Diagnostic Exome Sequencing Identifies Alterations in the Newly Characterized Gene, *COQ4*: Expanding the Phenotypic Spectrum

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BACKGROUND

- The *COQ4* gene (OMIM_612898) is located on chromosome 9q34.11 and consists of 7 coding exons.
- It is a newly characterized gene that encodes a mitochondrial protein involved in the organization of a multi-enzyme complex for the biosynthesis of Coenzyme Q10 (CoQ_{10}).
- CoQ_{10} is an essential mitochondrial inner membrane-associated lipid that acts as a carrier for electrons from respiratory complexes I and II to complex III.
- Previously reported patients with *COQ4*-associated CoQ_{10} deficiency have varied clinical presentations including acrocyanosis, bradycardia, hypotonia, lactic acidosis, respiratory insufficiency, hypertrophic cardiomyopathy, arthrogryposis, cerebellar hypoplasia, severe myoclonic epileptic encephalopathy, progressive ataxia, and scoliosis (Brea-Calvo *et al.* 2015, Chung *et al.* 2015).
- Early death in the neonatal period to months after birth occurred in the majority of the cases and none were reported to have dysmorphic features or microcephaly (Brea-Calvo *et al.* 2015, Chung *et al.* 2015).

CASE REPORT

- Here we report an additional non-consanguineous family with two affected siblings found to have compound heterozygous mutations in *COQ4.*
- The proband is a 16 year old Caucasian girl with infantile onset seizures, severe intellectual disability, microcephaly, short stature, visual cortical dysfunction, neuromuscular scoliosis with restrictive lung disease, pancytopenia, recurrent infections, and epistaxis.
- She also has dysmorphic features including a prominent nose, extended nasal columella, small mouth, small hands and feet with significant contractures, and small limbs (Figure 3).
- Consistent with a mitochondrial disorder, the patient's lactate and pyruvate normalized following CoQ_{10} supplementation.
- Her 18 year old brother is similarly affected with severe intellectual disability, dysmorphic features, multifocal intractable seizures, feeding problems, constipation, asthma, cortical blindness and contractures.

METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process.
- **Whole exome sequencing and data analysis:** Samples were prepared using the SeqCap EZ VCRome 2.0 (Roche NimbleGen, Madison, WI). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA). Data analysis were performed as previously described (Farwell *et al.* 2014).
- **Targeted Sanger sequencing** was performed to confirm the alterations and determine co-segregation in this family.

RESULTS

- The proband was found to have two missense alterations in *COQ4*: c.202G>C (p.D68H)/c. 469C>A (p.Q157K)
- Co-segregation analysis using Sanger sequencing revealed that the affected brother was also compound heterozygous for both alterations and an unaffected brother was a heterozygous carrier of the p.D68H alteration.
- Co-segregation analysis also revealed that her unaffected mother carried the p.D68H alteration and her unaffected father carried the p.Q157K alteration.
- **p.D68H (p.Asp68His)**
  - The amino acid at this position is completely conserved in available vertebrate species and the alteration is predicted to be probably damaging by Polyphen and deleterious by SIFT *in silico* analysis (Figure 1).
  - Based on data from the NHLBI Exome Sequencing Project (ESP), the alteration was not observed among 5,880 individuals tested.
  - A recent study reported two affected siblings with the p.D68H alteration in compound heterozygous with p.R66Q (Chung *et al.* 2015).
- **p.Q157K (p.Gln157Lys)**
  - The amino acid at this position is well conserved in available vertebrate species and the alteration is predicted to be possibly damaging by Polyphen and tolerated by SIFT *in silico* analysis (Figure 2).
  - Based on data from the NHLBI Exome Sequencing Project (ESP), the alteration was not observed among 6,503 individuals tested.

Figure 1. Conservation and *in silico* data for c. 202G>C (p.D68H)

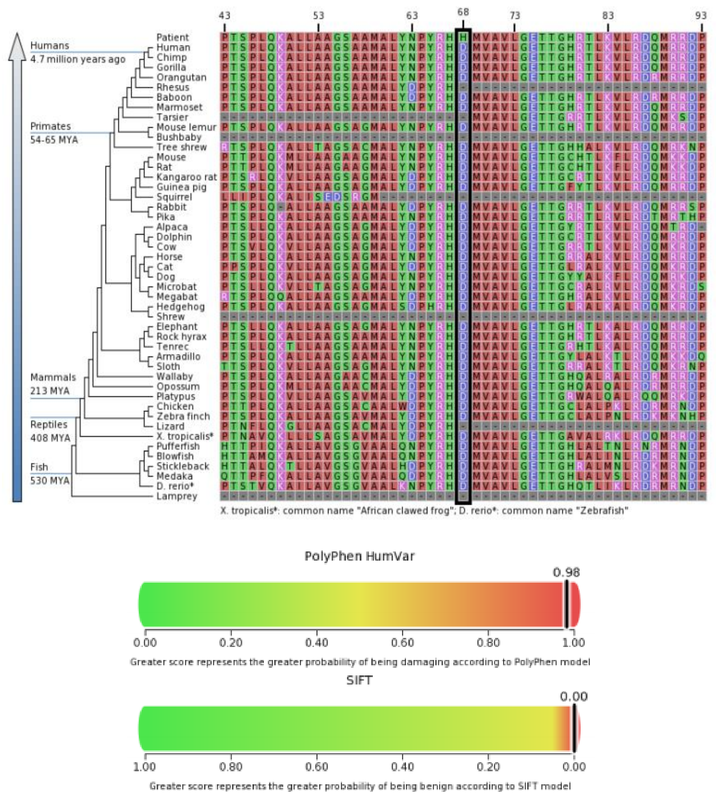


Figure 2. Conservation and *in silico* data for c. 469C>A (p.Q157K)

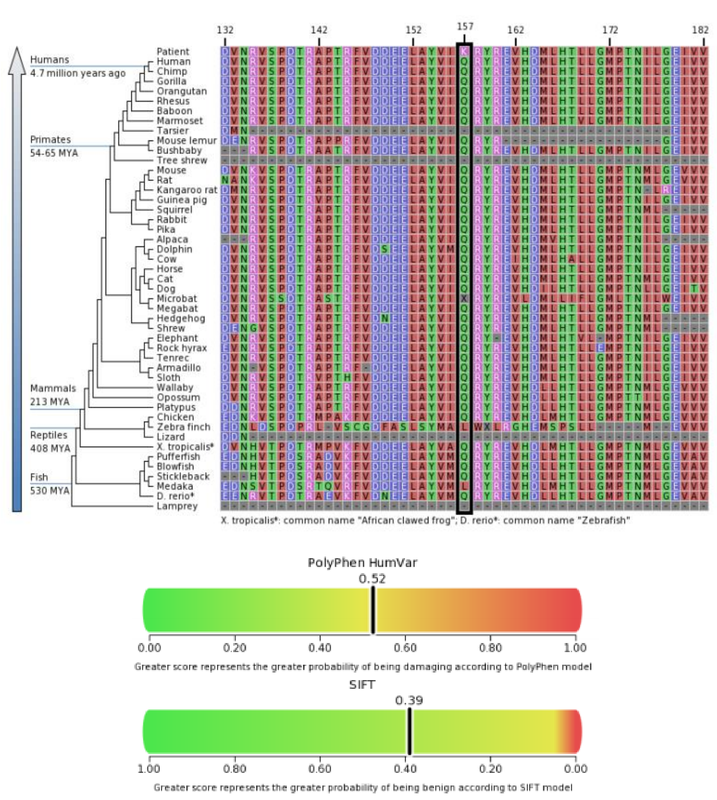


Figure 3. Proband and her brother with significant neurological deficits, microcephaly, contractures, and dysmorphic features.

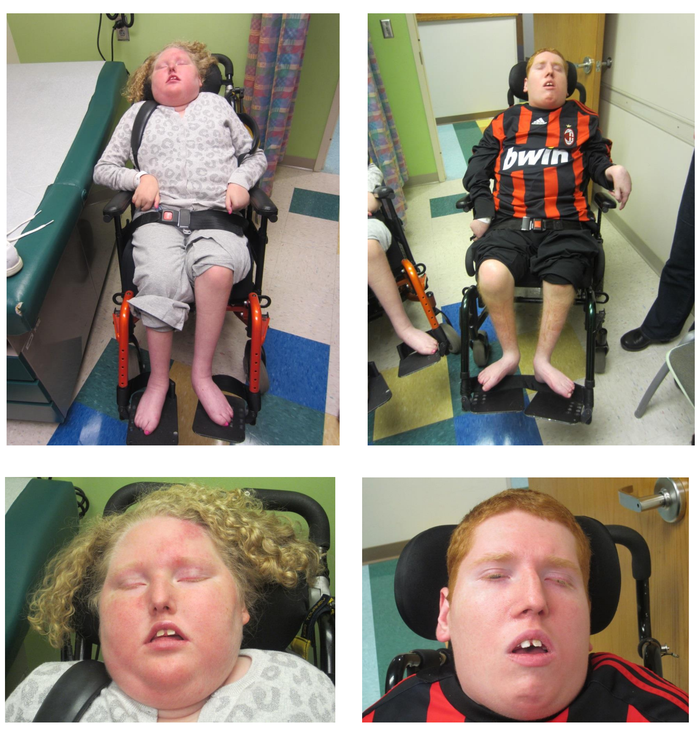
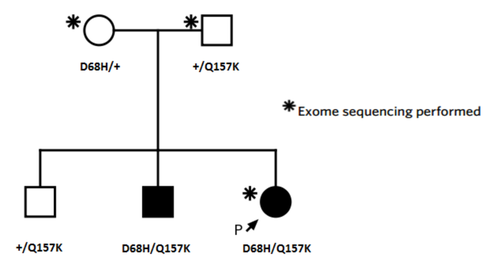


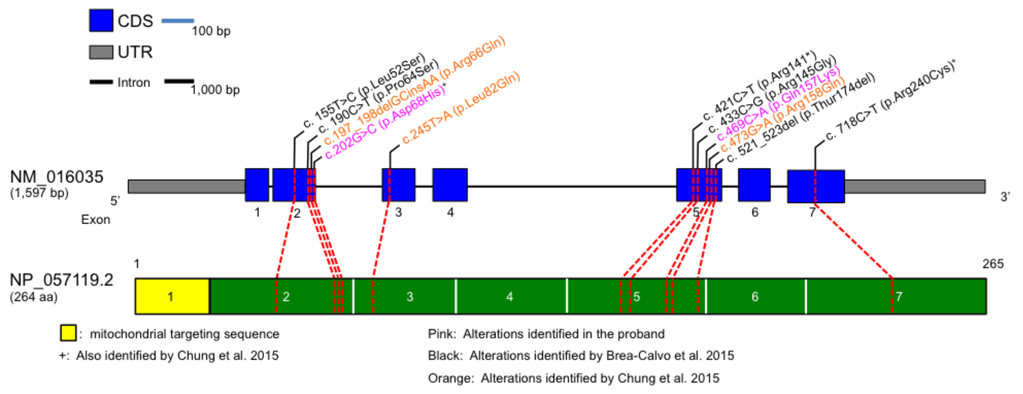
Figure 4. Co-segregation data



TAKE-HOME POINTS

- Family-centered diagnostic exome sequencing (DES) on the proband and her healthy parents revealed compound heterozygous c.202G>C (p.D68H) and c.469C>A (p.Q157K) missense alterations in *COQ4*.
- The alterations co-segregated in this family and were also present in her affected brother.
- Based on the available evidence, the c.202G>C (p.D68H) and c.469C>A (p.Q157K) alterations are classified as likely pathogenic variants.
- While the majority of previously reported patients with *COQ4*-associated primary CoQ_{10} deficiency had severe disease leading to early death and were not known to have dysmorphic features, the disease progression in this family is slower and includes dysmorphic features, thus expanding the phenotypic spectrum.

Figure 5. *COQ4* alterations reported to date
(Adapted from Brea-Calvo *et al.* 2015)



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