**44608 - RYR1 MUTATIONS IN CANADIAN SUBJECTS WITH MALIGNANT HYPERTHERMIA AND CENTRAL CORE DISEASE**

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INTRODUCTION: The RYR1 gene encodes the skeletal muscle calcium channel (ryanodine receptor isoform 1) which plays a crucial role in the process of excitation-contraction coupling and skeletal muscle calcium homeostasis.1 To date, more than 100 mutations in RYR1 have been found world-wide in patients with Malignant Hyperthermia (MH) and Central Core Disease (CCD)2 with different frequencies in different populations.3,4,5 Here we summarize the interim results of the first comprehensive RYR1 mutation screening conducted in the Canadian MH/CCD patients.

METHODS: Subjects representing the Canadian MH / CCD population were recruited for this study based on MH/CCD clinical manifestations and contracture test results. Following Institutional Research Ethics Board approval, and individual informed consent, DNA and RNA were extracted from peripheral blood, and RYR1 transcript was analyzed using reverse transcription (RT), polymerase chain reaction (PCR), and direct sequencing.6 Inheritance of detected mutations within a family was studied using Restriction Fragment Length Polymorphism (RFLP) analysis and sequencing of genomic DNA. The importance of each novel mutation was analyzed in silico on the basis of evolutionary conservation of the amino acid, changes in biochemical properties between wild type and mutant amino acid (SIFT7), and predicted structural localization within the RYR1 protein.

RESULTS: Screening of the complete 15 kilobase RYR1 transcript in RNA samples from 11 MH probands and of the 6 kilobase RYR1 mutation hotspot regions in 34 MH susceptible individuals revealed the presence of 21 RYR1 mutations in 20 of 45 subjects (44%). Nine mutations are newly discovered, and all 9 are predicted to be potentially pathogenic. Only 12 of the 21 mutations (57%) had been previously described in RYR1. Five out of 45 subjects (11%) were compound heterozygotes for two separate mutations. In addition, 15 synonymous silent polymorphic sites were identified in RYR1.

CONCLUSIONS: Our concerted effort so far has resulted in the most comprehensive DNA collection representative of the MH/CCD population in Canada. In this group, testing just for known RYR1 mutations would have detected only 57% of the 21 mutations so far discovered. Several novel MH mutations occur outside of the three accepted RYR1 hotspot regions. Moreover, the presence of more than one RYR1 mutation in some MH patients indicated that genetic screening for MH should not be limited to RYR1 hotspot regions, but should cover the

complete RYR1 coding sequence. Functional testing in a heterologous expression system will be required to verify functional significance.

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