 at 8 months, and to 4:1 at 13.5 months on diet. Blood 3-hydroxybutyric acid varied between 5.5 and 6.8 mM/L from 6 months on diet.

Results

MRI of the brain was performed five times before treatment (Fig. 2). There was a global lack of myelination in the cerebral hemispheres with a reduced supratentorial cerebral volume and markedly prominent cortical sulci, enlarged ventricles, and a thin corpus callosum (Fig. 2A,B,D,E). During the last two investigations before treatment, the findings were stationary corresponding to an arrested development at the age of around 6 months. In contrast, the cerebellum, brainstem, and thalami were essentially normal. MR single voxel spectroscopy was performed three times before treatment, of the left basal ganglia and thalamus, frontal white matter, and in the occipital cortex in the midline. Data were processed using the standard SAGE software (GE Medical Systems). All spectra showed severely reduced NAA peaks (Table S2). Normally, the ratio of NAA to creatine is >1 in all regions, at least after the age of 2 years.[14]

After 3 weeks on the KD, voluntary movements were noted in the patient's arms and some weeks later in the legs. She started to grind her teeth. Clinical examination at 3.5 months after diet start revealed clear improvement in psychomotor development. Eye contact was improved with a more intense gaze. There was a smiling response. The patient turned her head and eyes in the direction of sounds and followed moving objects. Vocal sounds were more diversified. She raised her arms, put her hands in her mouth, and could stretch legs and flex hips and knees. Parents and teachers noted increased consciousness about people and things around her. At clinical investigation at 8 months on the diet, the patient responded with a prompt smile and laughed intensely when tickled. She turned toward the person talking and communicated with sounds. She lifted her head from the headrest on the wheelchair but could not maintain head control. She made some movements in the upper part of her trunk, used her arms

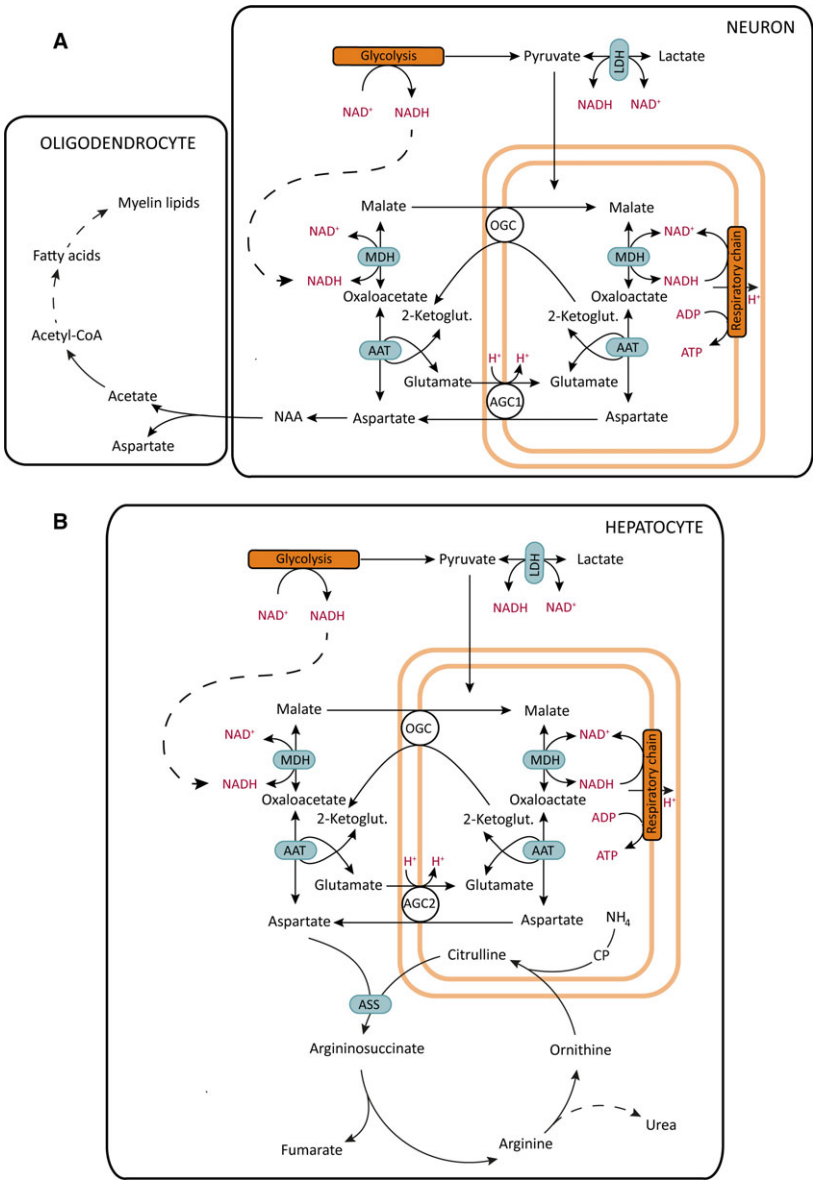

Figure 1.

The malate-aspartate shuttle (MAS) and its specific metabolic functions in the CNS and liver, respectively. The MAS translocates electrons produced during glycolysis across the inner mitochondrial membrane for oxidative phosphorylation. The shuttle is composed of two enzymatic functions present both in the cytosol and mitochondrial matrix (AAT and MDH), and two transporters located in the inner mitochondrial membrane (AGC and OGC). AGC exports aspartate from the matrix to the cytosol in exchange for glutamate plus a proton. In the cytosol, aspartate is converted by cytosolic AAT to oxaloacetate. MDH reacts with oxaloacetate to produce malate. MDH is an NADH-dependent dehydrogenase and is thus regulated by the NADH/NAD^+ ratio. The reduction of oxaloacetate to malate takes place in the presence of high levels of glycolytically generated NADH. Once malate is formed, it is imported by OGC from the cytosol into the mitochondrial matrix in exchange for 2-ketoglutarate. In the mitochondrial matrix, malate is oxidized by mitochondrial MDH into oxaloacetate. During this step, NADH is regenerated and can be used to pass electrons to the respiratory chain for ATP synthesis. Oxaloacetate is then transformed into aspartate by mitochondrial AAT, completing the cycle. NAD^+ in the cytosol can be reduced again by another round of glycolysis. In addition to its role in regulating redox balance, the efflux of aspartate by AGC has important metabolic functions. In neurons (A), AGC1 supplies aspartate for cytosolic NAA formation. NAA undergoes transaxonal transport into the oligodendrocyte where it is cleaved by aspartoacylase, liberating acetate for myelin lipid synthesis. Disrupted function of AGC1 therefore leads to reduced levels of aspartate, NAA, and myelin in the CNS. In hepatocytes (B), AGC2 supplies aspartate as a substrate for ASS in argininosuccinate synthesis. Impaired function of AGC2 results in increased ammonia and citrulline levels due to disruption of the urea cycle. AAT, aspartate aminotransferase; AGC, aspartate-glutamate carrier; ASS, argininosuccinate synthetase; CP, carbamoyl phosphate; OGC, oxoglutarate carrier; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; NAA, N-acetylaspartate.

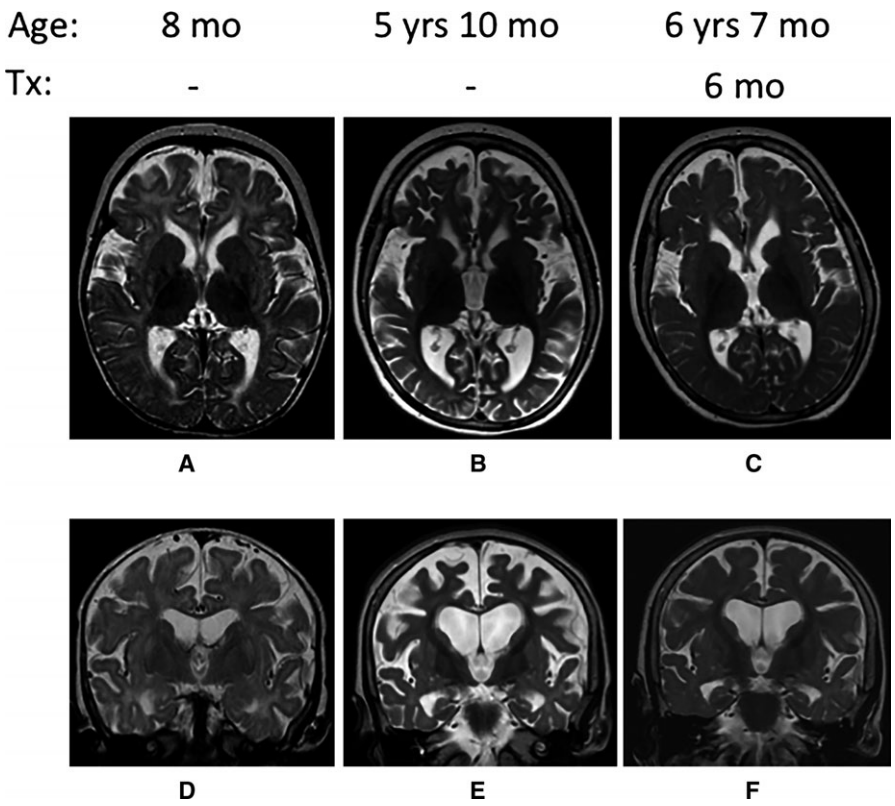

Figure 2.
Results from MRI investigations in the patient with AGC1 deficiency before and 6 months after treatment. Before treatment, T_2 axial imaging (A, B) and T_2 coronal imaging (D,E) showed lack of myelination and progressively reduced supratentorial cerebral volume. At 6 years and 7 months of age, after 6 months of treatment with the ketogenic diet, T_2 axial imaging (C) and T_2 coronal imaging (F) show that the previously high signal corresponding to white matter is lower, and that the ventricles and subarachnoid spaces are less prominent, indicating reversal of the volume loss. Tx, duration of treatment with the ketogenic diet.

more, and could touch a person standing beside her. She opened her hands and grasped objects; for example, she took off her glasses. She lifted her legs against gravity. At examination 13.5 months after the diet start she raised and held her head, turned the upper part of the trunk, and when lying on her back she could roll over to her abdomen. She pressed the button on her toy to make sounds. At 20 months on the diet she made diverse vocal sounds, grasped objects, and used her forearms to raise her head and the upper part of her trunk while lying on her stomach. She is now learning to sit without support. She is also seizure free (Table S1). At diet start she was treated with levetiracetam and oxcarbazepine, but both drugs could be tapered.

Thirty minute standard EEG studies were performed before and during the diet. EEG studies before diet showed general abnormality with slow background activity, a slight amount of interictal epileptiform activity, and six episodes of electrographic seizure activity accompanied by tonic and clonic seizures. EEG studies obtained at 9 and 12 months after KD start were normal.

MRI and magnetic resonance spectroscopy (MRS) were performed 6 and 19 months after start of treatment. After 6 months, there was a dramatic difference compared to the previous investigations, with a more mature signal in lobar as well as in peripheral white matter. Reduced ventricular size and less prominent subarachnoid spaces indicated increased volume, further illustrating resumed myelination (Fig. 2C,F). The NAA/creatine ratio was also improved. At the next investigation 19 months after KD start, MRI showed further improvement, with slight reduction in ventricular size and slightly less prominent subarachnoid spaces. However, the NAA/creatine ratio was not convincingly improved. We therefore retrieved raw data from MR spectroscopy, fitted spectra in the frequency domain, and quantified using LC Model software and LCMgui.[15] This revealed increases in both NAA and creatine, illustrating that the NAA/creatine ratio is insufficient for monitoring of the patient's progressive brain maturation (Table S2).

Discussion

The second unrelated child with AGC1 deficiency showed a profound global developmental delay with a static course as well as seizures during follow-up from infancy to 6.7 years of age.[2] Our patient had a similar, static course without any clinical improvement until she was offered treatment with KD at 6 years of age. She has now been on treatment for >20 months, with a dramatic response clinically as well as neuroradiologically.

The KD is a high-fat, adequate-protein and very low-carbohydrate diet that has been used for many years to treat medically refractory epilepsy in children. The KD is also an established therapy in specific IEM.[16] In the glucose transporter 1 deficiency syndrome and in pyru-

vate dehydrogenase complex deficiency, KD bypasses the metabolic block by providing ketone bodies as an alternative to glucose as a fuel for the brain. As in the above IEM, glucose cannot be efficiently used by neurons in AGC1 deficiency, since the increased NADH/NAD^+ ratio will prevent pyruvate from entering the tricarboxylic acid cycle (TCA) by reducing it to lactate.[17] Like in the IEM mentioned earlier, KD ameliorates neuronal energy deficiency in AGC1 deficiency by providing ketone bodies as an alternative to glycolytically generated pyruvate as a mitochondrial fuel for the brain. However, we also exploited a novel therapeutic mechanism of the KD: to manipulate the neuronal cytosolic redox status by reducing glycolytically generated NADH. Because MDH is an NADH-dependent dehydrogenase, this shifts the equilibrium of the MAS, resulting in cytosolic aspartate production and compensating for the abolished mitochondrial efflux. The pathway of generating myelin lipids utilizing neuronal aspartate and NAA is thus reactivated.

As discussed, the pathogenesis of AGC1 deficiency is complex including both neuronal energy deficiency and secondary hypomyelination due to lack of NAA. In addition, further studies on the *Agc1* knockout mouse have indicated that neuronal aspartate is also required as a nitrogen donor for de novo glutamate production in the neighboring astrocytes, leading to a gradual failure of the glutamate-glutamine cycle in the animals.[18] AGC1 deficiency thus seems to cause specific metabolic consequences in different cell types of the CNS.

The beneficial effect of the KD in AGC1 deficiency has potential relevance for secondary hypomyelination in general. The MAS is in direct contact with the TCA and with mitochondrial oxidative phosphorylation. The function of AGC is in fact driven by the proton gradient generated by the respiratory chain (Fig. 1).[19] It is therefore conceivable that defects in neuronal oxidative phosphorylation cause secondary hypomyelination through the same pathway. This warrants further investigations, for example, evaluating the KD in broader groups of patients using NAA as a biomarker for patient selection and treatment response.

Our patient showed a clear clinical response to KD. The future will tell how far her improvement will proceed, and how much irreversible damage she has sustained. In light of the successful treatment of this single case, identification of additional affected patients at a younger age has become extremely important.

Acknowledgments

We thank the family for participating in this investigation. This work was supported by the Swedish Research Council (12198), the Karolinska Institutet, the Stockholm County Council, the Swedish Brain Foundation, and the Knut and Alice Wallenberg Foundation.

Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Seizure types and frequency before the ketogenic diet as well as at 3, 8, 12, and 20 months after diet start.

Table S2. Results from MRS investigations in the patient with AGC1 deficiency before and after treatment with the ketogenic diet (KD).