Novel Mutation in *CLTC* Associated with Anomalous Development

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BACKGROUND

- *CLTC* encodes clathrin heavy chain 1 (CHC1), one of the components of clathrin, the vesicle coat protein involved in intracellular trafficking and endocytosis.
- *CLTC* is expressed at high levels in the brain, and it has been shown that inactivation of CHC1 in rat and fruit fly models prevents the recycling or release of vesicles in the pre-synaptic terminal.
- CHC1 is also associated with the mitotic spindle and complexes with 2 additional proteins to stabilize the kinetochore fibers.
- Mutations in *CLTCL1*, the second member of the Clathrin Heavy Chain family, have been associated with neurological disease: seizures, intellectual disability, autism and schizophrenia.

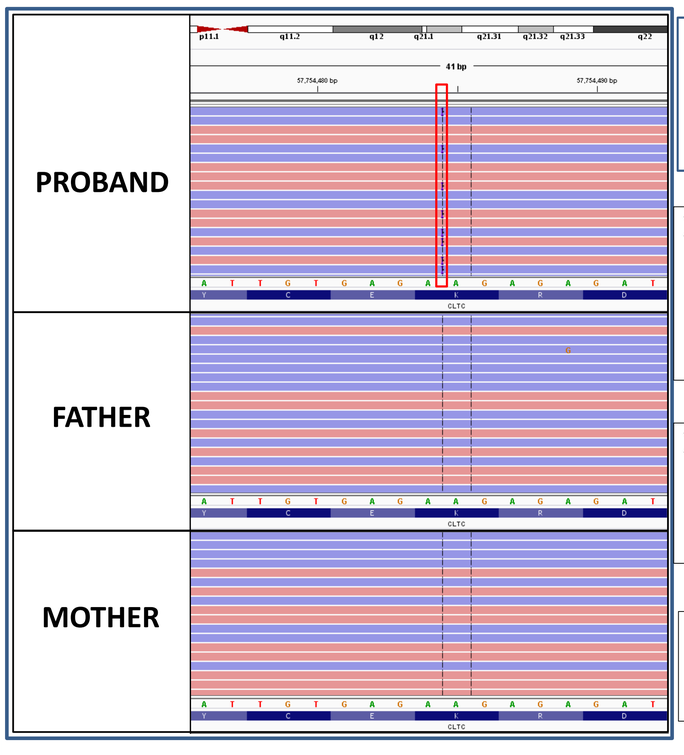
CASE REPORT

- This female was large for gestational age at 35 weeks and was delivered to a 27 year-old primigravid Caucasian whose pregnancy was complicated by pre-eclampsia.
- Neonatal period was notable for hypoglycemia, apnea, bradycardia, hyperbilirubinemia, grade I intraventricular hemorrhage, hypotonia and feeding difficulties.
- She presented in the emergency department at 5 months of age with lethargy, emesis and MRI revealing progressive ventricular enlargement with cerebral atrophy; VP shunt was placed promptly. Etiology of hydrocephaly was unknown. She has central apnea and hypothyroidism.
- Vitamin-K dependent clotting factor deficiency (VKCFD1) was diagnosed, and is now under good control with high dose vitamin K supplementation At 18 months of age, she was diagnosed with a neuroblastoma, for which she is undergoing treatment.
- She has numerous minor dysmorphic features. At two years of age, the patient has global developmental delays and nystagmus.

METHODS

- **Patients:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the proband and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process.
- **Whole exome sequencing:** Samples were prepared using the SeqCap EZ VCRome 2.0 (Roche NimbleGen, Madison, WI). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2500 (Illumina, San Diego, CA).
- **Characterized and Disease-causing (ChaD) and Novel gene databases**: The Characterized and Disease-causing (ChAD) gene database was curated on a weekly basis to include genes currently known to be responsible for causing human disease. The ChAD database included genes which are associated with syndromes listed in the Human Gene Mutation Database (HGMD) (Stenson, 2009) and the Online Mendelian Inheritance in Man (OMIM) database. Novel genes were defined as those not known to underlie a Mendelian condition at the time of data analysis. Any RefSeq gene not included in the ChAD database was included in the novel gene database.
- **Bioinformatics annotation, filtering of variants, and Family history Inheritance-based Detection (FIND):** HGMD, OMIM, the Single Nucleotide Polymorphism database (dbSNP) (Sherry, 2001), 1000 genomes, HapMap data (International HapMap, 2003) and online search engines (e.g., PubMed) were used to search for previously described gene mutations and polymorphisms. Stepwise filtering included the removal of common SNPs, intergenic and 3'/5' UTR variants, non-splice-related intronic variants, and lastly synonymous variants. Variants were then filtered further based family history and possible inheritance models using the informatics program "FIND" (Family history Inheritance-based Detection).
- **Personalized Medical Review with Enhanced and Comprehensive Assessment (PRECISE) of potentially causal variants:** Each candidate mutation was assessed by a molecular geneticist to identify the most likely causative mutation(s) using the "PRECISE" (Personalized Medical Review with Enhanced and Comprehensive Assessment) analysis method. In brief, interpretive filtering was 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. Candidate alterations undergo Sanger sequencing confirmation and familial co-segregation analysis.

FIGURE 1. Trio exome sequencing Identified an Apparently "*de novo*" Duplication, c.2737_2738dupGA (p.D913Efs*59) in the Proband's *CLTC* Gene



CLTC NM_004859 c.2737_2738dupGA p.D913EfsX59

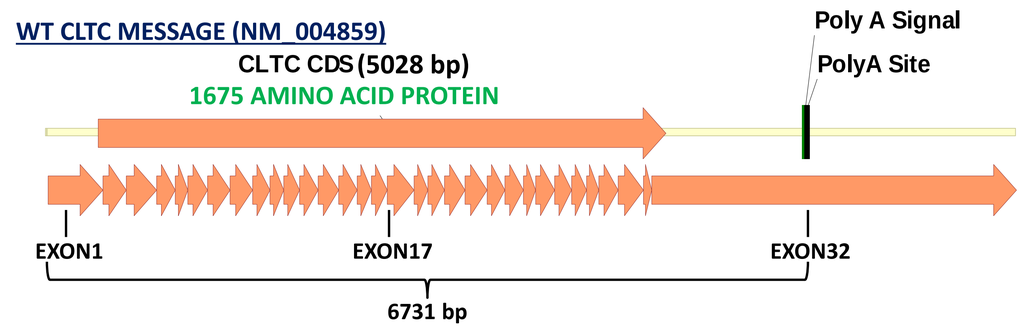
Nucleotides		Proband	Mother	Father
Reference	--	48.08% (50/104)	100.00% (94/94)	100.00% (100/100)
Mutation	GA	51.92% (54/104)	0.00% (0/94)	0.00% (0/100)
Q Score		2450	0	0

- **Red box spanning the proband's NGS reads (upper left panel) indicates the presence of the mutation; note that approximately half of the reads have the mutation, suggesting a heterozygous genotype (upper left and upper right panels).**
- **Alignment software classifies the mutation as c.2731_2732insAG, which is equivalent to the Human Genome Variation Society (HGVS) approved nomenclature of c.2737_2738dupGA (p.D913Efs*59).**
- **No other genetic changes were considered likely to explain the clinical phenotype except for the above mutation.**

FIGURE 2. Nature of the Mutation Suggests a Haploinsufficiency Model of Disease

WT *CLTC* ALLELE:

- **Generates wt message and full length protein with wt activity.**



ALLELE CARRYING *CLTC* c.2737_2738dupGA:

- **Generates aberrant message/ truncated protein subject to Nonsense Mediated Decay (NMD), effectively reducing the dose of CLTC activity by one half.**

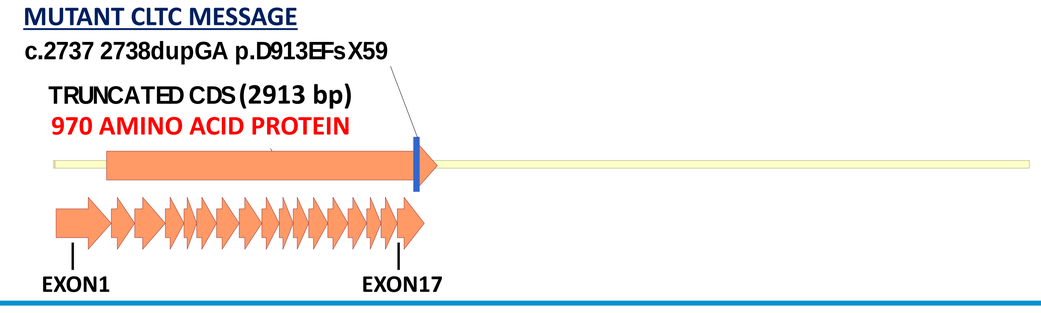
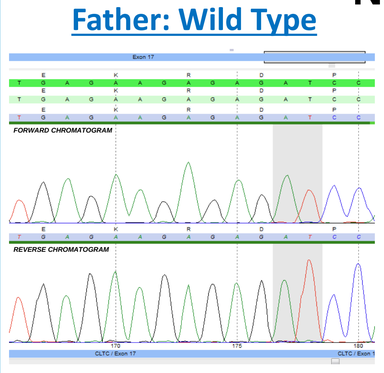
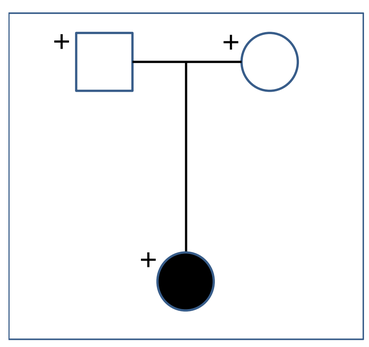


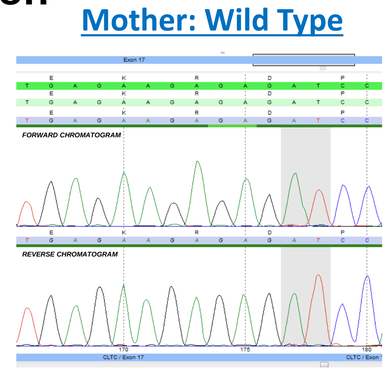
FIGURE 3. Sanger Sequencing Confirms the *de novo* Nature of the Mutation

Father: Wild Type

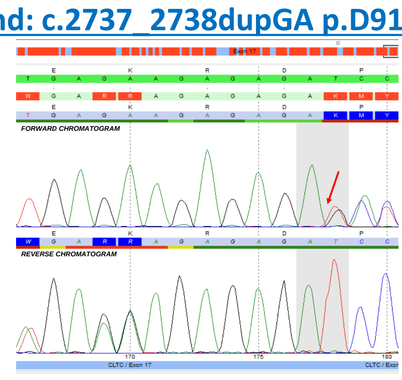




Mother: Wild Type



Proband: c.2737_2738dupGA p.D913EfsX59



+ indicates whole exome sequencing performed

Open shapes represent unaffected family members; black circle represents affected proband

Red arrow indicates the heterozygous c.2727_2738dupGA alteration in the proband's *CLTC* gene (lower two chromatograms)

CONCLUSIONS

- *CLTC* has not previously been associated with any diseases or developmental problems in humans.
- The mutation seen in this patient results in haploinsufficiency of the CHC1 molecule, which is needed for effective synaptic transmission and kinetochore stabilization.
- We propose that this mutation in the *CLTC* gene is the cause of neurological disease in this patient.

REFERENCES

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