James Gilfert[1], Bonnie Marr[2], Stuart Groskin[2], Adam Chamberlin[3], Sha Tang[3], Zoe Powis[3], Melissa Byler[1], Robert Roger Lebel[1]

1) Section of Medical Genetics, Department of Pediatrics, SUNY Upstate Medical University, Syracuse, NY
2) Crouse Hospital, Syracuse, NY
3) Ambry Genetics, Aliso Viejo, CA

Title: A novel autosomal recessive alteration in the *RYR1* gene in a patient with profound hypotonia

Homozygous and compound heterozygous mutations in the *RYR1* gene have been reported to cause hypotonia, facial weakness, nemaline myopathy, respiratory insufficiency, swallowing disturbances, and ophthalmoplegia. Heterozygous mutations in *RYR1* have been associated with malignant hyperthermia, central core disease, multiminicore disease, and spondylocostal dysarthrosis. We report a female neonate homozygous for c.14203C>T (p. R4735W) in exon 98 of the *RYR1* gene, a novel alteration. The phenotype was notable for decreased fetal movement, profound hypotonia, cerebral dysfunction (by EEG), and cerebral atrophy (by MRI). She did not have any skeletal anomalies appreciated on several chest and abdomen radiographs (normal spine, ribs and appendicular girdles, in AP views; humerus 64 mm, normal with normal metaphyses). She died at age 17 days, when artificial support was discontinued at the parents' request.

Arginine at amino acid position 4735 is located in the Inter S2-S3 domain, a highly conserved 114-aminoc acid loop (positions 4,666 – 4779) important for RYR1 gating. This amino acid is highly conserved in available vertebrate species and is present in the pH-regulated prokaryotic potassium channel KcsA. Structural remodeling based on homology to the known rabbit RYR1 structure revealed that the residue R4735 lies in the VSC (cytoplasmic subdomain of the voltage-sensing like domain, VSL) and at the interface between monomers and contacts residues in the EF-hand subdomain of the Central domain and lies at the end of bundles of helices forming the VSL domain. The residue appears to form contacts with residues on adjacent EF-hand domain and with a network of charged residues lying on the alpha-helices of the VSL domain.

p. R4735W is an extremely rare allele in the control population (not in ESP and 1000 genomes and observed in 2 out of 121,158 total alleles studied in ExAC). This alteration is predicted to be probably damaging and tolerated by PolyPhen and SIFT *in silico* algorithms, respectively. An allelic change p.R4735Q, as well as some neighboring missense changes (p.Y4733D, p.G4734E, p.R4737Q, p.R4737W), have been reported in patients with malignant hyperthermia and are currently considered variants of uncertain significance.