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EXPRESSION PROFILING AND IDENTIFICATION OF SLC2A1 AS A CANDIDATE GENE FOR VARIABLE RESPONSE OF KETOGENIC THERAPY IN UNCONTROLLED EPILEPSY PATIENTS

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ABSTRACT
Uncontrolled epilepsy is one such condition, which leads to recurrent seizures even after two to three trails of anti-epileptic drugs. Ketogenic diet has been validated as an effective treatment for uncontrolled epilepsy. Ketodiet helps in reduction of seizures and overall improvement of health of the patients. Inspite of all these, keto diet shows variable response rate in the patients. The variable response might be due to variable genetic makeup of the individual patients. The present study investigates the effect of the ketogenic diet through gene expression profiling using blood samples and qPCR techniques. 51 uncontrolled epilepsy patients and 50 healthy controls were included in this study, blood samples were collected in PAXgene tubes followed by RNA isolation from PAXgene blood RNA kit, cDNA conversion using Thermoscientific cDNA Reverse Transcription Kit, and qPCR using Maxima SYBR® Green qPCR master mix. The fold changes of gene expression was calculated using formula 2^- (ΔΔ Ct) Statistical analysis was done through Welch's t-test. Relative to controls (n=50), SLC2A1 expression was reduced to 0.382-fold in patients achieving complete seizure freedom (100% SRR, n=18), 0.111-fold in patients with ≥90% seizure reduction (90% SRR, n=12), 0.009-fold in patients with >50% seizure reduction (n=8), and 0.003-fold in patients with poor treatment response (<50% SRR, n=13), all reaching high statistical significance ($p < 0.0001$). These findings support SLC2A1 as a novel candidate biomarker for epilepsy treatment stratification. They further provide a molecular basis for evaluating ketogenic dietary therapy in SRR-defined, treatment-resistant patient subgroups....

KEYWORDS: keto diet, refractory epilepsy, drug resistant epilepsy, qPCR.

INTRODUCTION

Epilepsy is a chronic neurological disorder characterised by unprovoked, recurrent seizures, which are a result of abnormal or excessive neuronal activity in the brain. According to the world health organization, it affects around 50 million people worldwide, and an estimated 5 million people are diagnosed with epilepsy each year (WHO, 2019). The primary management of epilepsy relies on antiepileptic drugs (AEDs). However, many patients fail to attain a sustained seizure-free stage despite the trial of different adequate medications, which is defined by the International League Against Epilepsy (ILAE) as drug-resistant epilepsy (DRE) (Kwan et al., 2010). For these patients with uncontrolled epilepsy, non-pharmacological interventions are essential alternatives; one such intervention is the ketogenic diet.

A ketogenic diet (KD) is a high-fat, low-carbohydrate and moderate protein diet that induces a state of ketosis, where the primary energy substrate shifts from glucose to ketone bodies. It was originally introduced by Wilder in the 1920s as a diet that mimics fasting (D'Andrea Meira et al., 2019). It has been validated as an effective treatment for DRE, with approximately 50% of patients achieving more than a 50% reduction in seizure rate and up to 15% achieving a complete seizure-free status. It is especially effective with younger patients (Kossoff et al., 2018; Martin-McGill et al., 2020; Nathan et al., 2009).

The efficacy of the ketogenic diet is well documented, but there is a clinical observation of differential response variability among patients. While some individuals attain complete seizure-free status, some only achieve partial or no benefits at all (Kossoff et al., 2018; Nathan et al., 2009). Understanding this variability becomes a clinical priority. A study revealed that a greater fat ratio in the ketogenic diet, non-fasting induction, being female, and older age at the onset of seizures are associated with improved chances of seizure control at the six-month follow-up. Additionally, patients who initially experience a higher frequency of seizures and have less severe developmental delays demonstrate a positive response (Agarwal et al., 2017). Many studies show that genetic factors are the most important determinants of the response variability among patients. One cohort study of 226 children with refractory epilepsy demonstrated that certain causative

mutations in specific genes will predict if the patient responds favourably or unfavourably to KD. It was found that causative variants in transporter genes had the best response ($P = 0.009$), and variants in other membrane-related proteins (ion channels and neurotransmitter receptors) also showed good efficacy. However, the gene group related to cell structural integrity and/or homeostasis had the worst diet response ($P = 0.00006$) (Dahlin et al., 2025). This suggests that the molecular profile of the patient could inform the decision to initiate the ketogenic diet.

Causative variants specifically in the SLC2A1 (solute carrier family 2 member 1) and SCN1A (Sodium Voltage-Gated Channel Alpha Subunit 1) genes showed statistically significant associations with favourable KD response ($p < 0.05$) (Dahlin et al., 2025). SLC2A1encodes glucose transporter type 1 (GLUT1), the principal protein responsible for the facilitated transport of glucose across the blood-brain barrier (BBB). Pathogenic variants in SLC2A1 result in GLUT1 Deficiency Syndrome (Glut1DS), which presents as early-onset drug-resistant epilepsy. By providing ketone bodies as an alternative energy source that can bypass the dysfunctional GLUT1 transporter, the KD directly addresses the energy deficiency (Na et al., 2025; Wang et al., 2024).

The present study investigates the effect of the ketogenic diet on gene expression levels of patients with uncontrolled epilepsy to pre-determine the response rate of patients through gene expression profiling from blood samples using simple qPCR techniques.

METHODOLOGY

The patients, who are comfortable with the stated study, and who had completed the ketogenic therapy at least for 3 months were included in this investigation, complete clinical demographic data was collected for sub classification of the patients. Blood samples were collected in PAXgene vacutainers with informed consent of the patient, followed by taking signature of the guardian on the standard consent form. Blood samples of Age, gender and ethnically matched healthy controls were also collected. The collected blood samples stored at -80°C. Samples from the collected places were transported to the lab, according to the state protocol for the transport of the clinical material. Ethical clearance for this study was taken from The Karnataka Cancer Therapy and Research Institute, Hubballi, Karnataka, India (KCTRI/EC-01-B/2024).

The RNA from the blood was isolated according to standard PAXgene blood RNA kit. Formaldehyde agarose gel electrophoresis and Nanodrop UV Spectrophotometer was performed to check the quality and quantity of the isolated RNA. Thermoscientific cDNA Reverse Transcription Kit was used for the quantitative conversion of total RNA in a single 20 µL reaction to single stranded cDNA First, 10 µL of 2 X Rt master mixes was prepared by mixing 10X RT Buffer 2 µL, 25 X dNTP mixes (100 mM) 0.8 µL, 10 X RT random primers 2 µL, reverse transcriptase 1 µL, RNase Inhibitor 1 µL, Nuclease-free water 3.2 µL. To this mixture, 10 µL of RNA sample was added. The PCR programmed to the thermal cycles of one step each at 25°C for 5 min, 42°C for 60 min, 70°C for 5 min.

Maxima SYBR® Green qPCR master mix was used for real-time PCR analysis using SYBR® Green 1 Dye. The 20 µL reaction mixture containing Maxima SYBR Green PCR master mix (2X) 10 µL, forward and reverse primers (table 1) 1 µL (100 pmol), tamplet 1 µL (50 ng), and Nuclease-free water 7 µL. The PCR cycles were included 1 step of initial denaturation for 95°C for 10 min, 40 cycles of 95°C for 15 s, Tm 60°C for targeted genes primers for 30 s and 72°C for 30 s, were followed by melt curve.

Similarly and differentially expressed genes among different respondent groups were tabulated in terms of folds of up and down regulation. The relative gene expression were calculated based on the Ct (threshold cycle) value of each reaction. Relative gene expression level was calculated using the formula (2^- (ΔΔ Ct)):

Where ΔΔ Ct = ΔCt (patient) - avg. (Δ Ct (healthy control).

Δ Ct = Ct (GOI) – Ct (HKG)

GOI is each gene of interest.

HKG are the housekeeping genes

Statistical analysis Welch's t-test was used to assess the statistical significance of the data using SPSS software.

RESULTS

Total 51 uncontrolled epilepsy patients who were on the ketogenic therapy for minimum of 3 months were included in this study and 50 age, gender and ethnically matched healthy control samples were also included. 18 patients were further sub classified as seizure free with 100% SRR (seizure reduction rate), 12 as responders with 90% SRR, 8 as partial responders >50% SRR, and 13 as non-responders <50%. Clear two bands of 28s and 18s RNA confirms the successful isolation of total RNA from the blood samples (figure 1), and concentration RNA was approximately lying between 120 to 160 ng/ µL.

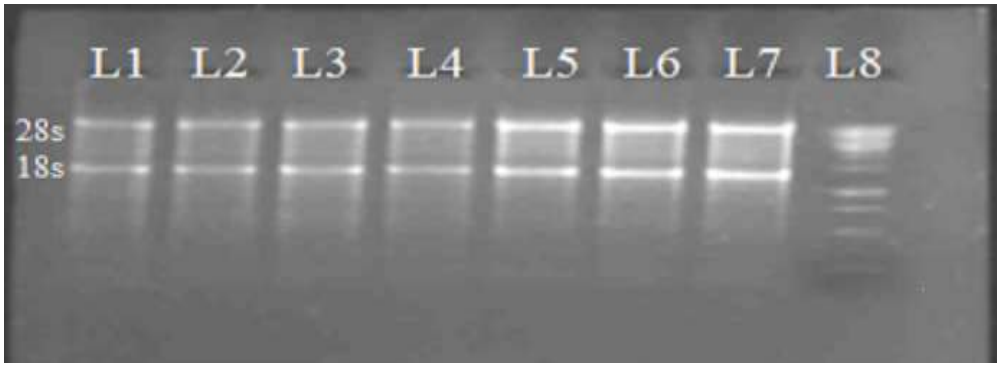

Figure 1. FA gel electrophoresis of isolated RNA with 1Kb ladder

Gene expression analysis of SLC2A1 gene along with the house keeping gene GAPGH for all the above sub classified groups and the healthy control samples was done using qPCR technique. Amplicon plots and 2 sharp melt curves represented successful amplification of target SLC2A1 and GAPDH genes (figure 2).

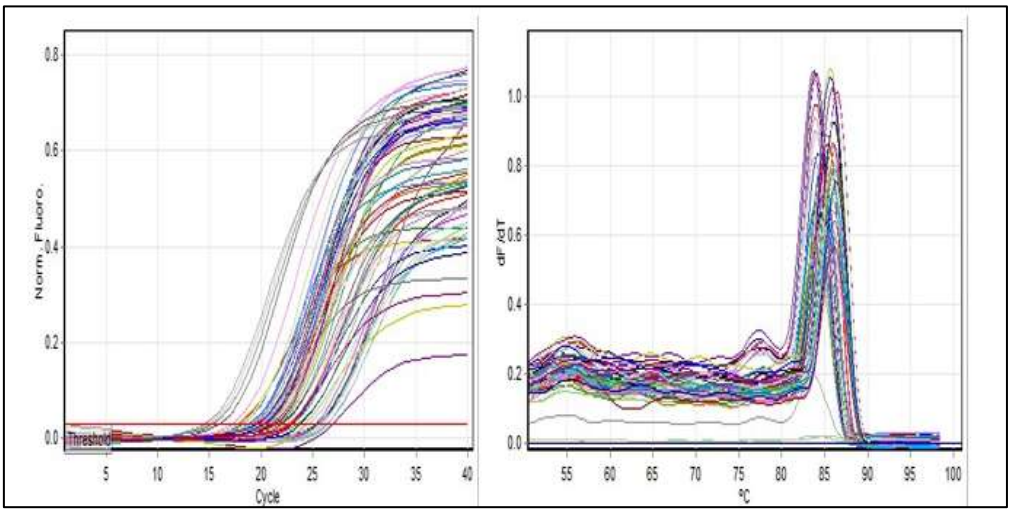

Figure 2. Amplicon plots and melt curves of SLC2A1 and GAPDH

Table 2 shows that, as the SRR increases there is a increase in the fold changes as well. SLC2A1 expression demonstrated a statistically significant and progressive decline with decreasing seizure reduction, with all treatment groups showing significantly lower expression compared to healthy controls (Welch's t-test, $p < 0.0001$ for all comparisons). Relative to controls (n=50), SLC2A1 expression was reduced to 0.382-fold in patients achieving complete seizure freedom (100% SRR, n=18), 0.111-fold in patients with ≥90% seizure reduction (90% SRR, n=12), 0.009-fold in patients with >50% seizure reduction (n=8), and 0.003-fold in patients with poor treatment response (<50% SRR, n=13), all reaching high statistical significance ($p < 0.0001$) (Figure 3).

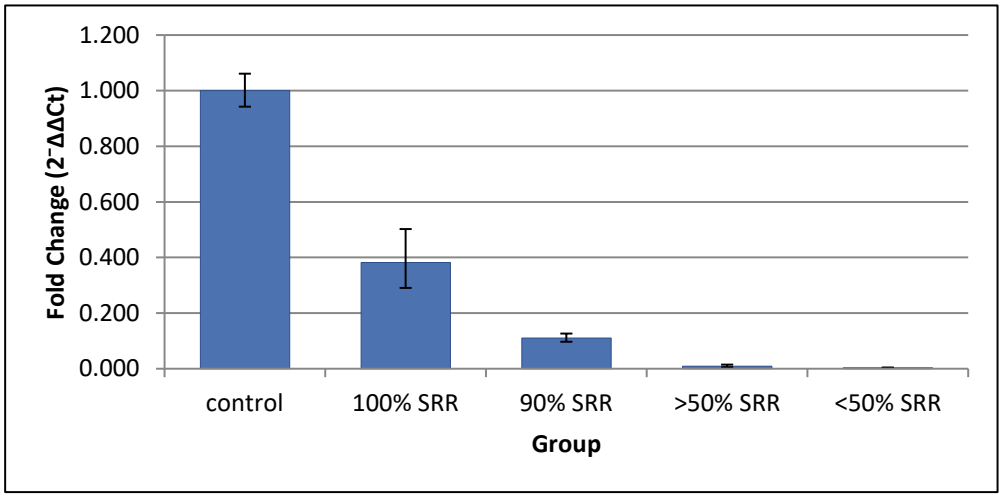

Figure 3. Graphical representation of fold changes of SLC2A1 gene in healthy control and sub classified groups

DISCUSSION

GLUT1, encoded by SLC2A1, mediates >90% of glucose transport across the blood–brain barrier and is the brain's primary energy supply route (Guo et al., 2005). Heterozygous SLC2A1 mutations cause GLUT1 Deficiency Syndrome (GLUT1-DS), characterized by drug-resistant infantile seizures, developmental delay, and movement disorders (Leen et al., 2010; De Giorgis and Veggiotti, 2012). The adult brain consumes up to 25% of systemic glucose at rest (Klepper and Leiendecker, 2007), so partial impairment of GLUT1 can be metabolically consequential. We report acquired, graded transcriptional suppression of SLC2A1 in non-responder epilepsy patients, extending the established genotype–phenotype relationship to a broader epilepsy population, reduced GLUT1 expression, genetic or acquired, may be a convergent mechanism driving seizure persistence.

Paradoxically, reduced neuronal energy supply increases seizure activity. Human iPSC-derived brain organoids with reduced GLUT1 expression couple cerebral hypometabolism to neuronal hyperexcitability (Mucha et al., 2026). In GLUT1-DS, seizures result from post-stimulus ATP depletion and impaired Na+/K+-ATPase function (Fernandez-Marmiesse et al., 2025), supporting a bioenergetic-failure model. Histopathology shows markedly reduced GLUT1 in focal cortical dysplasia (FCD) and co-localisation of cerebral hypometabolism with seizure-onset zones on

18F-FDG PET (Arslan et al., 2024). The progressive SLC2A1 suppression observed in our poorest responders (≥330-fold reduction) aligns with profound cerebral energy deficit sustaining seizure generation despite ketogenic therapy.

The ketogenic diet (KD) is the treatment of choice for GLUT1-DS because ketone bodies cross the blood–brain barrier via monocarboxylate transporters, bypassing defective GLUT1 (Kossoff et al., 2018). Given severe SLC2A1 suppression in our <50% seizure-reduction responders, KD is a rational, evidence-based therapy even without confirmed germline mutation. A 2024 network meta-analysis found KD, modified Atkins, and low-glycaemic-index therapy each achieved >50% seizure reduction in drug-resistant epilepsy (Mughal et al., 2024), and metabolomic changes during KD correlated with seizure reduction (Dahlin et al., 2024). Our findings provide a molecular rationale for selecting KD in SLC2A1-stratified subgroups.

Nearly 30% of epilepsy patients remain drug-resistant despite optimal therapy (Perucca et al., 2023), and no validated molecular biomarker predicts outcome at diagnosis. SLC2A1 is a strong candidate given its correlation with seizure-reduction rate (SRR), large effect sizes, and reproducibility. Limitations include modest sample sizes in SRR subgroups (especially >50% SRR, n=8), cross-sectional design preventing causal inference, and use of GAPDH alone. Future studies should validate reference genes. Independent, larger cohorts with rate of change ROC analysis, ΔCt cut-off optimisation, and longitudinal sampling are required before clinical translation.

Table 1. Designed primer sequence for SLC2 and GAPDH gene used in qPCR

Gene	Forward primer 5' – 3'	Reverse primer 5' – 3'
SLC2A1	GTCTGGCATCAACGCTGTCT	AACAGCGACACGACAGTGAA
GAPDH	CGACCACTTTGTCAAGCTCA	AGGGGTCTACATGGCAACTG

Table 2. Mean fold changes of all the sub classified groups and controls with their p values.

Group	n	Mean Fold Change	Error Bar (+)	Error Bar (-)	p-value	Significance
control	50	1.0000	0.0611	0.0576	ref	ref
100% SRR	18	0.3821	0.1202	0.0915	2.17×10^{-6}	***
90% SRR	12	0.1107	0.0157	0.0137	3.06×10^{-15}	***
>50% SRR	8	0.0090	0.0058	0.0035	2.53×10^{-7}	***
<50% SRR	13	0.0034	0.0010	0.0008	9.92×10^{-16}	***

CONCLUSION

This study identifies a robust inverse relationship between SLC2A1 expression and seizure control in human epilepsy patients, with poor treatment responders exhibiting profound transcriptional suppression of the brain's primary glucose transporter. These findings implicate bio energetic failure as a mechanism of treatment resistance and support SLC2A1 as a novel candidate biomarker for epilepsy treatment stratification. They further provide a molecular basis for evaluating ketogenic dietary therapy in SRR-defined, treatment-resistant patient subgroups.

Conflict of interest:

The authors declared no conflicts of interest concerning the research, authorship, funding and publication.

Data statement: Data is not available as it includes confidential information regarding the participants.

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