Clinical exome sequencing identifies a novel gene, *LINS*, associated with intellectual disability, failure to thrive, seizures, dysmorphology, and language regression.

Ira Lu[1], Layla Shahmirzadi[1], Ruth Baxter[1], Sha Tang[1], Kelly Gonzalez[1], Emily Jordon[2], Omar Abdul-Rahman[2]
[1]Ambry Genetics, Aliso Viejo, CA, 92656
[2]University of Mississippi Medical Center

BACKGROUND

- Extensive clinical evaluation and traditional genetic testing were unsuccessful in diagnosing an 8 year-old Caucasian female affected with intellectual disability (ID), failure to thrive, myopia, microcephaly, seizures, dysmorphic features, and language regression.
- Diagnostic Exome Sequencing identified two compound heterozygous alterations in the *LINS* gene.
- Mutations in *LINS* have been described in two previous cases of autosomal recessive ID.
- Phenotypic overlap with the current case include microcephaly and intellectual disability.
- Based on two previously reported cases, *LINS* mutations may result in a spectrum of clinical symptoms.

METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands 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 SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) or 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 2000 (Illumina, San Diego, CA).
- **Characterized and Disease-causing (ChaD) and novel gene databases**: The 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 on 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. Most candidate alterations undergo Sanger sequencing confirmation and familial co-segregation analysis.
- **Statistical analyses** were computed by chi^2 goodness of fit tests and Fisher's Exact Probability.

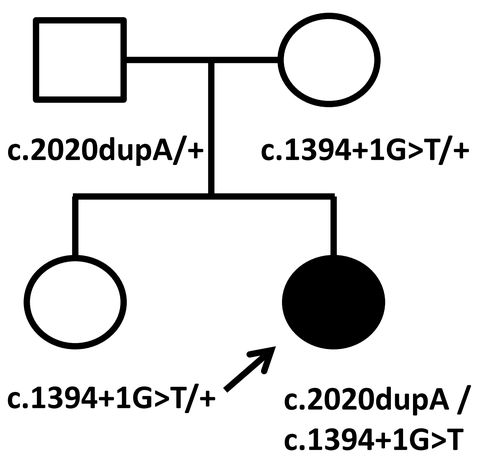
Analysis of Whole Exome Sequencing Data Identified One Notable Autosomal Recessive Candidate Gene: *LINS*

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

	Post-Inheritance Model Filtering	Post-Medical Review**		Notable Candidate Genes‡
		Post-alteration review	Post-clinical association review	
Autosomal Dominant Genes (Alterations)	33(36)	33(36)	4(4)	0(0)
Autosomal Recessive Genes (Alterations)	6(11)	6(11)	1(2)	1(2)
X-linked Recessive Genes (Alterations)	0(0)	0(0)	0(0)	0(0)
X-linked Dominant Genes (Alterations)	1(1)	1(1)	0(0)	0(0)
Y-linked Genes (Alterations)	N/A	N/A	N/A	N/A
TOTAL GENES (Alterations)	40(48)	40(48)	5(6)	1(2)

*First-Tier Exome analysis: Reported are genes that are known to be associated with a clinical phenotype based on the HGMD or OMIM-Morbid databases or the medical literature (the alterations within these genes are located in parentheses). "Clinically novel" genes are not analyzed or reported. Please inquire with the laboratory for more information about post-medical review variant filtered genes and alterations. **Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign. ‡Notable Candidate Genes: Gene alterations selected for co-segregation.

Family History



Proband Clinical Symptoms
Intellectual Disability
Failure to thrive
Language regression with aphonia
Myopia
Microcephaly
Seizures
Dysmorphic features

AUTOSOMAL RECESSIVE *LINS* MUTATION IN LITERATURE: TWO REPORTED CASES

- The *LINS* gene is a human homolog of the Drosophila segmentation lins protein, which is part of the Wnt signaling pathway.
- Two previously reported *LINS* cases involved consanguinity in families of Middle Eastern descent.
- **c.982_985delCATC : frameshift mutation†**
  - This mutation was observed in the homozygous state in four children from an Iranian family affected with moderate ID and microcephaly.
- **c.1219_1222+1delAAAGG: 5-nucleotide donor splice site deletion‡**
  - Results in exon skipping which splices exon 4 directly onto exon 6. A large deletion is observed in expressed mRNA transcripts.
  - This mutation was observed in the homozygous state in a Yemeni brother and sister pair affected with early onset ID, developmental delay, flattened midface, and head nodding behavior.

†Najmabadi H, *et al.* (2011). Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 478(7367):57-63.
‡Akawi NA *et al.* (2013). LINS, a modulator of the WNT signaling pathway, is involved in human cognition. *Orphanet J Rare Dis* 17;8:87.

TAKE-HOME POINTS

- *LINS* is a newly characterized gene involved in human cognition and brain development.
- Two alterations identified in the *LINS* gene though diagnostic exome sequencing, c.2020dupA and c.1394+1G>T, are a plausible cause of this patient's symptoms.
- *LINS* mutations are associated with a spectrum of clinical symptoms, which include intellectual disability, microcephaly, dysmorphology, developmental delay, and head-nodding behavior.
- Diagnostic exome sequencing is an effective method for identifying mutations and uncovering phenotypic spectrums of previously undiagnosed diseases.

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