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Mutation analysis of VLCAD gene in neonates — a sensitive and cost effective tiered approach

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ABSTRACT

Very-long-chain acyl-CoA dehydrogenase- VLCAD deficiency is an autosomal recessive disorder resulting from an inborn error of fatty acid oxidation. Fatty acid oxidation defects, including VLCAD deficiency, may account for as many as 5% of sudden infant death patients. VLCAD protein is loosely bound to inner mitochondrial membrane unlike the other acyl-CoA dehydrogenases-short, medium and the long. Over 150 mutations have been identified in the VLCAD gene. 40% of mutations seen in VLCAD gene are accounted by 779C>T, 830_832del and 848 T>C mutations in exon 9, it is imperative to screen the entire gene following an initial screen of three common mutations routinely for asymptomatic neonates. This tier screening approach would avoid false-negative diagnoses of VLCAD deficiency in newborns.

Genomic DNA isolated from samples have been analyzed with our tiered approach in which a total of 13 amplicons covering the entire ACADVL gene including coding regions and splice junctions are PCR-amplified with a high–fidelity DNA polymerase and analyzed by DNA double strand sequencing under fully optimized conditions for mutation screening. An initial single amplicon screen identifies the three most common mutations while the rest 12 amplicon screens identify any other mutation in the gene thus a cost-effective and sensitive screen. Advent of genotype-phenotype correlation in this disorder, the information derived from mutational analysis is essential in designing the appropriate follow-up and therapeutic regime for these patients. This screening process would also provide carrier frequencies of the most common ACADVL mutations in the population.

INTRODUCTION

Very long-chain acyl-coenzyme A dehydrogenase deficiency is a condition that prevents the body from converting certain fats to energy, particularly during periods without food (fasting). Normally, through a process called fatty acid oxidation, several enzymes work in a step-wise fashion to break down (metabolize) fats and convert them to energy. People with very long-chain acyl-coenzyme A dehydrogenase deficiency have inadequate levels of an enzyme that metabolizes a group of fats called very long-chain fatty acids.

Typically, initial signs and symptoms of this disorder occur during infancy or childhood and include low blood sugar (hypoglycemia), lack of energy (lethargy) and muscle weakness. People with an early onset of very long-chain acyl-coenzyme A dehydrogenase deficiency are also at risk of serious complications such as liver abnormalities and life-threatening heart problems. Symptoms that begin in adolescence or adulthood tend to be milder and usually do not involve heart problems. Episodes of very long-chain acyl-coenzyme A dehydrogenase deficiency can be triggered by periods of fasting, illness and exercise.

The breakdown of fatty acids takes place in the mitochondria found in each cell. The mitochondria are small, well-defined bodies that are found in the cytoplasm of cells and in which the body generates energy from the breakdown of complex substances into simpler ones (mitochondrial oxidation).

There appear to be two forms of VLCAD: an early-onset, severe form which, if unrecognized and undiagnosed, may lead to extreme weakness of the heart muscles (cardiomyopathy) and may be life-threatening (VLCAD-C) and a later-onset, milder form, sometimes referred to as VLCAD-H, that is characterized by repeated bouts of low blood sugar (hypoglycemia). Since the advent of expanded newborn screening programs using tandem mass spectrometry technology, more VLCAD infants are being detected earlier in the course of the disorder than in the past.

We present data from analysis of an example group with a sensitive, cost effective and less labor intensive approach. The exons and exon-intron boundaries of the ACADVL gene covering the most common mutations 779C>T, 830_832del and 848 T>C were amplified with universal PCR conditions followed by the rest of the exons. The PCR products were analyzed with bidirectional, double strand sequencing.

METHODS AND RESULTS

After PCR amplification of selected amplicons, amplicons were analyzed by bi-directional, double strand sequencing on an ABI 3100 automated sequencer (Applied Biosystems), see Figure 3. Variant identities were determined and labeled as pathogenic mutation or as a common single nucleotide polymorphism (SNP), see Figure 3.

Figure 1. The fatty acid b-oxidation metabolic pathway indicating the VLCADD block. In the mitochondria, as shown in the diagram the fatty acids in the acyl Co-A form are normally oxidized to acetyl-CoA which is used to produce the ketones that can supply the energy needs to compensate for the lack of adequate glucose. A deficiency of VLCAD however prevents ketone formation. The block at VLCAD also results in the accumulation of fatty acid intermediates that inhibit gluconeogenesis (thus preventing endogenous glucose production), have a toxic effect on the liver and produce metabolic acidosis. Muscle, particularly myocardium, requires a lot of energy and, therefore, becomes functionally impaired resulting in lethargy, hypotonia and hypertrophic cardiomyopathy.

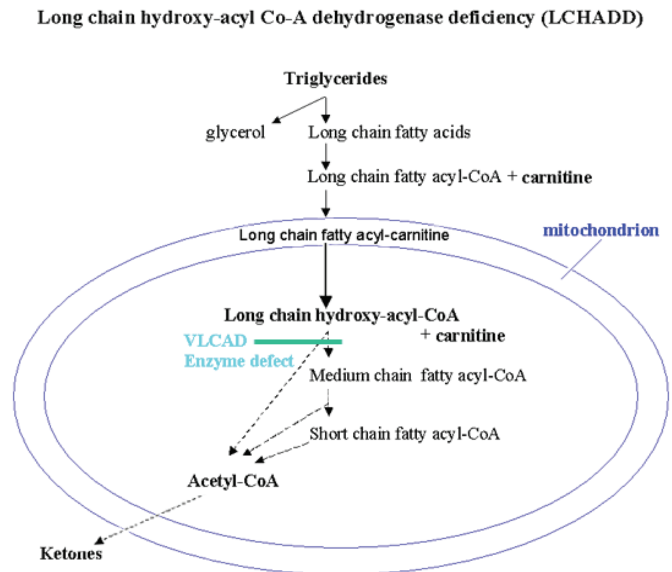

Table 1. VLCAD Mutations by Class and Exon Location

Mutation	Exon	Allele
Splice consensus sequence mutations		
G+1A	11	2
A-2C	8	1
G-1A	8	1
ΔG-1	6	1
Frame-shift mutations		
ΔG1621	17	1
Δ887-8	10	1
ΔT932	10	1
41-bp duplication	7	1
G1280A, W387ter	13	1
In-frame deletions		
Δ891-893, ΔK258	10	4
Δ386-8, ΔE89	6	2
Missense mutations		
C1837T, R573W	20	2
G1844A, R575Q	20	1
G1600A, E454K	16	2
G1468C, A450P	15	1
T1372C, F418L	14	1
G1322A, G401D	13	1
T848C, V243A	9	1
C779T, T220M	9	1
G637C, A173P	8	2
A739G, K207E	8	1

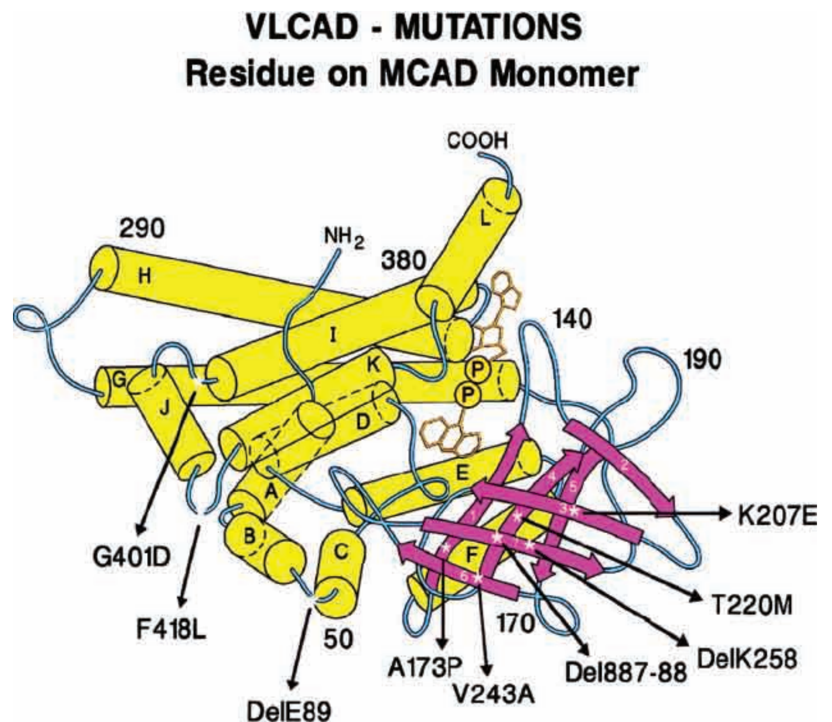

Figure 2. Positions of VLCAD missense mutations as predicted within structure of MCAD monomer. Yellow cylinders labeled A through L are -helixes. Purple arrows numbered 1 through 7 are ß-sheets. Blue lines are random coils. Stars identify positions of altered VLCAD residues. Flavin adenine dinucleotide cofactor is shown in dark yellow. Note that all VLCAD mutations are predicted to occur within ß-sheet domain or in random coils.

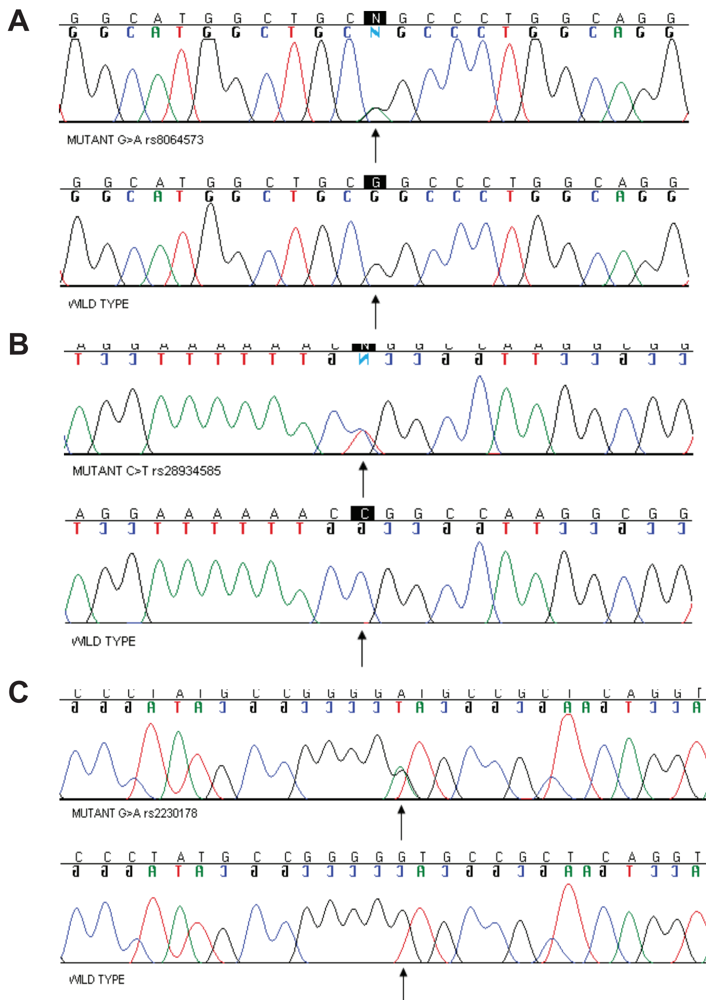

Figure 3. Sequence Analysis of PCR products amplified from exons 2, 3 and 10 in figure A, B and C respectively with SNP's indicated with arrows.

CONCLUSIONS

- Validation of this mutation screening approach to regions that harbor the most prevalent pathogenic mutations was confirmed by the successful detection of any and all reference mutations in test examples.

  Amplicons were designed and optimized for both accuracy and efficiency in the mutation screening process.

- PCR primers and conditions were designed and optimized to facilitate the use a single thermal cycling condition, while improving specificity and yield of all products for analysis. This heightened quality and minimized the resources required.

- Sequence analysis conditions were optimized for mutation detection in each amplicon with use of a tiered screening approach reducing cost with effective process time.

- The ACADVL gene was screened using this tiered approach with independent screening technologies. This ensured discovery of all variants and with greater sensitivity than by sequencing alone.

  We have developed and validated an in-depth screening methodology for mutational analysis of the ACADVL gene for mutations.

  This comprehensive analysis for testing can be completed within 2 weeks and is very competitive in both speed and cost.

REFERENCES

1. Bennett, M.J., Rinaldo, P. and Strauss, A.W. (2000) Inborn errors of mitochondrial fatty acid oxidation. *Crit. Rev. Clin. Lab. Sci.*, **37**, 1–44.

2. Vianey-Saban, C., Divry, P., Brivet, M., Nada, M., Zabot, M.T., Mathieu, M. and Roe, C.R. (1998) Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. *Clin. Chim. Acta*, **269**, 43–62.

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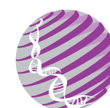

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