

BRAIN COMMUNICATIONS

Lunapark deficiency leads to an autosomal recessive neurodevelopmental phenotype with a degenerative course, epilepsy and distinct brain anomalies

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LNPK encodes a conserved membrane protein that stabilizes the junctions of the tubular endoplasmic reticulum network playing crucial roles in diverse biological functions. Recently, homozygous variants in *LNPK* were shown to cause a neurodevelopmental disorder (OMIM#618090) in four patients displaying developmental delay, epilepsy and nonspecific brain malformations including corpus callosum hypoplasia and variable impairment of cerebellum. We sought to delineate the molecular and phenotypic spectrum of *LNPK*-related disorder. Exome or genome sequencing was carried out in 11 families. Thorough clinical and neuroradiological evaluation was performed for all the affected individuals, including review of previously reported patients. We identified 12 distinct homozygous loss-of-function variants in 16 individuals presenting with moderate to profound developmental delay, cognitive impairment, regression, refractory epilepsy and a recognizable neuroimaging pattern consisting of corpus callosum hypoplasia and signal alterations of the forceps minor ('ear-of-the-lynx' sign), variably associated with substantia nigra signal alterations, mild brain atrophy, short midbrain and cerebellar hypoplasia/atrophy. In summary, we define the core phenotype of *LNPK*-related disorder and expand the list of neurological disorders presenting with the 'ear-of-the-lynx' sign suggesting a possible common underlying mechanism related to endoplasmic reticulum-phagy dysfunction.

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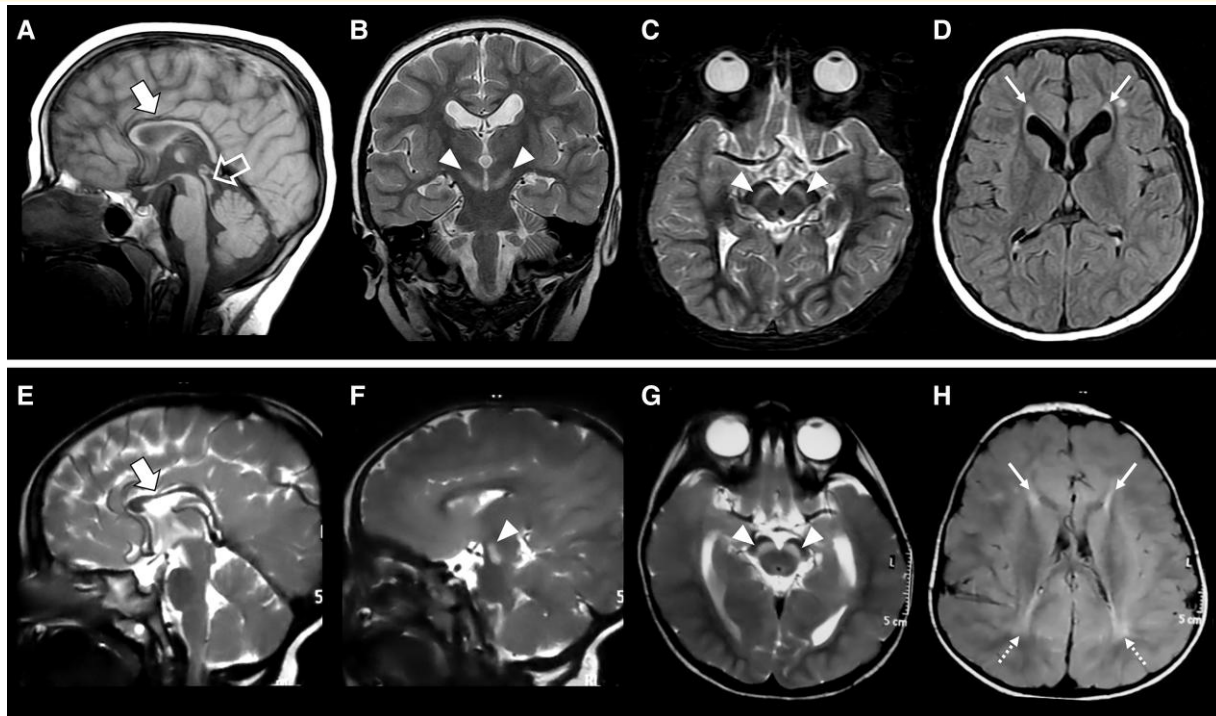


Figure 2 Neuroimaging features of LNP-related disorder. Brain MRI studies performed in Patient II:1 from Family 6 at 4 years of age (**A–D**) and in Patient II:2 from Family 7 at 2.5 years of age (**E–H**). Sagittal T₁- (**A**) or T₂-weighted (**E**) images demonstrate corpus callosum hypoplasia with prevalent involvement of the anterior portions (thick arrows). Coronal (**B**), axial (**C, G**) and sagittal (**F**) T₂-weighted images reveal symmetric marked T₂ hyperintensity of the substantia nigra (arrowheads). Note the ‘ears-of-the-lynx’ sign (thin arrows) on axial FLAIR images (**D, H**) consisting of hyperintense signal of the forceps minor bilaterally, which resembles the shape of the ears of a lynx with their characteristic apical hair tuft. Additional posterior periventricular white matter signal alterations are noted in Patient II:2 from Family 7 (dotted arrows). A short midbrain is also visible in Patient II:1 from Family 6 (empty arrow).

life like in the *ATP13A2*-related disorders, which could be potentially treated.

The effect of LNP deficiency on ER has previously been elucidated by knockout studies in *Saccharomyces cerevisiae*⁶ and mammalian cell lines,⁸ showing that its loss leads to a reduction of tubules and junctions and an increased sheet-like appearance at the cellular periphery, overall affecting the abundance of the three-way junctions. In humans, fibroblasts of patients harbouring a homozygous truncating variant in *LNP*K exhibited aberrant ER shape and increased luminal mass density.⁹ Likewise, we expect that the homozygous LoF variants identified in our patients result in a loss of protein function with consequent perturbation of ER morphology and homeostasis. However, the mechanism underlying impact on central nervous system development, resulting in cognitive impairment, epilepsy and brain malformations, is yet to be elucidated. The typical biphasic disease course with a neurodegenerative phase occurring on the background of a neurodevelopmental impairment may support at least in part a pathomechanism related to autophagy dysfunction as seen in other congenital disorders of autophagy.²² Of note, autophagosomes form at the ER in mammals, and ER membrane contacts are known to play a central role in regulating autophagosome formation.²³

Although we may speculate that LNP deficiency impairs ER homeostasis and function with consequent perturbation of autophagy, a direct functional linkage between LNP and autophagosomes remains elusive and related signalling pathways yet unknown.

Furthermore, it is unknown why spasticity is not a major finding in individuals with LNP deficiency in contrast to the SPG phenotype of individuals with pathogenic variants in other ER genes. Finally, deletion of the *LNP*K homologue (*lnp-1*) in *Caenorhabditis elegans* causes mislocalization of presynaptic proteins, suggesting a role of *Lnp-1* in synaptogenesis through regulation of vesicular transport or localization.²⁴ This finding is in line with the clinical presentation of refractory epilepsy in our cohort, pointing to a possible synaptic dysfunction due to LNP deficiency.

In summary, we outline the clinical features of the *LNP*K-related NDD, mainly characterized by moderate to profound ID, epilepsy and recognizable brain anomalies. Specifically, the ‘ear-of-the-lynx’ sign associated with corpus callosum hypoplasia and substantia nigra signal alterations are the key feature that could guide clinicians toward an early clinical diagnosis. Further studies are needed to elucidate the LNP’s role in ER of developing neurons and the exact pathomechanism leading to LNP deficiency.

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