Mitochondrial trifunctional protein (TFP) deficiency is one of the long-chain fatty-acid β-oxidation disorders. TFP is a multienzyme complex composed of four α-subunits containing the long-chain 2-enoyl-CoA hydratase and the long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) domains and four β-subunits harboring the long-chain 3-ketoacyl-CoA thiolase (LKAT) domain. TFP deficiency is defined by reduced activity of all three TFP enzymes and occurs due to heterogeneous α- or β-subunit mutations.1,19,24,25,28 In contrast, isolated LCHAD deficiency is defined by reduced LCHAD activity and is due to the common E474Q α-subunit mutation.

TFP deficiency is characterized by clinical heterogeneity24,25 and severe manifestations present similar to other long-chain fatty acid oxidation (FAO) defects with early-onset cardiomyopathy, recurrent Reye-like encephalopathy, hepatopathy, and neonatal or unexpected infant death. This phenotype has been most frequently reported,1,7,10,12,17 giving rise to the concept that TFP deficiency is often fatal. However, a milder form of TFP deficiency, characterized by peripheral neuropathy and myopathy with onset in later infancy that closely resembles the hereditary motor-sensory neuropathies (HMSN) or spinal muscular atrophy (SMA) has been described in patients with α- or β-subunit mutations.9,13,22,25 To date, the neuromyopathic manifestations of TFP deficiency have only been described in isolated case...
reports, leaving phenotype characterization incom-plete. We have collected clinical data in a series of 11 patients with neuromyopathic TFP deficiency with a special focus on the initial symptoms and progression of the disease through adolescence or adulthood. These 11 patients were recruited from a total of 27 families with TFP deficiency, 13 due to \( \beta \)-subunit mutations\(^{25} \) and 14 with \( \alpha \)-subunit mutations, that were referred to us for molecular and enzymatic analysis. The remaining 16 patients presented with cardiac or hepatic phenotypes.\(^{1,7,10,12,17,25} \)

We now describe the clinical presentation and long-term follow-up of 11 patients with neuromyopathic TFP deficiency arising from \( \alpha \)-subunit or \( \beta \)-subunit mutations in order to characterize this disease phenotype and to alert clinicians to its common occurrence in TFP deficiency.

**METHODS**

In 27 patients referred to us for molecular and enzymatic analysis, TFP deficiency was confirmed. Eleven presented with the neuromyopathic TFP phenotype. In 10, diagnosis was confirmed by enzyme assay in cultured skin fibroblasts derived from skin biopsies. In all 11 individuals, disease-causing mutations in the TFP genes were delineated.

The patients’ clinical and family histories were obtained from the referring physicians. All data were collected retrospectively. Patients 1 through 8 were previously reported by Spiickerkoetter et al.\(^{25} \) Patient 9 was reported by Ibdah et al.,\(^{9} \) patient 10 by Ibdah et al.,\(^{9} \) and Tein et al.,\(^{26,27} \) and patient 11, because of maternal HELLP syndrome in this family, by Isaacs et al.\(^{13} \)

**RESULTS**

Previous reports of TFP deficiency emphasized early-onset, severe phenotypes with cardiomyopathy, Reye-like symptoms, and sudden death.\(^{1,7,10,12,17} \) In this series of patients (Table 1), characteristic clinical features included progressive peripheral neuropathy and recurrent episodes of severe muscle weakness, myalgias, and rhabdomyolysis.

The first clinical symptoms occurred in all patients in early childhood, between 1 and 6 years of age (median, 19 months) (Table 1). Two patients

### Table 1. Disease-onset and follow-up in patients with TFP deficiency.*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at First symptom</th>
<th>Age at Onset</th>
<th>Age at Diagnosis</th>
<th>Age at Last follow-up</th>
<th>Age at First myoglobinuria</th>
<th>Age at Respiratory failure</th>
<th>Age at First symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>3 y</td>
<td>12 y</td>
<td>23 y</td>
<td>?</td>
<td>21 y</td>
<td>21 y</td>
<td>Hypoglycemia</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>6 m</td>
<td>10 m</td>
<td>3 y</td>
<td>1 y</td>
<td>1 y</td>
<td>1 y</td>
<td>Hypotonia</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>1–2 y</td>
<td>14 y</td>
<td>15 y</td>
<td>13 y</td>
<td>—</td>
<td>—</td>
<td>Motor delay</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>1–2 y</td>
<td>4 y</td>
<td>5 y</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Muscle weakness</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>6 y</td>
<td>6 y</td>
<td>10 y</td>
<td>6 y</td>
<td>10 y</td>
<td>—</td>
<td>Muscle weakness</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>3 y</td>
<td>18 y</td>
<td>25 y</td>
<td>?</td>
<td>18 y</td>
<td>—</td>
<td>Leg weakness</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>3 y</td>
<td>8 y</td>
<td>11 y</td>
<td>3 y</td>
<td>3 y</td>
<td>—</td>
<td>Respiratory failure(^1)</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>1–2 y</td>
<td>3 y</td>
<td>8 y</td>
<td>2 y</td>
<td>—</td>
<td>—</td>
<td>Motor delay, hypotonia</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>13 m</td>
<td>?</td>
<td>15 y</td>
<td>1–2 y</td>
<td>—</td>
<td>—</td>
<td>Muscle weakness</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>20 m</td>
<td>7 y</td>
<td>19 y</td>
<td>1–2 y</td>
<td>—</td>
<td>—</td>
<td>Motor delay</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>4 m</td>
<td>7 m</td>
<td>10 y</td>
<td>3½ y</td>
<td>—</td>
<td>—</td>
<td>Hypoglycemia, lethargy</td>
</tr>
</tbody>
</table>
presented initially with episodes of hypoketotic hypoglycemia in early infancy consistent with the hepatic phenotype of TFP deficiency and exhibited their first neuromyopathic symptoms at the age of 3 years (patients 1 and 11). The remaining nine patients became symptomatic with myopathic features (Table 1). Two independent neuromuscular features were found: episodic rhabdomyolysis and progressive peripheral neuropathy. In seven patients, the chronic neuropathic weakness preceded onset of recurrent episodes of rhabdomyolysis; in one patient with progressive peripheral neuropathy, episodes of rhabdomyolysis never occurred until the current age of 5 years (patient 4). In our cohort of 11 patients, only one presented with an episode of rhabdomyolysis with respiratory failure as the initial symptom (patient 7). Overall, the combination of episodic rhabdomyolysis and peripheral neuropathy was present in 10 of the 11 patients (Table 2). In one patient (patient 11), signs of peripheral neuropathy were not present, whereas pigmentary retinopathy was observed. Nerve conduction studies (NCS) to exclude a neuropathic component were not performed in this patient.

Ages at diagnosis varied between 10 months and 18 years (median, 8 years) as shown in Table 1. The average time between first symptoms and diagnosis was 5 years and 10 months. There was a predominance of affected males in our series (nine boys, two girls).

**Episodic Rhabdomyolysis.** Catabolic events such as prolonged exercise, fasting, illness, lack of sleep (patient 8), emotional stress (patient 10), or cold exposure (patient 9) triggered episodes of myoglobinuria with highly elevated serum creatine kinase (CK) concentrations (up to 60,000 IU/L; normal, <224 IU/L). In 5 of 11 patients (45%), transient respiratory failure due to severe muscle weakness was precipitated by an infection or exercise (Table 2). In patient 5, a varicella infection was the stressor.

The first episode of rhabdomyolysis was reported as early as in the 1st year of life and as late as at 13 years of age (Table 1).

**Neuropathy.** Peripheral neuropathy was clinically diagnosed in eight patients and was confirmed by nerve biopsies in three patients. In all individuals, the lower limbs were mainly involved. Secondary bilateral Achilles tendon contractures and planovalgus deformities were observed in four patients (Table 2). Two of them (patients 9 and 10) exhibited bilateral foot drop and also symmetric weakness in wrist and finger extensors. Another patient (patient 1) with severe progression of the neuropathy became immobile and wheelchair-dependent at the age of 22 years.

Mild facial muscle weakness was present in two patients (patients 3 and 10).

**Other Clinical Features.** Pigmentary retinopathy was observed in one patient (patient 11), with onset at the age of 3½ years. Electroretinogram changes were also noted in patient 10 in mid-adolescence without sign of night blindness.

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**Table 2. Clinical features in individuals with neuromyopathic TFP deficiency.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Progressive weakness</th>
<th>Episodic severe weakness</th>
<th>Symptoms induced by</th>
<th>Serum CK (IU/L) in myoglobinuric episodes&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Peripheral Neuropathy</th>
<th>Respiratory failure</th>
<th>Foot deformities</th>
<th>Improved with therapy</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>1000</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>?</td>
<td></td>
<td>elevated</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>40,000</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>60,000</td>
<td>+&lt;sup&gt;2&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>50,250</td>
<td>+&lt;sup&gt;2&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>+&lt;sup&gt;3&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>29,960</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>+&lt;sup&gt;3&lt;/sup&gt;</td>
<td>?</td>
<td>+</td>
<td>elevated</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>67</td>
</tr>
<tr>
<td>8</td>
<td>+&lt;sup&gt;3&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>50,000</td>
<td>+&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>67</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>elevated</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>165,000</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td>11</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>78</td>
</tr>
</tbody>
</table>

CK, creatine kinase; ?, not known; TFP, trifunctional protein.

Patients 3 and 4 are brothers.

<sup>1</sup>Normal serum CK is < 224; “elevated” indicates that the precise number was not available.

<sup>2</sup>Constant weakness without clear progression.

<sup>3</sup>Confirmed by sural nerve biopsy; see text for further details.
Whereas hepatic and cardiac symptoms were excluded in the majority of patients, patient 6 exhibited mild, reversible cardiomyopathy during episodes of metabolic derangement with rhabdomyolysis, diagnosed by standard echocardiographic criteria. Patient 10 was noted to have mild intraventricular conduction delays on electrocardiogram. The two patients who manifested with hypoketotic hypoglycemia in infancy did not present with further episodes of hypoglycemia in childhood and adolescence.

Maternal hemolysis, elevated liver function tests, and low platelets (HELLP) syndrome during pregnancy with affected probands occurred in 2 of 11 families (patients 8 and 11).

Nerve Conduction Studies and Sural Nerve Biopsies. NCS were performed in five patients and revealed markedly decreased amplitudes of compound muscle action potentials (CMAPs) and sensory nerve action potentials (SNAPs) with normal distant latency and mild decrease in conduction velocity in patients 3 and 4 suggesting axonal neuropathic changes. In patient 10, the SNAPs of the sural and radial sensory nerves were absent at age 7, and the CMAPs of the posterior tibial, peroneal, and median motor nerves were markedly decreased. At age 10, the CMAPs of the posterior tibial and peroneal motor nerves were also absent, and the CMAPs of the median and ulnar motor nerves were decreased. Sural nerve biopsy in patient 10 showed consistent findings, with axonal degeneration and loss with normal myelin. In patients 5 and 6, NCS revealed reduced amplitudes of motor and sensory action potentials, distant latencies, and reduced conduction velocities; sural nerve biopsies showed mixed axonal degeneration and loss of myelin (Table 2).

Electromyography and Muscle Biopsies. Electromyography (EMG) in patients 3–7 showed no evidence of myopathic changes. In patient 6, with disease-onset at 3 years of age, the EMG was normal at age 4, but showed signs of a neuropathic process by the age of 14 years. In patient 10, following an episode of myoglobinuria at 40 months of age, the EMG was mildly myopathic. Muscle biopsies performed in patients 5–7 revealed evidence of denervation with atrophic fibers and a preponderance of type I fibers. In patient 6, the predominance of type I fibers with focal type II fiber grouping was consistent with a denervation–reinnervation process. In patient 10, with disease-onset at 1–2 years, there was a mild increase in lipid and type I fiber predominance of 80% at 7½ years.

Biochemical Features. Elevation of plasma long-chain hydroxyacylcarnitines is suggestive of a defect of the mitochondrial trifunctional protein complex. During stable clinical conditions, a normal carnitine/acylcarnitine profile was observed in most patients, whereas during episodes of rhabdomyolysis, long-chain hydroxyacylcarnitines were significantly elevated. Only patient 3 also presented with normal acylcarnitines during such an episode.

During myoglobinuric episodes, serum CK concentrations were highly elevated up to 60,000 IU/L. Typically, the serum CK returned to normal within days, as the patients recovered.

Biochemical and Molecular Characterization. In 10 patients, TFP deficiency was confirmed by LCHAD and LKAT assays in cultured skin fibroblasts (Table 3). LCHAD activities ranged from 10% to 41% of normal controls with a mean of 21% (SD ± 9.2%), and LKAT activities were from 0 to 37% with a mean of 12% (SD ± 12.2%). Molecular genetic analysis of the TFP α- and β-subunits revealed α-subunit mutations in three and β-subunit mutations in eight patients (Table 3). One patient with α-subunit mutations was homozygous, and two were compound heterozygotes. Six patients were compound heterozygous for a β-subunit mutation. In two patients, only a single β-subunit mutation was delineated despite sequencing of all α- and β-subunit exons. The second mutation might be in intronic regions not amplified in our PCR products. Three of 10 unrelated patients presented with a premature termination codon on one allele and all other mutations were missense mutations.

Treatment. Because all patients were evaluated in different metabolic centers, there was no common treatment protocol. Regular meals, avoidance of fasting and other stressors, and reduced long-chain fat intake were the mainstay of therapy in all patients. Transient carnitine supplementation in daily doses from 25–100 mg/kg was reported in patients 1, 3, 4, 7, 10, and 11, but had no significant impact on the course of the disease. Steroid therapy was implemented in patient 10 with a sustained positive therapeutic effect on the limb-girdle myopathy and transient effect on the peripheral neuropathy.20,27 In all patients with the exception of patient 3, episodes of severe muscle weakness and myoglobinuria occurred less frequently after introduction of a high carbohydrate and low long-chain fat diet, and avoidance of precipitating factors such as fasting and prolonged exercise. Patient 3 was severely disabled by the neuropathy and almost wheelchair-bound prior to the
been associated with fatty acid oxidation defects to progressive sensory-motor neuropathy has not been observed in the case of TFP deficiency, which would be consistent with other FAO disorders. 

**DISCUSSION**

In FAO disorders, phenotypic heterogeneity is well known, primarily with severe early-onset and milder later-onset or adult presentations. Myoglobinuria triggered by exercise, fasting, or infection is typical of adult-onset presentations, although it may also occur in infantile forms and is particularly common in carnitine palmitoyltransferase II (CPT II) and very long-chain acyl CoA dehydrogenase (VLCAD) deficiencies. By contrast, weakness due to progressive sensory-motor neuropathy has not been associated with fatty acid oxidation defects other than in a few patients with isolated LCHAD deficiency, another disorder of the TFP complex. Progressive peripheral neuropathy is a significant feature of myopathic TFP deficiency and the combination of both progressive neuropathy and episodic myoglobinuria is present in more than 50% of patients with TFP β-subunit mutations (7 of 13 families from our cohort) and in about one-third of reported patients with TFP deficiency due to α-subunit mutations. In most patients, the neuropathic features precede rhabdomyolysis. Because this presentation has significant features in common with HMSN and SMA, some patients have carried these erroneous diagnoses for years before TFP deficiency was diagnosed, emphasizing the need for clinicians to develop a higher index of suspicion for FAO disorders in patients with neuromyopathic features, given the potential for treatment intervention.

Evidence of progression in the neuropathy was observed in 67% of our patients despite dietary treatment measures and avoidance of fasting. Sural nerve biopsies in three patients demonstrated axonal degeneration and, in two, additional myelin loss (Table 2). The etiology of axonal and myelin degeneration in TFP deficiency is unknown. Excessive elevation of long-chain L-3-hydroxyacylcarnitines and 3-hydroxy fatty acids may have neurotoxic effects. Studies of Schwann cell energy metabolism will be necessary to address whether energy depletion is an etiological factor in the neuropathy of TFP-deficient patients.

To slow progression of neuropathy, prednisone was administered to a patient with TFP deficiency in the belief that it may stabilize muscle and neuronal plasma membranes, as well as the fatty acid oxidati-
tion enzyme complex in the mitochondrial membrane, and it caused transient improvement.26

Deficiency of DHA may play a significant role in progression of neuropathy and has been documented in several children with LCHAD deficiency who presented with pigmentary retinopathy or peripheral neuropathy.27 A recent study demonstrated that peroxisomal β-oxidation enzymes are involved in the DHA biosynthesis, whereas deficiency of certain mitochondrial β-oxidation enzymes such as carnitine palmitoyltransferase I (CPT I), carnitine acylcarnitine translocase (CACT), CPT II, and VLCAD do not result in impaired DHA synthesis. This is consistent with the clinical finding that patients with CPT I, CACT, CPT II, or VLCAD deficiency do not present with neuropathy. LCHAD- and TFP-deficient fibroblasts were not tested for DHA biosynthesis. The long-chain L-3-hydroxyacylcarnitines and acyl-CoAs, which accumulate in LCHAD deficiency, may interfere with the successive desaturations or elongations of alpha-linolenic acid in the biosynthesis of DHA through substrate competition for the desaturases by accumulated metabolites.27

DHA supplementation in a boy with LCHAD deficiency and progressive sensorimotor axonopathy with demyelination resulted in marked clinical and electrophysiological recovery.27 In two patients from our study (patients 3 and 4), the same effect was observed with DHA supplementation. DHA depletion may therefore be related to the development of the neuropathy and retinopathy seen in LCHAD- and TFP-deficiency. Because a diet with reduced long-chain fat in patients with these disorders may also result in depletion of DHA or the n-3 fatty acid series precursor, alpha-linolenic acid, a secondary DHA deficiency could play a role in progression of disease. Whereas neuropathy is a chronic, progressive feature, episodes of myoglobinuria are triggered, transient, and preventable. Skeletal and cardiac muscle are highly oxidative tissues that utilize long-chain fatty acid substrates to generate energy. Therefore, during periods of fasting or prolonged exercise with increased bioenergetic demands, acute myopathy may be observed in long-chain FAO defects. Exercise-triggered myoglobinuria occurs with highly elevated serum CK, whereas between episodes these individuals demonstrate normal or only slightly elevated serum CK. Extreme elevation of serum CK during episodic metabolic derangements in TFP deficiency suggests necrosis and degeneration of muscle fibers, whereas normal CK concentrations during periods of clinical stability are consistent with regeneration and the histopathological findings of isolated atrophic muscle fibers. As in VLCAD and CPT II deficiency, severe energy depletion or toxic effects of long-chain 3-hydroxy fatty acids or acylcarnitines may contribute to the severe muscle cell destruction during acute episodes. Excessive amounts of palmitoylcarnitine have detergent properties on isolated canine myocytic sarcolemmal membranes and can potentiate free radical-induced lipid membrane peroxidative injury in ischemia.16

In our study, 90% of patients presented with exercise-induced, and 78% with illness-induced, myoglobinuria (Table 2). These episodes were accompanied by respiratory failure in 45% of patients, suggesting severe weakness of the diaphragm and other respiratory muscles. Stress-induced respiratory failure has also been described in a patient with CPT II deficiency14 and in TFP-deficient mice.11 Life-threatening respiratory failure and severe myoglobinuria potentially resulting in renal failure can be prevented by implementation of a high carbohydrate and low-fat diet with regularly scheduled meals and equidistantly-placed intermeal snacks. In long-chain fatty acid oxidation defects, limiting the intake of long-chain fats may be helpful; the substitution of medium-chain triglyceride oil for complete dietary fats has met with variable success.20

Major discoveries have been made in the genetic analysis of muscular dystrophies, spinal muscular atrophies, and hereditary neuropathies, which have changed the indications for use of muscle and nerve biopsies in the evaluation of children with presumed neuromuscular disorders.2 This applies to fatty acid oxidation defects as well. The diagnosis can be suspected on the basis of the clinical presentation, the plasma acylcarnitine profile,21 and on urinary organic acid analysis, and should be confirmed by enzymatic analysis in cultured skin fibroblasts and molecular genetic analysis. Between acute catabolic episodes, individuals with TFP deficiency may remain biochemically silent, emphasizing the need for a high index of clinical suspicion and for the collection of samples during an acute catabolic event.

We conclude that progressive peripheral motor-sensory neuropathy and episodic myoglobinuria, with onset between 1 and 6 years of age, characterize a major phenotype of TFP deficiency, and that the peripheral neuropathy can precede episodes of myoglobinuria by some years. Therefore, TFP deficiency must be included in the differential diagnosis of early-onset, progressive peripheral neuropathy with or without episodic myoglobinuria. With diagnosis, prevention of life-threatening rhabdomyolysis and acute respiratory failure is possible, and DHA sup-
plementation may reverse or slow progression of the associated neuropathy.

Many referring physicians, who suspected metabolic disorders, sent patients’ samples for analysis, and provided clinical data, made this work possible. We especially want to thank Dr. G.T. Berry, Gail Chuck, Dr. C. Dionisi-Vici, Dr. T. McGrew, Dr. Bryan E. Hainline, Dr. Joyce Kobori, Dr. Nicola Longo, Dr. Tony Persyk, Dr. Beverly Powell, Dr. Charles Roe, Diane Roe, Dr. Jerry Vockley, and Dr. Rebecca S. Wanner.

REFERENCES