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THE EFFECT OF UGT1A6 POLYMORPHISM AT TWO LOCI ON THE CLINICAL RESPONSE TO VALPROIC ACID IN EPILEPTIC CHILDREN

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ABSTRACT: Background and Aim: Valproic acid (VPA) is a widely used antiepileptic drug. The main pathway of its elimination is through conjugation by UDP-glucuronosyltransferases with several polymorphic forms. In this study, we aimed to examine the effect of polymorphism in UGT1A6 enzyme-coding gene at two loci, namely, 541A>G and 552A>C on clinical outcome in Egyptian children with idiopathic epilepsy. Clinical outcome investigations involved VPA serum level, seizure control and incidence of adverse drug reactions (ADR). **Methods:** Genetic polymorphisms were detected in 48 patients receiving VPA monotherapy by PCR-RFLP. Steady state concentrations at trough level were determined by homogenous enzyme immunoassay technique. Patients were monitored for ADR, seizure frequency as well as seizure severity (SS). **Results:** Variant genotype group (AC & CC) had lower concentration to dose ratios (CDR) than those with (AA) genotype for UGT1A6 552A>C (p=0.029). Variant allele carriers had significantly lower CDR than wild allele carriers for both 541A>G and 552A>C (p= 0.047 and p= 0.001, respectively). Wild genotype for 552A>C had higher SS scores on Chalfont scale (p=0.020) and showed significantly higher incidence of fatigue than variant genotypes (p=0.047). Children carrying variant genotype for 541 A>G showed a significantly higher incidence of difficulty in concentration reflected upon school work than wild genotype carriers (p=0.025). Neither loci could be found to affect seizure control. **Conclusion:** UGT1A6 polymorphisms may increase VPA metabolism in Egyptian epileptic children affecting both seizure severity as well as susceptibility to ADR. Further study with a larger sample size is strongly recommended.

Valproic acid (VPA) is a broad spectrum antiepileptic drug (AED) that is used in the treatment of primary generalised and partial seizures in both adult and pediatric populations.[1]

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However, VPA has been shown to have high interindividual variability in its pharmacokinetics and pharmacodynamics as well as a narrow therapeutic range (50–100µg/mL) which requires careful monitoring of its plasma level during therapy. [2, 3] Factors such as age, liver and renal function, co-medication, disease heterogeneity or nutritional status can generally affect individual response to AED therapy. [4] Genetic variation as well, may account partly for such observed interindividual variability in response.[5]

The major metabolic pathways of VPA comprise glucuronidation, β-oxidation and ω-oxidation.[6, 7] The former reaches up to 50% of the total metabolism of the initial dose. [8] A number of UGT isozymes including UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7 and UGT2B15 were reported to be involved in VPA glucuronidation.[6, 9]

Among these enzymes, UGT2B7, UGT1A6 and UGT1A9 account for the majority of VPA intrinsic hepatic clearance. [6, 8] The gene encoding for UGT1A6 enzyme was demonstrated to be highly polymorphic having at least four alleles characterized by three single nucleotide polymorphisms (SNPs).[10] These include polymorphisms 541A>G (rs2070959) and 552A>C (rs1105879), 19T>G (rs6759892) in its coding sequence affecting the pharmacokinetics of VPA, thereby altering its efficacy and adverse effect profiles.[11, 12]

Previous *in vitro* studies suggested that UGT1A6 polymorphism influences the glucuronidation rate of its substrate compounds [10, 11] while only few clinical studies have reported the effect of this genetic polymorphismon VPA level in epileptic patients. [13-18] They all included Asian populations of different ethnic backgrounds and the results were not entirely consistent. Children were included in only three of those studies. [14, 18, 19] Furthermore, none of the foregoing studies investigated the influence of this genetic polymorphism on drug response and treatment outcome.

Given that genetic differences affecting drug metabolizing enzymes contribute to variability in drug exposure and response, studying these factors may help understand, and possibly predict, the relationship between drug dose, the resulting drug concentration and pharmacologic effect. Ultimately, it may help guide VPA personalized therapy in epileptic children. Hence, the purpose of the current study was to evaluate the effect of UGT1A6 variants at 541A>G (rs2070959) and 552A>C (rs1105879) loci, on the responsiveness to VPA regarding both its serum level and clinical outcome in epileptic children treated at a University hospital in Egypt.

MATERIALS AND METHODS:

Patients, sampling and drug analysis: This study was conducted prospectively between April 2010–September 2011 at Pediatric Neurology Clinic, Children's Hospital, Ain Shams University, Cairo, Egypt. The study protocol was approved by the Hospital Ethical Medical Committee and signed informed consents were obtained prior to enrollment. A total of 50 randomly selected, consecutive patients were enrolled. Eligible patients were children (age, 2-18 years) diagnosed as idiopathic epilepsy according to International League Against Epilepsy (ILAE) criteria [20] and received VPA monotherapy. Exclusion criteria were: (1) history or evidence of hepatitis; (2) mental retardation; (3) unreliable record of seizure frequency; (4) documented noncompliance to antiepileptic drugs (AED); (5) receive concurrent medication known to either induce or inhibit VPA metabolism; and (6) suspected presence of neuro-degenerative, neuro-metabolic or neuro-cutaneous syndromes. Relevant data including age, gender, physical assessment, EEG, biochemical laboratory results, seizure history, and drug dosage were recorded.

Thirty patients have been on VPA therapy for at least 6 months at enrollment while 18 patients were diagnosed with new-onset epilepsy and started VPA when they first entered the study. VPA was started at an initial dose of 5 to 7.5 mg/kg/day with an increase of 5 – 7.5 mg/kg/day every 3–7 days up to a final dosage of 30 mg/kg/day. [21] The b.i.d. or t.i.d. schedules were standardized to a regimen of every 12 and 8 hours, respectively, for at least 7 days before blood sampling. For each patient, 5ml venous blood sample were withdrawn before morning dose at steady state (after 5 half-life periods) for measuring trough VPA level, when a stable dose at maintenance has been reached. For every patient, blood was collected into 2 sterile tubes; the first was allowed to clot at room temperature, centrifuged at 3000 rpm for 10 min then stored as serum at -20ºC for drug assay. The second tube (with EDTA) was frozen at -70 ºC for DNA extraction and genotyping. Drug levels were measured by homogenous enzyme immunoassay technique (Cobas®c 311, Roche diagnostics, USA) according to the manufacturer's instructions.[22] The assay was validated for bio sample analysis in a linear range of 2.8 – 150 µg/ml. A concentration to

dose ratio (CDR) was calculated for each patient by dividing steady state serum concentrations (C_{ss}) of VPA by the daily dose and body weight.

Genotyping:
Genomic DNA was isolated from human blood cells using QIA amp DNA Mini Kit supplied by Qiagen (Valencia, USA) according to manufacturer instructions. The UGT1A6 552A>C and 541A>G polymorphisms were analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, as described previously. [23] The extracted DNA was subjected to a consensus primer mediated PCR-RFLP, using the Stratagene Mx3000PThermocycler (Corbett Research, Australia) and the primers; 5′-GGAAAATACCTAGGAGCCCTGTGA - 3′ (forward) and 5′AGGAGCCAAATGAGTGAGG GAG-3′(reverse) (Bioneer, USA) producing DNA fragment of 992 bp containing both polymorphisms. PCR conditions comprised an initial denaturation step at 94°C (4 minutes), and 32 cycles consisting of denaturation at 94°C (30 seconds), annealing at 65°C (1minute), extension at 72°C (1 minute), then final extension at 72°C (6 minutes). The amplified product was then subjected to digestion with the restriction enzyme *NsiI* for 541A>G and *Fnu4HI* for 552A>C (New England Biolabs. Beverly, UK).

Amplified product of DNA samples and restriction fragments were run on a 2% agarose gel. DNA molecular weight marker was also run to identify the site of bands (100 bp DNA ladder supplied by Fermentas). After digestion with *NsiI*, a fragment of 992 bp was detected for the wild type, 992+616+376 bp fragments for the heterozygous variant and 616+376 bp fragments for the homozygous variant samples. Digestion with *Fnu4HI* yielded 833+159 bp products for the wild type, 833+464+369+159 bp products for heterozygous samples and 464+369+159 bp fragments for homozygous variant samples. Fragmentation patterns are shown in **Fig. 1**.

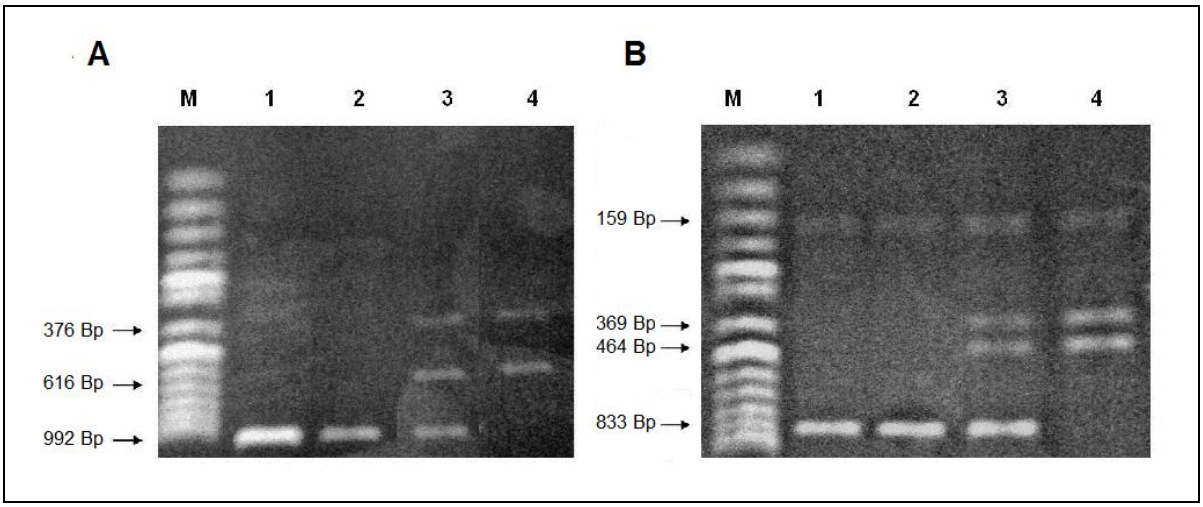

FIG. 1: FRAGMENTATION PATTERNS OF PCR PRODUCTS FOR DETECTION OF GENETIC POLYMORPHISMS IN UGT1A6. ELECTROPHORESIS PATTERNS OF PCR FRAGMENTS AFTER DIGESTING WITH NsiI fOR THE 541A>G POLYMORPHISM (A) AND WITH FNU4HI FOR THE 552A>CPOLYMORPHISM (B). M, 100 bp MARKER. LANES 2, HOMOZYGOSITY FOR COMMON ALLELES; LANES 3, HETEROZYGOSITY; LANES 4, HOMOZYGOSITY FOR VARIANT ALLELES.

Follow-up: Drug response involving both seizure control and adverse drug reactions was assessed every 1-2 months during therapy till a minimum of six months On each follow up visit, the number of seizures in the preceding period was recorded, drug compliance was checked and experienced ADR of VPA were recorded using a prepared checklist. [24] An average number of seizures per month was calculated for 6 months of follow up. A good response was defined as having a seizure frequency of <2 months for six consecutive months. [25] Seizure severity (SS) was rated using the Chalfont SS scale. [26] Complete blood count and serum ALT were performed routinely every 3-6 months. Additional laboratory tests were performed such as PT and PTT, blood bilirubin levels, blood ammonia, when a severe form of ADR were suspected.

Statistical Analysis: The normality of data such as age, weight, VPA dosage, C_{ss}, and CDR were tested before statistical analysis. Comparisons of

demographic characteristics including age, body weight, and gender among genotypic groups were done by Mann-Whitney test or Chi-square test. Association between VPA dosages, C_{ss}, CDR, Chalfont SS rating score, and number of ADR per patient, with genetic polymorphisms was examined using Mann-Whitney test. The relationship between seizure control, occurrence of ADR and genotypic groups was examined by the Chi-square test or the Fisher's exact test for small sample size. All p-values were two-sided. P-values < 0.05 were considered significant. Statistical analysis was performed using Statistical Package for Social Sciences version 17 (SPSS Inc, IL, USA).

RESULTS: Fifty pediatric patients were eligible but only 48 of them completed the study comprising 20 males (41.7%) and 28 females (58.3%). Patients had a median age of 7 years (range, 2–18 years) and a median weight of 24.5 kg (range, 12–96 kg). All of the participants were from the Egyptian population and were distinguished into two groups, established patients (n=30, 62.5%) with median duration of therapy with VPA of 2.6 years (range, 0.8–14years), and newly diagnosed patients (n=18, 37.5%) with median duration of treatment of 0.71 years (range, 0.5–1.6years) **Table 1.**

The mean VPA daily dose was 20.6 ± 7.1 mg/kg/day (range, 7–39.6 mg/kg/day). The maximum maintenance dose received averaged 22.2 ± 10 mg/kg/day (range, 7.7–39.6 mg/kg/day). Average C_{ss} was 59.96 ±27.18 µg/ml (range, 20–124 µg/ml).

TABLE 1: DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF EPILEPTIC CHILDREN (N=48)

Characteristics	N (%)
Age (years)	7 (2 – 18)§
Weight (kg)	24.5(12 – 96)§
Gender	
Male	20 (41.7)
Female	28 (58.3)
Patients groups	
Established	30(62.5)
Newly –diagnosed	18 (37.5)
Type of seizures	
Generalized tonic-clonic	28 (58.3)
Absence	3 (6.3)
Atonic	3 (6.3)
Tonic	1 (2.1)
Complex focal seizures	8 (16.7)
Simple focal	2 (4.2)
Focal with secondary generalization	3 (6.3)
Age at onset in years	
Groups	
0 – 4	21 (43.8)
5 – 9	20 (41.7)
> 10	7 (14.6)
Mean ± SD	5.82 ± 4.07
Baseline EEG	
Normal	7 (14.5)
Abnormal	41 (85.5)

§Data are expressed as median and range; SD: standard deviation.

Genotypic distributions were consistent with Hardy–Weinberg equilibrium proportions and genotype frequencies showed similar distribution at both UGT1A6 541 A>G and 552A>C loci (**Table 2**). The distribution of age, weight and gender was not significantly different among genotypic groups which comprised wild type versus variant (homozygous and heterozygous) genotypes at both SNPs (p>0.05). Genotypic comparisons using either dominant or codominant models demonstrated significantly lower CDRs for variant allele versus wild type allele carriers at both loci (**Table 2**) and so did allelic associations (**Table 3**). Also for the 552A>C polymorphism, patients with variant genotypes (AC& CC) had significantly lower VPA serum level than AA genotype (p=

0.010) (**Table 2**). However, no significant association was detected between either variant genotypes or allelic groups and the maximum maintenance dose at both loci (p>0.05) (**Table 2** and **3**).

TABLE 2: ASSOCIATION OF UGT1A6 541A>G AND 552A>C GENOTYPES WITH MAXIMUM VALPROIC ACID DOSAGE, CSS AND CDR IN EPILEPTIC CHILDREN

SNPs	Frequency N(%)	Maximum dose (mg/kg/day)	C_{ss} (µg/ml)	CDR (µg kg $ml^{-1}mg^{-1}$)
UGT1A6 541A>G				
Co-dominant model				
AA	25 (52.1)	20.1 (10.7-39.6)	60.0 (20.0-111.0) 58.5	3.0 (1.4-6.9)
AG	16 (33.3)	19.7 (10-38.9)	(26.0-110.0)	2.9 (2.0-5.5)
GG	7 (14.6)	27.7 (16.0-35.8)	36.0 (25 -124.0)	1.7 (1.2-4.0)
p-value		0.301	0.192	0.042
Dominant model				
AA	25 (52)	20.1 (10.7-39.6)	60.0 (20.0-111.0)	3.0 (1.4-6.9)
AG and GG	23 (48)	20.6 (10.0-38.9)	49.0 (25.0-124.0)	2.6 (1.2-5.5)
p-value		0.98	0.36	0.41
UGT1A6 552A>C				
Co-dominant model				
AA	25 (52.1)	22.7 (10.7-35.0)	66.0 (20.0-111.0) 49.5	3.1 (1.4-6.9)
AC	16 (33.3)	17.2 (10-38.9)	(24.0-124.0)	2.7 (1.5-5.4)
CC	7 (14.6)	23.3 (16.0-39.6)	36.0 (25 -80.0)	1.7(1.2-2.9)
p-value		0.177	0.014	0.005
Dominant model				
AA	25 (52)	22.7 (10.7-35.0) 20.0	66.0 (20.0-111.0)	3.1(1.4-6.9)
AC and CC	23 (48)	(10.0-39.6)	45.0 (24.0-124.0)	2.4(1.2-5.4)
p-value		0.342	0.010*	0.029*

***** Variant group's median is significantly lower than wildtype group's median; C_{ss}: steady state concentration of valproic acid; CDR**:** concentration to dose ratio.

TABLE 3: ASSOCIATION OF ALLELIC GROUPS OF UGT1A6 541A>G AND 552A>C POLYMORPHISMS WITH MAXIMUM VALPROIC ACID DOSAGE, C_{SS}AND CDR IN EPILEPTIC CHILDREN

Allele group	N	Maximum dose (mg/kg/day)	C_{ss} (µg/ml)	CDR (µg kg $ml^{-1}mg^{-1}$)
UGT1A6 541A>G				
A allele	66	20.1 (10-39.6)	59.5 (20-111)	3(1.4-6.9)
G allele	30	23.1 (10-38.9)	45 (25-124)	2.4 (1.2-5.5)
p-value		0.289	0 .094	0.047
UGT1A6 552A>C				
A allele	66	21.4 (10-38.9	60 (20-124)	3 (1.4-6.9)
C allele	30	20 (10-39.6)	42.5 (24-124)	2.1 (1.2-5.4)
p-value		0.825	0.001	0.001

C_{ss}: steady state concentration of valproic acid; CDR**:** concentration to dose ratio.

Regarding seizure frequency, no significant association was revealed between wildtype and variant groups at both loci (p>0.05, **Table 4**). As for the Chalfont SS score, variant group of 552A>C polymorphism had significantly lower score than wild genotype (p=0.02, **Table 4**). Comparing the prevalence of the screened ADR to VPA in variant and wild type genotypic groups at both loci, variant genotype (AG & GG) carriers showed significantly higher incidence of difficulty in concentration and/poor school achievement (p=0.047). On the other hand, variant genotype (AC& CC) had a significantly lower incidence of fatigue than wild type (p=0.025, **Table 5)**.

TABLE 4: ASSOCIATION OF UGT1A6 541A>G AND 552A>C GENOTYPIC GROUPS WITH SEIZURE FREQUENCY AND SEIZURE SEVERITY IN EPILEPTIC CHILDREN

Genotypic group	Seizure frequency<2/month (N, %)	Chalfont seizure severity score
UGT1A6 541A>G		
AA	18 (52.9)	0.0 (0.0-62.0)
AG & GG	16 (47.1)	0.0 (0.0-149)
UGT1A6 552A>C		
AA	15(44.1)	10.5 (0.0-149.0)*
AC & CC	19 (55.9)	0.0 (0.0-76.0)

*p=0.02.

TABLE 5: ASSOCIATION OF UGT1A6 541A>G AND 552A>C GENOTYPIC GROUPSWITH ADVERSE DRUG REACTIONS TO VALPROIC ACID IN EPILEPTIC CHILDREN

Adverse drug reaction (N, %)	UGT1A6 541A>G		UGT1A6 552A>C	
	Wild type group N=25	Variant group N=23	Wild type group N=25	Variant group N=23
Fatigue	11(68.8)	5 (31.2)	12(75.0)	4(25.0)*
Difficulty in concentration and/or poor scholastic achievement	2(22.2)	7(77.8)§	4(44.4)	5(55.6)
Hyperactivity	6(66.7)	3 (33.3)	5(55.6)	4 (44.4)
Decreased appetite +/- poor weight gain	12(63.2)	7(36.8)	11(57.9)	8(42.1)
Alopecia+/- curling	7(58.3)	5(41.7)	6(50.0)	6(50.0)
Insomnia	3(33.3)	6(66.6)	5(55.6)	4(44.4)
Drowsiness	7(46.7)	8(53.3)	8(53.3)	7 (46.7)
Obesity+/- increased appetite	3(50.0)	3(50.0)	3(50.0)	3(50.0)
Irritability	12(50.0)	12 (50.0)	12(50.0)	12 (50.0)
Headache	8(47.1)	9 (52.9)	8(47.1)	9 (52.9)
Number of adverse drug reactions per patient¶	3.0 (0.0-8.0)	4.0(0.0-10.0)	4.0(0.0-9.0)	3.0(0.0-10.0)

*p=0.047 (Chi-square test); §p=0.025 (Fisher exact test).
¶ Data are represented as median (minimum – maximum).

DISCUSSION: The effect of UGT1A6 polymorphisms on the pharmacokinetics of VPA have been investigated in several studies. [13-19] Few reported the association between genetic variants at 541A>G and 552A>C SNPs and VPA concentration and/or doses. [13-18] Despite the low power of the present study, an association between polymorphisms at 541A>G and 552A>C loci of the drug metabolizing enzyme UGT1A6 and CDR of VPA could be demonstrated. This was shown by significantly lower CDR in patients carrying the variant compared to those with wild type allele at both loci (p<0.05)when either genotypic group or allelic comparison was used.

These findings may imply an increased rate of VPA glucuronidation in the variant UGT1A6 carriers for the two investigated SNPs in accordance with former studies, [14-17] but not with three other studies that showed negative associations. [13, 18, 19]

In the study by Wang et al,[18] the authors investigated only 541 A>G polymorphism and concluded no association with adjusted serum level in epileptic children while Chu et al. reported that neither UGT1A6 552A>C nor 541A>G polymorphisms had a significant effect on VPA serum concentration in epileptic adults. [13] It is noteworthy that only 3 patients (out of 136) in this study had the GG or CC genotype (for 541A>G and 552A>C, respectively). A finding that could contribute to under representation of the homozygous variant forms and account for a lack of association that is partly explained by type II error. Jain et al. reported no association between the same polymorphisms and VPA doses or standardized VPA concentrations in epileptic children of the Indian population. The authors attributed their negative results to the small sample size of their study too.[19]

In the current study, neither UGT1A6 541A>G nor 552A>C genetic variants was found to have a significant effect on the maximum dose required to achieve seizure control contrary to those reported by Hung et al. in adult epileptic patients. [15] They found both 541A>G and 552A>C variant genotype carriers to require significantly higher dosage than wild-type carriers. In their study, VPA maintenance dose was set as the dosage not changed for at least 1 year under good patient's compliance and good seizure control providing no further definition for the latter. However, in the current study, the maximum VPA maintenance dose recorded for the patient was used instead to discern the genetic contribution to the whole long term dosage requirements of the patients, irrespective of the time of drug concentration analysis.

Differences in methodology, ethnicity, phenotype definition such as drug-responsiveness as well as patient age could partly explain the discordance between our results and those of Hung et al. Sample size limitation is an important, common problem in candidate gene association studies, including many of the previously mentioned studies, as well as the current one. All genetic association studies of VPA pharmacokinetics were performed in Asian populations including Chinese as well as Indian cohorts. Previously, Lampe et al. demonstrated that genotype and allele distributions of UGT1A6 polymorphisms including 541A>G and 552A>C (referred to as the haplotype UGT1A6*2) vary to a significant extent across different racial populations, especially among Caucasians and Asians. [27] Hence, extrapolating results from one population to another cannot be judiciously applied. Little is known about polymorphisms affecting VPA pharmacokinetics in Caucasians, let alone the clinical outcome. Besides, two of the previous studies were conducted in age-heterogeneous population combining pediatric with adult patients.[13, 17] Given that VPA metabolism can be significantly greater in children than in adults, [28] studies including heterogeneous population of children and adults might demonstrate significant variability.[29, 30]

Furthermore, the complexity of VPA elimination pathways may diminish the effect of UGT1A6 polymorphisms on VPA concentrations. [31] CYP P450 enzyme system, though accounting only for about 10% of VPA metabolism, is mediated by polymorphic isoforms such as CYP4B, CYP2C9 and CYP2A6.[6] Other UGTs involved such as UGT2B7, UGT1A3 have also polymorphic forms [9, 32] e.g. UGT2B7 161C>T polymorphism has been reported to affect VPA concentrations in pediatric epilepsy patients.[33] The altered activity of enzymes encoded by some genetic variants could be camouflaged by others in the same or different pathway of their substrate elimination.

Regarding seizure control, no significant association with seizure frequency as a measure of seizure control (p>0.05) was detected, which could be well attributed to our small sample size. On the other hand, it has been reported that both the clinical and toxic effects of the VPA are considered to be poorly correlated with total serum concentrations.[34] We found carriers of the minor allele at 552A>C locus having a significantly lower score for seizures on Chalfont SS scale than wild allele carriers (p=0.02). Although, not significantly different from each other in seizure control assessment, the wild genotype group was shown previously to have higher CDRs (median 3.1, range, 1.4-6.9) than the variant, reflecting the unclear relationship between concentration and clinical response.

None of the available association studies to date addressed the influence of these genetic variants on the clinical outcome to VPA therapy per se. It was tentatively approached by Hung et al. who preselected patients showing good drug response (seizure free/good seizure control) to evaluate multiple genetic influence including UGT1A6 gene on VPA pharmacokinetics. [15] Munisamy et al., also evaluated UGT1A6 genevariation in epileptic patients pre-identified with toxicity symptoms to therapy.[16]

Difficulty in concentration and poor school achievement was assessed as a subset of cognitive side effects of VPA. We found it of a significantly higher incidence in the variant allele group at 541A>G locus that previously showed significantly lower CDR than wild type group. Despite cognitive adverse effects being linked with higher AED dose, some studies report no relationship between them and the dose for valproate [35, 36] or its plasma level.[37] Altogether, factors such seizure type and frequency

may contribute to the patient's cognitive state.[38] We also found fatigue to be significantly higher in wildtype allele compared to variant allele carriers at 552A>C locus with the former having significantly higher CDR. Earlier, Fortscher et al. reported significantly higher VPA levels in patients complaining of tiredness than patients without this side effect. [39] When the number of ADR experienced per patient were compared among genotypes at both 541A>G and 552A>C loci, neither of them showed a significant association. Conversely, Jakovljevic et al. [24] observed a significant correlation between trough VPA level and number of ADR per patient while Hussein et al found no relationship between serum levels of AEDs including VPA and side effects in newly diagnosed patients. [40] The relationship between VPA concentration and effect remains yet unclear [34] and the mechanism by which these genetic variants affect the clinical outcome of therapy needs to be further elucidated.

Despite the low power of the study, it could be concluded that UGT1A6 polymorphic variants at 541A>G and 552A>C loci may be associated with increased VPA metabolism in Egyptian children having idiopathic epilepsy. Carriers for the variant allele tend to have lower CDR than wildtype allele counterparts at both loci. The investigated genetic variants may influence the clinical response profile of patients including both ADR to VPA as well as seizure severity.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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