ORIGINAL ARTICLE

POLG Mutations Are Probably Rare in the Han Chinese Population

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Objective Mutations in polymerase gamma gene (*POLG*) are believed to be an important cause of early and juvenile onset of non-syndromic intractable epilepsy. The aim of this study is to investigate the incidence/prevalence of *POLG* pathogenic variants in epilepsy patients of Han population through sequencing it.

Methods Han Chinese patients with seizures prior valproic acid (VPA) exposure at Shanghai Children's Hospital were collected from 2015 to 2019. The clinical diagnosis was based on the 2014 Consensus Statement of Epilepsy by the International League against Epilepsy (ILAE). Blood sampling were performed before VPA treatment. The *POLG* gene DNA was sequenced by either the first or the next generation sequencing (NGS). The *POLG* variant burden was illustrated. Liver functions were tested to describe whether they experienced VPA toxicity.

Results Totally 216 Han Chinese patients were included, aged from 1 month to 15 years old, 102 were male and 114 were female. The onset age was 1 month old to 13 years old, and the epilepsy course ranged from 2 weeks to about 3 years. VPA treatment was delivered for the generalized or intractable partial seizures at standard dosage. No patient experienced hepatic toxicity following VPA exposure. DNA sequencing data showed no patient had either a homozygous mutation or compound heterozygous mutation of *POLG*. Single heterozygous mutations of c.1150G>T and p.D384Y were found in 2 patients, and single heterozygous mutation of c.156_158dupGCA was found in 1 patient. None of these variants showed clinical significance.

Conclusions Functional modifying *POLG* homozygous mutations and compound heterozygous mutations were not detected and VPA toxicity was not seen in the current study. *POLG* mutation frequency might be rare in Han Chinese, and standard VPA therapeutic dosage might be safe for Han Chinese patients.

EPILEPSY is one of the most common neurologic disorders, affecting 1%-2% of the population worldwide. Valproic acid (VPA, 2-n-propylpentanoic acid) is believed to be a widely-used first-generation antiepileptic drug, which is prescribed mainly for epilepsy and psychiatric disorders. Its mechanism contains increase in γ -aminobutyric acid (GABA)-ergic and glutamatergic neurotransmission, calcium channel blockade, sodium channel modulation and HDAC inhibition, etc.^[1] VPA is relatively effective, pharmaco-economic and safe. Its most severe side effect is not common but potentially life-threatening because of idiosyncratic liver injury.^[2] Rare mutations in the polymerase gamma gene (*POLG*), which codes for the mitochondrial DNA polymerase γ ,^[3] lead to Alpers-Huttenlocher syndrome (AHS), a neurologic and metabolic disorder which presents as developmental delay, intractable epilepsy, and liver disease. Children with this syndrome are at high risk of developing life-threatening VPA liver toxicity. The use of VPA can be stopped in condition of either lack of efficacy or severe side effects. About 1/3 AHS patients develop liver toxicity within 3 months exposure to VPA. So, genetic variations in *POLG* may predict individuals who do not have obvious phenotype like AHS but are susceptible to VPA-related liver toxicity.^[4]

Stewart *et al.*^[5] suggested that prospective genetic testing of *POLG* could identify individuals at high risk of this potentially life-threatening liver toxicity induced by VPA exposure. As a method of molecular diagnosis and pre-treatment evaluation, genetic testing for *POLG* variations in patients with epilepsies is very important for clinical diagnoses, genetic counseling, and treatment decisions.^[6, 7] It is considered that VPA should not to be used to treat patients with *POLG* mutation for the high risk of VPA-caused liver toxicity.

The incidence of fatal VPA-caused liver failure decreases with the age increasing. The incidence is about 1:40,000 in adults while is as high as 1:600 in children under 2 year old in America.^[8] The most common variations in *POLG* are p.A467T, p.W748S, and p.G848S in European population, but such incidence/prevalence is not known for the Han Chinese population.

The incidence/prevalence of *POLG* pathogenic variants was discussed in this study through sequencing *POLG* from Han Chinese patients. We described the findings of 216 pediatric patients with epilepsy from the same ethnic background who underwent *POLG* variation evaluation by the first or next generation

sequencing (NGS) prior to VPA exposure. If the cases had no *POLG* homozygous or compound heterozygous mutation, they received VPA therapy at the standard therapeutic dosage (20-30 mg/kg/d). We observed whether these patients experienced VPA toxicity (liver injury or liver failure) within 2-3 months. If the cases had meaningful *POLG* homozygous or compound heterozygous mutations, we further examined peripheral blood of their proband's parents.

MATERIALS AND METHODS

Patients

This study was approved by the institutional review board of Shanghai Children's Hospital. We recruited patients of Han Chinese from the department of neurology in Shanghai Children's Hospital from 2015 to 2019 according to the following criteria: 1) diagnosed with epilepsy (either generalized or intractable partial seizures); 2) VPA treatment delivered; 3) no liver function abnormality (glutamic-pyruvic transaminase was normal) before VPA treatment; 4) had received a standard neurological examination. The diagnosis of epilepsy was based on the 2014 Consensus Statement of Epilepsy by the International League Against Epilepsy (ILAE).^[9] Epilepsy was defined by any of the following conditions: 1) at least two unprovoked (or reflex) seizure episodes happening longer than 24 hours apart; 2) one unprovoked (or reflex) seizure episode and a probability of further seizure episodes similar to the general recurrence risk (at least 60%) after two unprovoked seizure episodes, occurring over the next 10 years; 3) diagnosis of epilepsy syndrome.

Sequencing analysis

Once the diagnosis of epilepsy was made, parents were inquired by pediatric neurology consultants/registrar about DNA sequencing analysis on admission. We collected peripheral blood samples of patients after obtaining informed consent from parents. The sequencing method for each patient was applied based on their individual conditions. First generation sequencing was performed prior to VPA treatment when the diagnosis and etiology of epilepsy were confirmed. NGS was performed for patients whose etiology of epilepsy and treatment plan were vague and the frequency of seizure episodes was high. In case a patient was identified as a homozygous mutation or compound heterozygous mutation in *POLG*, we examined the proband's

biological parents' blood sample by using first or next generation sequencing after obtaining written informed consents.

First generation sequencing

The sequences of the amino acid coding regions and the adjacent regions of the *POLG* were amplified by PCR using the peripheral blood DNA as template. PCR products were confirmed by agarose gel electrophoresis. All DNA sequencings were performed by the Saiyin Company using the Sequencher software. We sequenced 22 exons and synthesized the primers, then supplied them to the sequencing company for preservation. The aligned reference sequence was a collection of amplified fragment sequences comprising a primer sequence. The Human Gene Mutation Database (HGMD, www.hgmd.cf.ac.uk) was reviewed to determine if any of the mutations were reported in the literature.

Next generation sequencing

Genomic DNA samples were sonicated, followed by hybridization with the NimbleGen 2.0 probe sequence capture array (Roche, Shanghai, China) to enrich the exon DNA (Joy Orient, China). Libraries were first tested for enrichment by quantitative polymerase chain reaction (qPCR) and for size distribution, and the concentration was tested using the Agilent Bioanalyzer 2100. The samples were sequenced on the sequencer (HiSeq2500, Illumina, California, USA). Each sample was performed for two parallel reactions. Data filtering, mapping, and variant detection were applied. Exon-enriched DNA was sequenced according to the manufacturer's instruction. Raw image files were processed using the BclToFastq (Illumina, California, USA) for base calling and generating raw data. Low-quality variations were filtered out using the quality score of Q20. The sequencing reads were aligned to the NCBI human reference genome (hg19) using the Burrows-Wheeler Aligner (BWA). Samtools and Pindel were used to analyze the single-nucleotide polymorphism (SNP) and the indexing of the sequence.

Data analysis

Synonymous changes and SNPs with minor allele frequency (MAF) higher than 5% were removed (Synonymous changes and SNPs can be searched on <http://www.ncbi.nlm.nih.gov/projects/SNP>); non-synonymous changes were filtered using SIFT software (<http://sift.jcvi.org>). The function of genes with muta-

tion was further analyzed for protein structure prediction.

VPA toxicity observation

We applied standard therapeutic VPA dosages after *POLG* variation evaluation. Aminotransferase, alkaline phosphatase (ALP), prothrombin time, and bilirubin levels were tested and recorded during treatment monthly for 2 or 3 months as follow-up. Patients were also monitored for hyperammonemia during their treatment.

RESULTS

Totally 216 patients of Han Chinese were included in the study. They were aged from 1 month to 15 years old, 102 (47.2%) were male and 114 (52.7%) were female. Their age at onset ranged from 1 month old to 13 years old, and the epilepsy course ranged from 2 weeks to about 3 years. NGS was performed in 178 patients (82.4%). None of the 216 patients experienced VPA toxicity after VPA exposure.

Sequencing the *POLG* in this cohort revealed no homozygous or compound heterozygous mutations. Single heterozygous mutations were found in 3 of this cohort (3/216). **Table 1** shows the clinical information of the 3 patients. Two patients had single heterozygous *POLG* mutation of c.1150G>T and p. D384Y (**Figure 1, 2**), 1 patient had single heterozygous mutation of c.156_158dupGCA (**Figure 3**). Two patients presented mild elevation of aminotransferase (41U/L). Both of them had upper respiratory infections when seizures occurred. The aminotransferase returned to normal when the infections were under control. Neither of them had a *POLG* variation.

DISCUSSION

It was reported that mild aminotransferase elevations were sometimes seen among VPA patients without *POLG* mutation in a prospective open-label clinical trial,^[10] but they returned to normal following discontinuation of VPA treatment. Hepatotoxicity was more common in patients with higher-dose VPA exposure.^[11] Two of 216 patients had mild aminotransferase elevation but it didn't lead to severe hepatotoxicity and they had no *POLG* variation. When the infections were under control, the aminotransferase returned to normal and VPA treatment continued.

Table 1. Clinical information of the 3 Chinese patients with single heterozygous mutation of *POLG* gene

Patient	Gender	Age	<i>POLG</i> variant	Family history	Clinical symptom	EEG	Brain MRI	Antiepileptics	Liver damage
1	Male	4 months and 11 days	c.1150G>T, p.D384Y	No	String of seizures onset (eyes gaze, upper limbs stretched forward, lower limbs elevation, limb stiffness for seconds, alleviated voluntarily), 10 times /string, 4-5 strings/day	No epileptic wave	Bilateral temporal frontal parietal space slightly widened	VPA,TPM	No
2	Female	1 month and 26 days	c.1150G>T, p.D384Y	No	String of seizures onset (lower limbs jitter, no eyes gaze), 3-4 times / string, a few strings / day	No epileptic wave	Normal	VPA,TPM	No
3	Female	5 months and 18 days	c.156_158dupGCA	Mother has febrile convulsion	Convulsions showed the eyes turned to the left or strabismus, limbs rigidity jitter, lips cyanosis, foaming at the mouth, occurred 3 times in 2 months	Refused	Refused	VPA	No

EEG, Electroencephalogram; VPA, Valproic acid; TPM, Topiramate.

Figure 1. Amino acid coding region sequence of *POLG* gene of the Patient 1 (male, 4 months and 11 days) with single heterozygous mutation of c.1150G>T and p. D384Y. **Figure 2.** Amino acid coding region sequence of *POLG* gene of the Patient 2 (female, 1 month and 26 days) with single heterozygous mutation of c.1150G>T and p. D384Y.

It is well known that mutations in *POLG* are important cause of early and juvenile onset epilepsy, an inherited mitochondrial disease in both children and adults.^[12, 13] They are also the most common cause of VPA toxicity following VPA exposure. AHS is the most severe phenotype of the *POLG*-induced disorders which exhibit intractable seizures, mental and motor disabilities, and liver problems, with catalytic deficiency in the mitochondrial activity.^[14] Due to the drug-resistant characteristic, VPA is ineffective and thus contraindicated in epileptic individuals carrying *POLG* mutations, as it can cause or worsen liver failure. A recent study by Jafarian *et al.*^[15] investigated in detail the effects of

VPA on mitochondrial metabolism in isolated rat liver, and found that VPA induced mitochondrial ROS production *via* impairment of respiratory chain complex II and mitochondrial permeability transition pore opening, which progressively disabled energy metabolism.^[16] The most common mutations in *POLG* are p.A467T, p.W748S and p.G848S in European population.^[17,18] The mutation of p.A467T was associated with the decreased activity of an enzyme, while another common *POLG* mutation, p.W748S, was also associated with lower DNA polymerase activity and a severe DNA-binding defect.^[19] The carrier frequency of the *POLG* mutation p.A467T is up to 0.6% in Belgium.^[20] Meanwhile

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