

Respiratory chain deficiency in nonmitochondrial disease

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Supplemental data
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ABSTRACT

Objective: In this study, we report 5 patients with heterogeneous phenotypes and biochemical evidence of respiratory chain (RC) deficiency; however, the molecular diagnosis is not mitochondrial disease.

Methods: The reported patients were identified from a cohort of 60 patients in whom RC enzyme deficiency suggested mitochondrial disease and underwent whole-exome sequencing.

Results: Five patients had disease-causing variants in nonmitochondrial disease genes *ORAI1*, *CAPN3*, *COLQ*, *EXOSC8*, and *ANO10*, which would have been missed on targeted next-generation panels or on MitoExome analysis.

Conclusions: Our data demonstrate that RC abnormalities may be secondary to various cellular processes, including calcium metabolism, neuromuscular transmission, and abnormal messenger RNA degradation. *Neurol Genet* 2015;1:e6; doi: 10.1212/NXG.000000000000006

GLOSSARY

ARE = AU-rich element; **CoQ10** = coenzyme Q₁₀; **LGMD2A** = limb-girdle muscular dystrophy type 2A; **mRNA** = messenger RNA; **mtDNA** = mitochondrial DNA; **RC** = respiratory chain.

Respiratory chain (RC) complex defects are hallmarks of mitochondrial disease that often provide the first diagnostic evidence for a mitochondrial disorder and guide subsequent DNA analysis of the mitochondrial DNA (mtDNA) and nuclear genes encoding mitochondrial proteins.¹ In a substantial proportion of cases, it is not possible to reach a molecular diagnosis, and it is assumed that most of these patients carry undetected mutations in genes coding for mitochondrial proteins. This report presents 5 patients with heterogeneous phenotypes and biochemical features of RC deficiency; however, the molecular diagnosis is not mitochondrial disease.

METHODS Patients with biochemical evidence of RC deficiencies measured by standard methods in accredited laboratories in whom mtDNA mutations had been excluded were selected from a cohort of 60 patients for whole-exome sequencing.¹ The diagnosis of a mitochondrial RC deficiency was based on the recently published consensus paper by the Mitochondrial Medicine Society (USA).² Coverage and depth statistics of exome sequencing for each patient and the bioinformatic prediction for each mutation are listed in tables e-1 and e-2 at Neurology.org/ng.

Standard protocol approvals, registrations, and patient consents. Informed consent was obtained from all participants in accordance with protocols approved by local institutions and research ethics committees.

RESULTS Patient 1 presented with generalized muscular hypotonia, failure to thrive, liver dysfunction, atrial septal defect, and severe immunodeficiency. Based on the biochemical defect of complex I (50%) and IV (80% rest-activity) in skeletal muscle, mitochondrial disease was suggested. Exome sequencing identified a homozygous mutation c.587T>C, p.Leu196Pro in the *ORAI1* gene, which is predicted to be deleterious with 6 different prediction programs (table e-2) and is located next to a pathogenic mutation (p.Leu194Pro).³ *ORAI1*

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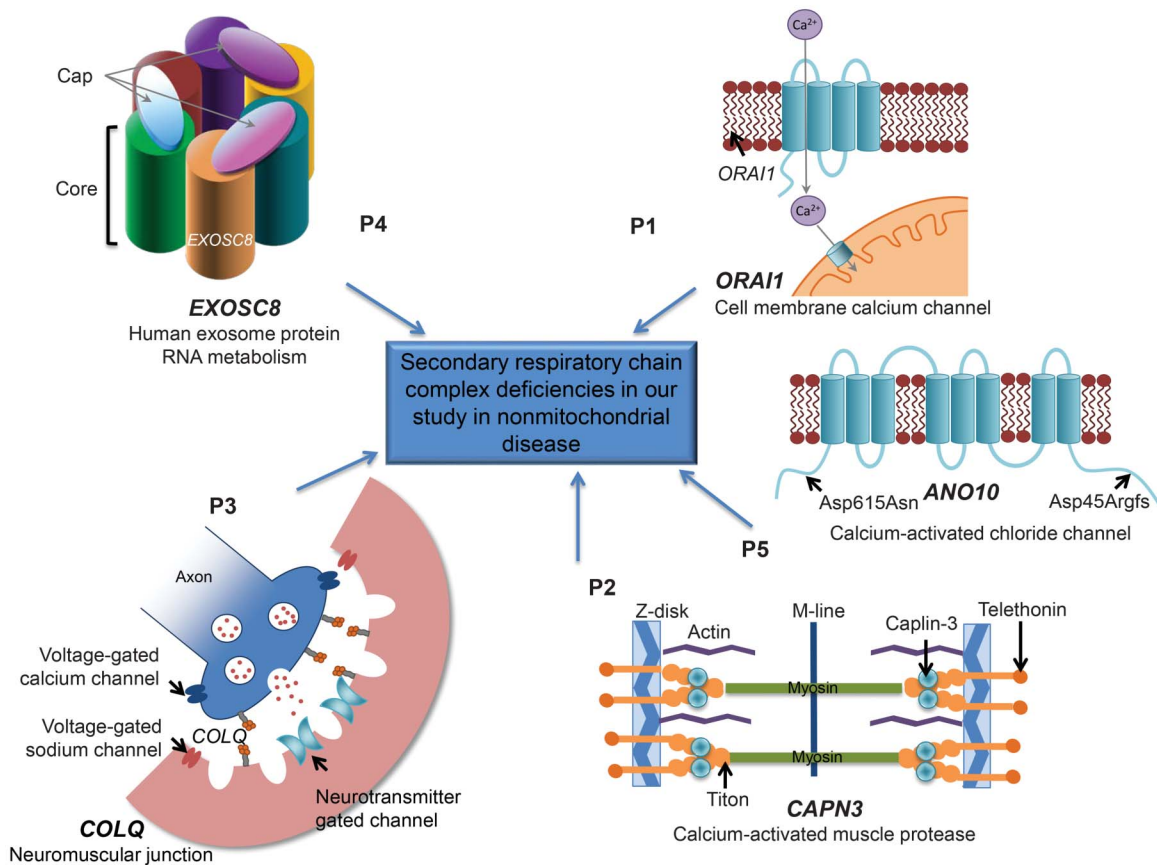
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Figure Detailed clinical and molecular genetic characteristics of patients in this study and localization of nuclear mutant proteins resulting in respiratory chain deficiencies

A

Patient	Family history	Age at onset/death or current age/Sex (†=Death)	Phenotype	Muscle biopsy	Gene	Variant
P1	Negative	Birth/ 9m†/F	Muscular hypotonia, rotary nystagmus, immunodeficiency, failure to thrive, hepatopathy, atrial septum defect	Complex I + IV ↓	<i>ORAI1</i>	Homozygous c.587T>C; p.Leu196Pro
P2	One affected sibling	14y/17y/F	Juvenile-onset slowly progressive myopathy with limb-girdle muscle weakness	Mild complex IV ↓	<i>CAPN3</i>	Homozygous c.1715G>A; p.Arg572Gln
P3	Consanguineous one affected sibling	Birth/6y/M	Axial muscle weakness, ptosis, and respiratory failure requiring ventilation	Complex I + IV ↓	<i>COLQ</i>	Homozygous c.636delA; p.Lys212fs
P4	Consanguineous 17 affected family members	2m/9m†/F	Muscle weakness, spastic tetraparesis, psychomotor retardation, visual loss, hearing loss, respiratory problems, hypomyelination, cerebellar hypoplasia, and thin corpus callosum	Complex I + IV ↓	<i>EXOSC8</i>	Homozygous c.815G>C; p.Ser272Thr
P5	Negative	7y/57y/F	Cerebellar ataxia and atrophy, dysarthria, mental retardation, seizures, and saccadic pursuit	Complex III ↓ low CoQ ₁₀	<i>ANO10</i>	Compound heterozygous c.132_133insT;p.Asp45Argfs c.1843G>A;p.Asp615Asn

B



(A) Table of clinical and molecular genetic characteristics of 5 patients with heterogeneous phenotypes and biochemical features of respiratory chain deficiency. (B) Crystal structure of the human exosome complex involved in RNA metabolism. *EXOSC8* highlighted in pink. *ORAI1*, calcium channel located on the cell membrane. *ANO10*, calcium-activated chloride channel located on the cell membrane. *CAPN3*, an intracellular calcium-activated neutral protease isoform located within the sarcomere. *COLQ* protein, a collagen-like subunit associated with acetylcholinesterase in skeletal muscle. CoQ₁₀ = coenzyme Q₁₀.

encodes a calcium release-activated calcium channel protein that allows cellular calcium influx³ and may alter mitochondrial calcium metabolism, leading to

RC deficiency. Autosomal recessive mutations in *ORAI1* have previously been described in association with severe combined immunodeficiency and

congenital myopathy and in dominantly inherited tubular aggregate myopathy with hypocalcemia.³

Patient 2 presented with a juvenile-onset slowly progressive myopathy and limb-girdle muscle weakness. The clinical diagnosis of a mitochondrial myopathy was based on the detection of cytochrome *c* oxidase–negative fibers and decreased complex IV activity in muscle (80% rest-activity). A known pathogenic homozygous c.1715G>A, p.Arg572Gln mutation was detected in *CAPN3* encoding a skeletal muscle-specific intracellular calcium-activated neutral protease, and its deficiency leads to oxidative stress and potentially a secondary RC deficiency.⁴ This is a previously published pathogenic mutation and the only variant that segregated with the disease in the family. *CAPN3* mutations are common causes of limb-girdle muscular dystrophy type 2A (LGMD2A).⁴ Muscle biopsy shows a dystrophic pattern; however, disorganized mitochondria and reduced complex V levels were occasionally reported in a few patients with LGMD2A.⁴

Patient 3 presented with axial muscle weakness, ptosis, respiratory failure requiring ventilation, and reduced IV (80% rest-activity) and borderline complex I activities. We detected a homozygous frameshift mutation c.636delA, p.Lys212fs in the *COLQ* gene. Mutations of the acetylcholinesterase collagen-like tail subunit gene (*COLQ*) cause congenital myasthenic syndromes due to the deficiency of acetylcholine esterase at the synaptic endplate.⁵ The clinical phenotype includes hypotonia, muscle weakness, delayed motor development, ophthalmoparesis, and respiratory failure. No involvement of mitochondria has been suggested in previous patients to date.⁵

Patient 4 belonged to a large Hungarian Roma pedigree presenting with hypomyelination, hypoplasia of the cerebellum and corpus callosum, and spinal muscular atrophy. Muscle biopsy revealed reduced complex I (60% rest-activity) and IV (80% rest-activity). We identified a homozygous c.815G>C, p.Ser272Thr mutation in a novel disease gene *EXOSC8* encoding an exosome subunit involved in AU-rich element (ARE)-containing messenger RNA (mRNA) degradation, and the altered myelination was due to abnormal degradation of ARE myelin proteins.⁶ The pathogenicity of the mutation was supported by the absence of *EXOSC8* protein in myoblasts of the patient and by segregation with the disease in the large pedigree.⁶ A secondary RC defect in these patients may be related to abnormal degradation of ARE mRNAs of mitochondrial disease genes.

Patient 5 presented in childhood with epilepsy and learning difficulties and developed cerebellar ataxia, brisk reflexes, and nystagmus from age 45 years. Complex III defect (60% rest-activity) and low coenzyme Q₁₀ (CoQ₁₀) (60% of low normal) were

detected in her muscle. We identified compound heterozygous pathogenic mutations in *ANO10* (c.132_133insT, p.Asp45Arg fs*53 and c.1843G>A, p.Asp615Asn).⁷ The pathogenicity of the missense variant c.1843G>A, p.Asp615Asn has been confirmed by its detection in additional patients.⁸ CoQ₁₀ supplementation resulted in clinical improvement, emphasizing the importance of low CoQ₁₀ in the pathomechanism.⁷ *ANO10* encodes an anoctamin protein forming calcium-activated chloride channels, implying that an abnormal calcium metabolism may cause mitochondrial dysfunction in ANO10 deficiency.⁷

DISCUSSION Typically, RC defects are associated with mutations in the mtDNA or in nuclear genes affecting mitochondrial proteins. The patients we present here had disease-causing (previously reported or nonsense) variants in nonmitochondrial disease genes, which would have been missed on targeted next-generation panels or on MitoExome analysis.⁹ Our data demonstrate that RC abnormalities may be secondary to various cellular processes, including calcium metabolism, neuromuscular transmission, and abnormal mRNA degradation; however, the role of mitochondria in these diseases needs further investigation (figure). Secondary RC defects in patients with neurogenetic disease have previously been reported. For example, Alexander disease is caused by nuclear mutations encoding non-RC chain proteins, but patients exhibit biochemical and MRI findings typically found with RC abnormalities.¹⁰

Faced with increasing numbers of published disease-causing variants, improved and more accessible sequencing techniques, and more comprehensive phenotype characterization, the clinician should keep an open mind when approaching genetic testing and not limit testing to predicted disease-causing variants in suspicion of mitochondrial disease.

AUTHOR CONTRIBUTIONS

Dr. A.P.: study concept and design, acquisition of data, and drafting of the manuscript. Dr. H.J.N.: study concept and design, acquisition of data, and drafting of the manuscript. Dr. H.G.: analysis and interpretation. Dr. A.A.: critical revision of the manuscript for important intellectual content. Dr. J.K.: acquisition of data. Prof. I.B.: acquisition of data. Dr. M.C.: acquisition of data. Dr. K.D.: analysis and interpretation. Ms. L.F.: analysis and interpretation. Dr. M.K.: acquisition of data. Dr. B.C.: acquisition of data. Dr. S.K.: acquisition of data. Dr. M.S.-K.: interpretation of data. Dr. V.K.: acquisition of data. Prof. E.H.-F.: critical revision of the manuscript for important intellectual content. Prof. P.F.C.: critical revision of the manuscript for important intellectual content. Prof. R.H.: study concept and design, acquisition of data, and drafting of the manuscript.

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DISCLOSURE

Dr. Pyle, Dr. Nightingale, Dr. Griffin, and Dr. Abicht report no disclosures. Dr. Kirschner has served on scientific advisory boards for PTC Therapeutics ISIS/Biogen Roche Pharma; has received funding for travel and/or speaker honoraria for ENMC workshop on neuromuscular diseases (nonprofit); has served as Associate Editor of the *Orphanet Journal of Rare Diseases*; and has received research support from the German Ministry of Research and Education European Union. Dr. Baric has received funding for travel and/or speaker honoraria from the Annual Multidisciplinary European Phenylketonuria Symposium; has served on the editorial board of *Journal of Inherited Metabolic Disease*; has served as principal investigator in clinical trials sponsored by Synageva BioPharma; and has received research support from Ministry of Science, Education and Sports of Republic of Croatia and Innermed. Dr. Cuk, Dr. Douroudis, Ms. Feder, Dr. Kratz, Dr. Czermin, and Dr. Kleinle report no disclosures. Dr. Santibanez-Koref has received research support from Medical Research Council, The Leverhulme Trust, and Sir Jules Thorn Charitable Trust. Dr. Karcagi and Dr. Holinski-Feder report no disclosures. Dr. Chinnery has served on the editorial board of *Brain* and has received research support from the Medical Research Council (United Kingdom). Dr. Horvath has received funding from the Medical Research Council (United Kingdom) (G1000848), the European Research Council (309548), and from the Mitochondrial European Educational Training (MEET), ITN MARIE CURIE PEOPLE, (317433). Go to Neurology.org/ng for full disclosure forms.

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