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Mutations in mRNA export mediator *GLE1* result in a fetal motoneuron disease

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Abstract

The most severe forms of motoneuron disease manifest *in utero* are characterized by marked atrophy of spinal cord motoneurons and fetal immobility. Here, we report that the defective gene underlying lethal motoneuron syndrome LCCS1 is the mRNA export mediator *GLE1*. Our finding of mutated *GLE1* exposes a common pathway connecting the genes implicated in LCCS1, LCCS2 and LCCS3 and elucidates mRNA processing as a critical molecular mechanism in motoneuron development and maturation.

Lethal congenital contracture syndrome 1 (LCCS1, MIM 253310) is an autosomal recessive condition characterized by total immobility of the fetus, detectable at the 13th week of pregnancy, accompanied by hydrops, micrognatia, pulmonary hypoplasia, pterygia and multiple joint contractures (Fig. 1). LCCS1 invariably leads to prenatal death before the 32nd gestational week1. Neuropathological analysis shows lack of anterior horn motoneurons, severe atrophy of the ventral spinal cord and hypoplastic, nearly absent skeletal muscles2. Another clinical phenotype, slightly milder but also characterized by fetal akinesia, arthrogryposis and motor neuron loss, is called lethal arthrogryposis with anterior horn cell disease (LAAHD)3. These fetuses often survive delivery, but die early as a result of respiratory failure. Neuropathological findings in LAAHD resemble those of LCCS1, but are less severe. Further clinical and autopsy data of fetuses have shown some overlap

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AUTHOR CONTRIBUTIONS
L.P. designed this study; M.K. and L.P. supervised this study; L.P., M.K. and R.H. obtained funding; M.K. and N.P. provided microsatellite marker analyses; H.O.N. and N.P. sequenced the candidate genes and analyzed sequences; H.O.N. and M.K. provided bioinformatics analysis; H.H. and S.K. provided *in situ* hybridization; J.T. and H.O.N provided functional studies; K.V., J.I. and R.H. performed phenotype assessment and sample collection; H.O.N. drafted the manuscript and H.O.N., M.K., N.P., J.I., R.H. and L.P. contributed to the writing of this paper.

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between these two fetal akinesia deformation sequence phenotypes, raising the possibility of an etiological relationship between them.

We previously mapped the LCCS1 locus in Finnish families to chromosome 9q34 (ref. 1). Here, we further restricted the critical chromosomal region to 1 Mb between markers D9S1827 and D9S752 (chr9:130041567-130991181, University of California Santa Cruz (UCSC) Genome Browser, March 2006) on the basis of historical recombinations in the shared haplotype among affected individuals. Through systematic sequence analyses of candidate genes in the region, we identified a previously uncharacterized SNP located in the 3′-UTR region of *SLC27A4* (c.*136A>G), which enabled us to further restrict the critical region to 800 kb (**Supplementary Methods** and Supplementary Table 1 online).

Potential mutations in LCCS1 and LAAHD cases were found in only one of the regional candidate genes, *GLE1*, which encodes a protein required for export of mRNAs from the nucleus to the cytoplasm in both lower and higher eukaryotic cells4. In humans, two different forms of this protein, GLE1a and GLE1b, have been identified. These isoforms represent splice variants and are identical except for their C-terminal regions5. Both GLE1 isoforms show a diffuse cytoplasmic localization, but GLE1b is also localized to the nuclear envelope and the nuclear pore complex4. GLE1 forms a heterotrimeric complex with nucleoporins NUP155 and CG1 *in vitro* and is required for the export of *HSP70* mRNA from the nucleus to the cytoplasm5,6.

Through sequence analyses of genomic DNA from LCCS1 cases, we identified a homozygous A→G substitution (c.432-10A>G) located within intron 3 of *GLE1*, ten nucleotides upstream of exon 4 (primer sequences are listed in Supplementary Table 2 online). This substitution creates an illegitimate splice acceptor site resulting in nine extra nucleotides in the *GLE1* cDNA (Supplementary Fig. 1 online). Because exon 3 ends in a full codon, the new splice site is predicted to result in the insertion of three amino acids (proline, phenylalanine and glutamine; T144_E145insPFQ) into the predicted coiled-coil domain of GLE1 (Fig. 2)5. All except one (51 of 52) of our LCCS1 cases in 29 unrelated families were homozygous for this substitution (LCCS1 Fin_{Major}). One was compound heterozygous for the Fin_{Major} mutation and a point mutation predicted to result in an R→H substitution in exon 12 (c.1706G>A, p.R569H; Fig. 2 and Supplementary Fig. 1). Typical LCCS1 features of this fetus were confirmed in the autopsy done after an induced abortion in the 14th gestational week. LCCS is enriched in the regional subisolate of Finland. When analyzing 200 healthy Finnish controls, we found that the carrier frequency of the LCCS1 Fin_{Major} mutation in the general Finnish population was 1%. The carrier frequency in northeastern Finland, where most of the families with LCCS1 originate, was 2%.

To investigate whether LCCS1 and LAAHD represent allelic disorders, we screened nine unrelated families with LAAHD, for a total of twelve affected individuals with mutations in *GLE1*. All twelve cases were compound heterozygotes: six were heterozygous for the LCCS1 Fin_{Major} mutation and a missense point mutation in exon 13 (c.1849G>A, p.V617M), and the remaining six carried the LCCS1 Fin_{Major} mutation and a missense mutation (c.2051T>C, p.I684T) in exon 16 (Fig. 2 and Supplementary Fig. 1). We further analyzed the DNA of one child with distinct congenital arthrogryposis but prolonged survival after birth. Initially, this individual was diagnosed with severe infantile spinal muscular atrophy (SMA type I or type 0)7, but no *SMN* gene deletions were found. The autopsy revealed typical neurogenic muscle atrophy and loss of anterior horn cells of the spinal cord. This individual was also compound heterozygous for the LCCS1 Fin_{Major} mutation and the p.I684T mutation in exon 16. Exons 15 and 16 of *GLE1* code for the CG1-binding domain of GLE1b. This domain is required for targeting GLE1 to the nuclear pore complex5 (Fig. 2). On the basis of our mutation analysis, we conclude that LCCS1 and

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LAAHD represent allelic disorders and that homozygotes for the Fin_{Major} mutation show a more severe phenotype than compound heterozygotes. All identified mutations and their predicted consequences on GLE1b structure are summarized in Supplementary Table 3 online.

To investigate *GLE1* expression during early development, we carried out RNA *in situ* hybridization of *Gle1* in sagittal sections of 11- and 13-d mouse embryos. We observed low ubiquitous expression in both 11- and 13-d embryos. We detected marked expression of *Gle1* in the neural tube of an 11-d-old embryo, specifically in the ventral cell population from which the motoneurons differentiate (Supplementary Fig. 2 online). Differentiation of neurons from this ventral cell population has been shown to be tightly regulated by the expression of specific homeodomain proteins8, including Nkx2.2 and Pax6. Notably, in our previous studies of global transcript profiles of spinal cords of fetuses with LCCS1 (ref. 9), we did not observe any significant change in the steady state transcript level of *GLE1*, whereas we detected marginal downregulation of *NKX2.2* and upregulation of *PAX6*. Our *in situ* data in mouse embryos also showed *Gle1* expression in lung epithelial cells, intestinal wall, esophagus and brain, specifically in the developing choroid plexus, of the 13-d mouse embryo (Supplementary Fig. 2). In regards to the LCCS1 phenotype, characterized by skeletal muscle atrophy and osteopenia, *Gle1* expression was also detected in somites, from which skeletal muscle and bone tissue differentiate.

Next, we expressed both wild-type GLE1b and the LCCS1 Fin_{Major} mutant GLE1b cDNA construct in HeLa cells. The diffuse cytoplasmic localization was similar for mutant and wild-type GLE1b (Supplementary Fig. 3 online), suggesting that the mutation does not dramatically alter the stability or localization of the protein. The LCCS1 Fin_{Major} mutation affects the highly charged middle region of GLE1, which is predicted to form a coiled-coil domain possibly involved in protein-protein interactions10 (Fig. 2). According to the Paircoil2 prediction program, the LCCS1 Fin_{Major} mutation considerably alters the GLE1 secondary structure, indicating disruption of the coiled-coil domain (Supplementary Table 4 online). As GLE1 is expressed ubiquitously, but the dramatic pathological changes are observed only in anterior horn motoneurons, it is likely that the predicted disruption of the coiled-coil domain prevents a critical interaction between GLE1 and a motoneuron-specific protein (or another ligand).

The causative mutations for LCCS2 and LCCS3 in two Bedouin kindreds have recently been identified11,12. LCCS3 is caused by a mutation in *PIP5K1C*, which encodes the enzyme PIPKγ of the phosphatidyl inositol pathway. The disease-causing mutation reduces the kinase activity of this enzyme to a barely detectable level12. LCCS2 was found to result from a loss-of-function mutation in *ERBB3*, which encodes HER3, a modulator of the phosphatidyl inositol pathway11. This is particularly noteworthy, as both proteins are involved in the synthesis of inositol hexakisphosphate ($InsP_6$), which binds directly to the yeast homolog of GLE1 (Gle1). Together, $InsP_6$ and Gle1 stimulate the ATPase activity of the DExD/H-box protein Dbp5 for nuclear mRNA export13,14 (Fig. 3). Further evidence connecting the phosphatidyl inositol pathway with nuclear mRNA export emerges from yeast data, which demonstrate reduced interactions between Dbp5 and Gle1 in strains lacking enzymes of the phosphatidyl inositol biosynthesis pathway13. Also, bioinformatics analyses carried out with the InterProScan program predicted a phosphatidylinositol 3-kinase p85 binding domain in the coiled-coil domain of wild-type GLE1—which is lost in the LCCS1 Fin_{Major} mutant protein and instead replaced by an actin-interacting domain—further supporting our hypothesis of impaired protein-protein interaction and providing an additional link to the phosphatidyl inositol pathway.

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Our study provides further insight into the molecular mechanisms underlying lethal motoneuron diseases and links all the genes implicated in LCCS disease to the $InsP_6$-Gle1-mediated mRNA export pathway. The post-transcriptional regulation of mRNAs is a complex process in which the final cellular destination of individual mRNAs is determined in the nucleus. There, molecular interactions designate subgroups of mRNAs to be transported to various subcellular regions, where they will eventually be translated into proteins15. This regulated translation of localized mRNAs enables the cell to respond quickly and effectively to extracellular stimuli, which is crucial for the normal development and function of neurons and, as suggested by our findings, especially critical for the early development and maturation of large anterior motoneurons.

Our identification of mutated *GLE1* as a cause of the most severe human motoneuron disease with an early block in the development of motoneurons of the spinal cord supports the critical role of normal mRNA processing in the development and survival of anterior motoneurons, and increases our understanding of the underlying mechanisms behind other devastating motoneuron diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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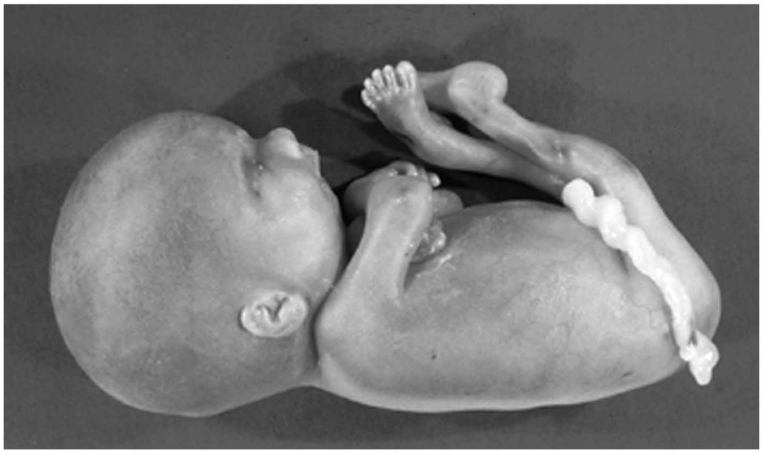

Figure 1.
A 23-week-old LCCS1 fetus with typical malpositioning of limbs and severe micrognathia.

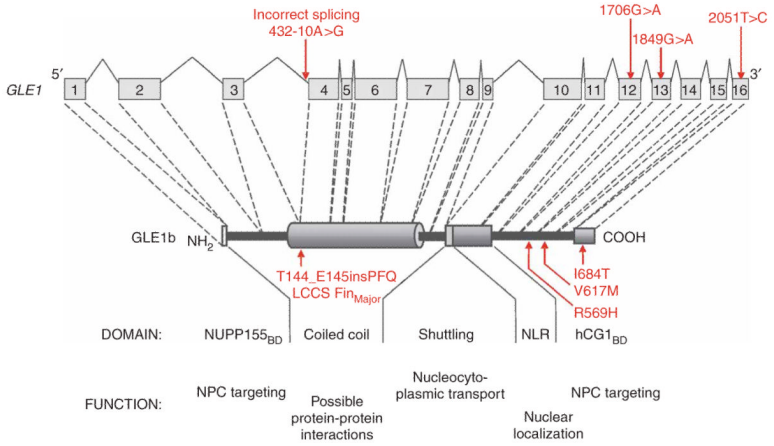

Figure 2.

Detailed structure of the *GLE1* gene and GLE1b protein, with identified mutations and predicted effects on the polypeptide. *GLE1*, comprising 16 exons, encodes a protein (GLE1b) with several functional domains. The mutations identified in LCCS1 and LAAHD cases affect the coiled-coil domain of GLE1b, as well as the C-terminal region, which contains the CG1-binding domain. Schematic modified from those previously reported4,5.

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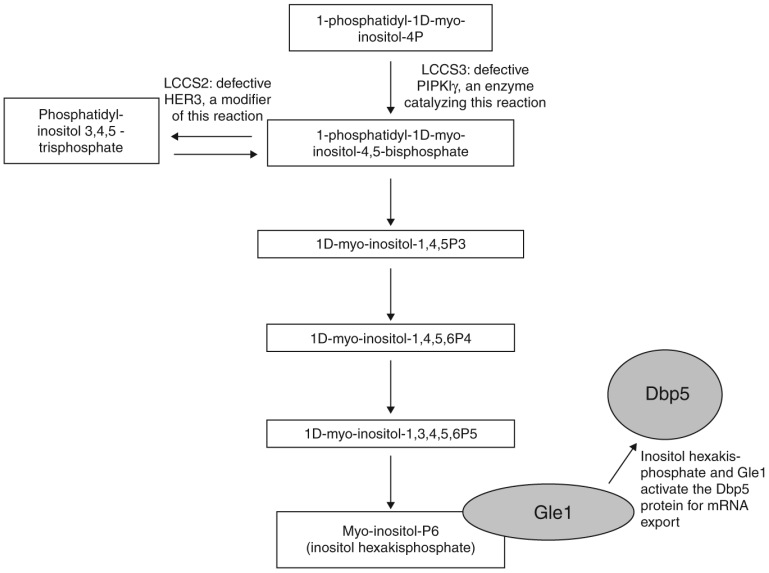

Figure 3.

The disease genes underlying LCCS2 and LCCS3 encode members of the phosphatidyl inositol pathway involved in the synthesis of inositol hexakisphosphate. In budding yeast, inositol hexakisphosphate and Gle1 are both required to activate the Dbp5 protein for mRNA export.

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