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ORIGINAL ARTICLE

AGC1 Deficiency Causes Infantile Epilepsy, Abnormal Myelination, and Reduced *N*-Acetylaspartate

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Abstract Background: Whole exome sequencing (WES) offers a powerful diagnostic tool to rapidly and efficiently sequence all coding genes in individuals presenting for consideration of phenotypically and genetically heterogeneous disorders such as suspected mitochondrial disease. Here, we report results of WES and functional validation in a consanguineous Indian kindred where two siblings presented with profound developmental delay, congenital hypotonia, refractory epilepsy, abnormal myelination, fluctuating basal ganglia changes, cerebral atrophy, and reduced *N*-acetylaspartate (NAA).

Methods: Whole blood DNA from one affected and one unaffected sibling was captured by Agilent SureSelect Human All Exon kit and sequenced on the Illumina HiSeq2000. Mutations were validated by Sanger sequencing

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in all family members. Protein from wild-type and mutant fibroblasts was isolated to assess mutation effects on protein expression and enzyme activity.

Results: A novel *SLC25A12* homozygous missense mutation, c.1058G>A; p.Arg353Gln, segregated with disease in this kindred. *SLC25A12* encodes the neuronal aspartate-glutamate carrier 1 (AGC1) protein, an essential component of the neuronal malate/aspartate shuttle that transfers NADH and H^+ reducing equivalents from the cytosol to mitochondria. AGC1 activity enables neuronal export of aspartate, the glial substrate necessary for proper neuronal myelination. Recombinant mutant p.Arg353Gln AGC1 activity was reduced to 15% of wild type. One prior reported *SLC25A12* mutation caused complete loss of AGC1 activity in a child with epilepsy, hypotonia, hypomyelination, and reduced brain NAA.

Conclusions: These data strongly suggest that *SLC25A12* disease impairs neuronal AGC1 activity. *SLC25A12* sequencing should be considered in children with infantile epilepsy, congenital hypotonia, global delay, abnormal myelination, and reduced brain NAA.

Introduction

Whole exome sequencing (WES) is gaining wide recognition as a powerful approach to rapidly and efficiently evaluate for coding mutations that cause a wide range of inherited monogenic diseases. In particular, there is a growing utilization of WES to evaluate individuals presenting for diagnostic evaluation of highly heterogeneous conditions, such as suspected mitochondrial disease (McCormick et al. 2013). In this study, a consanguineous Indian family presented in the Mitochondrial-Genetics Diagnostic Clinic for evaluation of a pathogenic etiology for two siblings with intractable epilepsy, global developmental delay, and congenital hypotonia in whom a mitochondrial disorder had previously been suspected but not confirmed by muscle-based enzymatic analysis. An extensive array of clinical diagnostic genetic testing performed to evaluate for known mitochondrial DNA and nuclear DNA causes of early infantile epileptic encephalopathy or mitochondrial diseases was unrevealing. Indeed, it is well known that muscle biopsies can be unrevealing of a specific histologic or biochemical abnormality in children even with known mitochondrial diseases (Haas et al. 2008) and that sequencing of all currently known mitochondrial disease genes is likely to identify a specific genetic etiology for only up to one-half of individuals with clinical manifestations of possible mitochondrial disease (Calvo et al. 2012). Following completion of mitochondrial DNA (mtDNA) genome sequencing and mtDNA deletion analysis in the proband's muscle, an autosomal recessive monogenic cause of disease was assumed to be present based on a similarly severe presentation in the proband's brother and known consanguinity. The family was therefore enrolled in a research study to pursue family-based whole exome sequencing to identify the specific genetic cause of their suspected mitochondrial disease.

Methods

Study participant description. Written informed consent for participation in The Children's Hospital of Philadelphia (CHOP) Institutional and Ethical Review Board–approved research studies, both in the CHOP Center for Applied Genomics (HH, PI) and in the CHOP Metabolism Division to evaluate the metabolic consequences of suspected mitochondrial disease (MJF, PI) was obtained from all immediate members of the study family, including two affected siblings, one healthy sister, and both unaffected parents (Fig. 1a). The two affected siblings were enrolled for research-based whole exome analysis based on having highly similar phenotypes that most notably included global developmental delay, epilepsy, hypotonia, and multiple dysmorphic features. The proband was a consanguineous Indian girl (Fig. 1a, individual II-1) who presented at age 6.7 years to the Mitochondrial-Genetics Diagnostic Clinic at The Children's Hospital of Philadelphia for diagnostic evaluation of refractory epilepsy with both focal and generalized seizures onset at 10 months controlled with topiramate and phenobarbital, global developmental delay without significant regression, absent speech, inability to follow commands, inability to sit unassisted or walk, and congenital profound hypotonia. Brain magnetic resonance imaging (MRI) performed at age 16 months (Fig. 1b–c) was significant for bilateral, symmetric abnormal signal in the putamina, consistent with suspected metabolic disease. Delayed myelination was also noted, as was prominence of the subarachnoid spaces and sulci that was consistent with cerebral volume loss. Brain magnetic resonance spectroscopy (MRS) at that time was significant for increased choline and myoinositol peaks and decreased *N*-acetylaspartate (NAA) peak, as well as increased lactate peaks in both the parenchyma and cerebrospinal fluid (CSF). Repeat brain MRI/MRS at age 5 years, 11 months (Fig. 1d–e), revealed normal-appearing basal ganglia with resolution of the signal abnormality previously seen in the putamen, and prominent ventricles and sulci consistent with cerebral volume loss versus hypoplasia. Brain MRS was consistent with previous findings including decreased NAA and increased lactate through the brain parenchyma and increased lactate in the CSF. Choline also appeared elevated in some areas (Fig. 1f). Extensive metabolic and genetic diagnostic evaluations, including muscle biopsy, were

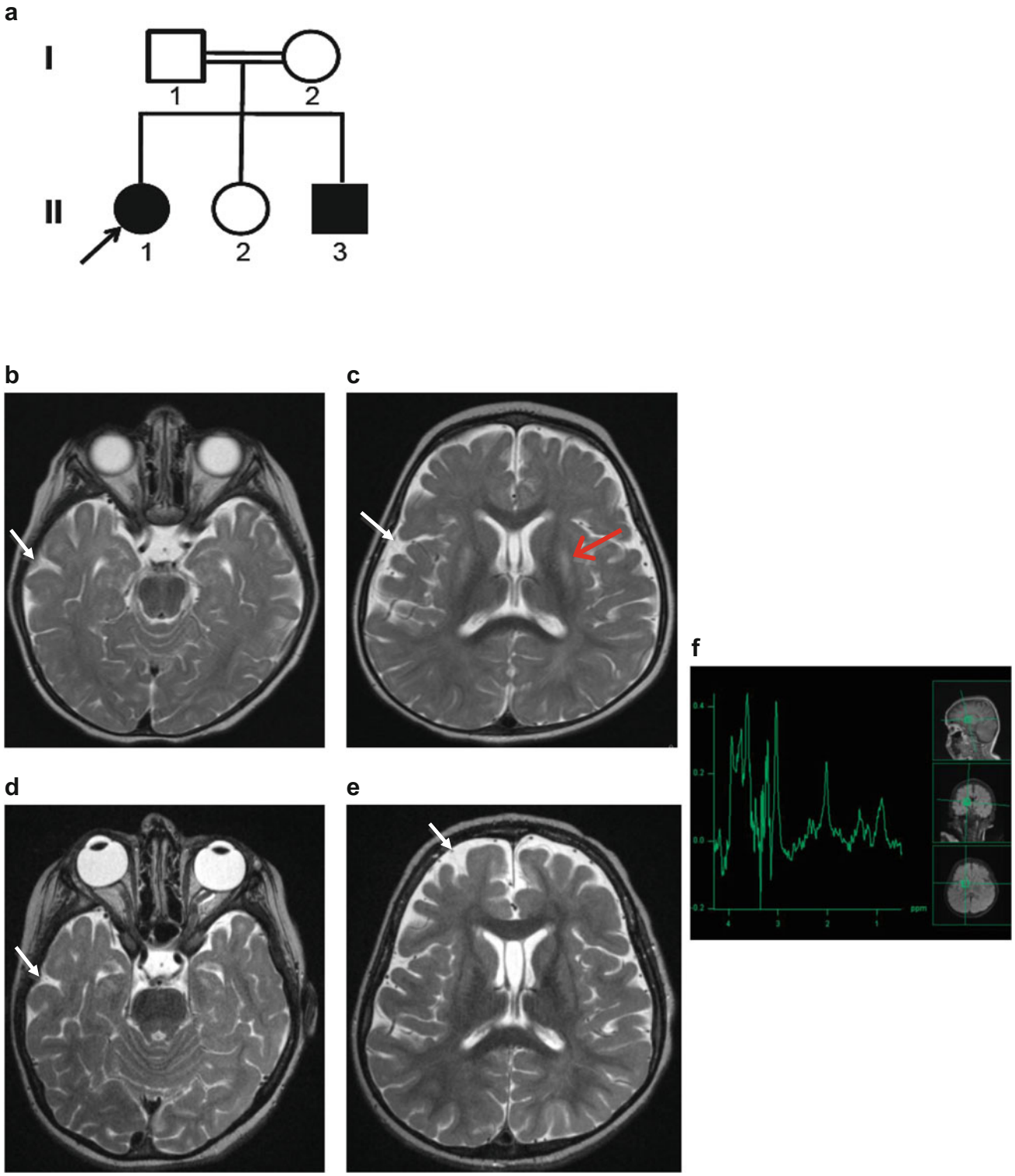


Fig. 1 Pedigree and neuroimaging findings of consanguineous Indian kindred. (a) Family pedigree. The parents are first cousins with two affected children (individuals II-1 and II-3) and one healthy daughter (individual II-2). **(b** and **c) Brain MRI of individual II-1 at age 16 months.** T2 axial images show increased extra-axial cerebrospinal fluid spaces, large sulci (white arrows), bright signal in putamen (red line arrow), and delayed myelination. **(d** and **e) Brain MRI of individual II-1 at age 5 years, 11 months.** As seen on the same sequences and cuts as shown at age 16 months, there is persistence of the signs of atrophy (white arrows), resolution of the T2 signal change in the putamena, and improved but still decreased myelination. **(f) Brain MRS of individual II-1 at age 5 years, 11 months** shows low *N*-acetylaspartate (NAA) peak at approximately 2 parts per million in a voxel placed over the basal ganglia

unrevealing of an underlying metabolic or genetic etiology in the proband. Her similarly affected younger brother (Fig. 1a, individual II-3) presented with global developmental delay at age 13 months, epilepsy onset at age 10 months, and had normal metabolic screening laboratory studies. No brain imaging or tissue biopsies were performed. Detailed descriptions of both children's clinical presentations, including their family history and prior diagnostic evaluations, are presented in the Supplemental File.

AGC1 activity analysis. Human wild-type and mutant AGC1 proteins were overexpressed as inclusion bodies in the cytosol of *Escherichia coli* C0214, solubilized, and purified as described (Palmieri et al. 2001; Fiermonte et al. 2009). The transport activities of the recombinant purified proteins were assayed as described (Palmieri et al. 1995, 2001). The amount of both wild-type and mutant AGC1 incorporated into liposomes was about 20 % of the protein added to the reconstitution mixture. Western blots were performed using the AGC1 B-2 monoclonal antibody (from Santa Cruz Biotechnology, Inc., USA) and porin monoclonal antibody (from MitoScience, USA).

Results

Family-based exome sequencing and bioinformatics analysis. We conducted whole exome sequencing on the affected boy (individual II-3) and his unaffected sister (individual II-2) (Fig. 2a and Supplemental File). We considered the exome variant profile under the assumption of an autosomal recessive model due to known consanguinity. An average coverage of 69-fold was established, where 89 % of the target region was covered at least 10-fold. Variants were filtered to exclude synonymous variants, variants having minor allele frequency exceeding 1%, variants with other occurrences in our in-house exome database, or benign variants, which collectively left variants in only two genes as possible disease-causing candidates (Fig. 2b). Additional whole exome sequencing analysis performed on the entire nuclear pedigree provided improved depth of coverage (Supplemental Table 1) but did not reveal any additional shared homozygous variants by both affected siblings. Both substitution variants were selected for Sanger validation (Supplemental File), with only the *SLC25A12* (solute carrier family 25 member 12) mutation showing complete segregation with the disease phenotype in this kindred (Fig. 2c). *SLC25A12* encodes the mitochondrial aspartate-glutamate carrier isoform 1 (AGC1). The specific *SLC25A12* homozygous mutation identified is c.1058G>A, which results in an Arg353Gln substitution in the 678 amino acid AGC1 protein (Palmieri et al. 2001). Moreover, *SLC25A12* is located in one of the large regions of homozygosity shared by both affected children that we had demonstrated by genome-wide SNP microarray analysis (Supplemental Table 2). The *SLC25A12* c.1058G>A mutation identified in this family appeared to be novel, as it was not detected in the 1000 Genomes Project, the NHLBI Exome Variant Server, or an additional 1200 exome samples previously analyzed in our in-house database.

The arginine at amino acid position 353 in the AGC1 protein is highly conserved in the mitochondrial carrier subfamily of the aspartate glutamate carrier (Fig. 2d), the glutamate carrier, and also in many other mitochondrial carrier subfamilies, thereby confirming its importance in the function and/or structure of mitochondrial carriers rather than in substrate binding (Palmieri 2013). The Arg353Gln mutation occurs in a different domain of the AGC1 transporter from the Gln590Arg mutation that was previously reported in one subject (Wibom et al. 2009). The Gln590Arg mutation involves an amino acid residue that protrudes into the internal cavity of the AGC1 carrier immediately above the substrate binding site (Wibom et al. 2009). In contrast, the Arg353Gln mutation involves an amino acid residue that is located just below the m-gate of the carrier and is thought to participate in closing and opening the carrier on the matrix side through an interaction with a highly conserved glutamate at residue 384 (Fig. 2d) (Pierri et al. 2014).

Functional validation of aberrant AGC1 activity. This carrier transports only L-aspartate and L-glutamate, and its physiological role is to catalyze the exchange of intramitochondrial aspartate with cytosolic glutamate plus a proton (Palmieri 2004). To investigate whether the R353Q mutation of AGC1 affects protein function, we overexpressed the wild-type and mutant AGC1 proteins in *Escherichia coli*, purified the proteins, and reconstituted these purified proteins into phospholipid vesicles (liposomes). We then followed the time courses of [^{14}C] aspartate/aspartate or [^{14}C]glutamate/aspartate exchange by adding radioactive aspartate or glutamate to proteoliposomes containing unlabeled aspartate and reconstituted with either the recombinant wild-type or mutant AGC1 (Fig. 3a). The proteoliposomal uptake of either [^{14}C]aspartate or [^{14}C]glutamate was much lower when the vesicles were reconstituted with the mutant AGC1 than with wild-type AGC1, although the amount of protein inserted in the liposomal membrane was the same for both recombinant proteins. Eighty-five percent inhibition of the initial transport rates of aspartate/aspartate and glutamate/aspartate exchanges was caused by the AGC1 mutation. Therefore, the transport activity of the R353Q mutant AGC1 was dramatically decreased but not completely abolished. Similar amounts of AGC1 and porin (a mitochondrial protein of the outer mitochondrial membrane) were seen in the mitochondrial protein fraction isolated from fibroblasts

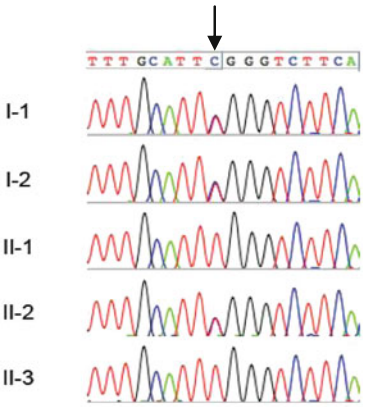
a

Filter	Homozygous	Compound heterozygous	All
NS/SS/I with at least 5X coverage	3149	1274	4065
MAF<0.01 in 1000 Genomes and ESP6500SI	105	63	162
Not present in in-house exome data*	14	28	42
Conserved and damaging	2	0	2

b

Gene	Mutation location (hg19)	cDNA alteration	Protein alteration
SLC25A12	chr2:172669962	c.1058G>A	p.R353Q
ALMS1	chr2:73777400	c.8075A>G	p.N2692S

c



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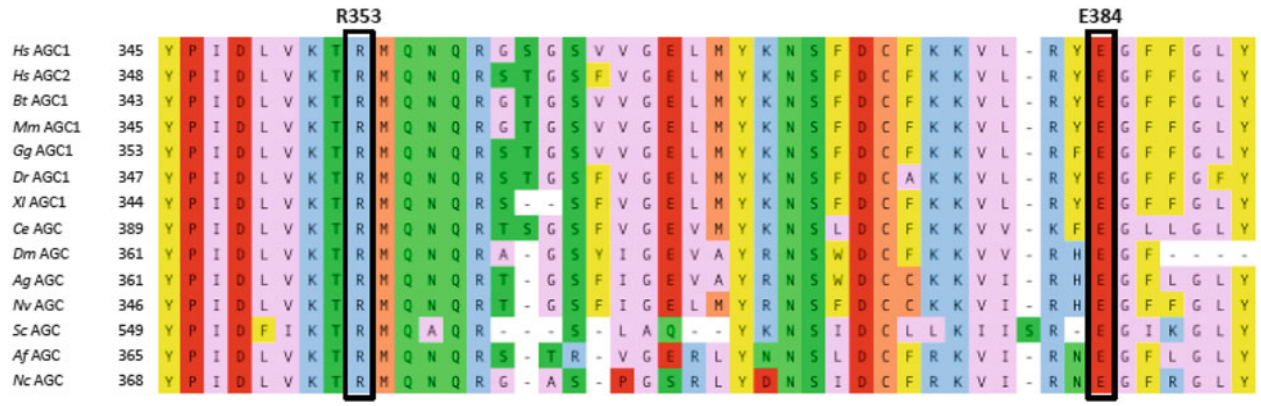

Fig. 2 (**a**) **Overview of candidate genes with potentially pathogenic alleles identified by whole exome sequencing in one affected sibling (individual II-3).** NS, non-synonymous variant. SS, acceptor or donor splice site variants. *I* coding indels, *MAF* minor allele frequency. ESP6500SI, 6503 exomes data from the NHLBI Exome Sequencing Project. *, Neither homozygous nor compound heterozygous variants were present in over 1,200 exome datasets that we had previously sequenced. (**b**) **Details of two candidate genes in which homozygous missense variants were identified by whole exome sequencing in individual II-3.** (**c**) **Mutation validation by Sanger sequencing** confirmed the *SLC25A12* mutation (c.1058G>A, black arrow) segregates with disease in individuals II-1 and II-3. Their healthy sibling and parents are each heterozygous carriers, consistent with autosomal recessive disease. (**d**) **Evolutionary conservation of the Arg353 residue in the AGC1 protein.** The Arg353 residue is located just below the m-gate of the AGC1 carrier, where it participates in closing and opening of the carrier on the mitochondrial matrix side through an interaction with a highly conserved glutamate at residue 384. *Hs Homo sapiens*, *Bt Bos taurus*, *Mm Mus musculus*, *Gg Gallus gallus*, *Dr Danio rerio*, *Xl Xenopus laevis*, *Ce Caenorhabditis elegans*, *Dm Drosophila melanogaster*, *Ag Anopheles gambiae*, *Nv Nasonia vitripennis*, *Sc Saccharomyces cerevisiae*, *Af Aspergillus fumigatus*, *Nc Neurospora crassa*

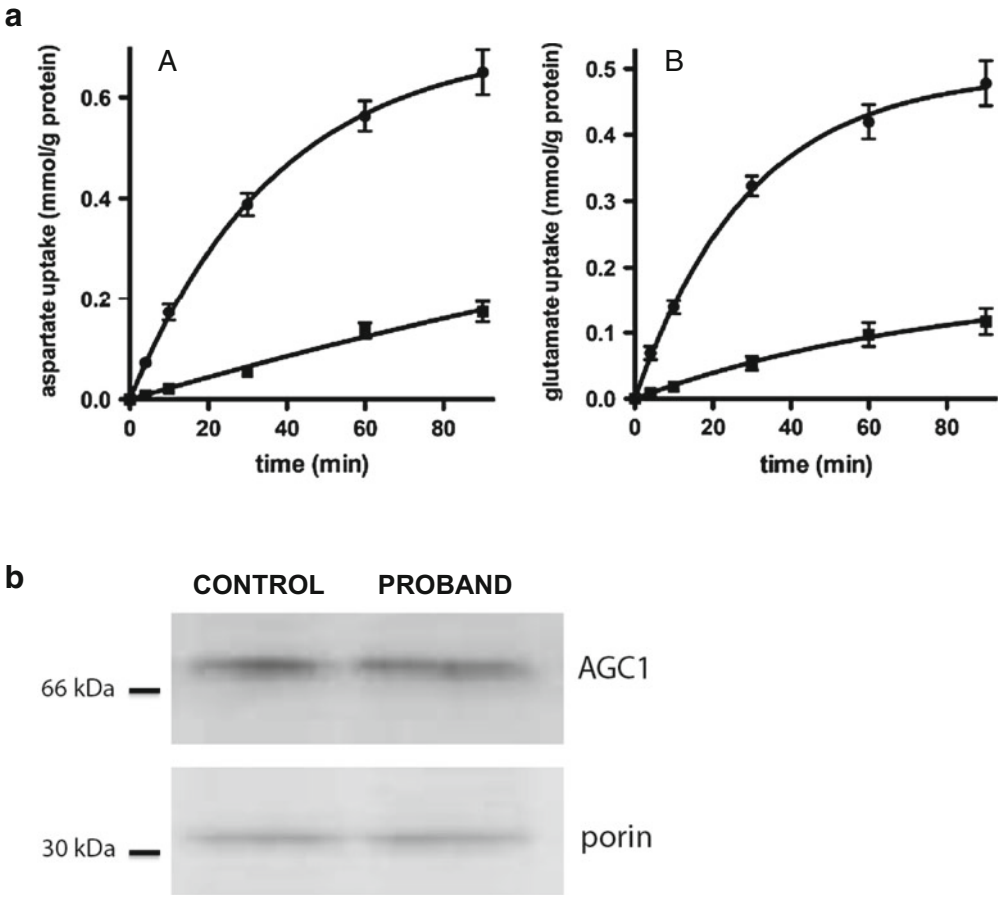

Fig. 3 Functional validation of AGC1 mutation in study proband's cells. (a) Transport assays of wild-type and mutant AGC1. Time courses of [^{14}C]aspartate/aspartate (*panel A*) and [^{14}C]glutamate/aspartate (*panel B*) exchanges in proteoliposomes reconstituted with the recombinant wild-type (*filled circles*) or R353Q mutant AGC1 from individual II-1 (*filled squares*) are shown. At time zero, 50 μM [^{14}C]aspartate (*A*) or 200 μM [^{14}C]glutamate (*B*) was added to proteoliposomes containing 20 mM aspartate. At the indicated times, the uptake of the labeled substrate was stopped by the addition of 15 mM pyridoxal 5′-phosphate and 10 mM bathophenanthroline. Data shown indicates mean and standard deviation of four independent experiments that were each performed in duplicate. **(b) Expression analysis of AGC1 in fibroblasts from AGC1 proband and unrelated healthy control.** Mitochondrial proteins (40 μg) were separated on 15 % SDS-PAGE, transferred onto nitrocellulose membrane, and immunodecorated with anti-AGC1 (*upper panel*) or anti-porin (*lower panel*) antibodies. Densitometry analysis revealed similar content of AGC1 and porin in the investigated mitochondrial extracts in three independent experiments

of the proband (individual II-1) and an unrelated healthy control that were immunodecorated with specific antibodies against AGC1 and porin (Fig. 3b), indicating that the R353Q mutation does not impede mitochondrial localization of AGC1. Finally, fluorescence microscopy of the proband's fibroblasts treated with 25 nmol MitoTracker Red (a mitochondria-specific fluorescent dye) revealed no alterations of the mitochondrial morphology as compared to control fibroblasts (data not shown).

Discussion

Whole exome sequencing analysis identified a novel homozygous c.1058G>A missense mutation in *SLC25A12* that segregated with disease in a consanguineous Indian kindred in which a brother and sister were similarly affected with severe global developmental delay, hypotonia, and intractable epilepsy with both focal and generalized seizures. Identification of *SLC25A12* as the disease gene highlighted the diagnostic relevance of the presumed nonspecific decrease in NAA and delayed myelination that had been identified on the proband's brain magnetic resonance spectroscopy and imaging studies. Indeed, this is only the second report of autosomal recessive *SLC25A12* mutations causing severe neurologic disease. The initial 2009 report linking *SLC25A12* to neurologic disease involved a Swedish girl who was found by candidate gene sequencing to harbor a homozygous AGC1 mutation that caused infantile-onset severe psychomotor retardation,

hypotonia, epilepsy, global cerebral hypomyelination, and dramatically decreased NAA (Wibom et al. 2009).

SLC25A12 encodes a neuronal-specific mitochondrial aspartate-glutamate carrier (aspartate-glutamate carrier isoform 1, AGC1). AGC1 is an important component of the neuronal malate/aspartate shuttle (Palmieri 2004), which is a crucial system to export intramitochondrial aspartate and to transfer the reducing equivalents of NADH from cytosol to mitochondria and hence to support oxidative phosphorylation (Lasorsa et al. 2003). A distinct disorder, AGC2 deficiency, results from *SLC25A13* mutations that reduce aspartate-glutamate carrier isoform 2 (AGC2) function with impaired malate/aspartate shuttle activity in the liver (Saheki and Kobayashi 2002). The prior report in the Swedish infant demonstrated complete loss of recombinant mutant AGC1 activity, resulting in no transport of aspartate or glutamate even after a 60 min incubation period (Wibom et al. 2009). In contrast, the homozygous c.1058G>A missense mutation identified in this consanguineous Indian family reduces recombinant mutant AGC1 activity to approximately 15 % of the initial wild-type rate, as determined when measuring either aspartate transport or glutamate transport. Delayed myelination and profound developmental delay were similarly observed both in our family and in the initially reported Swedish subject, where all three children now reported with AGC1 deficiency have had essentially no motor development through mid-childhood. The myelination defect primarily stems from neuronal loss attributable to the energy deficit caused by a lack of cellular reducing equivalents generated by the glutamate-aspartate shuttle (Wolf and van der Knaap 2009). The brain insult is possibly compounded by a lack of neuronal-generated NAA that is required as a precursor by oligodendrocytes to synthesize myelin and would lead to the secondary hypomyelination also observed in these patients (Wibom et al. 2009). As reduced NAA can be indicative of either decreased NAA production or neuronal loss, it is not possible to discern which mechanism predominates in AGC1 deficiency.

The complex, early-onset, and intractable epilepsy phenotype observed in the AGC1-deficient patients may be a feature present in individuals with many different primary and secondary mitochondrial disorders that involve decreased energy production in neurons (McCormick et al. 2013). Over the last few years, a growing number of genes involved in diverse cellular functions have been associated with early-onset intractable epilepsy together with global developmental delay. Recognized genes function as ion channels (*SCN1A*, *SCN2A*, *SCN8A*, *KCNQ2*, *KCNT2*), transcription factors (*ARX*, *ARHGEF9*, *CDKL5*, *PLCB1*), synaptic proteins (*PNKP*, *PCDH19*, *SPTAN1*, *STXBP1*), a mitochondrial glutamate symporter that transports glutamate and a hydrogen ion across the inner mitochondrial membrane (*SLC25A22*), and now another gene associated with inner mitochondrial membrane glutamate transport (*SLC25A12*). While *SLC25A22* mutation patients manifest both early myoclonic epilepsy and ohtahara-type early epileptic encephalopathy (Molinari et al. 2005, 2009; Poduri et al. 2013), the *SLC25A12* mutation patients' epilepsy resembles instead the non-myoclonic variant. Overall, this report establishes *SLC25A12* as a likely early-onset epileptic encephalopathy gene and emergence of altered glutamate handling as a functional subclass. While the precise cellular mechanism underlying seizures in these patients is not known, we conjecture that their seizures result from a combination of cellular injury and, possibly, although unproven, a cytosolic accumulation of glutamate that could impair synaptic uptake and trigger a hyperexcitable state. Interestingly, the AGC1 knockout mouse shows drastically reduced brain aspartate and NAA levels, global hypomyelination, and progressive failure of neuronal glutamatergic signaling (Ramos et al. 2011).

Should a primary energy deficiency be found to play a pathologic role in the etiology of AGC1-deficient seizures, use of the ketogenic diet might potentially offer a viable therapeutic strategy in these patients to reduce neuronal injury and improve epilepsy outcome. Although the role of the ketogenic diet for treatment of epilepsy in mitochondrial respiratory chain disorders is debatable, specifically relevant to the pathogenesis of neurologic disease in AGC1 deficiency is that although respiratory chain capacity is presumably normal, the reduction of the malate/aspartate shuttle results in a decrease of mitochondrial reducing equivalents that effectively generates an energy-deficient state despite normal respiratory chain capacity. Based on this consideration, the ketogenic diet might conceivably offer a viable therapeutic option for AGC1 deficiency through direct provision of acetyl CoA to the tricarboxylic acid cycle while bypassing the NAD^+-dependent glycolytic and pyruvate metabolism pathways.

This study also emphasizes the high heterogeneity in mitochondrial disease of both etiology and common biomarkers indicative of disease status. In these cases, clinical phenotypes and screening assays in blood, urine, and even muscle fail to provide diagnostic certainty for definitively categorizing an individual as having a primary mitochondrial disease. Indeed, while lactate was reported to be increased in the Swedish AGC1 proband in blood at 6 mmol and in CSF at 2.6 mmol each on one occasion, they were otherwise reportedly normal (Wibom et al. 2009). Similarly, concentrations of lactate and amino acids in blood in both affected siblings, and in CSF in our proband, were essentially normal, as is commonly true in known cases of mitochondrial disease (Haas et al. 2007). Interestingly, brain lactate was globally increased in our proband on brain spectroscopy performed both at 1 and 5 years of

age, which may result from the primary AGC1 deficiency or be a nonspecific finding in epilepsy, although the Swedish AGC1 proband did not have elevated lactate signal on MRS at 2 years, 9 months (Wibom et al. 2009). The muscle biopsy performed after the time of symptomatic onset in our proband at age 1 year showed normal histology and mitochondrial proliferation. Mitochondrial proliferation is a nonspecific finding that can be seen in the setting of reduced mitochondrial oxidative phosphorylation capacity, as is commonly seen in individuals with mitochondrial DNA (mtDNA) mutations involving mitochondrial tRNA genes or with mtDNA deletions (Wong 2010). While it is not clear what the basis for mitochondrial proliferation is in this case, as she had no evident reduction in muscle mitochondrial electron transport chain enzyme activity, it is possible that this was a response to a decrease in anaplerotic substrates due to the AGC1 defect reducing glutamate import into the mitochondria. While AGC1 does have neuronal expression, it is also known to be expressed in skeletal muscle and heart (Iijima 2001). The muscle biopsy was most useful in this case by facilitating demonstration that the children's disease was not due to a maternally inherited mitochondrial DNA genome mutation or deletion, nor due to a nuclear disorder that causes muscle-based mtDNA depletion. Thus, attention and diagnostic energy could be properly focused at that point on identifying the likely novel nuclear gene mutation underlying their now proven autosomal recessive disease. Ultimately, the recognition that *SLC25A12* mutations cause deficiency of a tissue-specific isoform of the mitochondrial AGC carrier explains the absence of more generalized multisystemic symptoms and metabolic findings in these patients.

In conclusion, it is clear that whole exome sequencing analysis facilitates diagnosis of individually rare causes of monogenic disease both in nuclear genes and, potentially, in the mitochondrial genome if it is targeted for analysis and the level of heteroplasmy is sufficient in the target tissue tested (Falk et al. 2012). Here, we have applied this approach to identify the second-ever reported cases of an autosomal recessive *SLC25A12* mutation that causes neuronal AGC1 deficiency. Differences in the three children affected in two unrelated kindreds demonstrates that AGC1 deficiency can variably lead to abnormal brain myelination, cortical atrophy, and/or neuronal energy deficiency with epilepsy and fluctuating basal ganglia involvement. Overall, AGC1 deficiency manifests as a severe, infantile-onset epilepsy syndrome with global developmental delay, congenital hypotonia, abnormal myelination, and reduced cerebral *N*-acetylaspartate levels, which could potentially be used as a diagnostic finding specific to this disease.

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One Sentence Synopsis

SLC25A12 mutations impair neuronal AGC1 activity and should be considered in children with infantile epilepsy, congenital hypotonia, global delay, abnormal myelination, and reduced brain *N*-acetylaspartate.

Compliance with Ethics Guidelines

Author disclosure statement. Marni J. Falk, Dong Li, Xiaowu Gai, Elizabeth McCormick, Emily Place, Francesco M. Lasorsa, Frederick G. Otieno, Cuiping Hou, Cecilia E. Kim, Nada Abdel-Magid, Lyam Vazquez, Frank D. Mentch, Rosetta Chiavacci, Jinlong Liang, Xuanzhu Liu, Hui Jiang, Giulia Giannuzzi, Eric D. Marsh, Yiran Guo, Lifeng Tian, Ferdinando Palmieri, and Hakon Hakonarson have no conflicts of interest to disclose.

Informed consent. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Animal studies. This article does not contain any studies with animal subjects performed by any of the authors.

Author contributions. MJF and HH designed and supervised all aspects of the study. MJF, EM, EP, and EDM clinically evaluated the subjects. MJF performed the skin biopsy to facilitate fibroblast analyses. FML, GG, and FP performed AGC1 activity studies and AGC1 expression analysis in fibroblasts. RC, FDM, YG, and EP coordinated research study subject enrollment. EM coordinated clinical results validation. FGO, CH, CEK, NA, LV, JL, HL, and XJ performed DNA sample extraction and handling, library preparation, whole exome sequencing, and data transfer. DL, LT, and XG performed bioinformatic analyses. MJF, DL, FP, EDM, and HH wrote the manuscript.

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