The evidence for sodium valproate toxicity in mitochondrial diseases: a systematic analysis

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The evidence for sodium valproate toxicity in mitochondrial diseases: a systematic analysis

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Abstract

We aimed to determine whether sodium valproate (VPA) should be contraindicated in all mitochondrial diseases, by systematically reviewing the published literature for mitochondrial DNA (mtDNA) and common nuclear genotypes of mitochondrial diseases using PubMed, Ovid Embase, Ovid Medline, and MitoPhen databases. We extracted patient-
level data from peer-reviewed articles, published till July 2022, using the Human Phenotype Ontology to manually code clinical presentations for 156 patients with genetic diagnoses from 90 publications. There were no fatal adverse drug reactions (ADRs) in the mtDNA disease group (35 patients), and only one out of 54 patients with a non-POLG mitochondrial disease developed acute liver failure. There were fatal outcomes in 53/102 (52%) POLG VPA-exposed patients who all harboured recessive mutations.

Our findings confirm the high risk of severe ADRs in any patient with recessive POLG variants irrespective of the phenotype, and therefore recommend that VPA is contraindicated in this group. However, there was limited evidence of toxicity to support a similar recommendation in other genotypes of mitochondrial diseases.

**Background**

Epilepsy often features in mitochondrial diseases, with myoclonic, generalised, and focal onset seizure types [1]. Sodium valproate (VPA) is effective in these seizure types in non-mitochondrial diseases, although not a first line treatment for focal onset seizures [2]. It is also used for migraines and bipolar disorder prophylaxis, which are found in mitochondrial diseases [3-5]. However, it is important to note that VPA has known teratogenic effects and associations with neurodevelopmental disorders, leading it to only be considered in the event of a lack of alternative options [6]. While VPA may cause transient liver enzyme increases in 15% of patients, severe adverse drug reactions (ADRs) like hepatic failure are rarer (0.01% of all patients) [7]. POLG-related mitochondrial disease, typically Alpers syndrome presenting with developmental regression and intractable seizures, is an
identified risk factor for VPA-induced hepatotoxicity [7]. POLG disease can present at different ages with seizures commonly presenting in early-onset and juvenile to adult-onset forms. However, liver involvement is prevalent in early-onset POLG disease and is associated with worse survival including in phenotypes where epilepsy is not a feature [8]. The severity of VPA-related ADRs in Alpers syndrome and the lack of systematic follow-up studies of VPA in other mitochondrial diseases has led clinicians to avoid VPA in all mitochondrial diseases without clear evidence, potentially discarding an effective and cheap medicine. To bridge this gap, we assessed VPA effects across various mitochondrial diseases.

MitoPhen [9], which contains published clinical data as human phenotype ontology (HPO) terms, was used to investigate whether there are any associated clinical features pre-empting VPA-related toxicity. Our aim was to evaluate the evidence base for VPA-induced toxicity in different mitochondrial diseases using published patient-level data.

**Methods**

**Search strategy and study selection**

A systematic review of the literature, published up to July 2022, was conducted using PubMed, Ovid Embase, Ovid Medline, and MitoPhen databases, according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [10] (Figure 1). Articles about mitochondrial diseases linked to migraine or seizures and VPA treatment were reviewed. We considered three groups: 1) POLG-related mitochondrial diseases, 2) mitochondrial DNA (mtDNA) diseases 3) other nuclear causes of mitochondrial disease based on PanelApp genes [11]. We supplemented the search with the ‘MitoPhen-Expanded’ dataset which currently contains data on 89 pathogenic mtDNA variants [9], and common
nuclear genotypes including POLG. Exclusion criteria were non-peer-reviewed articles, no documented VPA use, no genetic diagnosis, and lacking patient-specific information. An ADR was defined as a noxious or unintended response to VPA, where the causal relationship between VPA use and the reaction was strongly suspected [12]. ‘Symptom-control’ referred to reported seizure or symptom reduction with VPA. The search strategy and risk of bias assessment is detailed in Supplementary Information.

Survival analyses – POLG dataset

To investigate survival between patients who had and had not been exposed to VPA, we included the MitoPhen-Expanded POLG dataset (publications up to 01/06/2022), where patients with seizure phenotypes and without documented VPA use were coded as ‘no VPA’. The survminer R package [13] was used to create Kaplan-Meier plots and log-rank P-value was used for testing differences in survival between groups. ‘Event’ was defined as ‘death’ for survival analysis. Time in years was calculated by age at symptom onset till age at death or age at last follow-up.

HPO-based analyses

The OntologyX [14] and gplots [15] R packages were used to generate a matrix of phenotype similarity scores using the Lin similarity measure, and a heatmap displaying the hierarchically clustered scores per patient. Phenotype enrichment was explored within the POLG dataset by grouping for VPA and no VPA exposure, and between POLG and non-POLG datasets with VPA exposure, using one-sided Fisher’s exact test adjusted with the Benjamini-Hochberg procedure. HPO terms related to liver dysfunction were excluded to avoid over-representation from VPA-related toxicity.
Data availability

See Supplementary Information. MitoPhen is accessible on www.mitophen.org.

Results

The search strategy revealed a total of 156 patients from 90 articles with mitochondrial diseases who had reported VPA exposure (Figure 1).

**Group 1: POLG-related mitochondrial diseases**

Data collated from 46 articles on 102 patients with POLG-disease and VPA exposure (Figure 1) showed ADRs in 91 patients (89%), with 87 patients (85%) reported to have hepatotoxicity. There were no reported ADRs in seven and ADR status was unknown in four patients. There was no pre-existing liver disease in 92/102 patients (90%). There were fatal outcomes in 53/102 patients (52%), with eight being unrelated to VPA such as intractable seizures. Liver transplant was required in 15 patients, and six (40%) died post-transplant.

Age at onset was available for 98/102 patients (96%). Survival between male and female patients were similar ($p=0.7$), but the lower age at onset group (0-4years) had a lower survival compared with onset in later childhood: 5-18years ($p=0.05$). All patients exposed to VPA with reported genotypes (97/102) had recessive POLG variants. Compound heterozygous variants were associated with worse outcomes compared to homozygous variants ($p<0.01$) (Figure 2A).

There were 280 patients from 66 articles with recessive POLG variants, without documented VPA exposure included from the MitoPhen-Expanded dataset. There was a lower survival in the VPA exposure group compared with no VPA use ($p<0.0001$, Figure 2B).
Group 2: MtDNA diseases

In the mtDNA disease group, 28 articles mentioned VPA use in 35 patients without any documented fatal ADRs. Seizure control was reported to be effective in 10/13 (77%) patients with \textit{m.8344A>G} and presentations of myoclonic epilepsy and ragged red fibres (MERRF syndrome). Seizures were exacerbated in 3/8 (38%) \textit{m.3243A>G} patients who had features suggestive of mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS syndrome) (Figure 2C, Table S1). Patients with generalised myoclonic seizures, and/or focal myoclonic seizures, had reported good symptom control with VPA (Figure 2D). There was one report of VPA being instigated for migraine in a patient successfully, although it was a dominant symptom in five published patients. There were 3/35 (9%) cases with non-fatal hepatotoxic effects including pancreatitis (Table S1).

Group 3: Other nuclear-encoded mitochondrial diseases

The search strategy for 227 nuclear genotypes of mitochondrial diseases resulted in 16 articles documenting 19 patients (Figure 1). Four patients (21%) had VPA hepatotoxicity: one patient with a severe encephalopathy and epilepsy due to \textit{WARS2} variants developed acute liver failure after commencing VPA [16], two patients with recessive \textit{TWNK} variants and one with biallelic \textit{PARS2} variants had transient elevations in hepatic transaminases. Three patients had transient symptoms where VPA was thought to be a contributing factor, however a causal relationship was not clear. Therefore, 15/19 (79%) patients in this group had no definite VPA-induced ADRs (Table S2).

According to the Clopper-Pearson exact method [17], and pairwise Fisher’s exact tests with Bonferroni adjustment, VPA-induced hepatotoxicity proportions were significantly different between group 1: \textit{POLG} disease at 0.85 (95% CI: 0.77-0.92), and non-\textit{POLG} disease groups 2
and 3 at 0.13 (95% CI: 0.05-0.25, \(p<0.0001\)). In terms of VPA exposure, the data available for time from VPA use till ADR in 59 patients (53 diagnosed with \textit{POLG} disease) showed that this ranged from under 1 week to 1.33 years, with a median of 1 week.

**HPO-based analyses**

There were no enriched terms between the \textit{POLG} disease datasets grouped by VPA and no VPA exposure to distinguish those who had ADRs. Hierarchical clustering of all 156 patients exposed to VPA using phenotype similarity scores showed that patients with non-\textit{POLG} diagnoses clustered together (Figure S1A). Patients with early onset \textit{POLG} disease had a lower survival compared with other clusters (\(p<0.05\), Figure S1B). The clusters resemble clinically defined phenotypic groups relating to genotypes (Figure S1C). Status epilepticus was more frequent in \textit{POLG} disease (Figure S2), explaining why VPA was used in these patients.

**Discussion**

This comprehensive systematic review utilised several search engines, and MitoPhen-Expanded datasets. We assessed 228 nuclear genes and 27 mtDNA genes linked to mitochondrial diseases, incorporating patient-level data for 436 patients. No published patients with dominant \textit{POLG} variants exposed to VPA were found. Data confirm the significant risk in recessive \textit{POLG} disease (Figure 2), with fatal outcomes in 52% and ADRs in 89% of 102 patients, aligning with previous findings [4]. In mtDNA diseases, 10/13 patients with \textit{m.8344A>G}-induced seizures responded well to VPA with no reported ADRs. Non-\textit{POLG} genotypes without mtDNA depletion, including \textit{m.7472_7473insC}, \textit{TWNK}, and \textit{PARS2} variants, showed hepatic transaminase increases in 4/54 patients, comparable to rates in
non-mitochondrial diseases [7]. The lack of natural history data on rarer genotypes meant it was difficult to attribute VPA toxicity to the fatal neurological decline of a patient with a WARS2 diagnosis, following the resolution of their VPA-induced liver failure [16]. The reasons for significantly different VPA-induced hepatotoxicity proportions between POLG (87/102) and non-POLG mitochondrial diseases (7/54) ($p<0.0001$), remain unclear.

A limitation of this review is publication bias favouring POLG disease data, attributed to higher status epilepticus frequency in the POLG group (Figure S2), necessitating additional antiseizure medications like VPA. Also publication bias would factor in reporting of ADRs related to VPA over non-events, thereby giving a likely over-estimate of ADRs. Some authors changed VPA due to perceived ADR risks in non-POLG disease [18]. This contributes to uncertainty in clinical management of mitochondrial epilepsy due to lack of longer-term follow-up in non-POLG mitochondrial diseases with VPA exposure. Additionally, there were unclear causal relationships between VPA and clinical features which could be explained by natural progression of the mitochondrial disease [19]. Despite variability in reporting VPA dosage and time to ADR, consistent documentation of clinical features enabled survival analyses using HPO-driven clusters, revealing significantly different survival between POLG and non-POLG diseases (Figure S1). ADRs could not be predicted by phenotypic profiles in POLG disease.

Conclusions

The data showed a significantly lower proportion of hepatotoxicity with VPA in non-POLG versus POLG mitochondrial diseases. Additionally, VPA resulted in reported symptom control particularly in patients with m.8344A>G disease, with no reported ADRs. Longer-term follow-up studies are required to define the natural history of rare nuclear genotypes...
of mitochondrial diseases and the role of VPA in treating patients with non-POLG
mitochondrial diseases. We recommend VPA is contraindicated in patients carrying
recessive POLG variants.

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Declarations

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Ethical standards

The manuscript does not contain clinical studies or previously unpublished patient data.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

References


**Figure legends**

**Figure 1:** PRISMA approach to study inclusion in systematic review. Searches were performed separately for POLG-related mitochondrial disease, mtDNA disease, other nuclear-encoded mitochondrial diseases. *Nuclear genes associated with complex I-V deficiencies were only searched in the MitoPhen-Expanded dataset.

**Figure 2:** Survival in POLG disease and adverse reactions in mtDNA disease. A: Survival comparison in patients exposed to valproate acid (VPA) by genotype – the log-rank test revealed a significant difference between compound heterozygous and homozygous groups (p<0.01). B: Survival comparison between documented VPA treatment and no VPA exposure.
groups revealed a significant difference using the log-rank test (p<0.0001). C: VPA use by pathogenic mtDNA variant, adverse drug reaction (ADR) and reported symptom control. D: Symptom type and mtDNA variant with counts of patients where VPA was used (displayed as grey circles). ADR and symptom control status also displayed as red asterisk or coloured dot respectively.
Figure 1: PRISMA approach to study inclusion in systematic review. Searches were performed separately for POLG-related mitochondrial disease, mtDNA disease, other nuclear-encoded mitochondrial diseases.*Nuclear genes associated with complex I-V deficiencies were only searched in the MitoPhen-Expanded dataset.

350x251mm (300 x 300 DPI)
Figure 2: Survival in POLG disease and adverse reactions in mtDNA disease. A: Survival comparison in patients exposed to valproic acid (VPA) by genotype – the log-rank test revealed a significant difference between compound heterozygous and homozygous groups (p<0.01). B: Survival comparison between documented VPA treatment and no VPA exposure groups revealed a significant difference using the log-rank test (p<0.0001). C: VPA use by pathogenic mtDNA variant, adverse drug reaction (ADR) and reported symptom control. D: Symptom type and mtDNA variant with counts of patients where VPA was used (displayed as grey circles). ADR and symptom control status also displayed as red asterisk or coloured dot respectively.

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Supplementary Information

Methods:

Although this review was not prospectively registered with PROSPERO [1], the search strategy is detailed below.

Search strategy

Genotype groupings: 1) POLG-related mitochondrial diseases, 2) mitochondrial DNA (mtDNA) diseases 3) other nuclear gene causes of mitochondrial disease as per the list of 198 ‘green’ genes in the PanelApp mitochondrial disorders panel (downloaded 15/11/2023), including those associated with hepato-cerebral features [2]: AARS2, ABAT, ABCB7, ACAD9, ACO2, AFG3L2, AGK, AIFM1, ANO10, APOPT1, APTX, ATAD3A, ATP5A1, ATP5D, ATP5G3, BOLA3, BTD, C12orf65, C19orf70, C1QBP, CAT5A, CASR2, CHCHD10, CLPB, CLPP, COA6, COA7, COQ2, COQ4, COQ6, COQ7, COQ8A, COQ8B, COQ9, COX20, COX6A1, COX6A2, COX7B, CYC1, DARS2, DALT, DLD, DNA2, DNAJC19, DNM11, DNM2, EARS2, ECHS1, ELAC2, ETFDH, ETHE1, FAR52, FBXL4, FD2X, FDXR, FH, FLAD1, GARS, GDA1, GFER, GM1, GFM2, GLRX5, GTPBP3, HARS2, HCCS, HICB, HLC5, HSD17B10, HSPD1, HTRA2, IARS2, IBA57, ISCA1, ISCA2, ISCU, KARS, KIAA0391, LARS2, LIAS, LIG3, LIPT1, LIPT2, LONP1, LRPPRC, LYR4, LYRM4, LYRM7, MARS2, MDH2, MECR, MFF, MFN2, MGMEI, MUC1, MIPEP, MPC1, MRPL3, MRPL44, MRPS2, MRPS22, MRPS34, MSTO1, MTFFM, MTO1, MTPAP, NADK2, NARS2, NAXD, NAXE, NDUFA1, NDUF1A2, NDUF1A3, NDUF1A4, NDUF1A5, NDUF1A6, NDUF1A9, NDUF2A2, NDUF5A5, NDUF6A, NDUF6F3, NDUF6F5, NDUF6F6, NDUF6F8, NDUF1B10, NDUF1B11, NDUF1B3, NDUF1B8, NDUF1C2, NDUF1V2, NFS1, NFU1, NSUN3, OPA1, OPA3, PARS2, PC, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PET100, PMPCA, PMPCB, PNPLA8, PNPT1, POLRTM, PPAD, PPA2, PUS1, QRS1, RARS2, RMND1, RNASEH1, RTN4IP1, SACS, SARS2, SDHA, SDHAF1, SDHB, SDHC, SERAC1, SFXN4, SLC19A2, SLC19A3, SLC25A1, SLC25A12, SLC25A19, SLC25A26, SLC25A3, SLC25A32, SLC25A38, SLC25A42, SLC25A46, SPG7, SSBP1, SURF1, TARS2, TAZ, TFAM, TIMM50, TIMM8A, TIMMDC1, TMEM126B, TOP3A, TP1, TRIT1, TRMT10C, TRMT5, TRMU, TRNT1, TSFM, TUFM, UQCC2, UQCRB, UQCRC2, UQCRFS1, VARS2, WARS2, YARS2, DMPK.

Groups 1 and 3 were supplemented with searches of the ‘MitoPhen-Expanded’ dataset which contains data (updated to June 2022) on POLG, OPA1, TWWK, and 72 nuclear genes causing diseases arising from deficiencies of complexes I-V of the mitochondrial electron transfer chain:

ACDH9, FOXRED1, NDUF1A1, NDUF1A10, NDUF1A12, NDUF1A13, NDUF1A2, NDUF1A6, NDUF1A8, NDUF1A9, NDUF1A21, NDUF1F2, NDUF1F3, NDUF1F4, NDUF1F5, NDUF1F6, NDUF1F8, NDUF1B10, NDUF1B11, NDUF1B3, NDUF1B8, NDUF1C2, NDUF1F1, NDUF3, NDUF5A4, NDUF5A6, NDUF5A7, NDUF5A8, NDUF5F1, NDUF5F2, NDUF5F3, NDUF5F4, NDUF5F6, NDUF5F7, NDUF5F8, NDUF5V1, NDUFV2, NUBL1, TIMMDC1, TMEM126B, SDHA, SDHAF1, SDHB, SDHC, BDCT, CYC1, LYR4, TTC19, UQCC2, UQCRB, UQCRC2, UQCRFS1, APOPT1, COA6, COA7, COX10, COX14, COX15, COX20, COX4I1, COX6A1, COX6A2, COX6B1, COX7B, LRPPRC, NDUF4A4, PET100, SCO1, SCO2, SQOR, SURF1, TACO1, ATP5A1, ATP5D, ATP5G3, ATP5F2, TMEM70.

For group 2, we searched for 89 pathogenic mtDNA variants, as listed in MitoPhen [3], as these were manually curated to be pathogenic using the American College of Medical Genetics and Genomics guidelines [4].

Search terms used within PubMed, Ovid Embase and Ovid Medline alongside the genotypes above were: ‘headache’ or ‘migraine’ or ‘seizure’ or ‘epilep*’, and ‘mitochondrial disease*’ or ‘mitochondrial disord*’, and ‘sodium valproate’ or ‘valproic acid’.
MitoPhen-Expanded dataset: The search strategy used to compile this dataset is as per the MitoPhen database [3] – where PubMed is searched for ‘gene’ and ‘mitochondrial disease*’ or ‘clinical*’. Title and abstract reviews were performed to exclude articles which did not contain patient-specific information by co-author KS.

POLG dataset: Two data collectors (co-authors NE and AL) have additionally gathered data on treatments documented. The pathogenicity of POLG variants was checked using American College of Medical Genetics guidelines [4], the ‘Human DNA Polymerase Gamma Mutation Database’ [5], and through expert consensus between co-authors KS and RH. The MitoPhen-Expanded dataset was searched for any probands with human phenotype ontology (HPO) terms relating to headache, migraine, and seizures. Additional family members diagnosed with mitochondrial disease were included if sodium valproate (VPA) treatment was mentioned.

Nuclear genotypes associated with mitochondrial complexes I-V deficiency: PanelApp [2] was used to identify ‘green’ genes in these categories, and the search strategy is as above. Co-author CG collected patient-specific data including on treatments. Note: there were no mentions of sodium valproate in the MitoPhen-Expanded OPA1 and TWNK datasets, therefore we have not included these datasets in this paper.

Data extraction and review
Author TR screened the articles and collected the data. The data collected from articles documenting VPA use in patients with mitochondrial diseases included sex of patient, age at onset of symptoms, HPO terms (manually extracted), age at VPA use, age at adverse drug reaction (ADR), type of ADR, and whether the ADR was ultimately fatal. Author PFC performed a secondary review of included articles to ensure correct interpretation of data.

Risk of bias assessment summary
Author TR screened articles using the Joanna Briggs Institute case report critical appraisal tool [6]. Articles very infrequently reported dosages and frequency of administering VPA and did not report blood monitoring of VPA levels. Additional confounders such as other antiseizure medications were used in case reports, especially in the POLG disease datasets, which contributes to difficulty in ascertaining the true effect of VPA in ADR severity. However, the clinical presenting features and effects of suspected VPA toxicity were consistently reported across all groups. Therefore, the study was not suitable for a meta-analysis, but results could be synthesised using a phenotype-driven approach, and overall rate of ADRs could be commented on.

Literature Review Search terms:

**Group 1: Search strategy for POLG-associated mitochondrial disease**

Ovid Medline (1946 to September 21, 2022), Ovid Embase (1974 to September 22, 2022), PubMed (results to September 2022): 98 articles (once duplicates were removed)

MitoPhen-Expanded dataset: 12 articles

Following exclusion criteria: 46 articles included
Ovid MEDLINE(R) ALL <1946 to September 21, 2022>

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<td>119,014</td>
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Group 2: Search strategy for mtDNA disease
Search terms used were the same as for the POLG disease dataset above but each of the 89 pathogenic mtDNA variants within MitoPhen were reviewed.
Ovid Embase (1974 to June 09, 2022): 79 articles
PubMed (results to September 2022): 1 article
MitoPhen: 231 articles
Following exclusion criteria: 28 articles

Group 3: Search strategy for non-POLG nuclear genes
Search terms used were the same as for the POLG disease dataset above but each of the PanelApp genes listed above, were reviewed.
Ovid Medline (1946 to June Week 4 2022), Ovid Embase (1974 to July 01, 2022), PubMed (results updated to December 2022): 251 articles
MitoPhen-Expanded dataset: 3 articles
Following exclusion criteria: 16 articles

Results:

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<td>ND: 2</td>
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<td>Presentations requiring VPA</td>
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<td>treatment</td>
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<td>[7-14]</td>
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<td>Generalised myoclonic seizures: 18</td>
<td>[15-24]</td>
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<td>Generalised-onset and generalised myoclonic seizures: 4</td>
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<td>Focal-onset seizures: 4</td>
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<td>[32]</td>
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<td></td>
<td>Nausea and rise in liver transaminases- m.7472_7473insC</td>
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<td>Severe Reye-like syndrome- m.7472_7473insC</td>
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<td></td>
<td>Exacerbation of seizures- m.3243A&gt;G (3 patients)</td>
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<td>[8]</td>
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<td></td>
<td>m.3243A&gt;G – 2 patients</td>
<td>[21, 33]</td>
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<td></td>
<td>m.5537_5538insT – 1 patient</td>
<td>[11]</td>
</tr>
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<td></td>
<td>m.8356T&gt;C – 1 patient</td>
<td>[23]</td>
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<td></td>
<td>m.8993T&gt;G – 1 patient</td>
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<td>m.8363G&gt;A – 3 patients</td>
<td>[7, 14]</td>
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<td></td>
<td>m.8344A&gt;G – 10 patients</td>
<td>[10, 17-19, 24]</td>
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<td>Symptom control ineffective</td>
<td>m.10158T&gt;C – 1 patient</td>
<td>[28]</td>
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<td>m.8356T&gt;C – 1 patient</td>
<td>[23]</td>
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<td></td>
<td>m.13042G&gt;A – 1 patient</td>
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<td></td>
<td>m.8344A&gt;G – 3 patients</td>
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<td></td>
<td>m.3243A&gt;G – 5 patients</td>
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Table S1 Summary of data for published patients with mtDNA diseases and VPA exposure.
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<th>ADR</th>
<th>Ref</th>
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<td>ACAD9</td>
<td>1 patient: Treated at 18 months old with VPA for absence seizures, on a background of hypertrophic cardiomyopathy.</td>
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<td>[35]</td>
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<tr>
<td>COQ4</td>
<td>1 patient: presented with a progressive neurologic disorder, with tonic-clonic seizures at age 18 years, treated successfully with VPA.</td>
<td>None</td>
<td>[36]</td>
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<tr>
<td>ECHS1</td>
<td>1 patient: treated with VPA for seizures in infancy, also had features resembling Leigh syndrome. VPA thought to worsen abnormal eye movements, levetiracetam also worsened features. A valine-restricted diet was noted to improve neurological features.</td>
<td>Possible exacerbation of seizures</td>
<td>[37]</td>
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<tr>
<td>ETFDH and PHGDH</td>
<td>1 patient: Childhood-onset intractable seizures, treated with VPA, in combination with lamotrigine and clonazepam, achieved full seizure control. VPA withdrawn at three years old, when developmental regression noted. Clinical status improved after the amino acid therapy commenced to treat serine deficiency.</td>
<td>Unclear if developmental regression associated with VPA or disease progression</td>
<td>[38]</td>
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<td>NDUFS1</td>
<td>1 patient: Childhood-onset seizures and cerebellar ataxia, treated with VPA (at 12 months old). Death at three years old following acute metabolic acidosis and neurodegeneration.</td>
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<td>[39]</td>
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<td>OPA1</td>
<td>1 patient: Adult-onset occipital lobe epilepsy. VPA used in combination with several AEDs, no beneficial effects.</td>
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<td>[40]</td>
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<td>TRIT1</td>
<td>1 patient: Childhood-onset epilepsy triggered by febrile illness, including myoclonic epilepsy. VPA swapped to levetiracetam and clonazepam to avoid hepatotoxicity. 1 patient: childhood-onset generalised tonic-clonic seizures and myoclonic jerks. No improvement or worsening of seizure-control with VPA.</td>
<td>None</td>
<td>[41]</td>
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<td>TWNK</td>
<td>2 patients: Adult-onset epilepsy including episodes of status epilepticus. VPA was initiated but discontinued after about one month, due to a rise in liver transaminases.</td>
<td>Raised liver transaminases</td>
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<td>Mitochondrial aminoaeryl-tRNA synthetase genes:</td>
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<td>IARS2</td>
<td>IARS2: 2 siblings were treated for infantile spasms with VPA, without improvement in symptoms, and a subsequent diagnosis of Leigh syndrome. KARS: 1 patient was treated for seizures with VPA, with no further seizures noted.</td>
<td>None</td>
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<td>KARS</td>
<td></td>
<td>None</td>
<td>[45]</td>
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<tr>
<td>NARS2</td>
<td>NARS2: 1 patient with Leigh syndrome was treated with VPA at 1.7 years of age for generalised tonic and myoclonic seizures. She developed renal Fanconi syndrome which the authors attributed to VPA as she improved biochemically when VPA was stopped. Although, renal disease is documented in the natural history.</td>
<td>Suspected renal Fanconi syndrome</td>
<td>[46]</td>
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<td>PAR2</td>
<td>PAR2: 1 patient with a progressive neurodegenerative disease was treated with VPA at 7.5 years of age for seizures, but this was stopped within a few months due to transiently raised liver transaminases. He deteriorated with multiorgan failure and died at age 8.5 years. His sibling had a similar course.</td>
<td>Raised liver transaminases</td>
<td>[48]</td>
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<td>RARS2</td>
<td>RARS2: 2 siblings were treated with VPA in combination with other antiseizure medications for myoclonus with variable response.</td>
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<td>TARS2</td>
<td>TARS2: 1 patient was treated with VPA for seizures characterised by 'motion arrest', no symptom control.</td>
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<td>WARS2</td>
<td>WARS2: 1 patient with severe developmental delay, was treated at 6 years of age for a prolonged seizure with VPA. She developed acute liver failure a month later and VPA was stopped. She had severe encephalopathy despite liver enzymes normalising and died aged 6.5 years.</td>
<td>Acute liver failure</td>
<td>[51]</td>
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</table>

*Table S2 Articles where patients with nuclear genotypes of mitochondrial diseases have been treated with VPA.*
Figure S1 Phenotypic clustering across 156 patients with mitochondrial diseases and VPA exposure. A: Heatmap shows hierarchical clustering of phenotype similarity scoring, causative genes are displayed on the right, and the categories of gene are shown at the bottom – indicating clustering of non-POLG disease in cluster 5. Cluster numbers are coloured and shown at the top of the dendrogram. The row-side colours represent documented adverse drug reactions (orange) or not (green). B: Survival curves for each phenotypic cluster are presented, pairwise comparisons conducted using the Log-Rank test showed a significant difference in survival between Cluster 1 and Cluster 5 against other clusters (excluding Cluster 3) (p<0.05). Confidence intervals displayed at a 95% confidence level. C: Table highlights the frequent HPO terms seen in each cluster (with five or more patients documented to have the term), terms found in all clusters are shown at the bottom.
Figure S2 Frequency bar charts of top 20 human phenotype ontology (HPO) terms. 

A: Top 20 terms in 102 patients with POLG disease and VPA exposure - status epilepticus seen in >25%. 
B: Top 20 terms in 284 patients with POLG disease and no VPA exposure - status epilepticus seen in 50%. 
C: Top 20 terms in 54 patients with non-POLG disease and VPA exposure, status epilepticus not within top 20 terms.
References


The evidence for sodium valproate toxicity in mitochondrial
diseases: a systematic analysis

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Patrick F. Chinnery, FMedSci\textsuperscript{8,9}

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https://neurologyopen.bmj.com
Abstract

We aimed to determine whether sodium valproate (VPA) should be contraindicated in all mitochondrial diseases, by systematically reviewing the published literature for mitochondrial DNA (mtDNA) and common nuclear genotypes of mitochondrial diseases using PubMed, Ovid Embase, Ovid Medline, and MitoPhen databases. We extracted patient-
level data from peer-reviewed articles, published till July 2022, using the Human Phenotype Ontology to manually code clinical presentations for 156 patients with genetic diagnoses from 90 publications. There were no fatal adverse drug reactions (ADRs) in the mtDNA disease group (35 patients), and only one out of 54 patients with a non-\textit{POLG} mitochondrial disease developed acute liver failure. There were fatal outcomes in 53/102 (52\%) \textit{POLG} VPA-exposed patients who all harboured recessive mutations.

Our findings confirm the high risk of severe ADRs in any patient with recessive \textit{POLG} variants irrespective of the phenotype, and therefore recommend that VPA is contraindicated in this group. However, there was limited evidence of toxicity to support a similar recommendation in other genotypes of mitochondrial diseases.

\textbf{Background}

Epilepsy often features in mitochondrial diseases, with myoclonic, generalised, and focal onset seizure types [1]. Sodium valproate (VPA) is effective in these seizure types in non-mitochondrial diseases, \textit{although not a first line treatment for focal onset seizures} [2]. It is also used for migraines and bipolar disorder prophylaxis, which are found in mitochondrial diseases [3-5]. However, it is important to note recent cautions about the initiation of that VPA \textit{given the} \textit{has known} teratogenic effects and associations with neurodevelopmental disorders, leading it to be only \textit{be} considered in the event of a lack of alternative options [6]. While VPA may cause transient liver enzyme increases in 15\% of patients, severe adverse drug reactions (ADRs) like hepatic failure are rarer (0.01\% of all patients) [7]. \textit{POLG}-related mitochondrial disease, typically Alpers syndrome presenting with developmental regression
and intractable seizures, is an identified risk factor for VPA-induced hepatotoxicity [7]. POLG disease can present at different ages with seizures commonly presenting in early-onset and juvenile to adult-onset forms. However, liver involvement is prevalent in early-onset POLG disease and is associated with worse survival including in phenotypes where epilepsy is not a feature [8] other phenotypes such as myocerebrohepatopathy where paediatric patients present with hypotonia, failure to thrive and liver pathology, but epilepsy is not a feature and survival is comparable to children with Alpers syndrome [8]. The severity of VPA-related ADRs in Alpers syndrome and the lack of systematic follow-up studies of VPA in other mitochondrial diseases has led clinicians to avoid VPA in all mitochondrial diseases without clear good evidence, potentially discarding an effective and cheap medicine. To bridge this gap, we assessed VPA effects across various mitochondrial diseases. MitoPhen [9], which contains published clinical data as human phenotype ontology (HPO) terms, was used to investigate whether there are any associated clinical features pre-empting VPA-related toxicity. Our aim was to evaluate the evidence base for VPA-induced toxicity in different mitochondrial diseases using published patient-level data.

Methods

Search strategy and study selection

A systematic review of the literature, published up to July 2022, was conducted using PubMed, Ovid Embase, Ovid Medline, and MitoPhen databases, according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [10] (Figure 1). Articles about mitochondrial diseases linked to migraine or seizures and VPA treatment were reviewed. We considered three groups: 1) POLG-related mitochondrial diseases, 2)
mitochondrial DNA (mtDNA) diseases 3) other nuclear causes of mitochondrial disease based on PanelApp genes [11]. We supplemented the search with the ‘MitoPhen-Expanded’ dataset which currently contains data on 89 pathogenic mtDNA variants [9], and common nuclear genotypes including POLG. Exclusion criteria were non-peer-reviewed articles, no documented VPA use, no genetic diagnosis, and lacking patient-specific information. An ADR was defined as a noxious or unintended response to VPA, where the causal relationship between VPA use and the reaction was strongly suspected [12]. ‘Symptom-control’ referred to reported seizure or symptom reduction with VPA. The search strategy and risk of bias assessment is detailed in Supplementary Information.

Survival analyses – POLG dataset

To investigate survival between patients who had and had not been exposed to VPA, we included the MitoPhen-Expanded POLG dataset (publications up to 01/06/2022), where patients with seizure phenotypes and without documented VPA use were coded as ‘no VPA’. The survminer R package [13] was used to create Kaplan-Meier plots and log-rank P-value was used for testing differences in survival between groups. ‘Event’ was defined as ‘death’ for survival analysis. Time in years was calculated by age at symptom onset till age at death or age at last follow-up.

HPO-based analyses

The OntologyX [14] and gplots [15] R packages were used to generate a matrix of phenotype similarity scores using the Lin similarity measure, and a heatmap displaying the hierarchically clustered scores per patient. Phenotype enrichment was explored within the POLG dataset by grouping for VPA and no VPA exposure, and between POLG and non-POLG datasets with VPA exposure, using one-sided Fisher’s exact test adjusted with the
Benjamini-Hochberg procedure. HPO terms related to liver dysfunction were excluded to avoid over-representation from VPA-related toxicity.

Data availability

See Supplementary Information. MitoPhen is accessible on www.mitophen.org.

Results

The search strategy revealed a total of 156 patients from 90 articles with mitochondrial diseases who had reported VPA exposure (Figure 1).

Group 1: POLG-related mitochondrial diseases

Data collated from 46 articles on 102 patients with POLG-disease and VPA exposure (Figure 1) showed ADRs in 91 patients (89%), with 87 patients (85%) reported to have hepatotoxicity. There were no reported ADRs in seven and ADR status was unknown in four patients. There was no pre-existing liver disease in 92/102 patients (90%). There were fatal outcomes in 53/102 patients (52%), with eight being unrelated to VPA such as intractable seizures. Liver transplant was required in 15 patients, and six (40%) died post-transplant. Age at onset was available for 98/102 patients (96%). Survival between male and female patients were similar ($p=0.7$), but the lower age at onset group (0-4 years) had a lower survival compared with onset in later childhood: 5-18 years ($p=0.05$). All patients exposed to VPA with reported genotypes (97/102) had recessive POLG variants. Compound heterozygous variants were associated with worse outcomes compared to homozygous variants ($p<0.01$) (Figure 2A).
There were 280 patients from 66 articles with recessive POLG variants, without documented VPA exposure included from the MitoPhen-Expanded dataset. There was a lower survival in the VPA exposure group compared with no VPA use ($p<0.0001$, Figure 2B).

**Group 2: MtDNA diseases**

In the mtDNA disease group, 28 articles mentioned VPA use in 35 patients without any documented fatal ADRs. Seizure control was reported to be effective in 10/13 (77%) patients with $m.8344A>G$ and presentations of myoclonic epilepsy and ragged red fibers (MERRF syndrome). Seizures were exacerbated in 3/8 (38%) $m.3243A>G$ patients who had features suggestive of mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS syndrome) (Figure 2C, Table S1). Patients with generalised myoclonic seizures, and/or focal myoclonic seizures, had reported good symptom control with VPA (Figure 2D). There was one report of VPA being instigated for migraine in a patient successfully, although it was a dominant symptom in five published patients. There were 3/35 (9%) cases with non-fatal hepatotoxic effects including pancreatitis (Table S1).

**Group 3: Other nuclear-encoded mitochondrial diseases**

The search strategy for 227 nuclear genotypes of mitochondrial diseases resulted in 16 articles documenting 19 patients (Figure 1). Four patients (21%) had VPA hepatotoxicity: one patient with a severe encephalopathy and epilepsy due to WARS2 variants developed acute liver failure after commencing VPA [16], two patients with recessive TWNK variants and one with biallelic PARS2 variants had transient elevations in hepatic transaminases. Three patients had transient symptoms where VPA was thought to be a contributing factor,
however a causal relationship was not clear. Therefore, 15/19 (79%) patients in this group had no definite VPA-induced ADRs (Table S2).

According to the Clopper-Pearson exact method [17], and pairwise Fisher’s exact tests with Bonferroni adjustment, VPA-induced hepatotoxicity proportions were significantly different between group 1: POLG disease at 0.85 (95% CI: 0.77-0.92), and non-POLG disease groups 2 and 3 at 0.13 (95% CI: 0.05-0.25, p<0.0001). In terms of VPA exposure, the data available for time from VPA use till ADR in 59 patients (53 diagnosed with POLG disease) showed that this ranged from under 1 week to 1.33 years, with a median of 1 week.

HPO-based analyses

There were no enriched terms between the POLG disease datasets grouped by VPA and no VPA exposure to distinguish those who had ADRs. Hierarchical clustering of all 156 patients exposed to VPA using phenotype similarity scores showed that patients with non-POLG diagnoses clustered together (Figure S1A). Patients with early onset POLG disease had a lower survival compared with other clusters (p<0.05, Figure S1B). The clusters resemble clinically defined phenotypic groups relating to genotypes (Figure S1C). Status epilepticus was more frequent in POLG disease (Figure S2), explaining why VPA was used in these patients.

Discussion

This comprehensive systematic review utilised several search engines, and MitoPhen-Expanded datasets. We assessed 228 nuclear genes and 27 mtDNA genes linked to mitochondrial diseases, incorporating patient-level data for 436 patients. No published patients with dominant POLG variants exposed to VPA were found. Data confirm the
significant risk in recessive POLG disease (Figure 2), with fatal outcomes in 52% and ADRs in 89% of 102 patients, aligning with previous findings [4]. In mtDNA diseases, 10/13 patients with m.8344A>G-induced seizures responded well to VPA with no reported ADRs. Non-POLG genotypes without mtDNA depletion, including m.7472_7473insC, TWNK, and PARS2 variants, showed hepatic transaminase increases in 4/54 patients, comparable to rates in non-mitochondrial diseases [7]. The lack of natural history data on rarer genotypes meant it was difficult to attribute VPA toxicity to the fatal neurological decline of a patient with a WARS2 diagnosis, following the resolution of their VPA-induced liver failure [16]. The reasons for significantly different VPA-induced hepatotoxicity proportions between POLG (87/102) and non-POLG mitochondrial diseases (7/54) (p<0.0001), remain unclear.

A limitation of this review is publication bias favouring POLG disease data, attributed to higher status epilepticus frequency in the POLG group (Figure S2), necessitating additional antiseizure medications like VPA. Also publication bias would factor in reporting of ADRs related to VPA over non-events, thereby giving a likely over-estimate of ADRs. Some authors changed VPA due to perceived ADR risks in non-POLG disease [18]. This contributes to uncertainty in clinical management of mitochondrial epilepsy due to lack of longer-term follow-up in non-POLG mitochondrial diseases with VPA exposure. Additionally, there were unclear causal relationships between VPA and clinical features which could be explained by natural progression of the mitochondrial disease [19]. Despite variability in reporting VPA dosage and time to ADR, consistent documentation of clinical features enabled survival analyses using HPO-driven clusters, revealing significantly different survival between POLG and non-POLG diseases (Figure S1). ADRs could not be predicted by phenotypic profiles in POLG disease.
Conclusions

The data showed a significantly lower proportion of hepatotoxicity with VPA in non-POLG versus POLG mitochondrial diseases. Additionally, VPA resulted in reported symptom control particularly in patients with m.8344A>G disease, with no reported ADRs. Longer-term follow-up studies are required to define the natural history of rare nuclear genotypes of mitochondrial diseases and the role of VPA in treating patients with non-POLG mitochondrial diseases. We recommend VPA is contraindicated in patients carrying recessive POLG variants.

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Declarations

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Ethical standards

The manuscript does not contain clinical studies or previously unpublished patient data.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

References


Figure legends

Figure 1: PRISMA approach to study inclusion in systematic review. Searches were performed separately for POLG-related mitochondrial disease, mtDNA disease, other nuclear-encoded mitochondrial diseases. *Nuclear genes associated with complex I-V deficiencies were only searched in the MitoPhen-Expanded dataset.
**Figure 2: Survival in POLG disease and adverse reactions in mtDNA disease.**

A: Survival comparison in patients exposed to valproic acid (VPA) by genotype – the log-rank test revealed a significant difference between compound heterozygous and homozygous groups ($p<0.01$). B: Survival comparison between documented VPA treatment and no VPA exposure groups revealed a significant difference using the log-rank test ($p<0.0001$). C: VPA use by pathogenic mtDNA variant, adverse drug reaction (ADR) and reported symptom control. D: Symptom type and mtDNA variant with counts of patients where VPA was used (displayed as grey circles). ADR and symptom control status also displayed as red asterisk or coloured dot respectively.