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Valproic acid levels in neurodevelopmental disorders: correlation with CYP and SULT genes using LC-MS/MS

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Abstract

Background Valproic acid (VPA) is one of the most widely prescribed antiepileptic drugs worldwide, which is used to treat migraines, bipolar disorder, and anxiety. However, VPA is associated with a wide range of side effects. This study evaluates therapeutic drug monitoring (TDM) in individuals with neurodevelopmental disorders. It explores the correlation between valproic acid (VPA) plasma levels and genetic polymorphisms in cytochrome P450 (CYP) enzymes and cytosolic sulfotransferase (SULT) genes.

Methods A simple and accurate LC-MS/MS method was developed, validated, and applied in the TDM of 14 individuals on VPA therapy. Plasma VPA levels were measured, and genotyped genes SULT1A1, CYP2D64, CYP2D610, CYP3A5, and CYP2C19*2. Statistical analyses were conducted using SPSS.

Results Of the fourteen participants, two had toxic plasma VPA levels (≥ 100 µg/mL), one had a sub-therapeutic level (< 50 µg/mL), and eleven were within or slightly above the therapeutic range (50–100 µg/mL). No significant correlation was observed between VPA plasma concentrations and genotypes of SULT1A1 ($p = 0.522$), CYP2C192 ($p = 0.288$), *CYP2D64* ($p = 0.895$), or CYP2D6*10 ($p = 0.067$). While no direct associations were found, genotype-guided drug therapy remains a promising strategy for optimizing drug efficacy and minimizing toxicity.

Conclusions This study highlights the complexity of valproic acid (VPA) therapy in individuals with neurodevelopmental disorders and the limited influence of common genetic polymorphisms in CYP and SULT genes on VPA plasma levels. While therapeutic drug monitoring (TDM) remains an invaluable tool for optimizing VPA therapy, the lack of significant correlations between genetic variants and VPA concentrations suggests that routine pharmacogenetic testing for these specific variants may not be warranted in clinical practice. However, the observed toxic and sub-therapeutic VPA levels emphasize the importance of regular TDM to mitigate risks associated with overdose or insufficient dosing.

Keywords Therapeutic drug monitoring, Valproic acid, Neurodevelopmental disorders, Autism spectrum disorder (ASD), LC-MS/MS, CYP and SULT genes

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Background

Intellectual disability (ID) is characterized by deficiencies in general mental skills such as reasoning, problem-solving, planning, logical thought, judgment, academic learning, and learning from experience. The stated global prevalence of intellectual disability is 1%, ranging from 1–3% per country, with a male-to-female ratio of 2:1 [1]. An intelligence quotient (IQ) of 70 or lower indicates an ID diagnosis. Intellectual disorder is diagnosed before the age of 18, where the intensity of the condition has been described using the phrases "mild," "moderate," severe," and "profound" [2]. Additionally, data from the United States between 2019 and 2021 showed that boys were more than three times as likely as girls to be diagnosed with autism spectrum disorder, which often co-occurs with ID. These variations highlight the influence of diagnostic criteria, healthcare access, and reporting methods on prevalence estimates [3]. It is always a good idea to use inclusive language for neurodivergent conditions, such as autism spectrum disorder (ASD), which aims to employ respectful and accurate terminology; readers may refer to recent literature [4].

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by challenges in social communication and interaction, along with restricted, repetitive patterns of behavior, interests, or activities. According to the DSM-5 (2013), ASD now encompasses previous diagnoses, including autism, Asperger's syndrome, and pervasive developmental disorders not otherwise specified (PDD-NOS), under a single diagnostic category. In the past ten years, the number of individuals has risen dramatically, and it currently affects 1 to 1.5% of the population [2]. ASD is diagnosed more frequently in males than in females, with a reported diagnostic ratio of 4:1. The new diagnostic definition of ASD focuses on two central domains: social contact disorder and restricted interests and repetitive behaviors [5]. ASD can be detected as early as the age of 2 years [6]. In extreme cases, individuals with autism may show specific developmental disabilities, including cognitive impairment [7]. There is some proof of an elevated risk of epilepsy correlated with other causes, such as ASD etiology, seriousness of autistic traits, developmental regression, and family background [8]. Epilepsy is common in autism spectrum disorder (ASD), with prevalence estimates ranging from 5 to 46% depending on the study population, age group, and severity of ASD symptoms. A meta-analysis study reported an overall pooled prevalence of epilepsy in ASD to be approximately 21.4%, with higher rates observed in individuals with co-occurring intellectual disabilities (up to 46%) compared to those without intellectual disabilities (approximately 8%) [9].

Valproic acid (VPA) was used in clinical practice nearly 50 years ago. Its effectiveness and tolerability profiles have been well-documented in experimental and clinical trials. Because of its broad scope of efficacy over a wide variety of seizures and epileptic syndromes, it is a mainstay of anticonvulsant treatment [10]. VPA is available in various pharmaceutical formulations, including sodium valproate, valproic acid on its own, and valproic acid combined with valproic acid. A modified release formulation containing a 2.3:1 sodium valproate and VPA ratio is also provided. Valproate semisodium (divalproex semisodium in the United States) is a medication sold in the United Kingdom by Sanofi-Synthélabo under the brand name Depakote® [11]. It is an enteric-coated compound of sodium valproate and VPA in a 1:1 molar ratio, dissociating to release valproate ions in the gastrointestinal system.

VPA comes in several forms: immediate-release, enteric-coated, delayed-release (12 h), and extended-release (24 h). It may also be administered as an intravenous solution. Children's therapeutic regular doses vary from 15 to 60 mg/kg/day, while adults' doses range from 500 mg to 2 g a day [12]. VPA is associated with a wide range of side effects that are particularly concerning in polytherapy and long-term treatment. Gastrointestinal intolerance symptoms may be reduced when using an enteric-coated formulation or taking the medication at mealtime. Nervous system stimulation, such as nervousness, insomnia, and tremor, can occur regularly in some individuals. VPA has been linked to Parkinsonism and cognitive dysfunction. It can cause hyperammonaemia, encephalopathy, thrombocytopenia, and other blood diseases documented infrequently [13]. VPA has also been linked to toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome (SJS), and drug reactions with eosinophilia and systemic symptoms (DRESS), among other delayed hypersensitivity reactions [14]. Pancreatitis has been recorded in children and adults who have taken valproate; most cases have been identified in epileptic children or adults with kidney failure [15]. VPA has been linked to severe congenital malformations, most notably neural tube defects (e.g., spina bifida). Lower IQ scores in utero have been related to VPA exposure.

Drugs that increase the expression of hepatic enzymes can increase valproate clearance. Phenytoin, carbamazepine, and phenobarbital (or primidone) can, for instance, double VPA clearance. Since cytochrome P450 microsomal mediated oxidation is a minor secondary metabolic pathway compared to glucuronidation and beta-oxidation, drugs that inhibit cytochrome P450 isozymes, such as antidepressants, may have little impact valproate clearance. Valproate weakly inhibits some P450 isozymes, epoxide hydrase, and glucuronosyltransferases.

VPA's pharmacokinetics are non-linear due to their saturable binding to plasma proteins. The suggested VPA reference range for the treatment of epilepsy is

50–100 µg/mL. For bipolar disorder treatment, a higher dosage range of 50–125 µg/mL is recommended [16]. VPA's time to steady state is 2–4 days [17]. The T_{max} for valproate semisodium was 3.6 h, and for enteric-coated valproate, it was 3.8 h [11]. The maximum concentration reached for a medication, C_{max}, was 103 µg/mL for valproate semisodium at a dosage of 500 mg (the VPA equivalent of 500 mg) twice daily. In contrast, enteric-coated sodium valproate was 91.33 µg/mL at a dose of 500 mg (VPA equivalent: 433 mg) twice a day. In a study, depending on the dosage given (400–1500 mg/day), C_{max} after around 4 h ranged from 60 to 330 µg/mL [18]. In another study, following regular doses of 250–1000 mg of Depakote ER (equivalent to 250 mg of VPA), C_{max} for total VPA in children (ages 8–11) ranged from 55.7 to 132.7 µg/mL. Following regular doses of 500–1750 mg Depakote ER, C_{max} for total VPA in teenagers (ages 12–17) ranged from (31.7–120.6) µg/mL [19].

Therapeutic drug monitoring (TDM) of antiepileptic drugs (AEDs) has had a significant effect on epilepsy treatment for the last 10–20 years because of increased understanding of AED pharmacokinetics. VPA's ability to treat a variety of seizures with a single anticonvulsant has led to its widespread use, especially among children. Moreover, it's also increasingly used in the treatment of psychotic and schizoaffective conditions, neuropathic pain, and migraine headache prophylaxis. The clearance of VPA is stated to be concentration- or dose-dependent due to substantial inter-individual pharmacokinetic variation and concentration-dependent plasma protein binding pharmacokinetics of VPA [20]. Furthermore, the therapeutic effects of VPA are closely related to the drug's serum concentration. Its proper serum concentration is determined by various factors, including age, total body weight, VPA dosage, and co-administration of other medications that impair VPA's pharmacokinetics. Many methods of analysis for quantitating VPA in human plasma have been developed. Fluorescence polarization immunoassay (FPIA) has been used to monitor VPA in most of them. Additionally, other techniques, such as High-Performance Liquid Chromatography (HPLC) assays combined with either ultraviolet or fluorescence detection [21] and Gas Chromatography (GC) with mass spectrometric (MS) detection, have also shown satisfactory results. Most of these methods necessitate time-consuming analysis or running time. Analytical procedures requiring evaporation steps are undesirable due to the volatility of VPA. On the other hand, VPA could need a liquid-liquid extraction to be observed by ultraviolet or fluorescence, leading to a high processing time and poor recovery. So many methods have been used, including liquid chromatography-tandem mass spectrometric detection (LC-MS/MS). The primary benefit of such techniques would be that no prior chemical alteration of VPA is necessary.

Sulfonation has typically been regarded as a detoxifying pathway leading to even more water-soluble compounds and assisting their elimination through the kidneys or bile, as in the case of most xenobiotics and relatively insignificant endogenous substrates. Baumann originally discovered sulfonate conjugation in 1876, and it has subsequently been found to be a key process in the biotransformation of a wide range of xenobiotics and endobiotics, including medicines, chemical carcinogens, hormones, bile acids, neurotransmitters, peptides, and lipids. 3′-phosphoadenosine-5′-phosphosulfate (PAPS) is the widespread sulfonate donor for such processes. Thus, the transference of sulfonate (SO-3) toward a hydroxyl or amino group would be catalyzed via a supergene family of enzymes known as sulfotransferases (SULTs) [22]. SULTs seem genetically polymorphic and indicated in a diverse range of organs. Four leading SULT families are SULT1, SULT2, SULT4, and SULT6, with 13 human cytosolic SULT isoforms. The SULTs alloenzymes are encoded by this gene; SULT1A (SULT1A1*1 wild-type, SULT1A1*2, SULT1A1*3, and SULT1A1*4) were localized to chromosome 16p12.1–p11.2, with considerable biochemical changes in their activities. This polymorphism is particularly significant in the case of a mutation in exon seven at nucleotide 638 (codon 213), which results in a replacement of histidine by arginine (SULT1A1*2 allele), which is linked with lower enzymatic activity and heat resistance when compared to the wild-type allele (SULT1A1*1 allele) [23].

The current study aims to develop a simple and accurate LC-MS/MS method for determining VPA concentration in human plasma. The objective is to determine concentrations of VPA in individuals with neurodevelopmental disorders (intellectual disability and/or autism spectrum disorder), to evaluate TDM, and study the relationship between common genotypes of CYPs and SULTs genes and levels of VPA in these individuals by restriction fragment length polymorphism.

Methods

Human participants and study design

This research was carried out after receiving ethical approval from the Jordan Center of Pharmaceutical Sciences' inter-regulatory board on October 3, 2020. The Arabic Village for Special Education Center obtained parental approval separately. Fourteen participants were registered for the study. Blood samples were obtained from the Arabic Village for Special Education Center with the help of nurses. The selection criteria were based on individuals suffering from neurodevelopmental disorders (ID and/or ASD) and taking VPA at a steady state. In October 2020, blood chemistry data from the

Arabic Village for Special Education Center was collected, including kidney, liver, and thyroid function tests and hematologic analysis. A total of 1–5 mL of blood was obtained from each participant. Blood samples were collected at 12 h post-administration and centrifuged to give blood plasma samples using citrate tubes to analyze VPA. The samples were transferred within 30 min to the Jordan Center of Pharmaceutical Research for LC-MS/MS analysis. Half a milliliter of whole blood from each participant was withdrawn into a plain tube and frozen at -80 °C at the University of Petra until assay.

LC-MS/MS

The study used an LC-MS/MS system composed of high-pressure liquid chromatography (HPLC) (DIONEX Ultimate 3000) coupled with a mass spectrometer and detector (Thermo LCQ Fleet). Genotyping was carried out at the biotechnology laboratory of the Pharmaceutical Center of Petra University. The instruments used include: a UV spectrophotometry apparatus NanoDrop™ Thermo Fisher Scientific, Inc. (USA), a gel electrophoresis apparatus, nano PAC-300 from Cleaver Scientific (UK), a gel imaging instrument Bio-Rad gel Doc™ EZ imager (USA), and for DNA amplification, GeneAMP® PCR system 9700.

Under chromatographic conditions, the mobile phase consisted of an isocratic mixture of 0.1% acetic acid and acetonitrile 1:1 v/v. The pH was adjusted by adding 0.1% triethylamine. The internal standard (IS) was Ondansetron, and the chromatographic separation was achieved using analytical column C18 ACE (2.1 mm, 5 cm, 5 µm). The mobile phase flow rate was 25 mL/min, and the injection volume was 2 µL.

MS Conditions, the mass spectrometry detection mode for VPA was unfavorable, the VPA's mass transition (m/z) was 143, and the collision energy was zero. The mass spectrometry detection mode for Ondansetron was positive, the mass transition (m/z) was 294, and the collision energy was 25. Ion source temperature was maintained at 300 °C, Ionization voltage was kept at 5 Kv, and Sheath gas, auxiliary gas, and sweep gas flow rates were 50, 25, and 0 L/min, respectively.

Sample preparation: plasma extraction

Acetonitrile (precipitating agent) containing 10 µg/mL IS (Ondansetron) was added to a spiked plasma in a ratio of 3:2 v/v. The mixture was vortexed and centrifuged for 5 min at 14,000 rpm; the supernatant was transferred into an auto-sampler vial.

Standard calibration points and quality control sample preparation

100 mg of VPA raw material was weighed and dissolved in 5 mL methanol. The stock solution was mixed in a ratio of 1:1 v/v to prepare 20,000 µg/mL from VPA as a mixture solution. A serial dilution was prepared as calibrators of (50, 100, 200, 400, 700, 1000, and 1500) µg/mL, and quality control (QC) samples as follows: 150 low, 750 mid, and 1500 high µg/mL QC concentrations.

The validation fulfilled the guidelines set out by the European Medicines Agency [24]. The method was validated in accuracy, precision, sensitivity, specificity, and linearity.

The LC-MS/MS method used in this study to determine valproic acid (VPA) concentrations in plasma was thoroughly validated per EMA guidelines. The technique demonstrated excellent accuracy, with within-run and between-run accuracy values ranging from 97.5 to 102.9% across all quality control (QC) levels. Precision was confirmed with a coefficient of variation (CV) below 5.25%, meeting the acceptance criteria of ≤ 15% for QC samples and ≤ 20% for the lower limit of quantification (LLOQ). Linearity was established over a 5–150 µg/mL concentration range, with a correlation coefficient (R^2) of 0.9998. Sensitivity was defined at the LLOQ of 5 µg/mL, ensuring reliable quantification at low concentrations. Specificity tests showed no interference from endogenous or exogenous substances in the matrix. These robust validation parameters ensure that the LC-MS/MS method provides accurate, precise, and reliable results for therapeutic drug monitoring of VPA in clinical settings.

There were no interferences at the VPA and IS retention times. The peaks were well-formed and well-resolved from the plasma constituents. The matrix peak was less than 5% of the internal standard's peak area, deemed acceptable by the European Medicines Agency [25].

For genotyping, blood samples were collected from each participant using EDTA tubes. A total of 0.3 mL of each sample was utilized, with the remainder stored at -80 °C in case further testing is needed. QIAamp Blood mini kit was used to extract DNA from blood samples (Qiagen, Germany).

Results

Demography and VPA administration, the 14 individuals, all of whom were males, were diagnosed with a neurodevelopmental disorder (intellectual disability and/or autism spectrum disorder) within their first two years. The participants' actual weight ranged from 31.5 Kg to 87 Kg, their height ranged from 1.4 m to 1.7 m, and their ages ranged from 12 to 36 years old. Table 1 Shows the daily doses for every individual based on the sample number.

For blood screening, fourteen whole blood samples were withdrawn and centrifuged into plasma and serum. Blood serum samples were analyzed for blood chemistry analysis described in Supplementary Materials S1, S2, and S3. When the CBC results were compared to normal

Table 1 VPA dose regimens

Sample number	The total daily dose of VPA
1	250 mg
2	300 mg
3	500 mg
4, 5, 6, 7, 8 and 9	1000 mg
10, 11 and 12	1250 mg
13	1500 mg
14	2000 mg

ranges, the RBC, Hb, and HCT were lower than usual limits, as shown in Supplementary Materials S1. Some of the hematological adverse effects of VPA treatment that have been described include aplastic anemia, pure red cell aplasia, macrocytosis, leukopenia, and thrombocytopenia. This might contribute to the low RBC, Hb, and HCT levels in the sample numbers (2, 3, 4, 5, 9, 10, 11, and 14) [26]. ASD individuals with intellectual impairment had lower hemoglobin and hematocrit levels than ASD individuals with standard intellectual capability. Iron deficiency anemia was found to be higher in children with intellectual disabilities [27], and this might explain the low levels of RBC, Hb, and HCT. In sample numbers (5, 9, 10, 12, and 14), there is a decrease in neutrophil values that could be described as neutropenia. Neutropenia can also be caused by VPA treatment, which is reported in the literature. Numbers (1, 3, and 14) showed a decrease in their MCV values, which could be explained by iron deficiency anemia. Meanwhile, sample numbers (1, 4, 5, 9, 13, and 14) show decreased MCH values. A change in MCH usually corresponds to a change in MCV. Microcytic anemias are associated with a reduction in hemoglobin levels, whereas macrocytic anemias are associated with an elevation in hemoglobin levels. As a result, the MCH provides minimal information that is not already included in the MCV. There was a decrease in MCHC value in sample numbers (1, 3, 8, 9, and 10), indicating that RBCs were hypochromic, pale, and contained less Hemoglobin. Sample numbers (5 and 12) showed a decrease in WBCs, which is called leukopenia. Leukopenia is caused by a reduction in overall WBC production or an increase in WBC breakdown throughout the bone marrow [28].

In Supplementary Materials S2, sample numbers (1, 2, and 12) showed a low uric acid level. Long-term antiepileptic therapy, including VPA, has been shown to lower blood uric acid levels [29]. In sample number (11), there was an increase in uric acid blood and creatinine concentrations. Increased uric Acid production reported in some gout patients may be related to accelerated creatinine synthesis [30]. In sample numbers (7, 9, 11, and 13), there was a slight elevation of serum creatinine, reported in the literature as seen in individuals treated with VPA, and could be a sign that kidneys begin to dysfunction [31]. Sample number (2) had increased serum potassium, a condition called hyperkalemia. Hyperkalemia caused by VPA has rarely been documented in the literature. Also, in the sample, there was an increase in chloride serum levels. No correlation was mentioned between high chloride serum levels and VPA in the literature. Sample numbers (13 and 14) showed an increase in GGT levels, as shown in Supplementary Materials S2. Both samples are taking VPA along with carbamazepine. VPA and carbamazepine increase ALT, AST, and GGT, but carbamazepine can potentially increase GGT [32] more than VPA because of the enzyme induction [19]. Sample numbers (3 and 8) had an increase in globulin levels. There is no relation between globulin and VPA found in the literature. Sample number (5) had an increased level of direct bilirubin, which could be explained via chronic VPA treatment, alone or in combination. Acute hepatic impairment associated with prolonged VPA treatment has also been linked to an increase in plasma bilirubin levels. It is recommended for individuals on long-term VPA treatment to undergo frequent liver function testing [33]. Sample numbers (5 and 6) showed increased direct bilirubin level, also called conjugated bilirubin. Hepatocellular illness or cholestasis is the most common cause of conjugated hyperbilirubinemia (intrahepatic and extrahepatic) [34].

As shown in Supplementary Materials S3, sample numbers (1, 2, 6, and 13) had an increased TSH value. It was reported that VPA might cause changes in thyroid hormones [35]. Sample number (10) had a very low ferritin level. In children with ASD, there has been a high frequency of iron deficiency. However, research on the link between iron deficiency characteristics and ASD clinical symptoms is limited [27]. Sample numbers (4, 5, 6, 7, 8, 11, and 14) had a decreased level of vitamin D3. Vitamin D is a neurosteroid hormone that plays a vital role in brain development. As a result, its lack throughout pregnancy and early childhood might have a significant influence on the development of the brain, potentially leading to negative neuropsychological consequences such as autism spectrum disorder [36]. A study has revealed that there is a higher prevalence of vitamin D3 insufficiency in epileptic children under valproate therapy compared to healthy children [37].

Plasma Valproic acid (VPA) concentration and plasma concentrations of VPA were determined using LC-MS/MS, as shown in Table 2. When we Compare the results to the steady state mean concentration of VPA in the literature, we can classify them as sub-therapeutic, slightly high, high, or toxic [16].

The study identified various abnormal laboratory findings and VPA levels among the participants. Two participants (samples 8 and 14) exhibited toxic plasma VPA concentrations, exceeding 100 µg/mL, with levels

Table 2 VPA plasma concentration

Sample #	VPA Measured concentration (µg/mL) by LC-MS/MS
1	25.9
2	61.4
3	68.9
4	96.5
5	90.0
6	90.1
7	58.9
8	107.9
9	75.3
10	91.2
11	80.5
12	60.6
13	93.2
14	105.4
Average	79.0
CV%	28%

recorded at 107.9 µg/mL and 105.4 µg/mL, respectively. These toxic levels were accompanied by elevated gamma-glutamyl transferase (GGT) levels, suggesting potential liver damage or bile duct dysfunction. One participant (sample 1) had a sub-therapeutic VPA level of 25.9 µg/mL, which was significantly below the recommended therapeutic range of 50–100 µg/mL. Several participants showed hematological abnormalities: samples 3, 4, 5, and 9 demonstrated low platelet counts when VPA concentrations exceeded 80 µg/mL, consistent with thrombocytopenia observed in VPA treatment. Hypochromic and microcytic red blood cells were indicated by decreased mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values in samples 1, 3, and 9. Additionally, neutropenia was observed in sample 14, aligning with VPA's documented effects on white blood cells. Low serum vitamin D3 levels were recorded in multiple participants, with sample 10 showing particularly low levels, which could have implications for bone health and neurodevelopment. These results emphasize the complex interplay between VPA levels and associated biochemical abnormalities, reinforcing the need for routine laboratory monitoring in patients receiving VPA therapy.

Discussion

Individuals 3, 4, 5, 9, 10, 11, 12, and 14 showed signs of a potential correlation between VPA and haematological parameters, and sample numbers 4, 5, 6, and 13 showed signs of low platelet count when the plasma VPA level rose above 80 µg/mL, as documented in the literature [38]. In addition, there is a possible correlation between creatinine serum level and VPA serum level in individuals' numbers (7, 9, 11, and 13). Sample number (8) has normal liver enzyme levels, but these levels may surge as the individual's VPA level approaches toxic levels. Sample numbers (13 and 14) have elevated GGT levels, and there is a link between VPA plasma levels and GGT [32]. Sample numbers (3, 4, 5, 6, 8, 11, and 14) have low vitamin D3 levels, which could be linked to VPA plasma concentration.

Genotyping, as shown in Supplementary Materials S4, all participants were homozygous for SULT1A1 and CYP2D6-4, except for sample numbers (3 and 8) that were heterozygous abnormal. Sample number (8) has a toxic level of VPA, but sample number (3) has a therapeutic VPA level. Sample numbers (2 and 8) have heterozygous abnormal CYP 2C19*2; sample number (2) has a therapeutic VPA level, but sample number (8) has a toxic level of VPA. Sample number (3) has an abnormal heterozygosity for CYP6-10 and a therapeutic drug level. The classification of intellectual disability (ID) levels used in this study is based on established diagnostic criteria, categorizing ID into four severity levels: mild, moderate, severe, and profound. These classifications are determined by deficits in intellectual functioning, such as reasoning, problem-solving, and judgment, and adaptive functioning in conceptual, social, and practical domains. An IQ score below 70 is indicative of ID, with specific ranges defining the severity: mild (IQ 50–69), moderate (IQ 35–49), severe (IQ 20–34), and profound (IQ below 20). In this study, individuals were classified into these categories based on their clinical diagnoses, as shown in Supplementary Table S4. For example, participants 1, 2, and 5 were categorized as having moderate ID, while participants 3 and 4 were classified with mild ID. This classification provides a framework for understanding the variability in therapeutic outcomes and drug response among individuals with neurodevelopmental disorders.

Statistical analyses were conducted using SPSS version 28.0.0.0. Descriptive statistics were used to summarize demographic, clinical, and laboratory data, while inferential statistics were applied to evaluate the relationships between VPA plasma levels and genetic polymorphisms. Associations between categorical variables (e.g., genotype combinations and therapeutic drug monitoring (TDM) categories) were assessed using the chi-square (χ^2) test, with statistical significance set at a p-value of < 0.05. Continuous variables (e.g., VPA plasma concentrations) were analyzed using independent samples t-tests or one-way ANOVA, as appropriate.

Power calculations were performed retrospectively to assess the adequacy of the sample size ($n = 14$) for detecting significant associations. Given the small sample size, the study was underpowered for detecting small-to-moderate effect sizes (e.g., Cohen's $d < 0.5$ or odds ratios < 2.0). For example, a sample size of 14 provides only 30% power to detect a moderate effect size ($d = 0.5$) at a significance level of 0.05. This limitation highlights the exploratory

Table 3 Statistical analysis for the association between the genotype and TDM category

	SULT1A1	CYP3A5 + D	CYP2C19*2	CYP2D6*4	CYP2D6*10
P-value	0.522	Constant	0.067	0.288	0.895
χ2	3.217	Constant	8.782	2.492	1.098

nature of the study, with findings intended to generate hypotheses for future research rather than provide definitive conclusions.

The small sample size was determined by the availability of participants meeting the inclusion criteria at the study site, specifically individuals with neurodevelopmental disorders receiving stable VPA therapy. While this limited sample size restricts the generalizability of the findings, the study offers valuable preliminary data that can inform the design of larger, more adequately powered studies in the future.

Pearson's χ2 test was used to compare genotype and TDM category. The χ2 test was used to examine the statistical significance of the variations in SULT1A1, CYP3A5 + D, CYP2C19*2, CYP2D6*4, and 2D6*10 between individuals. Statistical significance was defined as a probability value of less than 0.05.

No statistically significant correlations were identified between VPA plasma concentrations and the genetic variants SULT1A1 ($p = 0.522$), CYP2C192 ($p = 0.288$), CYP2D64 ($p = 0.895$), or CYP2D6*10 ($p = 0.067$). Specifically, the p-values for SULT1A1 and CYP2D64 were 0.522 and 0.895, respectively, indicating no significant relationship as shown in Table 3. Similarly, the CYP2C192 variant showed a p-value of 0.288, and the CYP2D610 variant approached significance ($p = 0.067$) but did not reach the threshold for statistical significance. Confidence intervals for the observed associations further demonstrated the lack of a strong relationship, as the intervals overlapped extensively with the null value. The χ^2 test for the genotype groups and VPA levels did not reveal any significant deviations, confirming that these genetic variants are unlikely to contribute to the variability in VPA plasma levels within this cohort. These results align with previous studies suggesting minimal impact of these genotypes on VPA pharmacokinetics.

This investigation aimed to determine whether VPA and drug-metabolizing enzymes, the most significant cytochrome and sulfotransferases, were related in individuals with nervous system impairments or diseases. Our findings showed that specific genotypes' proportions were increasing in certain groups. Compared to previous studies' outcomes, these percentages from the harmful and regular TDM reference groups happened by accident rather than being statistically related. It was found that individualized VPA dosage regimens can be helpful for CYP2C19 genetic variations [39]. Allele CYP2C19*2 and CYP2C19*3 carriers exhibit greater trough plasma VPA concentrations than CYP2C19 wild-type patients, indicating that VPA plasma concentrations are strongly affected by CYP2C19 genetic variants and that the dose of VPA for intermediate and poor metabolizers may be lower than for extensive ones. Our study disagrees with them, and there are several explanations for this. One of these explanations could be the difference in the study community. They have females in their study, their mean age group is different, they have a larger sample size, and their nationality is various. Like our results, the CYP2C19 genotype was not clinically significant for VPA pharmacokinetic variability [40].

Therapeutic drug monitoring (TDM) of valproate (VPA) is a critical tool in clinical practice, enhancing the precision of treatment regimens and improving patient outcomes in neuropsychiatric disorders. A retrospective study in Saudi Arabia highlighted TDM's effectiveness in managing mood stabilizers like VPA, ensuring therapeutic levels, and minimizing adverse effects, particularly in bipolar disorder patients [41]. Similarly, a 5-year analysis conducted in Italy demonstrated the frequent underdosing of VPA in clinical settings, underscoring the role of TDM in preventing subtherapeutic levels and optimizing treatment efficacy [42]. Furthermore, the AGNP Consensus Guidelines strongly recommend TDM for valproate, recognizing its value in individualizing treatment and reducing the risk of toxicity [43]. These findings emphasize the indispensable role of TDM in ensuring the safe and effective use of valproate in diverse clinical scenarios.

The study has limitations that should be acknowledged to contextualize the findings. First, the sample size ($n = 14$) is small, which limits the statistical power to detect genetic associations and reduces the generalizability of the results to broader populations. The small sample size may increase the risk of type II errors, where significant associations could be missed. Second, the absence of female participants restricts the study's applicability to only male populations, neglecting potential sex-specific differences in the pharmacokinetics and pharmacodynamics of valproate. Additionally, the study does not account for potential confounding variables such as comorbid conditions, co-administered medications, or environmental factors that could influence drug metabolism and therapeutic outcomes. These limitations highlight the need for larger, more diverse studies to validate the findings and explore the broader implications of genotype-guided therapy for valproate in neurodevelopmental disorders.

Conclusions

This study highlights the complexity of valproic acid (VPA) therapy in individuals with neurodevelopmental disorders and the limited influence of common genetic polymorphisms in CYP and SULT genes on VPA plasma levels. While therapeutic drug monitoring (TDM) remains an invaluable tool for optimizing VPA therapy, the lack of significant correlations between genetic variants and VPA concentrations suggests that routine pharmacogenetic testing for these specific variants may not be warranted in clinical practice. However, the observed toxic and sub-therapeutic VPA levels emphasize the importance of regular TDM to mitigate risks associated with overdose or insufficient dosing.

Based on these findings, healthcare providers should prioritize individualized TDM and integrate laboratory assessments, such as liver function and hematological profiles, into routine monitoring for patients on VPA therapy. Future studies should explore larger, more diverse populations, including females and individuals with co-existing medical conditions, to validate these findings. Additionally, further research should focus on identifying other genetic, epigenetic, or environmental factors that might influence VPA pharmacokinetics and therapeutic outcomes. Finally, studies assessing the cost-effectiveness and clinical utility of genotype-guided VPA therapy would provide valuable insights for integrating pharmacogenomics into standard care.

Abbreviations

VPA	Valproic Acid
TDM	Therapeutic Drug Monitoring
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
CYP	Cytochrome P450
SULT	Sulfotransferase
ID	Intellectual Disability
ASD	Autism Spectrum Disorder
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
IQ	Intelligence Quotient
QC	Quality Control
LLOQ	Lower Limit of Quantification
RBC	Red Blood Cell
Hb	Hemoglobin
HCT	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
WBC	White Blood Cell
GGT	Gamma-Glutamyl Transferase
TSH	Thyroid-Stimulating Hormone
ALT	Alanine Transaminase
AST	Aspartate Transaminase
EMA	European Medicines Agency
PCR	Polymerase Chain Reaction
EDTA	Ethylenediaminetetraacetic Acid

Supplementary Information

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Supplementary Material 1

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Author contributions

Each author contributed to the analysis and interpretation of the findings. Additionally, the authors consented to accept the work in its submitted form. Conceptualization, E.M., and T.A.; methodology, A.A. and K.M.; software, A.A., E.M., L.A., and S.A.; validation, S.A., S.K., E.M., and A.A.; formal analysis, S.A., and S.K.; resources, T.A.; data curation, E.M., A.A., and S.A.; writing—original draft preparation, E.M., L.A., and K.W.O.; writing—review and editing, T.A., A.A., E.M., K.W.O., and O.M.; Verification, K.W.O. and O.M.; supervision, E.M., and L.A; project administration, T.A.; funding acquisition, T.A. The authors reviewed and approved the final version of the published manuscript for significant intellectual content.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The Helsinki Declaration was followed when conducting the study, the ICH HARMONISED GUIDELINE: INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE E6(R2) dated November 9, 2016. (ICH 2016). The Jordan Center of Pharmaceutical Research's Institutional Review Board (IRB) also approved it locally. All individuals and/or their legal representatives gave written informed consent before entering the study. All procedures, interventions, and laboratory tests were conducted per the Jordanian Ministry of Health's recommendations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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