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Clinical Diagnostic Exome Sequencing Identified a novel Mutation in *ACTG2*, Possibly Due to Germline Mosaicism, in Two Affected Children with Megacystis and Echogenic Bowel in a Chinese Family

BACKGROUND

- Over the last two years, clinical diagnostic exome (CDE) sequencing has been instrumental in successfully providing a molecular diagnosis for families who had previously been unsuccessful in their pursuit of the underlying disease etiology.
- The proband is a newborn Chinese boy with a prenatal diagnosis of enlarged bladder and echogenic bowel. He also had mild bilateral hydronephrosis.
- The proband's family history is remarkable for a similarly affected older sister who presented with megacystis and echogenic bowel dysmotility. The presence of two affected children of opposite genders in this family led to a suspicion of autosomal recessive (AR) inheritance of the disorder.
- Previous molecular workup only included SNP array analysis, which was uninformative. Clinical diagnostic exome test was then ordered within one month after the proband's birth.

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the patient and his parents. Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).
- Exome data undergoes alignment, base calling, and variant calling. Passing base calls have at least 7x coverage and quality scores of Q20 or higher, which translates to a base call error rate probability of 1:100, or a base call read accuracy of 99%. Exons plus at least 2 bases into the 5' and 3' ends of all the introns are analyzed and reported. Variants were filtered further based on family history and possible inheritance models. Data is annotated with the Ambry Variant Analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, predicted functional impact, and clinical disease associations (Human Gene Mutation Database (HGMD; Stenson, 2009)), OMIM, and several other databases).
- A molecular geneticist performed interpretive filtering based on the deleterious nature of the candidate alterations literature search and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype.
- Each candidate variant was analyzed by Sanger sequencing for mutation confirmation and co-segregation analysis.

Table 1. NUMBER OF GENES & ALTERATIONS IDENTIFIED

NUMBER OF GENES & ALTERATIONS IDENTIFIED BASED ON BIOINFORMATICS & INTERPRETATION*

	Post-Inheritance Model Filtering			Post-Medical Review			Notable Candidate Genes		
	HGMD/ OMIM-Morbid‡	Clinically novel**	TOTAL	HGMD/ OMIM-Morbid	Clinically novel	TOTAL	HGMD/ OMIM-Morbid	Clinically novel	TOTAL
Autosomal Dominant Genes (Alterations)	5 (5)	12 (12)	**17 (17)**	1 (1)	9 (9)	**10 (10)**	1 (1)	0(0)	**1 (1)**
Autosomal Recessive Genes (Alterations)	8 (23)	11 (26)	**19 (49)**	0 (0)	11 (18)	**11 (18)**	0 (0)	0 (0)	**0 (0)**
X-linked Recessive Genes (Alterations)	3 (3)	7 (7)	**10 (10)**	0 (0)	7 (7)	**7 (7)**	0 (0)	0 (0)	**0 (0)**
X-Linked Dominant Genes (Alterations)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Y-linked Genes (Alterations)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TOTAL GENES (Alterations)	**15 (30)**	**31 (46)**	**46 (76)**	**1 (1)**	**27 (34)**	**28 (35)**	**1 (1)**	**0 (0)**	**1 (1)**

‡HGMD/ OMIM-Morbid: Listed in this category are genes that have been described in either the HGMD or OMIM-Morbid databases (the alterations within these genes are located in parentheses)
**A clinically novel gene is a gene which is not currently known to underlie a genetic condition.

Figure 1. Parents-Proband Trio Exome Sequencing Identified An Apparently "*de novo*" Missense Change, c.770G>A (p.R257H), in the *ACTG2* Gene

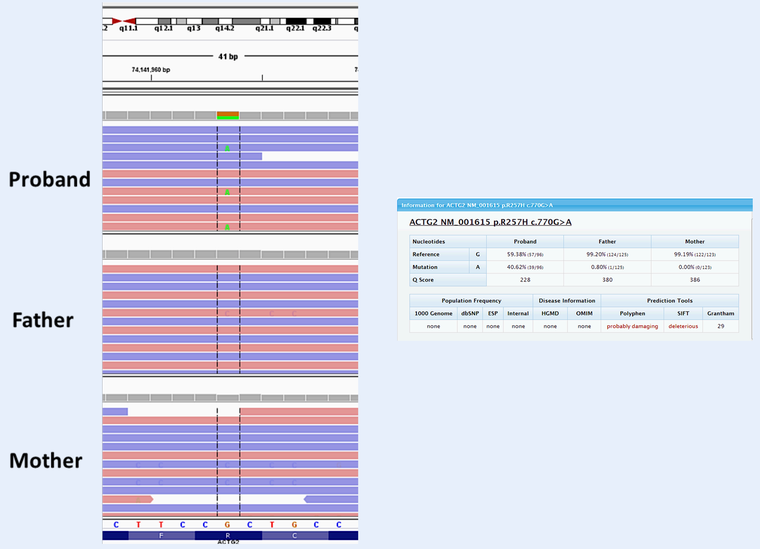
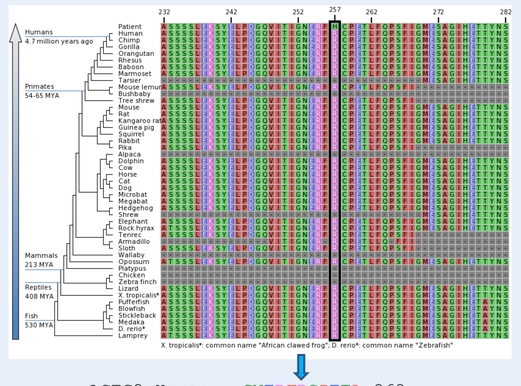


Figure 2. Amino acid R257 is Highly Conserved Throughout Evolution and Within the Actins

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ACTG2_Human    GNERFRCPETL 262
ACTA2_Human    GNERFRCPETL 263
ACTA1_Human    GNERFRCPETL 263
ACTC1_Human    GNERFRCPETL 263
ACTG1_Human    GNERFRCPEAL 261
ACTB_Human     GNERFRCPEAL 261
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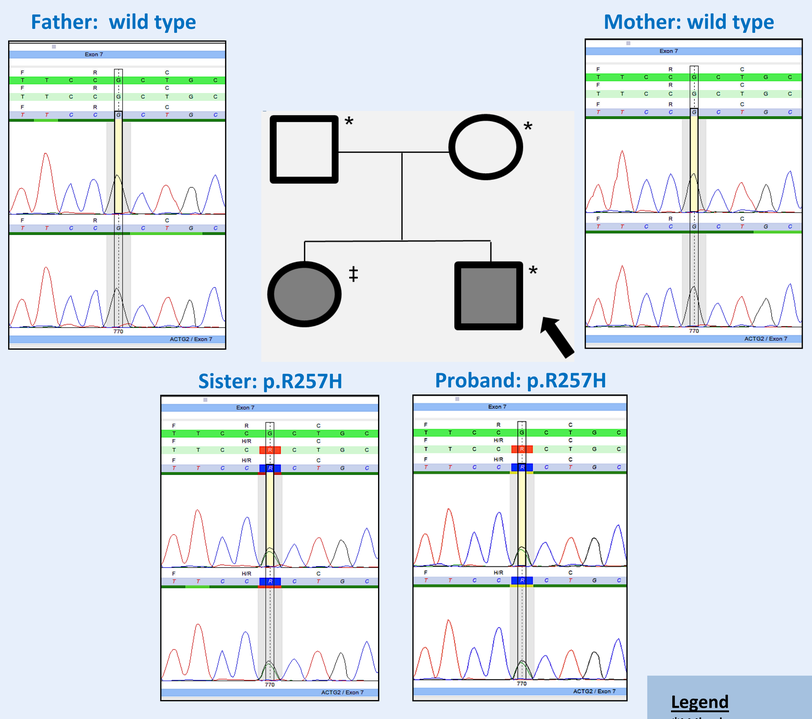
RESULTS/ DISCUSSION

- Full exome sequencing, bioinformatics analysis, and filtering based on autosomal and X-linked dominant and recessive inheritance models of the proband, mother, and father revealed 46 genes (76 unique alterations). Manual review to rule out sequencing artifacts and polymorphisms along with medical interpretation to rule out genes lacking clinical overlap with the patient's evaluated phenotype resulted in 28 genes (35 unique alterations) (**Table 1**).
- CDE performed on the proband-parents trio was unrevealing based on AR model while the autosomal dominant (AD) filter prioritized an apparently *de novo* novel change, c.770G>A (p.R257H), in the *ACTG2* gene (**Figure 1**).
- Sanger sequencing confirmation and co-segregation analysis revealed that both unaffected mother and father did not harbor the mutation while the affected sister is a heterozygous carrier of this change, indicating a possible gonadal mosaic origin of this mutation (**Figure 3**).
- *ATCG2* encodes for actin gammma 2, a smooth muscle actin found in enteric tissues. In the mice model, ACTG2 is abundantly expressed in the stomach, intestinal (both proximal and distal), colon, aorta, and bladder (Qian, 1996; Sun, 2009). It plays an important role in bladder smooth muscle formation in mice embryogenesis (Shiroyanagi, 2007). In vertebrates, six highly conserved actin isoforms have been identified. Various mutations in all the other five actin genes (*ACTA1, ACTA2, ACTB, ACTC1, and ACTG1*), mostly *de novo*, have been associated with a wide variety of genetic disorders. *ACTG2* is the least characterized locus in this gene family.
- The amino acid is highly conserved throughout evolution (**Figure 2**) and the alterations are predicted to be probably damaging and deleterious by Polyphen and SIFT *in silico* analyses. The arginine at this position is involved in cross-strand interactions within the actin filament and is conserved in all the six human actin proteins. Missense changes in the corresponding amino acid in *ACTA1* (p.R258H and p.R258L), *ACTA2* (p.R258H and p.R258C), and *ACTG1* (p.R256W) have been reported as disease-causing mutations (Nowak, 1999; Guo, 2007; Riviere, 2012). In the yeast model, substitution of this amino acid in ACTA2 resulted in altered filament conformation and consequent filament instability and misregulation by formin (Malloy, 2012). All these data further suggest the deleterious nature of the *ACTG2* c.770G>A (p.R257H) allele.
- Although not yet reported in HGMD/OMIM, mutations in the *ACTG2* gene have been recently described in patients with similar phenotype to the patient herein (Lehtonen, 2012). This study employed whole exome sequencing analysis identified a missense change, p.R148S, in *ACTG2* as the causative mutation in a Finnish family with 7 affected individuals in three successive generations with AD familial visceral myopathy (FVM), characterized by chronic intestinal pseudo-obstruction affecting especially the entire small bowel (Lehtonen, 2012).

Figure 3. Sanger Sequencing Confirmation and Co-segregation Analysis.
The affected sister also had this alteration, indicating the pathogenicity and a possible gonadal mosaic origin of this mutation.



Legend
*Whole exome sequencing
‡Co-segregation analysis

CONCLUSIONS

- Clinical diagnostic exome identified a "*de novo*" mutation in the *ACTG2* gene and established a molecular diagnosis for a patient in whom traditional testing methods were uninformative.
- The identified mutation is highly consistent with the patient's clinical symptoms which provides an opportunity for clinical intervention and surveillance as well as recurrence risks estimation and family planning.
- This report is the second case ever to prove *ACTG2* as a Mendelian disease-causing locus and reinforced that heterozygous mutations in *ACTG2* can cause bowel dysfunction.
- To our knowledge, this case represents the first report of a mutation arising by germline mosaicism for the actin genes and expands the clinical spectrum and inheritance pattern of *ACTG2* mutations.
- Our experience in this family also demonstrated that "bypassing" the traditional sequential molecular workup by going directly to CDE is a highly successful and cost-effective approach, especially in ultra-rare genetic diseases and less-studied disease loci.

References

1) Guo DC, *et al.* (2007) *Nat Genet* **39**:1488-1493.
2) Lehtonen HJ, *et al.* (2012) *Gastroenterology* 2012 Sep 6. [Epub ahead of print]
3) Malloy LE, *et al.* (2012) *J Biol Chem* **287**:28398-28408.
4) Qian J, *et al.* (1996) *Dev Dyn* **207**(2):135-144.
5) Riviere, *et al.* (2012) *Nat Genet* **44**:440-444.
6) Shiroyanagi Y, *et al.* (2007) *Differentiation* **75**(10):968-977.
7) Sun Q, *et al.* (2009) *J Biol Chem* **284**(47):32582-3290.