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CLP1 Founder Mutation Links tRNA Splicing and Maturation to Cerebellar Development and Neurodegeneration

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SUMMARY

Neurodegenerative diseases can occur so early as to affect neurodevelopment. From a cohort of more than 2,000 consanguineous families with childhood neurological disease, we identified a founder mutation in four independent pedigrees in *cleavage and polyadenylation factor I subunit 1* (*CLP1*). CLP1 is a multifunctional kinase implicated in tRNA, mRNA, and siRNA maturation. Kinase activity of the CLP1 mutant protein was defective, and the tRNA endonuclease complex (TSEN) was destabilized, resulting in impaired pre-tRNA cleavage. Germline *clp1* null zebrafish showed cerebellar neurodegeneration that was rescued by wild-type, but not mutant, human *CLP1* expression. Patient-derived induced neurons displayed both depletion of mature tRNAs and accumulation of unspliced pre-tRNAs. Transfection of partially processed tRNA fragments into patient cells exacerbated an oxidative stress-induced reduction in cell survival. Our data link tRNA maturation to neuronal development and neurodegeneration through defective CLP1 function in humans.

INTRODUCTION

tRNAs are abundantly expressed RNA molecules required to bring amino acids to the translating ribosome for protein synthesis. Of the 506 known human tRNAs, 32 are encoded as pre-tRNAs, containing introns that are spliced during maturation (Lowe and Eddy, 1997; Phizicky and Hopper, 2010). The nuclear-localized tRNA splicing endonuclease (TSEN) complex recognizes the secondary structure of pre-tRNA molecules and cleaves at the exon-intron boundaries to yield a 2′,3′-cyclic phosphate (i.e., phosphodiester) and 5′-OH terminal at the splice site (Peebles et al., 1983). Although still the subject of some debate, there are at least two proposed mechanisms of religating these "half" tRNAs in mammals (i.e., the 5′ and 3′ exons remaining after excision of the single intron). In the first, HSPC117 directly mediates the ligation of these ends, utilizing the phosphate from the phosphodiester bond in the linkage, and is inhibited by the presence of a 5′ phosphorylation (Popow et al., 2011). In the second, 5′ phosphorylation by CLP1, a component of the TSEN complex in mammals, is required (Paushkin et al., 2004; Weitzer and Martinez, 2007; Zillmann et al., 1991), but the ligase that follows has not yet been discovered. Utilization of both pathways in human cells is documented, but their relative importance and functional redundancy in development and homeostasis remain unclear.

Pontocerebellar hypoplasia represents a group of inherited progressive neurodegenerative disorders with prenatal onset, thus intersecting development with degeneration. All subtypes share common structural defects of the pons and cerebellum, evident upon brain imaging. Targeted therapy is nonexistent, and most patients die during infancy (Namavar et al., 2011b). Mutations in any of three subunits of the TSEN complex, the mitochondrial arginyl-tRNA synthetase gene, the RNA exosome component EXOSC3, and the vaccine-related kinase, are found in some cases (Budde et al., 2008; Edvardson et al., 2007; Renbaum et al., 2009; Wan et al., 2012). We recently implicated AMPD2 in PCH, causing a defect in protein translation due to guanosine triphosphate depletion (Akizu et al., 2013). The data implicate RNA maturation and protein synthesis defects in PCH but also suggest that further causes are yet to be identified.

Here, we demonstrate a requirement for *CLP1* in human brain development. We identify four independent families carrying a founder p.R140H mutation, which impairs affinity for the TSEN complex, kinase activity in a recombinant assay, and function in vivo. Consistent with its role in tRNA splicing, we find depleted mature tRNAs and excessive pre-tRNAs accumulating in patient-derived induced neurons (iNeurons). We demonstrate sensitivity of patient cells to oxidative-stress-induced death exacerbated by the addition of unphosphorylated 3′-tRNA-exon halves and partially corrected with 5′-phospho- 3′-tRNA exon addition. In sum, we uncover an evolutionarily conserved requirement for CLP1 during vertebrate neurogenesis and show that CLP1 is necessary for tRNA maturation, the loss of which leads to stress-induced cell death.

RESULTS

Patients Harboring *CLP1* Mutation Have Progressive Brain Atrophy

We collaboratively recruited more than 2,000 families, most with documented parental consanguinity, presenting a child with neurological disease. We performed exome sequencing on at least one affected member per family and then analyzed each for potentially deleterious homozygous mutations. Genome Analysis Toolkit (GATK) (DePristo et al., 2011) was used for variant identification and intersected with identity-by-descent blocks from HomozygosityMapper (Seelow et al., 2009). Rare potentially deleterious variants were prioritized against our cumulative in-house 4,000 patient exome database and across publicly available exome datasets, cumulatively numbering over 10,000 individuals. From this analysis, four independent consanguineous Turkish families with a neurodevelopmental/neurodegenerative disorder emerged (Figure 1A), all displaying an identical homozygous Chr11:57427367G>A (hg19) single-nucleotide transition in the *CLP1* gene, resulting in a p.ARG140HIS (p.R140H) amino acid substitution mutation.

Following the identification of the mutation, it became clear that the patients shared many clinical features, but it would have been difficult to separate them clinically from the rest of the cohort. After an unremarkable perinatal history, onset of slow, progressive, neurodegenerative features and/or static encephalopathy ensued by 6 months of age. Clinical features included failure to develop gross or fine motor skills, absent or delayed speech, progressive spasticity, and spontaneous epileptic seizures (Table 1 and Table S1 available online). Brain MRI demonstrated mild atrophy of the cerebellum, pons, and corpus callosum (Figure 1B), together with progressive microcephaly. Electromyography, although initially normal at a young age, demonstrated age-dependent muscle fibrillations and high-amplitude motor unit potentials in one patient, indicating progressive spinal motor neuron loss. Extensive testing for known metabolic or degenerative diseases was negative, suggesting a heretofore unknown condition.

The mutant allele was observed heterozygous twice in unrelated unaffecteds in our in-house exome database of collectively more than 2,000 independent exomes (including ~1,000 Turkish individuals) and was not reported in any public database, suggesting a carrier frequency of 1:1,000. In all families, we confirmed that the mutation occurred within a homozygous haplotype block (Figure S1A), suggesting a founder mutation. The mutated amino acid residue was highly conserved in all multicellular organisms (Figure 1C) and was predicted to be damaging (Adzhubei et al., 2010). No other potentially deleterious rare homozygous *CLP1* variants were present in the database. Comparison of exome allele calls among families suggested a minimal shared haplotype between Chr11:57317640–57461472 or 143,832 bp (Figure S1B), dated to a common ancestor ~16.2 generations in the past (± 8.7 generations, see Experimental Procedures), during the height of the Ottoman expansion.

Direct Sanger sequence analysis of all available family members, including deceased member 1810-VI-2 (from dried umbilical cord), demonstrated segregation according to a strict recessive mode of inheritance (Figure S1C), which was consistent with pathogenesis. Obligate carriers were entirely normal. We further found no other *CLP1* mutations in any other patient in our collective cohorts with overlapping clinical features nor any from an additional directly sequenced cohort of 100 cases with familial motor neuron disease.

CLP1^{R140H} Is Functionally Compromised

To determine whether the mutation was predicted to disrupt protein function, we modeled human CLP1 using the structure of the partially crystallized yeast nucleotide-bound Clp1 (Noble et al., 2007). In yeast, the p.140ARG is substituted for a LYS at the cognate position, which is also a polar basic residue (p.149LYS). Structure shows that the yeast p.149LYS is involved in the formation of an inferred hydrogen bond with the highly conserved p.59GLU residue (Figure 2A). This polar contact is predicted to be maintained in human but disrupted in the presence of the mutant p.140HIS residue, suggesting an alteration in protein structure or function. We found comparable CLP1 protein levels among all genotypes in primary fibroblast lysates derived from skin biopsy (Figure 2B), suggesting that protein stability was unaltered in the presence of the mutation.

The p.R140H mutation occurred right after the ATP-binding P loop (i.e., Walker A motif), conserved in all kinases. To determine whether the mutation alters CLP1 kinase activity, we tested recombinant wild-type (WT) and mutant GST-tagged human CLP1 protein against a poly(A) RNA oligonucleotide natural