TITLE: IDENTIFICATION OF NOVEL CCD MUTATIONS IN *RYR1*

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INTRODUCTION: Central Core Disease (CCD) is a rare, autosomal dominant severe subtype of Malignant Hyperthermia Susceptibility (MHS) that has a phenotype without anaesthetic exposure¹. CCD is a congenital myopathy, characterized by the presence of amorphous cores on histopathologic examination of skeletal muscle fibres, hypotonia, and sometimes life-threatening reactions triggered by commonly used anaesthetics. MHS and CCD can be caused by mutations in the ryanodine receptor gene (*RYR1*)¹. The protocol for sequence analysis of the large 15 kilobase *RYR1* transcript from human blood and muscle, previously used for detection of MHS mutations, was applied to search for *RYR1* mutations causative of CCD².

METHODS: Following Institutional Research Ethics Board approval, and subject informed consent, *RYR1* transcript RNA was extracted from peripheral blood and analyzed by Reverse Transcription (RT), Polymerase Chain Reaction (PCR), and direct sequencing. Inheritance of the mutation within a family was studied by restriction fragment length polymorphism (RFLP) analysis and sequencing of genomic DNA.

RESULTS: In three CCD families studied, one known, and two novel *RYR1* mutations were identified by direct sequencing of RT-PCR amplified transcripts. The previously reported³ mutation (Arg2435Leu) mutation was present in one CCD family. The novel mutations, Phe4808Leu and Leu4647Pro were each found in two other unrelated CCD families. Complete segregation was demonstrated with the CCD phenotype and the Arg2435Leu and the Phe4808Leu mutations. These mutations were absent in unaffected relatives and normal controls. The functional consequences of these mutations are being studied.

DISCUSSION: We have successfully identified *RYR1* mutations using peripheral blood from subjects with CCD, thus demonstrating that blood can be used as an alternative to muscle as a source of *RYR1* transcripts for genetic study. A causal role for each of the mutations identified in CCD patients is supported by evolutionary conservation of the mutated amino acid; their location in the MH/CCD regions of *RYR1*; their absence from unaffected subjects and normal controls; and by complete segregation with the disease phenotype. Our findings confirm the crucial role of *RYR1* in the aetiology of CCD.

REFERENCES:

1. Eur J Biochem 267: 5291-7
2. Anesthesiology 99: 289-296
3. J Med Genet 36: 115-118