E．結論

主に小児領域における診断システム整備を開始した。酵素解析、microscale oxygraphy 解析、遺伝子パネル、エクソーム解析等を連携して行うことが重要である。これらのシステムにより新規遺伝子の発見、病態解明、治療へのリンクが可能になってくる。

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H．知的財産権の出願・登録状況

（予定を含む。）

1．特許取得　　なし

2．実用新案登録　なし

3．その他　　なし

別紙３

厚生労働科学研究委託（難治性疾患実用化研究事業）
分担報告書

ミトコンドリア病診療の質を高める、レジストリシステムの構築、診断基準・診療ガイドラインの策定および診断システムの整備を行う臨床研究（H26-委託(難)-一般-072 ）

分担研究課題：　ミトコンドリア病診断システムの整備
ミトコンドリア病診療ガイドラインの策定

研究分担者：高嶋　博（鹿児島大学大学院医歯学総合研究科神経内科　教授）

研究要旨

ミトコンドリア病は、臨床的に多様な疾患であり、通常で考えられている以上に軽症の成人ミトコンドリア病が存在すると考えられる。また遺伝形式も母系遺伝だけでなく多様である。発症年齢は出生時～老年期発症まで拡がりがあり、小児期と成人期での表現型の違いも本症の確定診断を困難にしている。また、ミトコンドリア病が母親から由来するミトコンドリア DNA の異常でも通常の遺伝病と同じく核遺伝子の変異でももたらされ、そのミトコンドリア関連核遺伝子が 1500 以上と膨大である。我々は、次世代シークエンサーを用いることで、ミトコンドリア病の効率的診断方法の確立を目指した。また、診療ガイドラインの策定に関してはKSS/CPEO を担当し、診療ガイドラインを策定するために、論文の収集と評価を行なった。

研究協力者

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（鹿児島大学大学院医歯学総合研究科神経内科）

A．研究目的

ミトコンドリア病は、臨床的に多様な疾患である。我々は通常で考えられている以上に軽症の成人ミトコンドリア病が存在すると考えている。また遺伝形式も母系遺伝だけでなく多様である。発症年齢は出生時～老年期発症まで拡がりがあり、小児期と成人期での表現型の違いも本症の確定診断を困難にしている。そしてなにより一番の問題はミトコンドリア病が母親から由来するミトコンドリア DNA の異常でも通常の遺伝病と同じく核遺伝子の変異でももたらされ、そのミトコンドリア関連核遺伝子が 1500 以上と膨大なことである。しかし近年、次世代シークエンサーを利用することにより遺伝学的診断効率は格段にあがっている。我々は本法を用いた遺伝学的診断について、既に Charcot-Marie-Tooth 病で十分な経験を有している。本法を用いたミトコンドリア病の効率的診断方法の確立を目指す。

B．研究方法

1992 年から 2013 年、臨床的にミトコンドリア病と診断され、筋病理でミトコンドリア異常を確認した 53 例を次世代シークエンサー （MiSeq & HiSeq 2000）を用いて解析した。 Exome-sequencing は、パイロットスタディーとして 10 例の症例に行なった。方法として米国 Baylor 医科大学が用いている NGS パネルを基本に、いくつかのオリジナルの検索遺伝子を追加し評価した。まず、ミオパチー/横紋筋融解に関連 40 個の first panel を調べ、 次に追加でミトコンドリア関連 127 個の second panel を調べた。　抽出された変異は, Polyphen, SIFT などの公的ソフトを用いて病的意義について検討し

た。

C．研究結果

核遺伝子の検討では first panel で RRM2B, DGUOK, SUCLG1, CPT2 などに SNPs が検出されたが，病的意義としては、RRM2B が唯一原因遺伝子である可能性があった。Second panel でも８個の遺伝子が候補として導出されたが，病的遺伝子といえるものはなかった．また上記の遺伝子パネルを用いた方法でない手法で鉄芽球性貧血を伴った兄妹例に候補遺伝子として ABCB7 が同定された．

D．考察

今回の検討では新規原因遺伝子の探索も期待して、特殊例も含めて検討したため、期待していた以上に診断効率が上がらなかった。ミトコンドリア関連の核遺伝子は 1500 以上とされる。ミトコンドリア病はミトコンドリアのヘテロプラスミーがあるため、DNA 採取臓器により結果が異なる可能性もある。核遺伝子検査まで行う必要があるかどうかについての基準を設けることも診断効率の上昇に関係するものと考えられた。また原因同定のためのワークフローの確立についてはさらなる検討を要する．

診療ガイドラインの策定に関しては KSS/CPEO を担当。本年度は診療ガイドラインを策定するために、クリニカルクエスチョン形式に問題点を抽出できるように仮のクエスチョンを設定し、論文の収集と評価を行なった。希少疾患のため症例報告レベルの報告が多いが、一つ一つの報告は臨床的に示唆に富むものも多い。生活習慣病のガイドラインのようにエビデンスレベルで文献を選択することがないように注意が必要であると思われた。また偏った報告の収集にならないように、同一の疾患に対して、複数のガイドライン委員の関与が必要と思われた。

E．結論

Exome sequencing では *RRM2B* に変異を認め家系間内の解析を進めているところである。*ABCB7* は鉄芽球性貧血の原因としては確立されているが、ミオパチーとしての報告がこれまでになく、ミトコンドリアミオパチーの新規原因遺伝子の可能性が高い。今後、生化学的検査や機能解析を要する。

G．研究発表

なし。

H．知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得　　なし。
2. 実用新案登録　なし。
3. その他　なし。

Ⅲ．研究成果の刊行に関する一覧表

様式第１９

委託業務題目　「ミトコンドリア病診療の質を高める、レジストリシステムの構築、診断基準・診療ガイドラインの策定および診断システムの整備を行う臨床研究」

機関名　千葉県がんセンター

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
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Pathophysiology of stroke-like episodes in MELAS: a 23-year observational study. The 55th Annual Meeting of the Japanese Society of Neurology. International Workshop and Oral Presentation 15: Stroke 2,	Iizuka T, Tominaga N, Ishima D, Kaneko J, Nishiyama K.	第 55 回日本神経学会学術大会	2014.5.23	国内
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Neuropathology of MELAS in the acute stage of stroke-like episode.	Daita Kaneda, Masayuki Shintaku, Mie Kubota-Sakashita, Tadafumi Kato, Yu-ichi Goto	国際神経病理学会	2014.9.14-18	国外
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掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
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各論 III ミトコンドリア代謝異常症・Mitochondrial Disease 4.各疾患について (2) ミトコンドリア呼吸鎖異常症 b) Complex II(ミトコンドリア呼吸鎖複合体 II) 欠損症	村山　圭	代謝性ミオパチー	2014	国内
各論 III ミトコンドリア代謝異常症・Mitochondrial Disease 4.各疾患について (2) ミトコンドリア呼吸鎖異常症 c) Complex III (ミトコンドリア呼吸鎖複合体 III) 欠損症	村山　圭	代謝性ミオパチー	2014	国内
各論 III ミトコンドリア代謝異常症・Mitochondrial Disease 4.各疾患について (2) ミトコンドリア呼吸鎖異常症 d) Complex IV (ミトコンドリア呼吸鎖複合体 IV) 欠損症	村山　圭	代謝性ミオパチー	2014	国内
各論 III ミトコンドリア代謝異常症・Mitochondrial Disease 4.各疾患について (2) ミトコンドリア呼吸鎖異常症 e) Complex V (ミトコンドリア呼吸鎖複合体 V) 欠損症	村山　圭	代謝性ミオパチー	2014	国内
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小脳萎縮症	小坂　仁	新領域別症候群シリーズ No.29「神経症候群（第２版）IV、日本臨牀社	2014	国内
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MERRF	井川正道，米田誠	代謝性ミオパチー	2014	国内
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頭痛とミトコンドリア病	飯塚高浩	神経眼科	2014	国内
L-アルギニン（MELAS）	古賀靖敏	引いて調べる先天代謝異常	2014	国内
ミトコンドリア機能の臨床生化学的評価	古賀靖敏	代謝性ミオパチー	2014	国内
ミトコンドリア代謝異常症のトピックス	古賀靖敏	代謝性ミオパチー	2014	国内
ミトコンドリアにおける代謝	古賀靖敏	代謝性ミオパチー，診断と治療社	2014	国内
ミトコンドリア病の診断の進め方	古賀靖敏	代謝性ミオパチー	2014	国内
ミトコンドリア代謝異常症の臨床的病型による分類　MELAS	古賀靖敏	代謝性ミオパチー	2014	国内
ミトコンドリア病の新しいバイオマーカーFGF21,	八ツ賀秀一、古賀靖敏	Clinical Neuroscience　偏桃体—up to date	2014	国内
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GDF-15 is a novel biomarker to evaluate efficacy of pyruvate therapy for mitochondrial diseases.	Fujita Y, Ito M, Kojima T, Yatsuga S, Koga Y, Tanaka M	Mitochondrion	2014	国外
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New TRPM6 mutation and management of hypomagnesaemia with secondary hypocalcaemia.	Katayama K, Povalko N, Yatsuga S, Nishioka J, Kakuma T, Matsuishi T, Koga Y	Brain Dev	2014	国外
Cdk5rap1-Mediated 2-Methylthio Modification of Mitochondrial tRNAs Governs Protein Translation and Contributes to Myopathy in Mice and Humans.	Wei Fan-Yan, Zhou Bo, Suzuki Takeo, Miyata Keishi, Ujihara Yoshihiro, Horiguchi Haruki, Takahashi Nozomu, Xie Peiyu, Michiue Hiroyuki, Fujimura Atsushi, Kaitsuka Taku, Matsui Hideki, Koga Yasutoshi, Mohri Satoshi, Suzuki Tsutomu, Oike Yuichi, Tomizawa Kazuhito.	Cell Metabolism.	2015	国外
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Fever of Unknown Origin as the Initial Manifestation of Valproate-Induced Fanconi Syndrome.	Nozaki F, Kumada T, Kusunoki T, Fujii T, Murayama K, Ohtake A	Pediatr Neurol	2014	国外

（注１）　発表者氏名は、連名による発表の場合には、筆頭者を先頭にして全員を記載すること。

（注２）　本様式は excel 形式にて作成し、甲が求める場合は別途電子データを納入すること。

Ⅳ. 研究成果の刊行物・別刷

COQ4 Mutations Cause a Broad Spectrum of Mitochondrial Disorders Associated with CoQ_{10} Deficiency

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Primary coenzyme Q10 (CoQ_{10}) deficiencies are rare, clinically heterogeneous disorders caused by mutations in several genes encoding proteins involved in CoQ_{10} biosynthesis. CoQ_{10} is an essential component of the electron transport chain (ETC), where it shuttles electrons from complex I or II to complex III. By whole-exome sequencing, we identified five individuals carrying biallelic mutations in *COQ4*. The precise function of human COQ4 is not known, but it seems to play a structural role in stabilizing a multiheteromeric complex that contains most of the CoQ_{10} biosynthetic enzymes. The clinical phenotypes of the five subjects varied widely, but four had a prenatal or perinatal onset with early fatal outcome. Two unrelated individuals presented with severe hypotonia, bradycardia, respiratory insufficiency, and heart failure; two sisters showed antenatal cerebellar hypoplasia, neonatal respiratory-distress syndrome, and epileptic encephalopathy. The fifth subject had an early-onset but slowly progressive clinical course dominated by neurological deterioration with hardly any involvement of other organs. All available specimens from affected subjects showed reduced amounts of CoQ_{10} and often displayed a decrease in CoQ_{10}-dependent ETC complex activities. The pathogenic role of all identified mutations was experimentally validated in a recombinant yeast model; oxidative growth, strongly impaired in strains lacking COQ4, was corrected by expression of human wild-type *COQ4* cDNA but failed to be corrected by expression of *COQ4* cDNAs with any of the mutations identified in affected subjects. *COQ4* mutations are responsible for early-onset mitochondrial diseases with heterogeneous clinical presentations and associated with CoQ10 deficiency.

Coenzyme Q (CoQ), or ubiquinone, is a lipophilic component of the electron transport chain (ETC), where it shuttles electrons derived from NADH and $FADH_2$ to ETC complex III (cIII) or ubiquinone-cytochrome c reductase. The main electron donors to CoQ are ETC complexes I (cI) and II (cII) but also include other mitochondrial flavoproteins, for instance, electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial (ETF-dehydrogenase [ETFDH]), which is the terminal component of fatty acid β-oxidation and branched-chain amino acid oxidation pathways. CoQ can also act as an antioxidant and a membrane stabilizer, is a cofactor of additional mitochondrial enzymes (e.g., uncoupling protein UCP1),[1,2] and plays an indispensable role in the de novo pyrimidine biosynthesis as the electron acceptor from dihydroorotate dehydrogenase.[3–5]

CoQ is a 1,4-benzoquinone with a tail of 10 isoprenyl units in humans (CoQ_{10}) but of variable length in other species (e.g., CoQ_6 in yeast). The synthesis of the isoprenoid moieties proceeds via either mevalonate or

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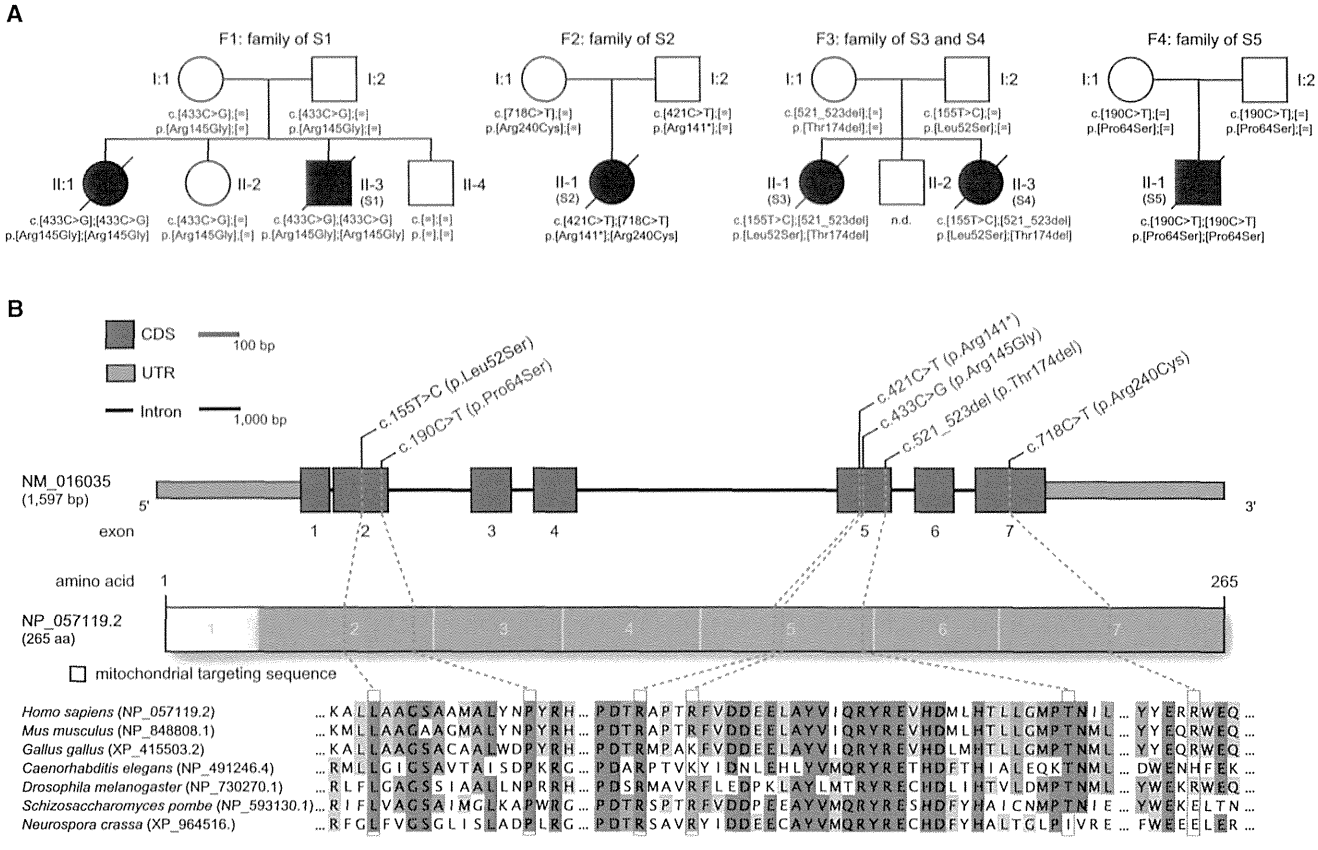

Figure 1. Pedigrees of Investigated Families and *COQ4* Structure and Conservation of Identified Mutations
(A) Pedigrees of four families affected by mutations in *COQ4*. The mutation status of affected and unaffected family members is indicated by closed and open symbols, respectively.
(B) *COQ4* structure showing the identified mutations. The structure of the gene product, COQ4, is also shown with known domains and localization and conservation of amino acid residues affected by the mutations. Intronic regions are not drawn to scale.

2-C-methyl-D-erythritol 4-phosphate pathways, whereas the aromatic precursor of the CoQ benzoquinone ring is p-hydroxybenzoate, derived from tyrosine.[6] After the isoprenoid "tail" is bound to the aromatic "head," the ring undergoes sequential modification. At least ten enzymes participate in CoQ biosynthesis; in yeast, and possibly mammals as well, these enzymes are all localized in mitochondria.

Primary CoQ_{10} deficiency is the biochemical signature of a group of rare, clinically heterogeneous autosomal-recessive disorders caused by mutations in several genes encoding proteins involved in CoQ_{10} biosynthesis.[7] Mutations in *COQ2* (MIM 609825), *COQ6* (MIM 614647), *ADCK3* (*COQ8* [MIM 606980]), *ADCK4* (MIM 615573), *COQ9* (MIM 612837), *PDSS1* (MIM 607429), and *PDSS2* (MIM 610564) have been reported in subjects with severe infantile mitochondrial syndromes associated with severe tissue CoQ_{10} deficiency, whereas the genetic bases underpinning adult-onset CoQ_{10} deficiency remain mostly undefined.[8,9] *COQ4* (MIM 612898) codes for a ubiquitously expressed 265-amino-acid protein that is peripherally associated with the mitochondrial inner membrane on the matrix side;[10] the precise function of human COQ4 is not known, but the yeast ortholog seems to play a structural role crucial in the stabilization of a multiheteromeric complex including several, if not all, of the CoQ biosynthetic enzymes.[11]

We report here the identification of pathogenic biallelic *COQ4* mutations in a total of five individuals from four families; these subjects were part of a cohort of severe mitochondrial cases where the CoQ_{10} defect was not anticipated. The family pedigrees are shown in Figure 1A.

Subject 1 (S1; II-3, family 1), a boy, was the third of four siblings and was born to healthy, non-consanguineous Italian parents after an uncomplicated pregnancy and elective cesarean delivery. His oldest sister (II-1), who presented with bradycardia and hypotonia, died at birth, and his 16-year-old second sister and his 5-year-old brother are alive and well. At birth, S1 had a weight of 3,410 g, a length of 49.5 cm, and a head circumference of 34.5 cm. Apgar scores were 7 and 10 at 1 and 5 min after birth, respectively. At birth, his condition appeared critical, given that he showed severe hypotonia, areflexia, acrocyanosis, bradycardia, and respiratory insufficiency. Ultrasound examination revealed markedly decreased motility of the left ventricle with an ejection fraction of 20%–25%. No evidence of hepatic or renal impairment was observed. Dobutamine infusion via an umbilical venous catheter was

Table 1. Mitochondrial ETC Activities in Muscle

	Subject	cI/CS[a]	cI+III/CS[a]	cII/CS[a]	cII+III/CS[a]	cIII/CS[a]	cIV/CS[a]	CS[b]
Muscle biopsy	S1[c]	36	24	N	34	N	N	64
	S2[c]	6	ND	42	43	10	30	57
	S3	N	N	N	55	N	50	54
	S4	145	N	N	N	222	189	109
	S5	<5	ND	N	30	50	N	65

Abbreviations are as follows: N, value in the control range; ND, not done; cI, complex I; cII, complex II; cIII, complex III; cIV, complex IV; cI+III, coupled activity of complexes I and III; and cII+III, coupled activity of complexes II and III. The analyses were performed in different laboratories, and the reference values are diverse (they usually range between 60% and 140% of the mean control value). The values of ETC complex activities out of the control range (specific to each enzymatic activity and to each laboratory) are reported.
[a]Mean control value (%) of CS-normalized ETC complex activities.
[b]Percentage of mean control value.
[c]Sample from autopsy.

ineffective, and the baby died 4 hr after birth. His blood glucose level was normal, as were renal and hepatic parameters; plasma creatine kinase was moderately elevated (861 U/l; normal value [n.v.] < 400), and blood lactate was extremely high (20.1 mM; n.v. < 2). Analysis of urinary organic acids showed elevated levels of 2-OH glutaric acid, whereas plasma and urinary amino acids were within normal ranges. The autopsy examination revealed left ventricular hypoplasia with septum hypertrophy and a patent ductus arteriosus. No brain examination was performed.

The activities of the ETC complexes in autoptic skeletal-muscle homogenate showed severe defects of both coupled cI+cIII and cII+cIII reactions, normalized to citrate synthase (CS), and a decrease in CS-normalized cI (Table 1). In both liver and cultured fibroblasts, the CS-normalized activities of each of the individual ETC complexes were in the control range. Although the coupled cI+cIII activity cannot be reliably assayed in cultured cells,[12] the coupled cII+cIII activity was clearly decreased in S1 fibroblasts (65% of the control mean).

S2 (II-1, family 2) was born at the 34th week of gestation and was the female first child of non-consanguineous Japanese parents. Her birth weight was 1,120 g (−2.2 SDs). Apgar scores were 7 and 8 at 1 and 5 minutes after birth, respectively. There was no family history of neurological or cardiac disease. The pregnancy was complicated by severe intrauterine growth delay and ultrasound-documented hypertrophic cardiomyopathy. On S2's first day of life, she became apnoeic and was intubated as a result of respiratory failure. She initially displayed moderate lactic acidosis, but soon after her admission to Neonatal Medical Center, her lactic acidosis rapidly worsened (blood lactate = 11.2–18.8 mM; n.v. < 2); her hypertrophic cardiomyopathy evolved into severe heart failure, leading to death at the age of 1 day.

The metabolic profile (urinary and plasmatic amino acids, organic acids, and acylcarnitines) showed no significant findings. A liver autoptic specimen showed a severe deficiency of cI (cI/CS ratio = 2.9%); autoptic skeletal-muscle homogenate also showed a cI deficiency together with less pronounced reductions of other ETC complexes (Table 1).

Sisters S3 (II-1, family 3) and S4 (II-3, family 3) are the first and third, respectively, of three siblings and were born to healthy, non-consanguineous Austrian parents. Their brother (II-2) is a healthy, unaffected boy. S3 and S4 were born prematurely at gestational ages of 32 weeks (birth weight = 1,550 g) and 34 weeks (birth weight = 2,170 g), respectively.

Performed at the 20th week of gestation, prenatal organ screening of S3 revealed a suspected malformation of the cerebellum. A postnatal cranial ultrasound showed cerebellar hypoplasia. After birth, she showed distal arthrogryposis, but no other dysmorphic features. At birth, she suffered from respiratory-distress syndrome, and a few hours later, a severe myoclonic epileptic encephalopathy ensued; blood lactic acid at 36 hr of age was 6.4 mM and rose to 14 mM prior to her death by multiorgan failure on the third day of life. Echocardiography showed a normal heart. Metabolic investigations (amino acids in plasma, acylcarnitine profile, and standard newborn screening) were essentially normal. Analysis of organic acids in urine showed excretion of glycerol and 2-OH-glutarate. In frozen postmortem muscle (obtained within 30 min after death), ETC enzyme activities were slightly decreased (Table 1). An autopsy of the brain revealed severe olivopontocerebellar and thalamic hypoplasia and scattered cavitations in the white matter; the visceral organs appeared normal for the gestational age.

Six years later, prenatal organ screening of the sister, S4, showed cerebellar hypoplasia, suggesting the same disease as in S3. Similar to her sister, S4 suffered from neonatal respiratory distress. No dysmorphic features were present. Echocardiography was normal. A cranial ultrasound confirmed cerebellar hypoplasia. Six hours after birth, epileptic encephalopathy ensued; blood lactic acid was 3.5 mM at 2 hr of age and rose to 9 mM at death on the second day of life. Metabolic investigations showed normal newborn-screening results and a normal acylcarnitine profile. Amino acids in plasma were grossly elevated but showed no specific pattern. Analysis of urinary organic acids showed excretion of a "mitochondrial dysfunctional pattern" with malate, fumarate, and 2-OH-glutarate, as

well as vitamin B6 metabolites and N-acetyl-tyrosine. Analysis of frozen postmortem muscle showed elevated levels of ETC activities (Table 1). In both girls, blood glucose concentration and renal and hepatic parameters were in the normal range.

S5 (II-1, family 4) is an 18-year-old young man and is the only offspring of healthy Italian parents who deny consanguinity and originate from a medium-size town in southern Italy. Pregnancy was normal, and delivery was via cesarean section because of a podalic presentation. He was born at term, and his weight at birth was 4,100 g. Weight and motor development were reportedly normal in his first year of life, but he started to show slowly progressive motor deterioration after the age of 10 months, when he manifested unsteadiness in maintaining acquired sitting position. He achieved the ability to walk with a spastic ataxic gait at 3 years of age but lost ambulation by 6 years of age and has been wheelchair bound since then. At 12 years of age, he started manifesting epileptic seizures in the form of prolonged right-side hemiclonic seizures. MRI showed bilateral increased signal intensity in fluid-attenuated-inversion-recovery and T2-weighted sequences in both occipital-cortical and juxtacortical areas (Figures S1A–S1D). Around the same period, he started to have swallowing difficulties. He was admitted for extensive investigation. Thorough blood tests excluded liver and kidney involvement and did not show lactic acidosis. A specific pattern of organic aciduria was excluded. Electrophysiological examination showed a sensory motor polyneuropathy with slowed conduction velocities. During a 5-year follow-up, he showed a slowly progressive downhill course with recurrent treatment-resistant seizures, worsened swallowing impairment, progressive scoliosis, and cognitive deterioration. A muscle biopsy was performed when he was 12 years old. Spectrophotometric assays of the ETC complexes in muscle homogenate showed virtually undetectable cI/CS ratios and reduced cII+cIII/CS and cIII/CS ratios. The other ETC complex activities were within control limits (Table 1). Since the age of 15 years, he has used a percutaneous-endoscopic-gastronomy tube and has developed severe scoliosis with a Cobb angle of 75°. Control MRI performed when he was 17 years old showed cerebellar atrophy, widening of ventricular brain spaces, and scars from cortical necrotic lesions in both occipital areas (Figures S1E–S1H).

In agreement with the Declaration of Helsinki, informed consent for genetic and biochemical studies was signed by the parents of all subjects, and the ethics committee of the Technische Universität München approved the study.

We performed whole-exome sequencing (WES) to investigate the molecular bases of the mitochondrial disease presentations of S1, S4, and S5, as described previously.[13] Coding DNA sequences were enriched with a SureSelect Human All Exon 50 Mb V4 or V5 Kit (Agilent) and subsequently sequenced on a HiSeq2500 system (Illumina). Read alignment to the human reference assembly (UCSC Genome Browser hg19) was done with the Burrows-Wheeler Aligner (version 0.7.5), and single-nucleotide variants and small insertions and deletions were identified with SAMtools (version 0.1.19). On the basis of the rare disease phenotype and a pattern concordant with autosomal-recessive inheritance, we sought genes carrying rare (minor allele frequency [MAF] < 0.1% in 4,500 control exomes) variants predicted to be compound heterozygous or homozygous. We then prioritized variants in genes coding for proteins with known or predicted mitochondrial localization.[14] This filtering strategy led to the identification of recessive variants in *COQ4*, coding for a mitochondrial protein involved in CoQ_{10} biosynthesis,[10] in all three subjects. In S2, we used the SeqCap EZ Library (version 1.0; Roche NimbleGen). Details on the bioinformatics pipeline and variant filtering have been reported recently.[15] Sequencing statistics are provided in Table S1.

We identified *COQ4* mutations (RefSeq accession number NM_016035.3) in four individuals (Figure 1). In S1, we identified a homozygous missense variant, c.433C>G (p.Arg145Gly). Both parents and a healthy sister are heterozygous carriers, and a healthy brother has two reference alleles. No material was available from the deceased sister. S2 was found to be compound heterozygous for a nonsense variant on the paternal allele and a missense variant on the maternal allele: c.[421C>T];[718C>T], p.[Arg141*];[Arg240Cys]. S4 was found to be compound heterozygous for a missense mutation and an exon 5 in-frame deletion: c.[155T>C];[521_523delCCA], p.[Leu52Ser];[Thr174del]. Both variants were also confirmed in the DNA of S3, whereas the parents are heterozygous for only one variant each (the father carries the missense mutation, and the mother carries the deletion). In S5, we identified a homozygous mutation, c.190C>T (p.Pro64Ser). Both parents are heterozygous for this mutation.

None of the identified variants are present in our exome database, which contains 4,500 samples, or in public SNP databases, including dbSNP, the NHLBI Exome Sequencing Project Exome Variant Server, and the Exome Aggregation Consortium (ExAC) Browser. The only exception is the c.718C>T variant (rs143441644), which is reported to have an extremely low frequency (MAF = 0.00023; 28/12,0330 alleles) in the ExAC Browser. Moreover, all missense changes are predicted to be deleterious by several bioinformatics tools (Table S2).

Because of the identified genetic defects, we tested CoQ_{10} levels in available specimens from the subjects. In a muscle biopsy from S1, we detected a clear reduction of CoQ_{10} (32.9 nmol CoQ_{10}/g protein; n.v. = 101–183; 1.16 nmol CoQ_{10}/CS; n.v. = 1.75–3.46). In fibroblasts from S1, the levels of CoQ_{10} were also lower than CoQ_{10} levels in neonatal control fibroblasts (54% of control mean). In frozen muscle from S3, CoQ_{10} was reduced (13.5 nmol CoQ_{10}/g protein; n.v. = 160–1,200; 0.3 nmol CoQ_{10}/CS; n.v. = 2.7–7); in muscle from S4, CoQ_{10} was profoundly reduced (25.7 nmol CoQ_{10}/g protein; n.v. = 160–1,200; 0.1 nmol CoQ_{10}/CS; n.v. = 2.7–7), whereas in S5 muscle, the amount of CoQ_{10} was slightly decreased