Clinical and genetic characteristics of patients with fatty acid oxidation disorders identified by newborn screening

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Abstract

Background: Fatty acid oxidation disorders (FAODs) include more than 15 distinct disorders with variable clinical manifestations. After the introduction of newborn screening using tandem mass spectrometry, early identification of FAODs became feasible. This study describes the clinical, biochemical and molecular characteristics of FAODs patients detected by newborn screening (NBS) compared with those of 9 patients with symptomatic presentations.

Methods: Clinical and genetic features of FAODs patients diagnosed by NBS and by symptomatic presentations were reviewed.

Results: Fourteen patients were diagnosed with FAODs by NBS at the age of 54.8 ± 4.8 days: 5 with very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, 5 with medium chain acyl-CoA dehydrogenase (MCAD) deficiency, 1 with primary carnitine deficiency, 1 with carnitine palmitoyltransferase 1A (CPT1A) deficiency, 1 with long-chain 3-hydroxyacyl-CoA dehydrogenase or mitochondrial trifunctional protein (LCHAD/MTP) deficiency, and 1 with short chain acyl-CoA dehydrogenase (SCAD) deficiency. Three patients with VLCAD or LCHAD/MTP deficiency developed recurrent rhabdomyolysis or cardiomyopathy, and one patient died of cardiomyopathy. The other 10 patients remained neurodevelopmentally normal and asymptomatic during the follow-up. In 8 patients with symptomatic presentation, FAODs manifested as LCHAD/MTP deficiencies by recurrent rhabdomyolysis or cardiomyopathy (6 patients), and VLCAD deficiency by cardiomyopathy (1 patient), and CPT1A deficiency by hepatic failure (1 patient). Two patients with LCHAD/MTP deficiencies died due to severe cardiomyopathy in the neonatal period, and developmental disability was noted in CPT1A deficiency (1 patient).

Conclusions: NBS helped to identify the broad spectrum of FAODs and introduce early intervention to improve the clinical outcome of each patient. However, severe clinical manifestations developed in some patients, indicating that careful, life-long observation is warranted in all FAODs patients.

Keywords: Fatty acid oxidation disorders, Newborn screening, Genotype-phenotype correlation, Treatment outcome
Background

Fatty acid oxidation (FAO) is a key metabolic pathway for maintaining energetic substrates used to maintain metabolic homeostasis. FAO is important for some high-energy-requiring organs and provides the main energy supply during prolonged fasting, febrile illness, cold exposure, or muscular exertion. The prime pathway for the degradation of fatty acids is mitochondrial fatty acid oxidation, which is composed of the uptake and activation of fatty acids, carnitine cycles, beta-oxidation cycle, and electron transfer [1].

More than 15 distinct disorders have been described as affecting FAO; These include glutaric aciduria type 2, primary carnitine deficiency and deficiencies of carnitine palmitoyltransferase 1A (CPT1A), carnitine acylcarnitine translocase (CACT), very long chain acyl-CoA dehydrogenase (VLCAD), long chain hydroxyacyl-CoA dehydrogenase or mitochondrial trifunctional protein (LCHAD/MTP), medium chain acyl-CoA dehydrogenase (MCAD), medium/short chain hydroxyacyl-CoA dehydrogenase (M/SCHAD), and short chain acyl-CoA dehydrogenase (SCAD) [2–4].

After the introduction of newborn screening by tandem mass spectrometry analysis of acylcarnitines, the detection of FAO disorders (FAODs) has increased. The estimated combined incidence of all FAODs is 1 in 9000, which is calculated from reports out of Australia, Germany, and the USA, but it seems to be much lower in Asian countries [5]. The estimated prevalence of long chain fatty acid oxidation disorders in Korea is 1 in 15,800, which is more frequently diagnosed following the introduction of tandem mass spectrometry newborn screening [6].

FAODs present with a heterogeneous clinical phenotype at various ages of onset, from neonate to adulthood. The most severe form manifests with hypertrophic cardiomyopathy, hepatic encephalopathy, or severe hypoketotic hypoglycemia in the neonatal period or infancy. The less-severe, later-onset myopathic form is characterized by exercise-induced myopathy and rhabdomyolysis [7, 8].

The diagnosis of FAODs are based on the measurement of abnormal acylcarnitines and confirmed by enzyme assay or molecular analysis. Early identification of FAODs became possible using expanded newborn screening using tandem mass spectrometry, which measures acylcarnitine levels in dried blood spots. Identification of FAODs in newborn screening is very important because intervention in the presymptomatic period helps improve patient prognosis [9].

In this respect, here we describe fourteen patients with diverse FAODs identified by newborn screening, and compare their clinical outcome and genetic characteristics with those of 8 patients diagnosed by symptomatic presentation. Our experience indicates the effectiveness of newborn screening for the early diagnosis of FAOD, especially in the presymptomatic period. However, careful observation and appropriate management is warranted to improve the clinical outcome of the affected patients.

Methods

Patients

A total of fourteen patients were diagnosed with FAODs by newborn screening between May 2002 and February 2016 at the department of Medical Genetics, Asan Medical Center Children’s Hospital, Seoul, Korea. Clinical features of these patients were compared with nine FAOD patients identified by symptomatic presentation, including rhabdomyolysis, cardiomyopathy, or developmental delay.

The Institutional Review Board at Asan Medical Center approved this study. Appropriate written informed consent was obtained from the parents of all participants.

Methods

Presenting manifestations, biochemical findings including plasma acylcarnitines, molecular analysis, and clinical course of each patient were reviewed retrospectively.

All patients diagnosed by newborn screening tests were referred to our department due to abnormal newborn screening results. Dried blood spot samples were obtained at the hospital where the patients were born and tandem mass spectrometry analysis was performed at commercial biochemical laboratories. Newborns with abnormal initial screening result were requested for repeated tandem mass spectrometry. If a second screening results were still exceeding the cut off value, molecular genetic analyses was performed and the diagnosis of FAOD was confirmed after receiving a positive confirmation test.

Acylcarnitine was measured by Liquid Chromatography-Tandem Mass Spectrometry, as previously described [6]. Genomic DNA was isolated from peripheral blood leukocytes. PCR was performed for all coding exons and exon–intron boundaries of SLC22A5 for primary carnitine deficiency, CPT1A for CPT1A deficiency, ACADVL for VLCAD deficiency, HADHA and HADHB for LCHAD/MTP deficiencies, ACADM for MCAD deficiency, and ACADS for SCAD deficiency. Direct sequencing was performed on a ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator cycle sequencing kit (Applied Biosystems). In silico prediction analyses were performed for novel missense and splicing variants, using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) and SIFT (http://sift.jcvi.org).
Results
Clinical characteristics of patients with FAODs identified by newborn screening
FAODs were identified in 14 patients by newborn screening: VLCAD deficiency (5 patients), MCAD deficiency (5 patients), primary carnitine deficiency (1 patient), CPT1A deficiency (1 patient), LCHAD/MTP deficiencies (1 patient), and SCAD deficiency (1 patient). Newborn screening using tandem mass spectrometry was performed at 3.2 ± 0.2 days after birth and the diagnosis of FAOD was confirmed by molecular genetic analyses at the mean age of 54.7 ± 40.8 days (range: 16–153 days). Plasma C14:1 (p.[Pro35fs*27]), c.[104_105ins10] (p.[Pro35fs*27]), c.[104_105ins5] (p.[Pro35fs*25]), c.[103_112dup] (p.[Arg38-Profs*26]), c.[996_997ins(T)] (p.[Ala333Cysfs*26]), and c.[552C > G] (p.[Ile184Met])). Two novel mutations, c.[748G > T] (p.[Val250Leu]) and c.[1015C > T] (p.[Arg339Ter]), were detected in CPT1A deficiency. All other mutations were previously reported (Table 1) [10–20].

Molecular characteristics of patients with FAODs identified by newborn screening
Germline mutations of the gene responsible for each FAOD were identified in 90% of alleles (9 out of 10 alleles) in VLCAD deficiency, 90% (9 out of 10 alleles) in MCAD deficiency, 100% (2 out of 2 alleles) in LCHAD/MTP, primary carnitine, CPT1A, and SCAD deficiencies (Table 1). In VLCAD deficiency, 5 out of 7 mutations were novel mutations, c.[104_105ins10] (p.[Pro35fs*27]), c.[104_105ins5] (p.[Pro35fs*25]), c.[103_112dup] (p.[Arg38-Profs*26]), c.[996_997ins(T)] (p.[Ala333Cysfs*26]), and c.[552C > G] (p.[Ile184Met])). Two novel mutations, c.[748G > T] (p.[Val250Leu]) and c.[1015C > T] (p.[Arg339Ter]), were detected in CPT1A deficiency. All other mutations were previously reported (Table 1) [10–20].

Clinical outcomes of patients with FAODs identified by newborn screening
The mean age at last follow-up for the 14 patients was 2.5 ± 2.0 years (range: 49 days–6.6 years of follow-up. At the age of 7, the patient’s intelligence quotient (IQ) was less than 35 and Korean Childhood Autism Rating Scale (K-CARS) score was 44, suggesting severe intellectual disability and severe autism.

Discussion
The current study described the clinical and genetic features of 14 patients with FAODs identified by newborn screening, compared to those of 8 patients diagnosed with FAODs based on their symptomatic presentations. The results of our current study indicate some important findings. Newborn screening helped to identify the broad spectrum of FAODs compared to FAODs with symptomatic presentation. These findings
were comparable to previous reports regarding the newborn screening program [8, 22]. In addition, the relative frequency of each FAOD was reflected; VLCAD, LCHAD/MTP, and MCAD deficiencies were common FAODs identified by newborn screening. Alternatively, other FAODs, including primary carnitine deficiency, CPT1A or SCAD deficiencies, were identified in a small number of patients. Of note, VLCAD or LCHAD/MTP deficiencies were the most common among the FAODs identified either by newborn screening or by symptomatic presentation, whereas all patients with another common FAOD, MCAD deficiency, were identified by newborn screening, as symptomatic presentation of MCAD deficiency is expected to be very rare.

### Table 1: Clinical, biochemical, and genetic characteristics of patients with fatty acid oxidation disorders diagnosed by newborn screening

| No | Age at diagnosis | Age at last follow-up | Phenotype | Acylcarnitine | Gene | Allele 1 | Allele 2 |
|----|------------------|-----------------------|-----------|---------------|------|----------|----------|
|    |                  |                       |           | Sample Elevated acylcarnitine (value) |      |           |          |
| 1  | 39 days          | 3.7 years             | recurrent rhabdomyolysis and hypertrophic cardiomyopathy after 7 months old | DBS | C14:1 (2.504 μM; ref., 0.006–0.166), C14:1 (1.097 μM; ref., 0.006–0.166) | ACADVL | c.[104_105ins10] (p.[P35fs*27]) | c.[104_105ins5] (p.[P35fs*25]) |
| 2  | 33 days          | 5.8 years             | recurrent rhabdomyolysis after 11 months | DBS | C14:1 (n.a.), C14 (n.a.), C14:2 (n.a.) | ACADVL | c.[1349G > A] (p.[R450H]) | c.[1349G > A] (p.[R450H]) |
| 3  | 25 days          | 10 months             | hypertrophic cardiomyopathy | DBS | C14:1 (0.581 μM; ref., 0.006–0.166), C14:1 (1.391 μM; ref., 0.034–0.599) | ACADVL | c.[103_105dup] (p.[R450H]) | c.[1349G > A] (p.[R450H]) |
| 4  | 49 days          | 3.3 years             | 1 episode of rhabdomyolysis | DBS | C141 (6.62 μM; ref., < 0.85) | ACADVL | c.[996_997ins(T)] (p.[A333C*26]) | c.[552C > G] (p.[I184M]) |
| 5  | 48 days          | 2.0 years             | asymptomatic | Plasma | C14 (0.184 μmol/L; ref., < 0.15), C14:2 (0.215 μmol/L; ref., < 0.13) | ACADVL | c.[1349G > A] (p.[R450H]) | ? |
|    |                  |                       |           | Sample Elevated acylcarnitine (value) |      |           |          |
| 6  | 16 days          | 4.5 years             | asymptomatic | DBS | C8 (0.68 μM; ref., < 0.31) | ACADVL | c.[617G > A] (p.[R206H]) | c.[1189 T > A] (p.[Y397N]) |
| 7  | 36 days          | 3.5 years             | asymptomatic | DBS | C6 (n.a.), C8 (n.a.), C10:1 (n.a.), C10 (n.a.) | ACADVL | c.[1085G > A] (p.[G362E]) | c.[1189 T > A] (p.[Y397N]) |
| 8  | 51 days          | 6.9 years             | asymptomatic | DBS | C6 (0.46 μM; ref., < 0.22), C8 (1.66 μM; ref., < 0.35) | ACADVL | c.[449_452del] (p.[Y150Rfs*4]) | c.[1189 T > A] (p.[Y397N]) |
| 9  | 56 days          | 1.4 years             | asymptomatic | DBS | C8 (2.98 μM; ref., < 0.37), C10:1 (0.58 μM; ref., < 0.40) | ACADVL | c.[449_452del] (p.[Y150Rfs*4]) | c.[1085G > A] (p.[G362E]) |
| 10 | 153 days         | 1.4 years             | asymptomatic | Plasma | C8 (0.868 μmol/L; ref., < 0.18), C8 (5.067 μmol/L; ref., < 0.27), C10:1 (1.387 μmol/L; ref., < 0.46) | ACADVL | c.[1189 T > A] (p.[Y397N]) | ? |
| 11 | 53 days          | 3.2 years             | mild CK elevation, normal development | Plasma | C0 (4.1 μmol/L; ref., 12–46), Total carnitine (6.1 μmol/L; ref., 19–59) | SLC22A5 | c.[396G > A] (p.[W132*]) | c.[1400C > G] (p.[S467C]) |
| 12 | 41 days          | 5 months              | normal development | Plasma | C0 (88.839 μmol/L; ref., < 62.10), C0/C16+C18 (123.3) | CPT1A | c.[748G > T] (p.[V250 L]) | c.[1015C > T] (p.[R399*]) |
| 13 | 26 days          |                       | Family history of sibling who died of lactic acidemia during the neonatal period. Died at age 49 | DBS | C16O1H (n.a.), C16OH/C16 (n.a.), C18:1OH (n.a.), C14 (n.a.), C14OH (n.a.) | HADHA | c.[1689 + 2 T > G] (deletion of exon 16) | c.[1689 + 2 T > G] (deletion of exon 16) |
| 14 | 141 days         | 5 months              | asymptomatic | Plasma | C4 (4.51 μmol/L; ref., < 1.06) | ACADVS | c.[164C > T] (p.[P55L]) | c.[1041A > G] (p.[E344G]) |

*Indicates novel mutations. DBS dried blood spot samples, n.a. not available
The purpose of newborn screening is to identify patients with inborn metabolic disorders in their pre-symptomatic period and intervene to prevent a metabolic crisis and improve their clinical outcome. In our current study, a fair outcome was observed in the patients with MCAD, CPT1A or primary carnitine deficiency identified by newborn screening. However, clinical outcomes were not significantly different among patients with long-chain FAODs, including VLCAD deficiency and LCHAD/MTP deficiencies identified either by newborn screening or by symptomatic presentation; most long-chain FAOD patients developed recurrent rhabdomyolysis and hypertrophic cardiomyopathy regardless of presymptomatic management.

| No | Age at diagnosis | Age at last follow-up | Phenotype                                      | Acylcarnitine Sample | Elevated acylcarnitine (value) | Gene | Allele 1  | Allele 2 |
|----|------------------|----------------------|------------------------------------------------|----------------------|-------------------------------|------|-----------|----------|
| 1  | 2.7 years        | 11.3 years           | Recurrent rhabdomyolysis, sensorimotor polyneuropathy, difficulty running and climbing stairs | DBS                  | C10OH (n.a.), C18OH (n.a.)    | HADHB | c.[340A > G] (p.[N114D]) | c.[739C > T] (p.[R247C]) |
| 2  | 2.1 years        | 11.9 years           | Recurrent rhabdomyolysis, sensorimotor polyneuropathy, difficulty running, positive Gowers' sign | DBS                  | C10 (n.a.), C12 (n.a.), C14:1 (n.a.), C14OH (n.a.), C16OH (n.a.), C18:1OH (n.a.) | HADHB | c.[340A > G] (p.[N114D]) | c.[919A > G] (p.[N307D]) |
| 3  | 4.8 years        | 6.8 years            | Recurrent rhabdomyolysis, sensorimotor polyneuropathy, difficulty running | DBS                  | C14OH (n.a.), C16OH (n.a.), C18OH (n.a.), C18:1OH (n.a.) | HADHB | c.[340A > G] (p.[N114D]) | c.[1148C > T] (p.[S383 L]) |
| 4  | 10.6 years       | 23.3 years           | Recurrent rhabdomyolysis, sensorimotor polyneuropathy, walk with assistance | DBS                  | C14OH (0.156 μM; ref., 0.003–0.87), C16OH (0.228 μM; ref., 0.003–0.083), C18OH (0.072 μM; ref., 0.003–0.055) | HADHB | c.[919A > G] (p.[N307D]) | c.[1165A > G] (p.[N389D]) |
| 5  | 1 day            | –                    | Severe cardiomyopathy at first day of life. Died of lactic acidosis at 4 days old | DBS                  | C16OH (0.86 μM; ref., < 0.15), C18OH (0.33 μM; ref., < 0.1), C18:1OH (0.48 μM; ref., < 0.08), C14:1 (0.66 μM; ref., < 0.35), C14 (1.35 μM; ref., < 0.86), C16:1 (0.54 μM; ref., < 0.25) | HADHA | c.[1793_1974del] (p.[H598Rfs*33]) | c.[1793_1974del] (p.[H598Rfs*33]) |
| 6  | 5 days           | –                    | Presented with tachypnea and metabolic acidosis at 5 days old. Died at 9 days old due to cardiomyopathy | DBS                  | C14 (n.a.), C14OH (n.a.), C16OH (n.a.), C18OH (n.a.), C18:1OH (n.a.) | HADHB | c.[1136A > G] (p.[H379R]) | c.[1211dup] (p.[G404 fs*2]) a |
| 7  | 2 months         | 3.9 years            | Hypertrophic cardiomyopathy, recurrent rhabdomyolysis | DBS                  | C14:1 (n.a.), C14 (n.a.) | ACADVL | c.[997_998ins(T)] (p.[A333*]) a | c.[1770_1773del] (p.[S590*]) a |
| 8  | 33 months        | 6.8 years            | Recurrent hepatic failure, nephromegaly, hemolytic anemia, rhabdomyolysis, developmental delay | Plasma               | C0 (68.86 μmol/L; ref., < 62.10), C0/C16 + C18 (1639) | CPT1A | c.[837,838insT] (p.[I279P]) | c.[947G > A] (p.[R316Q]) |

Table 2 Clinical, biochemical, and genetic characteristics of patients with fatty acid oxidation disorders diagnosed by clinical signs and symptoms.

*Indicates novel mutations. DBS dried blood spot samples, n.a. not available.
inactivating or null alleles [25–28]. This correlation became more meaningful when the phenotype-genotype correlation was evaluated in all patients with VLCAD or LCHAD/MTP deficiencies, irrespective of the mode of identification. Seven patients with severe types of mutations, such as frame-shift, nonsense, or splicing mutations, experienced severe phenotypes. Particularly, early death from severe cardiomyopathy was noted in 3 patients with severe mutations in LCHAD/MTP deficiencies. On the other hand, patients with missense mutations only either remained asymptomatic or experienced milder phenotypes (Tables 1 and 2).

As a long-term complication, peripheral polyneuropathy developed in four patients with LCHAD/MTP deficiencies, which has been reported in up to 80% of cases. In addition, pigmentary retinopathy develops in up 15–30% of patients [29, 30]. The mechanism responsible for these complications is not fully understood, although accumulation of 3-hydroxy fatty acid intermediate may be responsible [8]. Because the follow-up period was short for the patients in our current report, observation for these long-term complications is necessary.

The benign clinical course of MCAD deficiency can also be explained in part by the mutation type; most of the mutations were missense and only a small proportion of severe mutations such as frame-shift were noted. However, a life-long follow-up evaluation is required for patients with MCAD deficiency, considering the development of late-onset metabolic episodes. Between 3 and 24 months of age, patients may experience hypoketotic hypoglycemia, vomiting, lethargy, encephalopathy during febrile illness, or prolonged fasting. Sudden unexplained death may be the first presentation of MCAD deficiency [31, 32]. Even some adult patients may suffer from rhabdomyolysis, hepatic failure, encephalopathy or cardiac arrest triggered by alcohol consumption, pregnancy, or prolonged fasting [9, 33].

SCAD deficiency has also been classified as a benign condition because most of the newborns with SCAD deficiency as identified by newborn screening do not develop a clinical phenotype without any medical intervention. There exist controversies whether SCAD deficiency is benign biochemical phenotype, a clinical disorder with incomplete penetrance, or a clinically relevant part of multi-factorial or a multi-genetic disorder [34, 35]. Previously, some patients reported as having severe developmental delay, dysmorphic features and epilepsy, which would have been attributed to unknown genetic defects rather than to SCAD deficiency.

The CPT1A deficiency patient identified by newborn screening harbor heterozygous of two novel mutations. The patient remained asymptomatic during follow-up period, which was relatively short considering that CPT1A deficiency patients usually present by the age of 2 years [36]. The major manifestation of CPT1A deficiency is hepatic encephalopathy followed by febrile illness or prolonged fasting [21]. Even for a patient with neonatal pre-symptomatic presentation, late-onset hepatic failure develops, requiring lifelong evaluation. There exists a possibility that the novel missense mutation may not be deleterious, yet longer follow-up is needed in our patient in case of developing the symptomatic manifestations.

Conclusions
Newborn screening for FAODs revealed the relative frequency of each disease subtype and their general clinical characteristics. This screening helped to reduce the mortality and morbidity of each patient with FAODs, but their broad spectrum of disease severity was also encountered regardless the mode of diagnosis, which was explained in part by their respective genotype. Careful, life-long observation of patients with FAODs is required to improve clinical outcome.

Abbreviations
CPT1A: Carnitine palmitoyltransferase 1A; FAO: Fatty acid oxidation; FAODs: Fatty acid oxidation disorders; LCAH/D/MTP: Long-chain 3-hydroxyacyl-CoA dehydrogenase or mitochondrial trifunctional protein; MASHAD: Medium/short chain hydroxyacyl-CoA dehydrogenase; MCAD: Medium chain acyl-CoA dehydrogenase; NBS: Newborn screenings; SCAD: Short chain acyl-CoA dehydrogenase; VLCAD: Very long chain acyl-CoA dehydrogenase

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Availability of data and materials
The authors declare that the data supporting the findings of this study are available within the article.

Authors’ contributions
EK, HWY and BHL designed the study. EK and BHL drafted the manuscript. All authors (EK, YMK, MK, SHI, GHK, HJC, JHC, HWI, and BHL) were involved in analyzing and interpreting data. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This study was conducted after obtaining appropriate written informed consent from the parents of all participants, and the Institutional Review Board (IRB) at Asan Medical Center approved this study (IRB number: 2016–1098).

Consent for publication
A written informed consent for publication was obtained from each patient or responsible family member.

Competing interests
No potential conflict of interest relevant to this article was reported.

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