Using the *C. elegans* lem-2 Gene to Reconstruct the Human LEMD2 Mutation Associated with Hutterite-type Cataract/Cardiomyopathy

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**Abstract**

Figure 1: A. Needleman-Wunsch alignment of the N-terminal region of the Human LEMD2 protein and the *C. elegans* LEM-2 protein. Highlighted in yellow is the conserved Leucine residue that is changed to Arginine in the human mutation. B. Schematic summary of the CRISPR-induced changes in the *C. elegans* *lem-2* gene introducing the Leucine to Arginine mutation at amino acid residue 16. Protein sequence is shown above the DNA sequence. The leucine ttg codon is changed to arginine cgg codon (red), thereby introducing a BsrBI restriction site (underlined). The reverse complement of the CRISPR/Cas9 PAM sequence is highlighted in yellow.

C. Differential interference contrast (DIC) image of typical arrested *lem-2(ca19)* embryos whose mothers had been treated with *emr-1* RNAi. D. DAPI-stained JM311 *lem-2(ca19)* embryo whose mother had been treated with *emr-1* RNAi. E. Total brood sizes measured for 4-5 days following the L4 stage for strains N2 (wildtype); VC1317 *lem-2(ok1807)* and JM311 *lem-2(ca19)*. “***” indicates t-test probability <0.001. Beeswarm-boxplots were assembled in RStudio. F. Body length measurements (microns) measured for arrested non-Green L2 larvae segregating from strains JM312 and JM313, i.e. *emr-1(gk119)*; *lem-2(ok1807)* or *emr-1(gk119)*; *lem-2(ca19)* larvae segregating from mothers homozygous for the respective *lem-2* allele but heterozygous for *emr-1(gk119)*. “*” indicates the (one-sided) t-test probability that *lem-2(ca19)* larvae are shorter than *lem-2(ok1807)* < 0.05. Beeswarm-boxplots were assembled in RStudio. G. Rates of pharyngeal pumping measured in strains N2 (wildtype), VC1317 and JM311. Beeswarm-boxplots were assembled in RStudio. The three lowest pumping rates for each data set were assessed...
The human LEMD2 protein and its homologs in other animals are associated with the inner nuclear membrane, the nuclear lamina and with functions such as chromatin organization and nuclear repair (Barton et al. 2015). The human mutation (c.38T>G; L13R) changes a single amino acid in the highly conserved LEM domain and, when homozygous, is associated with juvenile cataracts and with a greatly increased incidence of early onset cardiac arrest (Shokeir and Lowry 1985; Boone et al. 2016; Abdelfatah et al. 2019). The carrier frequency of this mutation in the North American Hutterite population is estimated to be as high as 12% (Abdelfatah et al. 2019).

The LEMD2 homolog in C. elegans is LEM-2. The lem-2 gene has been well characterized and appears to be largely redundant with the gene emr-1 (Lee et al. 2000; Liu et al. 2003; Barkan et al. 2012; Cohen-Fix and Askjaer 2017). Although lem-2 knockouts, whether by gene deletion or by administration of RNAi, show only mild phenotypes, ablation of both lem-2 and emr-1 genes causes complete lethality: if both zygotic and maternal contributions are removed, animals arrest as early embryos; maternally rescued animals arrest at ~ the L2 larval stage (Lee et al. 2000; Liu et al. 2003; Barkan et al. 2012; Cohen-Fix and Askjaer 2017). We have reconstructed the “Hutterite-type cataract/cardiomyopathy” mutation in the C. elegans lem-2 gene and now compare mutant phenotypes to the phenotypes produced by complete lem-2 knockouts. A longer term aim will be to exploit this reconstructed mutation in C. elegans to identify LEM-2 interacting factors, both biochemically and genetically.

A partial sequence alignment (Figure 1A) shows that amino acid leucine 16 in the C. elegans LEM-2 protein corresponds to amino acid leucine 13 in human LEMD2. As shown in Figure 1B, we used CRISPR-Cas9 methods (Dokshin et al. 2018) to convert C. elegans leucine 16 to Arginine 16 (codon change from TTG to CGG); the mutation, designated lem-2(ca19), introduced a Bsrl restriction site that was used to follow the mutant gene through genetic crosses, including two initial outcrosses. The lem-2(ca19) mutation acts similarly to a complete lem-2 gene knockout: treating the otherwise quite healthy strain JM311 lem-2(ca19) with emr-1 RNAi by feeding (Kamath and Ahringer 2003; Kamath et al. 2003) leads to 100 % lethality, as previously reported for the lem-2 deletion allele tm1582 (Barkan et al. 2012) and that we now also confirm for the lem-2 deletion allele ok1807 used as our positive control. The key phenotype (Figure 1C) is embryonic arrest with fewer than 100 cells, frequent vacuoles and no obvious sign of differentiation (standard gut granule birefringence assay); a typical arrested embryo stained with DAPI (1 µg/ml) shows irregular condensed nuclei and the nuclear membrane and even DNA (Barton 2012).

In summary, we have produced a mutation in the C. elegans lem-2 gene reconstructing a LEMD2 mutation that causes juvenile cataracts and premature cardiac arrest in the North American Hutterite population. Our main conclusion is that, in C. elegans, this single amino acid mutation acts similarly to a complete loss of function mutation; however, it also appears to show a mild antimorphic character. LEM-domain proteins are known to be multifunctional, binding to other proteins (e.g. BAF and lamins), the nuclear membrane and even DNA (Barton et al. 2015). Thus, if the ca19 mutation compromises one LEM-2 function but not others, the mutant protein could form non-productive complexes that could interfere with wildtype function or with the redundant function of EMR-1, in other words act as an antimorph; we have not tried to test this model by assessing phenotypes of heterozygotes. In any case, even if this mutation can act like an antimorph in C. elegans, there is no guarantee that the corresponding mutation acts as an antimorph in humans; for

Methods

**LEM-2 antimorph classification will be explained and defended below.** A mild antimorphic nature of allele lem-2(ok1807) changes a single amino acid in the highly conserved LEM domain and, when homozygous, is associated with juvenile cataracts and with a greatly increased incidence of early onset cardiac arrest (Shokeir and Lowry 1985; Boone et al. 2016; Abdelfatah et al. 2019). The carrier frequency of this mutation in the North American Hutterite population is estimated to be as high as 12% (Abdelfatah et al. 2019).

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by RStudio boxplot as “outliers” and were omitted from significance tests. T-test probabilities are indicated as follows: “*” < 0.05; “**” < 0.01; “***” < 0.0001.
Examples, molecular interactions with other components of the inner nuclear membrane could be different in humans and in worms.

**Methods**

**Request a detailed protocol**

Strain JM311 *lem-2(ca19)* was produced as described above (Figure 1B and (Dokshin et al. 2018)), including two outcrosses to N2 wildtype worms and validation by sequencing of PCR amplified fragments. Strain VC1317 *lem-2(ok1807)* was obtained from the Caenorhabditis Genetics Centre and outcrossed once (the strain designation was not changed). To assess the phenotype of maternally rescued *emr-1; lem-2* larvae, we constructed strains in which the *lem-2* allele was homozygous but the *emr-1* deletion allele was heterozygous and balanced by a reciprocal translocation for which we use the shorthand *hT2green*. The proper designation of *hT2green* is *[hT2[blil-4(e937) let-?(q782) qIs48] (I;III)* where the integrated chromosomal insertion *qIs48 [Pmyo-2::gfp; Pes-10::gfp; Pes-1::gfp]* results in GFP expression. The relevant strains used in this experiment are as follows: JM312 *emr-1(gk119)/hT2green I; lem-2(ok1807) II; +/hT2green III*. JM313 *emr-1(gk119)/hT2green I; lem-2(ca19) II; +/hT2green III*. To measure the tip-to-tail length of arrested larvae, small non-Green animals (picked at a time when the rescued Green animals on the plate were young adults) were suspended in egg buffer containing 0.2% Tricaine + 0.02% Tetramisole + 5mM Sodium Azide and mounted on agarose pads; DIC images were analyzed using ImageJ. Pumping rates were measured at room temperature with one day adults in the presence of 10mM serotonin in 10% M9 buffer mixed with an equal volume of overnight culture of *E. coli* OP50 (Weeks et al. 2018). Pumping rates were measured by video recording at a magnification of 20X and analyzed at slower frame rates. RNAi by feeding was performed as described by (Kamath and Ahringer 2003), using library clone M01D7.6. Double stranded RNA was made by *in vitro* transcription of the same plasmid and injected at a concentration of 1 mg/ml as previously described (Fukushige et al. 2005; Goszczynski and McGhee 2005).

**Reagents**

Strains JM311, JM312 and JM313 will be made available at the Caenorhabditis Genetics Center.

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**Funding:** The authors gratefully acknowledge funding support from the following organizations: The Canadian “Rare Diseases:Models and Mechanisms” Network (RDMM), the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Alberta Children’s Hospital Research Institute (ACHRI). Some strains were provided by the Caenorhabditis Genetics Centre (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

**Author Contributions:** Ayaa AlKhaleefa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - review and editing. Frances L. Snider: Investigation, Methodology, Writing - review and editing. Henry J. Duff: Conceptualization, Funding acquisition, Supervision, Writing - review and editing. James D. McGhee: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing - original draft.

**Reviewed By:** Anonymous

**History:** Received June 15, 2020 Accepted June 23, 2020 Published June 29, 2020

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**Citation:** AlKhaleefa, A; Snider, FL; Duff, HJ; McGhee, JD (2020). Using the C. elegans lem-2 Gene to Reconstruct the Human LEMD2 Mutation Associated with Hutterite-type Cataract/Cardiomyopathy. microPublication Biology. https://doi.org/10.17912/micropub.biology.000273