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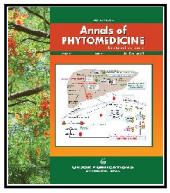

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A cohort study on UGT1A3 polymorphism in pediatric epileptic patients

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Abstract

This study aimed to investigate the relationship between UGT1A3 genetic variations and the clinical outcomes, including effectiveness and tolerability of sodium valproate monotherapy in pediatric patients with epilepsy. In this prospective cohort study, we enrolled one hundred pediatric patients with epilepsy aged between 2 and 18 years who had been receiving sodium valproate as the sole treatment for at least one month. We obtained consent from the patients and their guardians for genetic polymorphism analysis and collected blood samples to assess serum drug concentrations, biochemical parameters, and platelet counts. Genomic DNA was isolated from whole blood using the phenol-chloroform extraction and ethanol precipitation method. Subsequently, it underwent PCR amplification and sequencing for mutation analysis. Hematology cell counters were used for biochemical investigations, and the clinical outcomes were evaluated in terms of sodium valproate's effectiveness and safety. Our findings revealed that most individuals with the wild-type variations of A17G and C133T experienced either generalized tonic-clonic seizures (GTCS) or partial seizures, indicating that these mutations might be advantageous in reducing the risk of seizures. Conversely, the mutant variations of T31C, G81A, T140C, and A477G were associated with epilepsy in patients, suggesting that these mutations could pose a risk. There were no significant differences in liver function tests at baseline, 6 months, and 1 year, and these results were consistent across different genotypes. Serum creatinine levels remained within the normal reference range. While insignificant correlation of gene polymorphisms and serum sodium valproate concentrations, the drug levels did vary significantly over different time intervals. UGT1A3 gene polymorphism studies may prove valuable as a predictive tool before initiating sodium valproate treatment to anticipate treatment responses. Our study demonstrated that valproate was tolerated well by pediatric patients with epilepsy, with no substantial changes in hepatic, renal, or pancreatic parameters. These results support the use of sodium valproate as an effective antiepileptic drug for pediatric patients.

1. Introduction

Epilepsy is a chronic neural condition characterized by recurrent, uncontrolled seizures (Thurman *et al.,* 2011). Seizures are caused by abberant electrical activity in the brain, might results in unconsciousness and treated in 70% of patients through drugs (Saloni *et al.,* 2022; Palla *et al.,* 2022). Statistics indicate an annual occurrence of epilepsy in 40 to 70 individuals per 100,000 people (Kotsopoulos *et al.,* 2002). Pediatric epilepsy is prevalent in approximately 22 out of every 1,000 children (Hackett *et al.,* 1997). However, research conducted in India suggests a lower prevalence, estimated at around 4 to 8 cases per 1,000 people (Gadgil *et al.,* 20011; Amudhan *et al.,* 2015).

Sodium valproate (VPA), a broad-spectrum antiepileptic medication, is commonly prescribed for both mild and intractable seizures. Nevertheless, it exhibits significant variability in pharmacokinetics and pharmacodynamics among individuals. This means that even when administered the same VPA dosage, the serum levels of the medication vary. To ensure optimal treatment outcomes, monitoring the serum concentration of VPA is essential (Chadwick *et al.,* 1985).

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The variations in serum levels may be attributed to genetic factors, underlying medical conditions, and individual responses (Hung *et al.,* 2001; Downing *et al.,* 2010; Franciotta *et al.,* 2011).

Pharmacogenetics is the field of study that explores genetic variations' impact on metabolism of drug, pharmacological targets, pathogenesis, leading to variations in drug efficacy and side effects (Dlugos *et al.,* 2006; Szoeke *et al.,* 2006). This has led to the evolution of "individualized medicine," replacing the traditional approach of "one-size-fits-all" with a focus on prescribing the right drug to the right patient at the right dose and time. Therefore, it is of paramount importance to investigate how genetic polymorphisms may influence the pharmacokinetics and pharmacodynamics of antiepileptic medications.

The metabolism of VPA occurs predominantly in the liver through various processes, including cytochrome P450 (CYP)-dependent reactions, mitochondrial oxidation, and microsomal glucuronide conjugation initiated by uridine 5'-diphospho (UDP) glucuronosyl transferase (UGT). Only a small fraction of VPA is excreted unchanged in urine, with the majority undergoing metabolic transformation. Several well-studied CYP isoforms, such as CYP2C9, CYP2A6, CYP2B6, and possibly CYP2C19, are involved in VPA metabolism.

UGT genes are categorized into four families: UGT1, UGT2, UGT3, and UGT8, and they participate in the glucuronidation of VPA. The UGT1 and UGT2 families are primarily involved in the glucuronidation

of endogenous and exogenous substances. Among the UGT isozymes, UGT1A3, UGT1A6, and UGT2B7 play vital roles in the formation of VPA glucuronides, accounting for approximately 50% of VPA metabolism.

There is limited research on UGT polymorphisms concerning sodium valproate within the Indian context. Existing studies have shown that the UGT1A6 552 A>C polymorphism significantly affects the steady-state concentration of VPA. However, these studies are constrained by small sample sizes, a focus on specific UGT isoforms, and limitations in representing the general population. Consequently, they do not provide conclusive evidence of a direct relationship between specific UGT1A6 polymorphisms and serum valproic acid levels.

Similarly, research on UGT1A3 polymorphism is limited. However, studies outside India have identified associations between UGT1A3 polymorphism and plasma valproic acid levels, suggesting that specific UGT1A3 polymorphisms may influence VPA metabolism. These studies imply that carriers of certain UGT1A3 polymorphisms may require higher VPA dosages to maintain therapeutic levels.

The substantial variability in VPA dosages among individuals can be attributed to genetic variations in genes responsible for drug metabolism. The concept of "individualized medicine" recognizes that a one-size-fits-all approach is inadequate and emphasizes the importance of tailoring drug treatments based on individual genetic profiles. Therefore, it is essential to investigate the potential involvement of genetic polymorphisms in VPA metabolism. Notably, there is a scarcity of studies examining the impact of UGT1A3 gene polymorphism on VPA metabolism, particularly within the Indian context.The study aimed to achieve the following objectives:

i. Assess the UGT1A3 polymorphism patterns in pediatric epileptic patients receiving valproate monotherapy and investigate their association with plasma valproate concentrations.

ii. Examine the relationship between genetic polymorphisms of UGT1A3 and the clinical effectiveness of sodium valproate, as well as its adverse effects.

iii. Evaluate the adverse effects of sodium valproate by analyzing parameters related to liver function (LFT), hematology, and clinical factors, and establish correlations with genetic polymorphisms.

2. Materials and Methods

2.1 Methodology

The research took place at the Central Research Laboratory of K S Hegde Medical Academy and the Department of Pediatrics at Justice K S Hegde Charitable Hospital in Mangalore, Karnataka, India.

The study employed a prospective cohort design and included one hundred pediatric epileptic patients aged between 2 and 18 years. These patients were diagnosed based on their seizure history and electroencephalogram tests, and they were all receiving sodium valproate as monotherapy for a minimum of one month.

Exclusion criteria were applied to exclude children receiving other antiepileptic drugs, medications that could interfere with valproate metabolism, or herbal or alternative medicines. Patients with hepatic and renal abnormalities, as determined through clinical and laboratory investigations, were also excluded.

In order to gather samples, patients were advised to omit their medication from the night before. Blood samples were then obtained the next morning to estimate trough-level concentrations. The collected blood samples were divided into three segments: 2 ml of EDTA whole blood was preserved at –80ºC for the analysis of genetic polymorphisms, another 2 ml of whole blood in a plain vial underwent centrifugation to obtain serum, which was subsequently stored at –80ºC for the assessment of serum drug concentration and biochemical parameters, and 1 ml of EDTA whole blood was employed for platelet estimation.

2.1.1 DNA isolation

Genomic DNA was extracted from whole blood using a Phenol-chloroform extraction and ethanol precipitation method. The quantity and purity of the DNA were evaluated using a nanodrop spectrophotometer, and the isolated DNA was then stored at –20°C until it was ready for further analysis.

2.1.2 Genotyping

The Polymerase Chain Reaction (PCR) method was employed to amplify the UGT1A3 gene, resulting in a 519-base pair product, utilizing specific primers. The amplified DNA samples were validated on a 2% agarose gel stained with ethidium bromide, and sequencing was utilized to detect any mutations in the gene sequence.

2.1.3 High performance liquid chromatography (HPLC)

The steady-state trough serum concentration of sodium valproate was determined through high-performance liquid chromatography (HPLC) analysis. HPLC is an precisely used tool for the quality assessment (Srivani *et al.,* 2022). This analysis involved utilizing a reverse-phase column under specific conditions, and concentration standards for valproic acid were prepared. Chemicals were used such as hydrochloric acid, HPLC grade acetonitrile, methanol of HPLC grade, phosphoric acid and hydrogen peroxide (Padmabhusana *et al.,* 2022; Sujatha *et al*., 2022). Biochemical investigations, encompassing various parameters such as albumin, total protein, AST, ALT, ALP, total bilirubin, direct bilirubin, serum amylase, creatinine, urea, and platelet count, were conducted using a fully automated clinical chemistry analyzer.

Clinical outcomes were assessed in terms of the efficacy and safety of sodium valproate. Responders were characterized as patients who exhibited no relapse or fewer than two seizure episodes in one year of follow-up and who tolerated sodium valproate monotherapy without encountering adverse effects. Non-responders, on the other hand, were individuals who experienced two or more seizure episodes in one year or developed adverse drug effects following therapy initiation.

Before commencing the study, ethical approval was obtained from the Central Ethics Committee (NU), and written informed consent was acquired from the parents or first-degree relatives of the patients.

The gathered data were summarized by calculating frequencies and percentages for qualitative information, and means with standard deviations (mean ± SD) for quantitative data. To assess Hardy-Weinberg equilibrium (HWE), an online "wpcalc" calculator was employed.

Comparisons between gene polymorphism and sodium valproate levels were conducted using either One-Way ANOVA or the Student's "t" test, depending on the specific analysis requirements.

The association between genetic polymorphism and clinical outcomes was evaluated using the Chi-Square test. The statistical significance of these analyses was determined by the "*p*" value.

3. Results

Out of the initial 122 screened patients, 100 met the inclusion criteria and provided informed consent to participate in the study. At the time of enrollment, samples were collected from all 100 patients. Subsequently, during the six-month follow-up, samples were obtained from 81 patients. The 1-year follow-up was conducted with 72 patients.

Eleven patients transitioned from sodium valproate treatment to alternative anti-seizure drug therapy. In eight instances, an additional anti-seizure drug was prescribed alongside sodium valproate to address recurrent seizures. Unfortunately, single patient passed away due to complications arising from congenital heart disease. Additionally, samples could not be collected from eight patients at the six-month and one-year follow-ups; however, it is noteworthy that they remained seizure-free during this period (Figure 1).

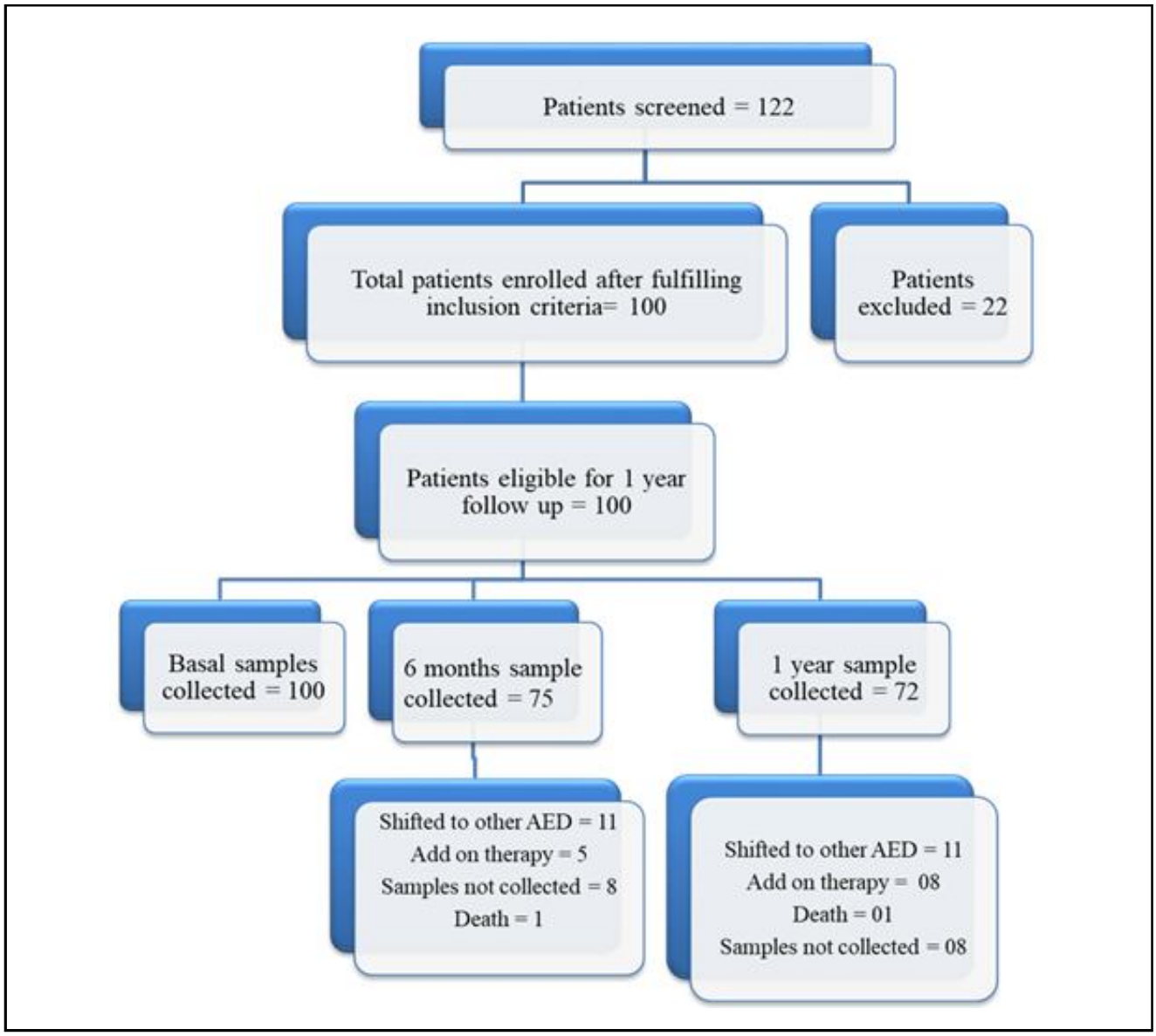

Figure 1: Strobe flow chart.

The mean age of the study participants was 8.5 ± 4.3 years, ranging from 2.2 to 17.3 years, and the average BMI during enrollment was 16.5 ± 4.3, with a range of 7.81 to 32.84. Among the participants, 57 were males, and 43 were females. The patients were classified into three groups based on age: 2-6 years, 6-12 years, and 12-18 years. A statistically significant difference in BMI was observed among the different age groups. However, it is noteworthy that the BMI values fell within the normal range for patients across the various age groups (Table 1).

Table 1: Demographic data of patients in different age groups at baseline

Characteristics	2-6 years (N=25)	6-12 years (N=60)	12-18 years (N=15)	*p*-value
Gender - Male	17	32	08	-
Female	08	28	07	-
Seizure type - GTCS	17	55	11	-
CPS	08	05	04	-
BMI	14.08 ± 3.5	16.08 ± 3.8	21.04 ± 4.4	0.000*
Sodium valproate concentration (µg/ml)	95.7 ± 43.6	104.2 ± 28.5	112.3 ± 23.3	0.315

UGT1A3 A17G on sequencing showed that wild was 99% and mutant allele was only 1%. 100%of the patients showed mutation in T31C genotype. Sequencing of G81A showed that 14% were wild type and 86% were mutant. There was no mutation at all for C133T genotype. T140C analysis revealed 6% of the patients had wild type and 94% mutant variety. A477G showed 27% wild variety and 73% mutant type (Table 2). Sequencing results are depicted in 3A-3E.

Table 2: UGT1A3Sequencing results of study participants

UGT1A3											
A17G		T31C		G81A		C133T		T140C		A477G	
Wild A	99	Wild T	0	Wild G	14	Wild C	100	Wild T	6	Wild A	27
Mutant G	01	Mutant C	100	Mutant A	86	Mutant T	0	Mutant C	94	Mutant G	73

Patients with wild alleles of A17G showed a decline in VPA concentrations, (11.3% at 6 months 20.02% at 1 year) (Table 3). Patients with T31C also followed the similar pattern but alleles were of mutant variety. Both wild and mutant varieties of G81A exhibited decline in the VPA concentration at 6 months and 1 year. Patients with other genotypes, C133T, T140C and A477G also had a fall in VPA concentration (both wild and mutant) in the similar pattern. However, the p values were not calculated as some of the groups had minimum number of values needed for statistical analysis.

Table 3: Steady state sodium valproate concentration in different genotype

Genotype	Basal (n=100) (µg/ml)		6 months (n=81) (µg/ml)		1 year (n=72) (µg/ml)	
	Wild	Mutant	Wild	Mutant	Wild	Mutant
UGT1A3 (A17G)	103.3 ± 34.28	104.0	92.53 ± 42.09	63.00	77.16 ± 34.70	142.0
UGT1A3 (T31C)	-	103.3 ± 34.10	-	92.15 ± 41.95	-	78.03 ± 35.27
UGT1A3 (G81A)	99.36 ± 29.98	104.0 ± 34.84	86.50 ± 47.21	93.20 ± 41.21	77.17 ± 41.69	78.19 ± 34.29
UGT1A3 (C133T)	103.3 ± 34.10	-	92.15 ± 41.95	-	78.03 ± 35.27	-
UGT1A3 (T140C)	103.5 ± 38.13	77.25 ± 45.75	98.75 ± 54.76	91.78 ± 41.58	77.25 ± 45.75	78.07 ± 35.00
UGT1A3 (A477G)	98.89 ± 31.87	105.0 ± 34.96	93.74 ± 41.87	91.50 ± 42.35	82.95 ± 31.21	75.98 ± 36.91

It can be observed in Table 4 that majority of wild varieties of A17G and C133T suffered either with GTCS or partial seizures suggesting that the mutation would be beneficial in reducing the risk of seizures. Vice versa holds good for T31C, G81A, T140C and A477G. Patients with mutant varieties were epileptic in these genotypes suggesting that these mutations were dangerous.

Table 4: Pattern of UGT1A3 gene polymorphisms with seizure types

Genotype	GTCS (N=83)		Partial seizure (N=17)	
	Wild	Mutant	Wild	Mutant
UGT1A3 (A17G)	82	1	17	-
UGT1A3 (T31C)	-	83	-	17
UGT1A3 (G81A)	12	71	2	15
UGT1A3 (C133T)	83	-	17	-
UGT1A3 (T140C)	5	78	1	16
UGT1A3 (A477G)	23	60	4	13

Table 5: Comparison of biochemical and hematological parameters in different age groups at baseline

Characteristics	2-6 years (Mean ± SD) (N=25)	6-12 years (Mean ± SD) (N=60)	12-18 years (Mean ± SD) (N=15)	*p*-value
Albumin (g/dl)	4.31 ± 0.31	4.47 ± 0.37	4.62 ± 0.20	0.023*
Total protein (g/dl)	6.98 ± 0.50	7.45 ± 0.68	7.56 ± 0.29	0.003*
SGOT (IU/l)	38.5 ± 17.1	30.5 ± 13.6	21.8 ± 5.7	0.047*
SGPT (IU/l)	14.2 ± 5.01	13.7 ± 4.88	13.4 ± 5.26	0.951
Alkaline phosphatase (IU/l)	208.3 ± 72.6	198.4 ± 51.3	141.7 ± 64.2	0.010*
Direct bilirubin (mg/dl)	0.11 ± 0.03	0.11 ± 0.04	0.10 ± 0.03	0.524
Total bilirubin (mg/dl)	0.22 ± 0.09	0.30 ± 0.13	0.41 ± 0.19	0.020*
Blood urea (mg/dl)	22.16 ± 7.9	20.6 ± 7.13	20.7 ± 8.5	0.696
Serum creatinine (mg/dl)	0.33 ± 0.14	0.50 ± 0.19	0.68 ± 0.19	0.000*
Amylase (IU/l)	76.28 ± 30.2	89.13 ± 41.3	83.8 ± 40.2	0.528
Platelets (cells/mm^3)	305760 ±75870	271230 ±59830	288670 ±55967	0.074

Albumin, total protein, SGOT, alkaline phosphatase, bilirubin total, and serum creatinine values showed a significant difference in patients of different age groups during enrolment (Table 5).

Comparison of drug concentration, biochemical and hematological parameters at diverse time intermissions were depicted in Table 6. VPA concentrations significantly decreased over the 1 year period. Serum creatinine was in the biological reference range even though significant difference was there when basal, 6 months and 1 year values.

Table 6: Drug concentration, biochemical and hematological parameters

Characteristics	Basal (n=100) Mean ± SD	6 months (n=81) Mean ± SD	1 year (n=72) Mean ± SD	*P*-value
BMI	16.51 ± 4.30	16.69 ± 3.71	17.37 ± 3.60	0.354
Sodium valproate concentration (µg/ml)	103 ± 34.10	92.15 ± 41.95	78.03 ± 35.27	<0.0001**
Albumin (g/dl)	4.44 ± 0.38	4.43 ± 0.34	4.43 ± 0.35	0.99
Total protein (g/dl)	7.34 ± 0.64	7.49 ± 0.615	7.49 ± 0.71	0.241
SGOT (IU/l)	29.41 ± 16.71	27.25 ± 8.30	28.87 ± 8.36	0.512
SGPT (IU/l)	13.55 ± 8.173	12.63 ± 4.88	12.40 ± 5.26	0.464
Alkaline phosphate (IU/l)	192.7 ± 72.75	204 ± 79.90	206 ± 80.08	0.461
Direct bilirubin (mg/dl)	0.10 ± 0.11	0.108 ± 0.054	0.113 ± 0.08	0.860
Total bilirubin (mg/dl)	0.29 ± 0.11	0.297 ± 0.16	0.309 ± 0.186	0.917
Blood urea (mg/dl)	20.94 ± 7.55	19.23 ± 7.28	19.85 ± 6.65	0.284
Serum creatinine (mg/dl)	0.48 ± 0.21	0.397 ± 0.137	0.390 ± 0.124	0.0002**
Amylase (IU/l)	85.19 ± 43.04	79.71 ± 32.83	77.5932.80	0.383
Platelet (cells/cubic mm)	281960 ± 65080	27733 ± 42979	295845 ± 30500	0.715

**p* highly significant

80% of the patients with wild alleles of A17G were responders, implying that even though they were at risk of epilepsy, enzyme UGT1A3 was effectively metabolizing VPA in them (Table 7) whereas patients with T31C, G81A, T140C and A477G mutation was beneficial in maintaining VPA concentration in therapeutic range and have majority of the patients were responders. However, Table 8 shows that there was no significant association between the gene polymorphism and clinical outcome in pediatric epileptics.

Table 7: Pattern of UGT1A3 gene polymorphisms with clinical outcome

Genotype	Clinical response (Efficacy)			
	Wild		Mutant	
	Responders (%)	Non- responders (%)	Responders (%)	Non- responders (%)
A17G	80	19	-	1
T31C	-	-	80	20
G81A	10	4	70	16
C133T	80	20	-	-
T140C	5	1	75	19
A477G	23	4	57	16

Table 8: Association of UGT1A3 gene polymorphisms with clinical outcome

UGT1A3 Genotype	Polymorphism pattern	Polymorphism in percentage	Clinical outcome		*p* value
			Responders (n=80)	Non responders (n=20)	
UGT1A3 (A17G)	Wild	99	8019	01	-
	Mutant	1			
UGT1A3 (T31C)	Wild	0	080	020	-
	Mutant	100			
UGT1A3 (G81A)	Wild	14	1070	416	0.38
	Mutant	86			
UGT1A3 (C133T)	Wild	100	800	200	-
	Mutant	0			
UGT1A3 (T140C)	Wild	6	575	119	0.83
	Mutant	94			
UGT1A3 (A477G)	Wild	27	2357	416	0.43
	Mutant	73			

There was no association between UGT1A3 and seizure type patients (Table 9)

Table 9: Association of UGT1A3 gene polymorphisms with seziure type

UGT1A3 Genotype	Polymorphism pattern	Polymorphism in percentage	Seizure type		*p* value
			GTCS(n=83)	Focal seizures(n=17)	
UGT1A3(A17G)	Wild	99	821	170	-
	Mutant	1			
UGT1A3(T31C)	Wild	0	083	017	-
	Mutant	100			
UGT1A3(G81A)	Wild	14	1271	215	0.77
	Mutant	86			
UGT1A3 (C133T)	Wild	100	830	170	-
	Mutant	0			
UGT1A3 (T140C)	Wild	6	578	116	0.98
	Mutant	94			
UGT1A3 (A477G)	Wild	27	2360	413	0.73
	Mutant	73			

There was insignificant difference in the liver function tests at Basal, 6 months and 1 year as well as between the genotypes. Even though serum creatinine levels were in the biological reference range. There was a significant decline in the levels at 6 months and 1 year for patients with genotypes A17G, T31C, G81A, C133T. Creatinine levels did not differ significantly in patients with T140C and A477G at different intervals.

Majority of the non-responders were of wild type in patients with A17G and C133T. Whereas for other genotypes (T31C, G81A, T140C, A477G). Majority of the non-responders were of mutant alleles (Table 10)

The Naranjo scale comprises ten questions designed to gather information related to various aspects of adverse drug reactions and the medications prescribed to the patient. These questions are answered with 'yes,' 'no,' or 'do not know.' The total score is used to categorize the adverse drug reaction: a score of ≥9 indicates a 'definite ADR,' while a score between 5 and 8 is classified as a 'probable ADR.' A score between 1 and 4 is labeled a 'possible ADR,' and a score of 0 corresponds to a 'doubtful ADR.'

The Naranjo Algorithm, developed by Naranjo and colleagues, serves as a questionnaire to determine whether an adverse drug reaction (ADR) is genuinely caused by the drug or other factors. The values derived from this algorithm are sometimes utilized in peer reviews to validate the author's conclusions regarding adverse drug reactions.

Causality assessment was carried out using the Naranjo algorithm by a Senior Research Fellow under the supervision of the treating paediatrician. The Naranjo score for this specific adverse drug reaction (acute pancreatitis) was found to be +6, indicating the causality of the ADR as 'Probable' (Table 12).

Table 10: Pattern of UGT1A6 gene polymorphism in non-responders (N=20)

	Pattern	Frequency	% of patients
A17G	AA	19	95
	AG	1	5
	GG	0	-
T31C	TT	0	-
	TC	11	55
	CC	9	45
G81A	GG	4	20
	GA	15	75
	AA	1	5
C133T	CC	20	100
	CT	-	-
	TT	-	-
T140C	TT	1	5
	TC	18	90
	CC	1	5
A477G	AA	4	20
	AG	16	80
	GG	-	-

Table 11: Comparison of sodium valproate drug concentration with clinical outcome

Clinical outcome	Basal (µg/ml)	6 months (µg/ml)	1 year (µg/ml)	*p* value
Responders	101.4 ± 34.23	92.82 ± 42.21	78.52 ± 35.00	0.0011**
Non- responders	111 ± 33.35	87.16 ± 41.97	73.89 ± 39.69	0.0481*

ANOVA test, $p<0.05$ considered significant

Table 12: Reply to the framed questions of Naranjo scale

Sl.No.	Questions	Reply	Assessment Score
1.	Have there been prior definitive findings or reports regarding this reaction?	No	0
2.	Did the adverse event manifest subsequent to the administration of the suspected drug?	Adverse event appeared after initiation of Sodium valproate treatment.	+2
3.	Did the adverse response show improvement upon discontinuation of the drug or following the administration of a specific antagonist?	Adverse event improved when treatment was discontinued. No specific antagonist was administered. Levetiracetam treatment was started.	+1
4.	Did the adverse event resurface upon the reintroduction of the drug?	Drug was not re-administered again.	0
5.	Are there alternative factors, aside from the drug, that could independently have triggered the reaction?	No	+2
6.	Did the reaction recur when a placebo was administered?	Do not know, because placebo was not prescribed.	0
7.	Were toxic concentrations of the drug detected in the blood or other fluids?	Drug was not found to be in toxic range.	0
8.	Did the severity of the reaction intensify with an increase in dosage or diminish with a decrease in dosage?	Rechallenge was not done.	0
9.	Did the patient experience a comparable reaction in any previous exposure to the same or similar drugs?	No	0
10.	Was there any objective evidence validating the occurrence of the adverse event?	Biochemical and ultrasonography finding were suggestive of acute pancreatitis. Brief report:- Patient was on Sodium Valproate treatment (T. Enchorate Chrono 500mg BD) since Day 1 Developed pain abdomen, more in peri-umbilical region, from Day 5 USG was done on Day 12 Impression: Reactive mesenteric lymphadenopathy, Appendix was normal, Head of pancreas – Bulky. De-challenge was done on Day 13, and the patient recovered completely within a week. The patient was shifted to levetira cetam. On further follow up visit, patient was doing well with levetiracetam.	+1
	Total score		6

The correlation study revealed that, at the basal level, only creatinine levels exhibited a significant negative correlation with sodium valproate concentration (p=0.011). Conversely, parameters such as albumin, total protein, direct bilirubin, total bilirubin, alkaline phosphatase, amylase, and platelet count showed insignificant negative correlations. Additionally, an insignificant positive correlation was identified between basal sodium valproate concentration and SGPT, SGOT, and urea (Table 13).

After six months of follow-up, there was a negative correlation between sodium valproate concentration and parameters like albumin, total protein, SGPT, direct bilirubin, total bilirubin, creatinine, and amylase. However, these correlations remained statistically insignificant. Notably, a significant negative correlation was observed between sodium valproate concentration and platelet count at the six-month follow-up (p=0.023). Furthermore, an insignificant positive correlation was noted between sodium valproate and SGOT, alkaline phosphatase, and urea at this stage (Table 13).

At the one-year follow-up, a significant correlation was detected between sodium valproate concentration and SGPT, urea, and amylase (p=0.005, 0.049, 0.020, respectively). Nevertheless, the other parameters did not exhibit statistically significant correlations at the one-year follow-up (Table 13).

Table 13: Correlation between Sodium valproate concentration and biochemical and hematological parameters

	Basal		Six months		One year	
	r-value	*p*-value	r-value	*p*-value	r-value	*p*-value
Albumin (g/dl)	-0.054	0.592	-0.056	0.633	0.113	0.347
Total protein (g/dl)	-0.024	0.810	-0.052	0.660	-0.005	0.969
SGOT (IU/l)	0.082	0.416	0.043	0.711	0.158	0.189
SGPT (IU/l)	0.052	0.604	-0.052	0.655	0.327	0.005*
ALP (IU/l)	-0.112	0.268	0.074	0.530	0.156	0.194
BID (mg/dl)	-0.026	0.795	-0.120	0.304	-0.043	0.724
BIT (mg/dl)	-0.070	0.490	-0.090	0.440	-0.076	0.528
Urea (mg/dl)	0.006	0.951	0.068	0.561	0.235	0.049*
Creatinine (mg/dl)	-0.254	0.011*	-0.161	0.168	0.096	0.425
Amylase (IU/l)	-0.124	0.218	-0.044	0.711	0.276	0.020*
Platelet (lakh/mm^3)	-0.080	0.428	-0.262	0.023*	-0.131	0.277

Pearson correlation coefficient test used. *p-value <0.05 considered statistically significant

4. Discussion

The liver, along with the biliary and gastrointestinal tracts, serves as the primary location for the expression of UGT1A3. The UGT1A3 gene is known for its high mutation rate, with numerous mutation sites identified. In the UGT1A3 gene's promoter region and first exon, there are currently 31 single nucleotide polymorphisms (SNPs), most of which result from alkaline substitutions (Zhang *et al.,* 2019).

In our current research, it was observed that there were almost no mutations for A17G and C133T, while the majority of alleles exhibited the mutant type in the case of the remaining four mutations (as shown in Table 2). Previous studies have indicated that mutations are detected in four out of seven loci of UGT1A3, with no mutations found in the other three loci (*i.e*., C133T, A808G, and G342A) (Zhang *et al*., 2019).

While there was no significant association between gene polymorphisms and serum VPA concentrations, VPA levels did exhibit significant variations at different intervals, as detailed in Table 6. It was noted that when compared to homozygotes with only the T31C mutation in the wild type, children with heterozygous mutations had considerably lower standardized plasma concentrations of sodium valproate. This suggests that the high allele frequency of T31C (36.67%) has a significant impact on the plasma concentration of sodium valproate in children with epilepsy, indicating that children with heterozygous mutations may require higher doses of sodium valproate to achieve the target plasma concentration range (Zhang *et al.,* 2019). These findings align with those from Cho et al. (Chao *et al*., 2012) and suggest that the UGT1A3 gene can influence the activity of its own transcription enzymes. It is important to note that the limited sample size and variations in drug metabolism between adults and children may contribute to the inconsistency in these findings. Research on the effects of UGT1A3 on AEDs is still limited, and further investigations are needed to better understand how UGT1A3 influences changes in medication plasma concentration.

As for the mutations observed, A17G and C133T appeared to be beneficial, while T31C, G81A, T140C, and A477G mutations had potentially harmful effects as shown in Table 4. However, the observed alleles were helpful in metabolizing VPA, as indicated by the proportion of responders as shown in Tables 4, 7, 8. Notably, no significant association was found between polymorphism and seizure type as detailed in Table 9.

UGT1A3 is an enzyme responsible for glucuronidating both xenobiotic and endobiotic compounds. A study by Iwai *et al.* 2004 identified polymorphisms in exon 1 of UGT1A3 in the DNA of 100 healthy Japanese volunteers, revealing six single nucleotide polymorphisms (SNPs). Among these, two were silent alterations at codons 27 and 159, while the remaining four (Q6R, W11R, W45R, and V47A) resulted in amino acid modifications. The population under study presented five polymorphisms that encoded for five different forms of UGT1A3 proteins. The most common wild type allele for UGT1A3 had amino acid sequences: Q6-W11-R45-V47. This study found that 36% of the population was homozygous for the wild form of UGT1A3 and identified two parallel SNP pairings (Q6A-W11R and W11R-V47A), leading to amino acid changes on single alleles.

Our study reported that most patients did not experience gastrointestinal disturbances like nausea, vomiting, or abdominal pain. There were no cases of transient hair loss or changes in hair color or texture during the therapy. While some subjects showed an insignificant weight gain, it was not statistically significant. No alterations were observed in sleep patterns, sleep duration, or patients feeling drowsy due to the therapy. Skin rashes were also not observed in patients undergoing treatment. However, there was one case of acute pancreatitis in a patient, which was resolved through de-challenge, and the patient fully recovered. No challenge was given, and the patient was switched to levetiracetam. Causality assessment, using the Naranjo algorithm, indicated a score of +6, suggesting the probable nature of the adverse drug reaction due to sodium valproate.

In our study population, sodium valproate proved to be an effective and well-tolerated treatment for controlling seizures. Educating parents and caregivers about potential adverse drug effects, their early detection, management, and preventive measures could enhance treatment adherence. Timely identification and management of these pharmacological side effects can significantly improve the patient's

quality of life. The variations between our findings and those of other studies may be attributed to differences in methodology, ethnicity, sample collection methods, drug concentration estimation techniques, sample sizes, and phenotypic definitions, such as drug responsiveness.

A limitation common to many candidate gene association studies, including those previously mentioned and our own, is the constraint imposed by sample size. Given that children often exhibit significantly different sodium valproate metabolism than adults, our study primarily focused on pediatric epileptic patients.

The UGT exhibits highest activity in the human liver and is extensively disseminated in various human tissues and organs, including the kidney, heart, brain, and skin (Ke *et al.,* 2021;Chen *et al* 2021). UGT is predominantly located in the hepatic tissue and possesses substrates, including metabolic reactions of both exogenous substances like drugs and endogenous substances like steroid hormones, bile acids, and tretinoin (Sun *et al.,* 2015). UGT plays a critical role in the phase II metabolic response in the body, making its substrates more water-soluble through glucuronidation, thus aiding in their excretion. The UGT gene is polymorphic, leading to the encoding of enzymes with different rates and levels of substrate metabolism (Nakamura *et al.,* 2008). In humans, the UGT genes are primarily found in two families: UGT1, which contains the subfamily UGT1A, and UGT2, which includes the subfamilies UGT2A and UGT2B (He *et al.,* 2014).

Presently, several major hospitals in China have extensively integrated drug-metabolizing gene identification into the clinical management of epilepsy. Patient oral mucosal epithelial cells are regularly sampled for DNA to identify target genes, which are then used to select appropriate AEDs for patients based on the phenotypes of various target genes, reducing the risk of side effects and ensuring high bioavailability. These findings contribute to the development of a comprehensive genetic database for the Chinese people, enabling provision of personalized clinical recommendations based on genetic distribution traits. Additionally, the collection of substantial data supports the development of novel AEDs. With the ease of collecting oral mucosal epithelial cells, it is highly probable that comprehensive recommendations for personalized AED types, doses, and administration frequencies will be provided in the future.

Epilepsy is a severe condition with significant impacts on human health, and research into effective treatments is ongoing worldwide. Despite the considerable adverse effects, conventional AEDs remain commonly used in the current epilepsy treatment.

5. Conclusion

In conclusion,We observed higher prevalence of mutant carrier alleles for the UGT1A3 gene at loci T31C, G81A, T140C, and A477G, indicating a notable pattern of genetic polymorphism in the study population.Sodium Valproate Concentration: While there was insignificant association of UGT1A3 genotypes and valproate levels, it was noted that the mean valproate level was higher in individuals with wild genotypes compared to those with mutant genotypes. This suggests that genetic polymorphism in the UGT1A3 gene may influence the metabolism of sodium valproate. Clinical Outcome: Different patterns of UGT1A3 gene polymorphism did not exhibit a significant association with the clinical outcome of valproate treatment.These findings imply, UGT1A3 gene polymorphism may not be a reliable predictor of the clinical response to sodium valproate. Predictive Potential: UGT1A3 gene polymorphism studies could potentially be utilized before initiating sodium valproate treatment to predict how individuals may respond to the treatment. This could aid in tailoring treatment plans for patients based on their genetic profiles. Tolerability and Safety: Our study demonstrated that valproate was tolerated well in our pediatric study population, with minimal changes in hepatic, renal, and pancreatic parameters. This suggests that sodium valproate can be considered a safe and effective anti-epileptic drug for use in pediatric patients with epilepsy.

These findings contribute to our understanding of the relationship between UGT1A3 gene polymorphism, sodium valproate metabolism, and its clinical effects in pediatric patients with epilepsy. Further research is needed to validate and expand upon these observations and to explore the potential for personalized treatment approaches in the field of epilepsy management.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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