Diagnostic Exome Sequencing (DES) of a Female Patient with Features of Autism Spectrum Disorder (ASD) Identifies a Novel Gene, *RYR3*, which Lies at the Human 15q14 Schizophrenia/ Autism Susceptibility Locus

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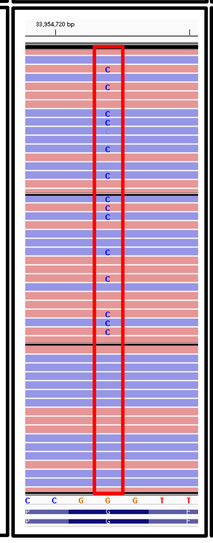
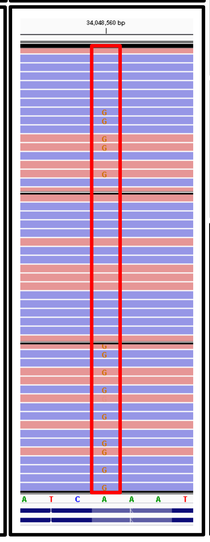
BACKGROUND

- Diagnostic exome sequencing (DES) is ideally suited to be the standard of care for diagnostic medicine in the 21st century because it offers a high diagnostic rate, guides clinical management, and lowers the overall cost of medical care (Farwell, 2014; Soden, 2014; Srivastava, 2014).
- The proband was a 4 year-old girl who presented multiple traits suggestive of ASD: she had very limited expressive language, made intermittent eye contact, and failed to consistently play with other children.
- The proband's parents are first-cousins and the family history is negative for other similarly affected individuals. SNP chromosome microarray did not identify any pathogenic copy number variants but did show multiple areas of homozygosity, which is consistent with the known consanguinity.
- Previous evaluation included a head MRI which showed a faint high signal in the periventricular white matter and the cerebellar white matter.
- DES was recommended because clinical evaluation and SNP chromosome microarray analysis failed to identify a causative genetic lesion underlying the patient's symptoms.

METHODS

- **Patients:** Genomic deoxyribonucleic acid was isolated from whole blood extracted from the proband and relatives who were referred to Ambry Genetics (Aliso Viejo, CA) for DES. Informed consent was obtained from all family members involved in the testing process.
- **Exome library preparation,** sequencing, bioinformatic processing, and data analysis were performed as previously described (Farwell , 2014).

FIGURE 1. Trio Exome Sequencing Identifies Compound Heterozygous Alterations in the Proband's *RYR3* Gene

SAMPLE	Exon 35 (c.4991) Chr15:33954722	Exon 59 (c.8569) Chr15:34048560
PROBAND COMPOUND HETEROZYGOTE c.4991G>C (p.G1664A) c.8569A>G (p.K2857E)		
FATHER HETEROZYGOTE c.4991G>C (p.G1664A)		
MOTHER HETEROZYGOTE c.8569A>G (p.K2857E)		

RYR3 NM_001036 p.G1664A c.4991G>C

Nucleotides		Proband	Father	Mother
Reference	G	48.28% (28/58)	50.98% (78/153)	100.00% (109/109)
Mutation	C	51.72% (30/58)	49.02% (75/153)	0.00% (0/109)
Q Score		245	457	350

RYR3 NM_001036 p.K2857E c.8569A>G

Nucleotides		Proband	Father	Mother
Reference	A	52.78% (38/72)	100.00% (96/96)	40.32% (25/62)
Mutation	G	47.22% (34/72)	0.00% (0/96)	59.68% (37/62)
Q Score		271	317	228

- **Red boxes (left) spanning the NGS reads indicate the presence of alterations in Exon 35 and Exon 59 of *RYR3.***
- **Note that the father and mother are heterozygous for *RYR3* c.4991G>C and c.8569A>G, respectively (lower two panels, left).**
- **As the proband (upper panels, left) inherits c.4991G>C from her father and c.8569A>G from her mother, she is compound heterozygous.**
- **No other genetic changes were considered likely to explain the clinical phenotype except for the aforementioned mutations.**

FIGURE 2. Sanger Sequencing Reveals That Compound Heterozygosity of the Two *RYR3* Alterations Co-Segregates with Affected Status Among the Five Siblings

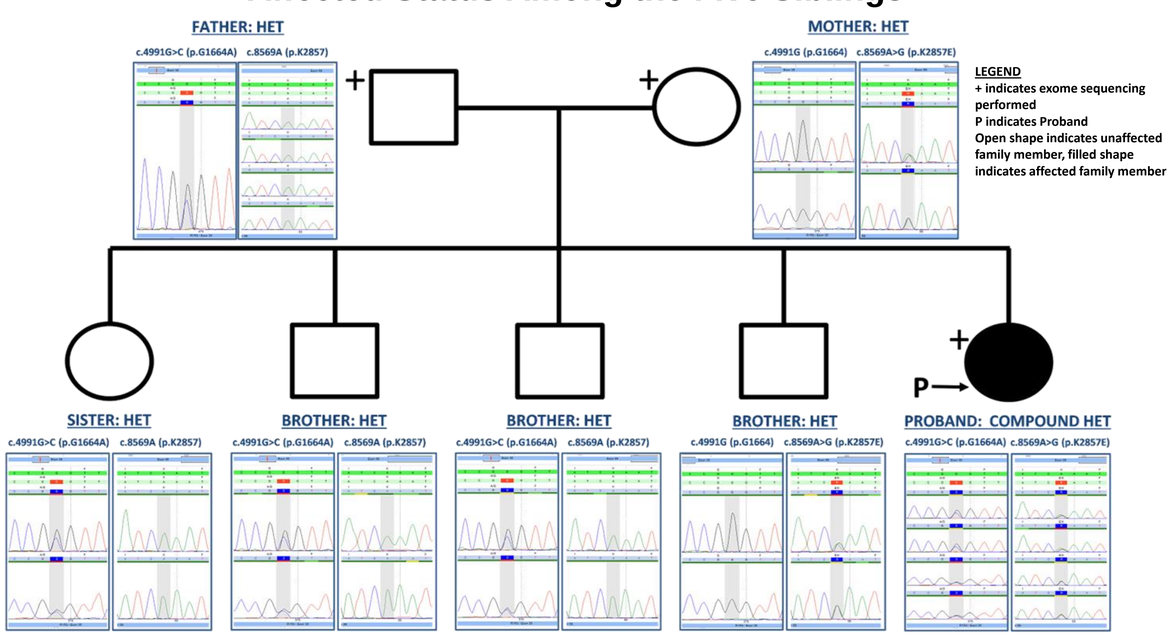
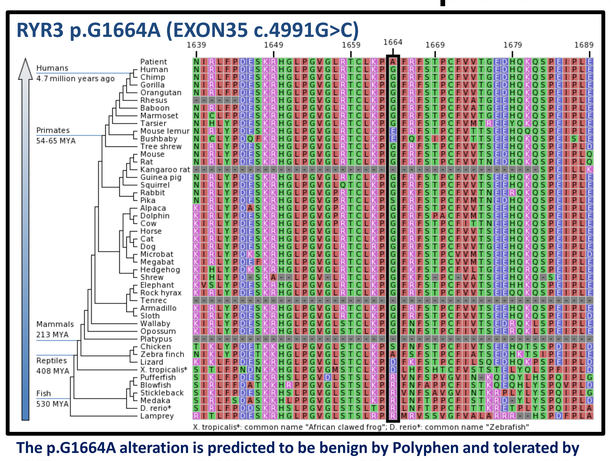
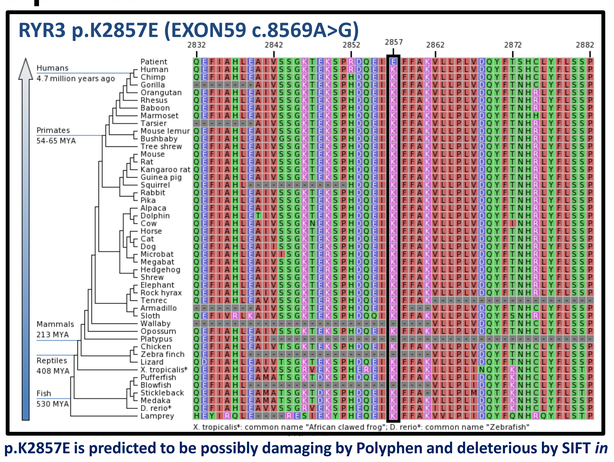


FIGURE 3. Amino Acid Conservation and *In Silico* Predictions for RYR3 p.G1664A and p.K2857E

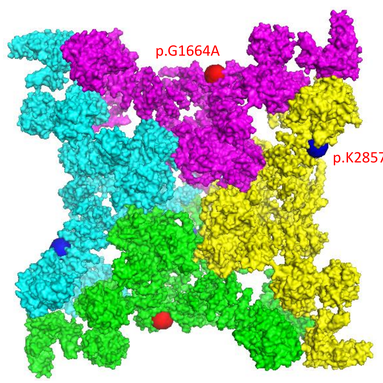


The p.G1664A alteration is predicted to be benign by Polyphen and tolerated by SIFT *in silico* analyses; p.G1664A is not found in ESP, 1000 Genomes, or dbSNP.



p.K2857E is predicted to be possibly damaging by Polyphen and deleterious by SIFT *in silico* analyses; pK2857E is not found in ESP, 1000 Genomes, or dbSNP.

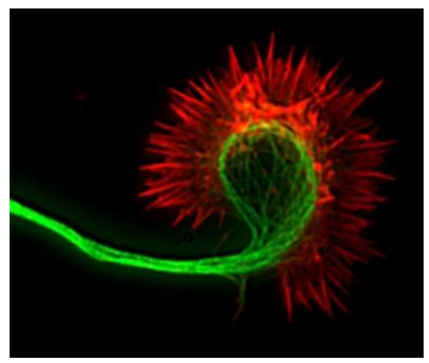
Evidence Suggests that *RYR3* Alterations Cause the Patient's Phenotype



VARIANT LOCATIONS ON THE RYANODINE RECEPTOR CALCIUM CHANNEL TETRAMER (Efremov, 2015)

- The three *RYR* genes encode transmembrane proteins that assemble into ~2.2 MDa homotetrameric calcium channel-receptors. They are members of ***A)*** the Ryanodine-Inositol 1,4,5-triphosphate Receptor Ca^{2+}Channel (RIR-CaC) Family and ***B)*** the Voltage-Gated Ion Channel (VIC) Superfamily (Uniprot **Q15413**).
- RYRs localize to the endoplasmic reticulum (ER) membrane where they serve to regulate intracellular Ca^{2+} homeostasis.
- Although Ca^{2+} is the major physiological ligand that triggers RYR channel opening and the subsequent influx of Ca^{2+} from the ER lumen into the cytoplasm (a process known as Ca^{2+}-induced Ca^{2+}-release, or CICR), a vast array of other molecules have been shown to modulate RYR activity (Van Petegem, 2012).
- The observed variants map to the surface of the protein (p.G1664- red sphere and p.K2857-blue sphere) in locations known to bind different RYR inhibitory modulators (Petegem, 2015).
- **RYR3** is preferentially expressed in brain (Matsuo, 2009; OMIM # 180903; Unigene: Hs.709373).
- ***RyR3*** knock-out mice exhibit altered social behavior that mimics ASD-like symptoms in humans: ***RyR3-/-*** mice exhibit statistically significant reductions in social contact and the animals tend to sleep alone. Further, such mice display increased locomotor activity and tend to run in circles (Balschun, 1999).

- ***RYR3*** plays a crucial role in neuronal development by guiding the steering of developing axons in response to external cues such as cell adhesion molecule-receptor binding. Such binding triggers specific signaling cascades that alter the activity of the intracellular cAMP-Protein Kinase A axis; this in turn modulates RYR3 activity. Activated RYR3 then triggers an influx of cytosolic Ca^{2+} within the growth cone of the migrating axon that causes the cone to turn toward the Ca^{2+} gradient in a process known as **attractive turning** (Ooashi, 2005).
- *De novo* missense alterations in ***RYR3*** and Ca^{2+} channel ***ITPR1*** have been reported in several unrelated patients with schizophrenia (Fromer, 2014; HGMD CM142577 , CM142578 and CM142712).
- There are multiple reports of patients who display ASD and schizophrenia symptoms and who harbor gross deletions and duplications that span the ***RYR3*** locus (DECIPHERv8.3).
- Alterations in other VIC superfamily members (***CACNA1D, CACNA1E, CACNA1H***) have been associated with neurological disease in humans, including ASD and schizophrenia (HGMD CM124574, CM124575, CM062487, CM062488, CM062489, CD142877).
- In support of the model proposed herein, ***RYR1*** alterations inherited in an autosomal recessive manner are known to cause Central Core Disease of Muscle (OMIM #117000).



RYR3 PLAYS A KEY ROLE IN AXONAL GROWTH CONE STEERING (IMAGE FROM KALIL, 2011)

CONCLUSIONS

- Clinical DES of a parents-proband trio, in conjunction with Sanger co-segregation analysis, revealed that two unique compound heterozygous ***RYR3*** missense alterations are the likely cause of disease in a child presenting ASD-like symptoms. Neither her parents nor her siblings, who are all unaffected, possess the alterations in combination.
- The fact that the alterations cause disease when present in compound heterozygous form owes to the quaternary structure of the **RYR3** Ca^{2+} channel. We propose that the alterations, each of which is situated in the cytoplasmic topological domain of a separate **RYR3** subunit, exert an effect *in trans* that alters interaction between structural moieties present on different and adjacent subunits to the point that typical channel function and/ or regulation of activity are compromised.
- To our knowledge, this is the first report of ***RYR3*** missense alterations causing ASD in humans. Ongoing functional studies will further define the impact of *RYR3* alterations on neurodevelopment and disease.

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