Short Research Article ISSN: 2689-1077

Genetics & Molecular Medicine

Is the *ACADVL* c.1226C>T (p.T409M) Variant a Mutation from the Pacific Islands? A Hawaiian Newborn Screening Dried Bloodspots Study

Nathaniel Barasa[1], Kejian Zhang[1], Victor Wei Zhang[2,3], Ryan M. Lambert[4], Carlos E. Prada[1] and C. Alexander Valencia[1,5,6,7*]

[1]*Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH 45229, USA and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA.*

[2]*Amcare Genomic Laboratory, Guangzhou, China.*

[3]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, 77030, USA.*

[4]*Lake Erie College Osteopathic of Medicine, Erie, PA 16509, USA.*

[5]*Interpath Laboratory, Pendleton, OR, 97801 USA.*

[6]*Department of Geriatrics, West China Hospital, Sichuan University, Chengdu, Sichuan, China.*

[7]*NewGenia, Stockholm, Sweden.*

***Correspondence:**
Dr. C. Alexander Valencia, Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH 45229, USA and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA Interpath Laboratory, 2460 SW Perkins Ave. Pendleton, OR 97801.

Received: 10 May 2021; **Accepted:** 05 June 2021

Citation: Barasa N, Zhang K, Zhang VW, et al. Is the *ACADVL* c.1226C>T (p.T409M) Variant a Mutation from the Pacific Islands? A Hawaiian Newborn Screening Dried Bloodspots Study. Genet Mol Med. 2021; 3(1): 1-2.

Keywords
Bloodspot, Very long chain acyl-coenzyme a dehydrogenase deficiency**,** Newborn screening, *ACADVL* gene.

Introduction

Very long chain acyl-coenzyme a dehydrogenase deficiency (VLCADD), a genetic disorder that has newborn screening (NBS) state programs, that affects 1 in 30,000 people. This disorder has mild to severe clinical features, namely, hypertrophic or dilated cardiomyopathy, hypotonia, hepatomegaly, intermittent hypoglycemia and other clinical manifestations. Pathogenic variants in the *ACADVL* gene have been associated with VLCADD whereby a strong phenotype to genotype correlation exists. Generally, a severe phenotype is observed when two null mutations are present, childhood-onset disease, and a less severe phenotype is seen when two mutations result in some residual enzymatic activity [1-4].

The c.1226C>T (p.T409M) variant was reported as a pathogenic variant and was seen more often in Pacific Island populations. In the Hawaiian (HI) newborn screening (NBS) program 99.8% of infants are included. The Oregon State Public Health Laboratory processes and stores the HI bloodspots for 1 year for quality improvement (QI) purposes. In the HI NBS Program, 44 VLCADD positive children were detected from 2007 to 2013. One copy of the c.1226C>T variant was detected in 19 HI/Pacific Islander/ Asian cases with 11 homozygotes, four heterozygotes, and four compound heterozygotes. Although c.1226C>T was reported as pathogenic, most HI children had no clinical signs or symptoms.

The HI c.1226C>T homozygotes (11/~133,000 births in the years 2007-2013) were higher (2.5 times) compared to the national homozygotes with a 1/30,000 prevalence. The HI NBS T allele frequency was 0.25% (2007 to 2013). The objective of this study was to assess the HI DBS sample allele (T) frequency at position 1226 of the *ACADVL* gene to assistant in variant classification.

Methods

The HI Department of Health Institutional Review Board approved this project as a QI activity. About one-thousand randomly sampled HI NBS bloodspots from patients of Asian and/or Pacific Islander ancestry were collected in January (600) and February (400) of 2014, deidentified and sent to the Cincinnati Children's Hospital Medical Center Molecular Genetics Laboratory from the Oregon

Laboratory for genotyping analysis. DNA was extracted from the NBS bloodspot samples and genotyped [(*ACADVL* c.1226C>T (p.T409M)] by Sanger sequencing due to technical issues with the equivalent TaqMan assay. Several samples failed the genotyping analysis due to poor DNA quality and/or quantity.

Results and Discussion

In sum, 998 NBS bloodspots were received by Cincinnati Children's Hospital Medical Center Molecular Genetics Laboratory and genotyping analysis were performed on 850 samples due to the quality and quantity issues for the remaining samples. Genotyping results revealed 807 CC wildtype homozygotes (94.9%), 43 CT heterozygotes (5.1%), and zero TT homozygotes (0%). The allele frequency was 97.5% and 2.5% for C and T, respectively.

The increased frequency of heterozygotes or homozygotes for the T allele may be partly due to a founder effect in the Asian and/or Pacific Island groups. Although the T allele was expected to be elevated, based on HI NBS results from 2007 to 2013 with a T allele frequency of 0.25% (from 11 c.1226C>T homozygotes, 4 heterozygotes, and 4 compound heterozygotes), the results of this study were 10X higher than anticipated (2.5%). This study possibly concentrated the allele frequency by only including infants of Asian and/or Pacific Islander descent. Approximately 48% of the HI population is made up of Asian and Pacific Island ethnic groups only. When two or more ethnicities are summed, an additional 23% of the HI population is partly of Asian and/or part Pacific Island ethnicity (United States Census Bureau, 2013). The T allele frequency in this study was elevated compared to the previous HI NBS screening results.

The c.1226C>T variant substitutes threonine for a methionine amino acid at codon 409 (p.Thr409Met) of the ACADVL protein. Physiochemically, the threonine residue is moderately conserved. A population database search demonstrated a low minor allele frequency for the c.1226C>T change (rs113994169, gnomAD ALL: 0.0049% - Latino/Admixed American: 0.0056% - East Asian: 0.035% - Other: 0.069%). In New Zealand, this c.1226C>T variant was reported in asymptomatic HI, Maori and Pacific Islander newborns. These patients had an abnormal newborn screening result for VLCAD deficiency [5,6]. Importantly, four of 6 asymptomatic homozygous patients had a normal confirmatory plasma acylcarnitine profile [5]. Similarly, fatty acid oxidation probe analysis was normal in homozygous newborns; however, newborns with compound heterozygous (c.1226C>T variant + pathogenic variant) changes had mild fatty acid oxidation results [6]. In addition, a Maori population retrospective review of newborn screening cases was below the New Zealand C14:1 cutoff but above the R4S cutoff (0.9 - 2.4 mol/L). The study showed the higher prevalence of this variant in the Maori population. Elevated NBS C14:1 levels ($p<0.0001$) were observed in c.1226C>T homozygotes but did not have classical clinical VLCAD deficiency symptoms which implied an attenuated phenotype. Consistently, in our HI Asian and/or Pacific Islander homozygote newborns had no clinical features of the disease. Currently, in ClinVar this variant had two classifications; unknown significance and pathogenic [7]. Functional prediction algorithms are inconsistent about this missense change: SIFT - Deleterious, PolyPhen-2 - Benign, Align-GVGD - Class C0. Altogether, these results bring into question the current classification of this variant as "pathogenic". In lieu of the current evidence and the fact that studies have not examined the presence of late-onset VLCAD symptoms during adolescence or adulthood, this variant should be classified as a variant of uncertain significance [8].

In summary, this study will (1) facilitate conversations across HI NBS Program and the Oregon State Public Health Laboratory to ensure correct interpretation of VLCADD variants on the HI NBS panel; (2) help HI's clinical genetics professionals in discussing c.1226C>T "abnormal" NBS results with parents; (3) provide information to reclassify this variant as uncertain significance due to its high frequency, mostly normal biochemical assays and potentially attenuated or benign disease phenotype. The necessity of a long-term study involving a larger dataset for further clarification of the nature of c.1226C>T variant and (5) c.1226C>T may be added as a second line screening in the HI/Maori population after abnormal VLCAD NBS to stratify patients.

References

1. Fukao T, Watanabe H, Orii K, et al. Myopathic form of very-long chain acyl-coa dehydrogenase deficiency: evidence for temperature-sensitive mild mutations in both mutant alleles in a Japanese girl. Pediatr Res. 2001; 49: 227-231.
2. Gobin-Limballe S, Djouadi F, Aubey F, et al. Genetic basis for correction of very-long-chain acyl-coenzyme A dehydrogenase deficiency by bezafibrate in patient fibroblasts: toward a genotype-based therapy. Am J Hum Genet. 2007; 81: 1133-1143.
3. Andresen BS, Olpin S, Poorthuis BJ, et al. Clear correlation of genotype with disease phenotype in very-long-chain acyl-CoA dehydrogenase deficiency. Am J Hum Genet. 1999; 64: 479-494.
4. Leslie ND, Valencia CA, Strauss AW, et al. Very Long-Chain Acyl-Coenzyme A Dehydrogenase Deficiency. GeneReviews®. University of Washington, Seattle. 1993.
5. Merritt JL, Vedal S, Abdenur JE, et al. Infants suspected to have very-long chain acyl-CoA dehydrogenase deficiency from newborn screening. Mol Genet Metab. 2014; 111: 484–492.
6. Abstracts of the SSIEM 2014 Annual Symposium, 2-5 September, 2014, Innsbruck, Austria. J Inherit Metab Dis. 2014; 37 Suppl 1: 27-185.
7. VCV000021013.3 - ClinVar - NCBI.
8. Nykamp K, Anderson M, Powers M, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. Genet Med. 2017; 19: 1105-1117.