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Survival Beyond the Perinatal Period Expands the Phenotypes Caused by Mutations in *GLE1*

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Abstract

Mutations in *GLE1* underlie Lethal Congenital Contracture syndrome and Lethal Arthrogryposis with Anterior Horn Cell Disease. Both Lethal Congenital Contracture syndrome and Lethal Arthrogryposis with Anterior Horn Cell Disease are characterized by reduced fetal movements, congenital contractures, and a severe form of motor neuron disease that results in fetal death or death in the perinatal period, respectively. We identified bi-allelic mutations in *GLE1* in two unrelated individuals with motor delays, feeding difficulties and respiratory insufficiency who survived beyond the perinatal period. Each affected child had missense variants predicted to result in amino acid substitutions near the C-terminus of *GLE1* that are predicted to disrupt protein-protein interaction or *GLE1* protein targeting. We hypothesize that mutations that preserve function of the coiled-coil domain of *GLE1* cause Lethal Arthrogryposis with Anterior Horn Cell Disease whereas mutations that abolish the function of the coiled-coil domain cause Lethal Congenital Contracture syndrome. The phenotype of Lethal Arthrogryposis with Anterior Horn Cell Disease is now expanded to include multiple individuals surviving into childhood suggesting

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that Lethal Arthrogyrosis with Anterior Horn Cell Disease is a misnomer and should be re-named Arthrogyrosis with Anterior Horn Cell Disease.

Keywords

phenotype expansion; *GLE1*; motor neuron disease; developmental delay; respiratory difficulties; arthrogyrosis

INTRODUCTION

Exome sequencing (ES) is a powerful tool for dissecting the genetic basis of Mendelian conditions, including both the identification of novel disease genes and the diagnosis of monogenic diseases [Chong et al., 2015a]. Exome sequencing has been particularly useful for making a precise molecular diagnosis of phenotypically and genetically heterogeneous disorders. In turn, this has broadened the spectrum of phenotypes of many known Mendelian conditions and the number of conditions associated to single genes [Dyment et al., 2015; Thevenon et al., 2016].

Mutations in *GLE1* have been reported to underlie two autosomal recessive conditions, Lethal Congenital Contracture Syndrome 1 (LCCS1; OMIM #253310) and Lethal Arthrogyrosis with Anterior Horn Cell Disease (LAAHD; OMIM #611890), which result in death in the fetal and perinatal period, respectively [Nousiainen et al., 2008]. Lethal Congenital Contracture Syndrome 1 is characterized by a lack of fetal movements in the second trimester of pregnancy. Affected individuals typically present with intrauterine growth retardation (IUGR), fetal hydrops, micrognathia, pulmonary hypoplasia, and multiple joint contractures [Herva et al., 1985]. Individuals with LAAHD present with a similar albeit “milder” phenotype, with prenatal onset of diminished fetal mobility and contractures, and post-natal respiratory failure resulting in perinatal death [Vuopala and Herva, 1994].

Herein we report the delineation of two unrelated individuals with a phenotype similar to LAAHD, who survived beyond four years of life, and were either homozygous or compound heterozygous for missense variants in *GLE1*. These findings expand the phenotype of LAAHD and suggest that either some mutations in *GLE1* are associated with better outcomes and / or differences in intervention early in life can improve outcomes.

CLINICAL FINDINGS

The study was approved by the Ethics Committee of the University Medical Center Hamburg-Eppendorf, Malta, and the Institutional Review Board of the University of Washington.

FAMILY A

Proband 1 (Figure 1; Supplementary Material, Table I) was the first-born male of healthy non-consanguineous parents of European and Maltese ancestry. Due to fetal bradycardia with initial uterine contractions he was delivered by emergency Caesarian at 38 weeks

gestation with a birth weight of 2,480 g (-2.0 SD), a birth length of 46 cm (-2.3 SD) and an OFC of 35 cm (0 SD). Severe respiratory distress required resuscitation and admission to the neonatal intensive care unit where he was ventilated. Abnormal facial characteristics included a prominent forehead, down-slanting palpebral fissures, tent-shaped mouth, prominent frenulum, micrognathia and low-set ears (Figure 1A). Congenital contratures included bilateral camptodactyly of both hands, adducted thumbs, and bilateral clubfeet. His neurological exam at birth was notable for hypertonia of his upper and lower limbs with startling and jerky movements.

At the age of 12 months, he had a flexion contracture of the left hip, decreased abduction of both shoulders and decreased extension of both elbows. He sat unsupported at 10 months and walked with a broad base gait at 3 years 8 months. Examination at 4 years 8 months of age revealed continued expressive speech delay due mainly to micrognathia but his receptive language was normal. His muscle bulk was markedly diminished. He could walk without assistance but occasionally used a K-walker.

FAMILY B

Proband 2 (Supplementary Material, Figure 1 and Table 1) was the first-born female of healthy non-consanguineous parents of European ancestry. A previous pregnancy was terminated at 20 weeks because of generalized non-immune hydrops fetalis, however no genetic analysis was performed and fetal DNA was not obtained. Proband 2 was born at 41 weeks gestational age via vacuum extraction due to fetal bradycardia. Her birth weight was 2,930 g (-1.4 SD), birth length 48 cm (-1.8 SD) and OFC 34.5 cm (-0.4 SD).

She was evaluated at 12 months of age because of suspected developmental delay. She sat unsupported at age of 12 months, crawled at 15 months and stood at 15 months. At 2 years and 5 months, she could speak ten words, but not a full sentence. Examination at age of 3 years and 3 months (Figure 1B) revealed a restless girl with truncal hypotonia, dystonic movements of the upper limbs, involuntary facial grimacing and eye movements, and unmotivated yelling. She was unable to sit or walk unsupported, and protective reflexes were depressed. Supported gait was ataxic. Her weight, height and OFC were within the normal range.

Starting at the age of 14 months she developed frequent febrile infections that each resulted in loss of motor skills and extended recovery time. At the age 3 years 8 months, she had parainfluenza pneumonia which progressed to severe hypoxemic respiratory failure requiring ventilation. After prolonged weaning from the ventilator, she developed delirium and motor abnormalities with flailing of arms and legs, grimacing and writing movements, even while asleep. These involuntary movements improved over the next weeks but she had lost her gross motor skills including sitting and crawling. Repeated MRI of brain showed generalized parenchymal volume loss and a small brainstem. After her fourth bout of pneumonia she died of respiratory insufficiency at the age of four years.

GENETIC RESULTS

Exome sequencing and analysis were performed as described in Families A [Chong et al., 2015b] and B [Hempel et al., 2015]. A homozygous variant in *GLE1* (GenBank: NM_001003722.1), c.2078C>T [p.(Ser693Phe)], was considered the best candidate given the gene's known function and the similarity of phenotypes previously reported to be caused by mutations in *GLE1*. This variant is a founder mutation as it lies within a large 7.7 Mb run of homozygosity and the parents originate from the same island. In family B, bioinformatic filtering did not detect *de novo* variants. Filtering for rare candidate variants (alternate allele frequency <0.01, according to ExAC) under an autosomal recessive model of inheritance identified compound heterozygous alterations, c.1706G>A [p.(Arg569His)] and c.1750C>T [p.(Arg584Trp)] in only a single gene, *GLE1*.

The p.(Ser693Phe) variant in proband 1 has not been observed in over 76,000 population controls from multiple public databases. The variant p.(Arg569His) identified in proband 2, has been reported in individuals with a LCCS1/LAAHD-like phenotype [Nousiainen et al., 2008; Ellard et al., 2014] and has a global allele frequency of 0.0004 (ExAC). The second variant, p.(Arg584Trp), has a global allele frequency of 5×10^{-5} . All three variants have high phred-scaled CADD (v1.3) scores (which incorporates conservation and other pathogenicity predictors such as PolyPhen-2 and SIFT), consistent with pathogenic recessive variants (Supplementary Material, Table I). Taken together, both the genetic and phenotypic data suggest that the *GLE1* variants identified (Figure 2 and Supplementary Material, Table I) are causal for a phenotype overlapping LAAHD and LCCS1. Investigators studying the two affected individuals described here connected via GeneMatcher [Sobreira et al., 2015], a member of MatchMaker Exchange (MME) [Philippakis et al., 2015].

DISCUSSION

We identified two families without known Finnish ancestry who each had a child with a phenotype similar to LAAHD and LCCS1 and were either compound heterozygous or homozygous for missense variants in *GLE1* predicted to be deleterious. In contrast to LAAHD and LCCS1, both children survived the perinatal period and one is still alive (Supplementary Material, Table II). Indeed, no fetus with LCCS1 surviving beyond 35 weeks EGA or infant with LAAHD surviving beyond eight weeks after birth has been reported until the recent publication of single exception [Smith et al., 2016]. Accordingly, the natural history of the children reported herein is considerably different from that of children with either LCCS1 or LAAHD (Supplementary Material, Table II). This observation suggests that either the children reported herein have a condition distinct from both LCCS1 and LAAHD, that mutations in *GLE1* produce a single condition with a broad distribution of phenotypes, or that these children represent phenotypic expansion of LCCS1 or LAAHD.

Smith et al. [2016] recently reported a family with two affected brothers, one of whom died at two weeks of age while the other survived to 12 years, who were compound heterozygotes for *GLE1* variants, c.100-7_100-3delTCTCT [p.(D34_K107del)] and c.1882-2A>G [p.(V238_Nfs*2)]. Smith et al. [2016] proposed that LCCS1 and LAAHD constitute the same

clinical entity with variable severity because the two disorders have “nearly indistinguishable phenotypes.” However, the observation that all individuals homozygous for p.T144_E145insPFQ died prenatally and exhibit no fetal movements during gestation (Supplementary Material, Table II) suggests that LCCS1 represents a distinct clinical entity as the prognosis is different than for individuals who have biallelic *GLE1* mutations. This is similar to other human genes in which a specific or small number of mutations result in a distinct phenotype (e.g., *FGFR3* and achondroplasia).

GLE1 is an essential mRNA export factor [Watkins et al., 1998] that also plays a role in both initiation and termination of protein translation in eukaryotes [Bolger et al., 2008; Folkmann et al., 2013]. *GLE1* encodes at least two nearly identical isoforms, *GLE1A* and *GLE1B*. Both contain a N-terminal domain required for localization to the nuclear pore complex (NPC), a nucleocytoplasmic shuttling domain, and a coiled-coil domain that is necessary for oligomerization of *GLE1*—oligomerization is needed for NPC localization and mRNA shuttling [Kendirgi et al., 2003]. A C-terminal 43 amino acid segment unique to *GLE1B* (Figure 2b) is required for localization of *GLE1* to the NPC [Kendirgi et al., 2003; 2005]. Nevertheless, this difference leads to distinct functions: *GLE1B* exports mRNAs from the nucleus to the cytoplasm via the NPC, while *GLE1A* localizes to the cytoplasm and is required for stress granule assembly and disassembly, thereby regulating protein translation [Aditi et al., 2015].

Each of the variants we identified affects a residue(s) located in the C terminus of *GLE1* (Figure 2 and Supplementary Material, Table I), suggesting a possible relationship between affected domain(s) or affected isoform(s) and phenotype. However, other than proband A, who is homozygous for p.(S693F), a variant that lies in the domain unique to *GLE1B*, all individuals with *GLE1* mutations reported to date have either one or two mutations predicted to affect both isoforms (Supplementary Material, Table 1). Therefore, we cannot readily distinguish any relationships between observed phenotype features, severity, and type or location of a variant.

We predict that biallelic mutations that truncate or otherwise result in loss of function of the coiled-coil domain of both *GLE1* isoforms cause LCCS1. In contrast, individuals with mutations that preserve the function of the coiled-coil domain in both isoforms cause LAAHD. We also suggest that because the phenotype of LAAHD is now expanded to include multiple individuals surviving into childhood, LAAHD should be re-named Arthrogyposis with Anterior Horn Cell Disease (AAHD).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Photographs of individuals with LAAHD

Images show facial features of proband 1 at 8 weeks (A-1) and 9 months (A-2, A-3) and proband 2 (B-1, B-2) at 3 years 5 months of age. Features include prominent forehead, down-slanting palpebral fissures, and micrognathia. See Supplementary Material, Table I for detailed clinical information on each affected individual.

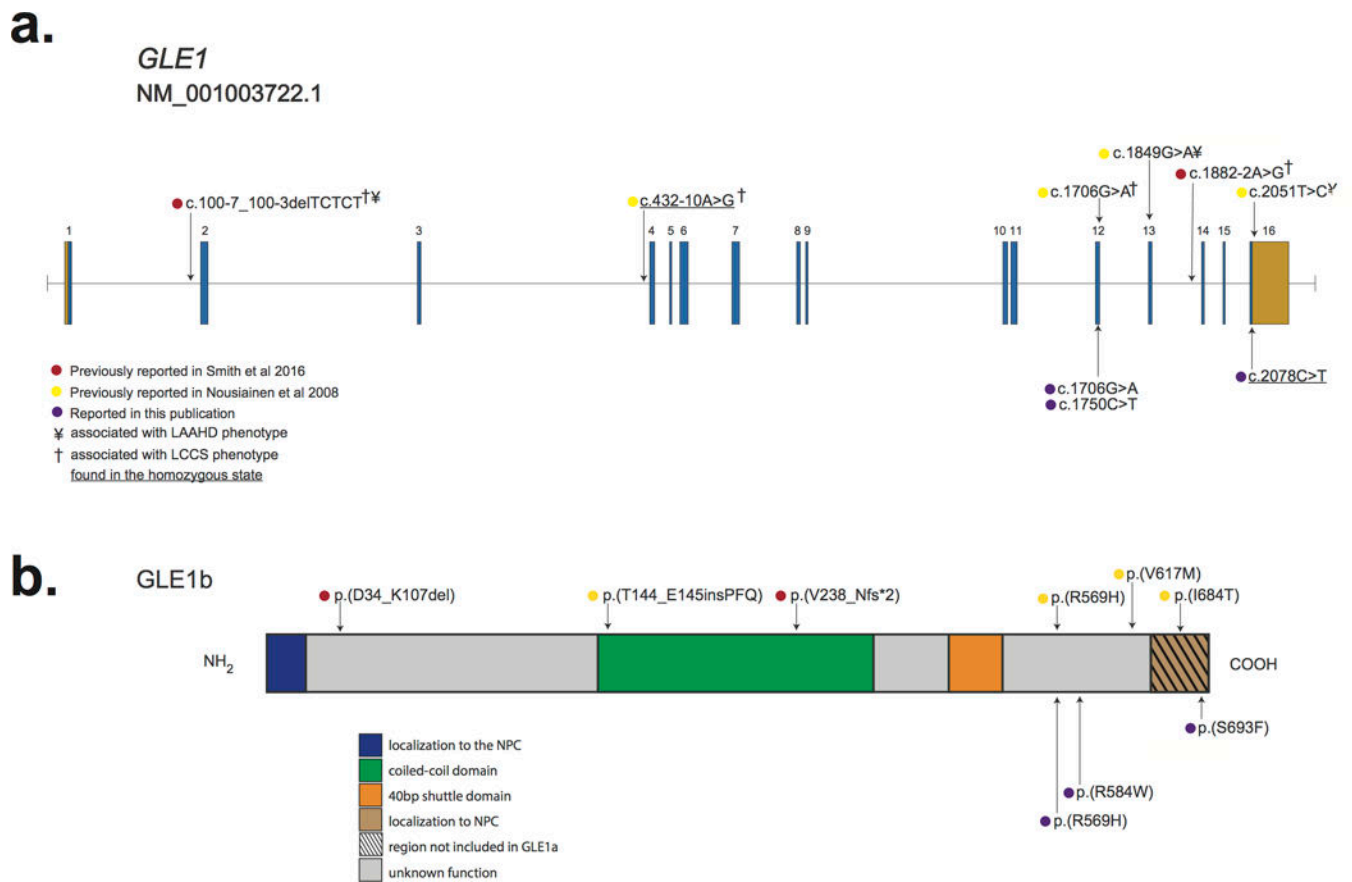


Figure 2. Genomic and protein structure of *GLE1* and spectrum of pathogenic mutations in *GLE1*

a.) *GLE1* is composed of 16 exons, all which are protein-coding (blue) and two of which also contain non-coding sequence (orange). Lines with attached dots indicate the approximate locations of eleven different recessive variants that cause LCCS1 and LAAHD. The color of each dot reflects the report of each mutation. b.) Protein topology of *GLE1* is comprised of six domains. Most mutations lie in the C-terminal portion of *GLE1*. No mutations in the N-terminal Nup155 binding domain or in the nucleocytoplasmic shuttling domain have been reported. The approximate positions of variants that cause LCCS1 and LAAHD are indicated by colored dots. Only p.(V238_Nfs*2) has been shown to result in complete lack of *GLE1*; all other variants have been shown or are expected to be expressed.