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Authors

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Zaki, Maha S
Al-Owain, Mohammed
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Lunapark deficiency leads to an autosomal recessive neurodevelopmental phenotype with a degenerative course, epilepsy and distinct brain anomalies

Andrea Accogli[1,2], Maha S Zaki[3], Mohammed Al-Owain[4], Mansour Y. Otaif[37], Adam Jackson[5,6], Emanuela Argilli[25], Kate E. Chandler[5,6], Christian G.E.L. De Goede[7], Tülün Cora[8], Javeria Raza Alvi[9], Atieh Eslahi[10,11], Mahsa Sadat Asl Mohajeri[10], Setareh Ashtiani[12], PY Billie Au[12], Alicia Scocchia[13], Kirsi Alakurtti[13], Alistair T. Pagnamenta[14], Mehran Beiraghi Toosi[15,16], Ehsan Ghayoor Karimiani[17,18], Majid Mojarrad[10,11,19], Fatemeh Arab[20], Fahrettin Duymuş[8], Morris H. Scantlebury[21], Gözde Yeşil[22], Jill Anne Rosenfeld[23,24], Ayberk Türkyılmaz[26], Safiye Güneş Sağer[27], Tipu Sultan[9], Farah Ashrafzadeh[15], Tatheer Zahra[9], Fatima Rahman[9], Shazia Maqbool[9], Mohamed S. Abdel-Hamid[28], Mahmoud Issa[3], Stephanie Efthymiou[29], Peter Bauer[30], Giovanni Zifarelli[30], Vincenzo Salpietro[29,38] Zuhair Al-Hassnan[31], Siddharth Banka[5,6], Elliot H. Sherr[25], Joseph G. Gleeson[32,33], Pasquale Striano[34,35] Henry Houlden[29], on behalf of the Genomics England Research Consortium, Mariasavina Severino[36]*, Reza Maroofian[29]*

[1]Division of Medical Genetics, Department of Specialized Medicine, McGill University, Montreal, Canada
[2]Department of Human Genetics, McGill University, Montreal, QC, Canada
[3] Clinical Genetics Department, Human Genetics and Genome Research Institute, National Research Centre, Cairo, Egypt
[4] Department of Medical Genomics, Center for Genomics Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
[5] Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK
[6] Manchester Centre for Genomic Medicine, University of Manchester, St Mary's Hospital, Manchester Academic Health Science Centre, Manchester, UK
[7] Department of Paediatric Neurology, Royal Preston Hospital, Preston UK
[8] Selcuk University School of Medicine, Department of Medical Genetics, Turkey
[9] Department of Pediatric Neurology, Institute of Child Health, Children Hospital Lahore, Lahore, Pakistan.
[10] Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[11] Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[12]Alberta Children's Hospital Research Institute Department of Medical Genetics, University of Calgary, Alberta, Canada
[13] Blueprint Genetics Inc, Seattle, WA, USA
[14] NIHR Biomedical Research Centre, Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK
[15] Pediatric Neurology Department, Mashhad University of Medical Sciences, Mashhad, Iran
[16] Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[17] Molecular and Clinical Sciences Institute, St. George's, University of London, Cranmer Terrace, London, UK
[18] Department of Medical Genetics, Next Generation Genetic Polyclinic, Mashhad, Iran
[19] Genetic Center of Khorasan Razavi, Mashhad, Iran
[20] Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[21] Departments of Pediatrics and Clinical Neuroscience University of Calgary; Alberta Children's Hospital Research Institute, Hotchkiss Brain Institute & Owerko Center, University of Calgary, Alberta, Canada
[22] Istanbul University Istanbul Medical Faculty Department of Medical Genetics, Turkey
[23] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA
[24] Baylor Genetics Laboratories, Houston, Texas, USA
[25] Department of Neurology, University of California, San Francisco, San Francisco, California, USA
[26] Department of Medical Genetics, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey
[27] Clinics of Pediatric Neurology, Kartal Dr. Lütfi Kırdar City Hospital, İstanbul, Turkey

[28] Medical Molecular Genetics Department, Human Genetics and Genome Research Institute, National Research Centre, Cairo, Egypt
[29] Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, Queen Square, London, UK
[30] CENTOGENE AG, Rostock, Germany
[31] Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia; Department of Medical Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
[32] Department of Neurosciences, University of California, San Diego, La Jolla, USA
[33] Rady Children's Institute for Genomic Medicine, San Diego, USA
[34] Department of Neurosciences Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), University of Genoa, Genoa, Italy.
[35] Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto "Giannina Gaslini", Genoa, Italy
[36] Neuroradiology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy
[37] Kingdom of Saudi Arabia Abha – Asser Region Abha Maternity and Children hospital, Saudi Arabia
[38] Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy

*Mariasavina Severino and Reza Maroofian contributed equally to this work.

Correspondence:

Reza Maroofian PhD
Department of Neuromuscular Disorders, Queen Square Institute of Neurology, University College London, WC1N 3BG, London, UK.
r.maroofian@ucl.ac.uk

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Abstract

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 non-specific 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 eleven families. Thorough clinical and neuroradiological

evaluation was performed for all the affected individuals, including review of previously reported patients.

We identified twelve distinct homozygous loss-of-function variants in sixteen 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.

Keywords: endoplasmic reticulum, LNPK, ear-of-the-lynx sign, substantia nigra, corpus callosum hypoplasia.

Introduction

The endoplasmic reticulum (ER) is involved in diverse biological functions, including protein synthesis, folding and transport, carbohydrate metabolism, lipid and steroid synthesis, and calcium homeostasis[1,2]. The progressive understanding of ER structure and function in recent years has unraveled the role of ER dysfunction in several neurodegenerative disorders in humans, such as hereditary spastic paraplegia (SPG)[3] and Parkinson disease[4].

Lunapark (Lnp) is a conserved membrane protein that localizes preferentially to the three-way junctions connecting the tubular ER network.[5,6] Through its ubiquitin ligase activity it ubiquitinates atlastin-2 for the tubular network formation and stabilization of the junctions[7,8]. In higher eukaryotes, phosphorylation of Lnp may contribute to the conversion of the ER from tubules to sheets during mitosis[8].

Recently, three homozygous loss-of-function (LoF) variants in *LNPK* were shown to cause a neurodevelopmental disorder (OMIM#618090) in four patients displaying global developmental delay (GDD)/intellectual disability (ID), epilepsy, corpus callosum hypoplasia and variable impairment of cerebellar development[9,10].

Here, we present 16 new individuals from 12 different families harboring 11 novel homozygous LoF variants in *LNPK*, outlining the molecular and phenotypic spectrum of *LNPK*-related disorder.

Material and methods

Patients and genetic analysis

Sixteen previously unreported patients from 12 families of different ancestries (Egyptian, Iranian, Turkish, Saudi Arabian, Afghan, Pakistan and British) as well as additional follow-up data from reported 4 patients from 3 families (Egyptian, Pakistani and Turkish) were included in this study after obtaining written informed consent (Figure 1A).

Clinical data were collected using standardized proforma from around 10 different hospitals and clinics, for all individuals. Brain magnetic resonance imaging (MRI) of these and of the previously reported patients[9,10] were reviewed by an experienced neuroradiologist (MS). Exome sequencing (ES) or genome sequencing (Family 2) was performed in probands in the respective collaborating centers using slightly different analysis platforms according to the BWA/GATK's based pipelines. Sanger sequencing with standard methods was performed for candidate variants' validation and familial segregation. All *LNPK* variants are reported according to the transcript NM_030650.3 and classified according to the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) variant classification system. This study was approved by the Medical Ethical Committee of UCL Queen Square Institute of Neurology.

Results

Genetic findings

Exome sequencing or genome sequencing revealed 12 novel or ultra-rare *LNPK* variants homozygous in affected individuals as follows: a nonsense variant c.19C>T p.(Arg7*) in Family 1, a splicing variant c.-62-1G>T in Family 2, a nonsense variant c.428C>A p.(Ser143*) in the Families 3 and 4, a frameshift c.359_362del p.(Leu120Glnfs*14) in Family 5, a frameshift variant c.402_405del p.(Leu134Phefs*24) in Family 6, a frameshift variant c.726del p.(Pro243Leufs*2) in family 7, the splicing variant c.1054+1G>T in Family 8, a frameshift variant c.355dup p.(Ile119Asnfs*3) in Family 9, a nonsense variant c.889C>T p.(Arg297*) in Family 10, a frameshift variant c.431dup p.(Lys145Glufs*6) in Family 11 and a nonsense variant c.757C>T p.(Arg253*) in Family 12 (Figure 1A-D). Ten of these variants were novel, while the c.726del p.(Pro243Leufs*2) was previously reported in an unrelated family from Egypt[9].

For Family 2, homozygosity was due to uniparental isodisomy involving the entire chromosome 2, and only the mother was a heterozygous carrier. Sanger sequencing confirmed segregation of the variants with the phenotype within these families.

All variants were classified as pathogenic according to the ACMG/AMP criteria and are extremely rare in human population variant databases (allele frequency ranging from 0 to 0.000003995 in gnomAD, UK biobank, and Queen Square genomics database). None of the variants were reported in a homozygous state in healthy individuals.

All nonsense and frameshift variants are predicted to result in a premature truncation of the transcript, likely leading to nonsense-mediated mRNA decay. The variants c.-62-1G>T the and c.1054+1G>T are predicted to severely impair the protein structure through aberrant mRNA splicing (acceptor loss - 0.98 score and donor loss - 0.99 score respectively, according to the SpliceAI tool)[11]. No other pathogenic/likely pathogenic variants were identified in the currently known neurodevelopmental or neurodegenerative disorders (NDDs)-related genes.

Clinical and neuroradiological findings

All 16 patients (9 females, 7 males; mean age 8.2, range 2 - 19) had GDD and moderate-to-profound ID (moderate=5; severe=8; profound=3). Only one individual was able to walk with support at the last follow-up visit, and all were mostly non-verbal. Developmental regression was observed in seven, mostly occurring after seizure onset. One individual (II:2 of Family 5) died at the age of 9.5 years due to status epilepticus in the context of respiratory infection. Twelve individuals had epilepsy, experiencing different seizure types with a predominance of myoclonic and tonic-clonic seizures, and the age of onset was between 2 months and 6 years. For nine of them epilepsy was refractory to anti-seizure medications. Review of available EEG for 11 patients (including 2 previously reported) did not identify a specific electrographic pattern. Additional details about EEG findings are available on Table 1 and Supplemental material. Two patients were diagnosed with autism spectrum disorder while no major behavioral abnormalities were noted in other children. Neurological exam demonstrated axial hypotonia (n=16), hyporeflexia (n=6), limb hypertonia (n=4), cerebellar tremor (n=3), and ataxic gait in one of the two patients who were able to walk prior to regression. Ophthalmological findings included strabismus (n=5), nystagmus (n=4), bilateral cataracts (n=2) and optic atrophy (n=1). Two individuals had postnatal microcephaly, and another two showed mild macrocephaly, while the majority had normal head circumference. Subtle and non-specific dysmorphic features were noticed in those individuals for whom photos were available (Figure 1C).

Brain MRI studies were available for review in 18 patients (14 from the present cohort and 4 from previous publications[9,10]; mean age at MRI: 4.6 years, range 1-14 years) (Supplemental Figure 1). In all patients (18/18, 100%) we found callosal hypoplasia with prevalent anterior involvement and focal signal changes of the forceps minor of the corpus callosum reminiscent of the "ear-of-the-lynx" sign (Figure 2). Additional prominent features included bilateral symmetric T2-FLAIR hyperintensity of the substantia nigra (13/18, 72.2%), enlargement of the cerebral CSF spaces (11/18, 61.1%), a short midbrain (10/18, 55.5%), white matter volume loss with an antero-posterior gradient (9/18, 50%), mild inferior vermis hypoplasia (8/18, 44.4%), and other periventricular white matter signal alterations (8/18, 44.4%). Mild cerebellar atrophy (3/18, 16.6%) was detected in a subset of patients

(Supplemental Fig. 2). Clinical features are summarized in Table 1, Fig. 1D and extensively available on Supplemental Table 1.

Discussion

All affected individuals of our and previous cohorts[9,10] harbor LoF homozygous variants in *LNPK,* resulting in a neurodevelopmental phenotype characterized by moderate to profound DD/ID, refractory epilepsy and a recognizable neuroradiological pattern. Interestingly, brain MRI analysis including review of previously published patients led us to identify a consistent neuroimaging phenotype characterized by callosal hypoplasia and abnormal signal 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. Of note, the "ear-of-the-lynx" sign has been typically described in SPG11 (MIM#604360) and SPG15 (MIM#270700)[12], linked to pathogenic variants in genes encoding spatacsin (*SPG11*) and spastizin (*ZFYVE26*) respectively, which play pivotal roles in intracellular trafficking and are part of a multiprotein complex important for ER function[13-15]. The presence of this sign in the LNPK-related disorder further underscores the importance of ER for axon development and function[3]. Moreover, signal alterations of the forceps minor with an "ear-of-the-lynx" or "ear-of-the-grizzly" morphology have been recently described in AP-4-associated hereditary spastic paraplegia (AP-4-SPG)[16], and in the allelic disorders SPG78 (MIM#617225) and Kufor-Rakeb syndrome (MIM#606693), due to biallelic variants in *ATP13A2*[17]. The "ear-of-the-lynx" sign has been also occasionally reported in patients with variants in the *SPG7* and *CAPN1* genes, linked to SPG7 (MIM#607259) and SPG76 (MIM #616907), respectively[18,19]. Notably, several genes associated with the "ear-of-the-lynx" sign such as *SPG11*[20], *ZFYVE26*[20], *ATP13A2*[17] and *LNPK*[21] have been implicated in autophagy, raising the suspicion for a possible common underlying mechanism related to ER-phagy dysfunction. Interestingly, myoclonic seizure is frequently observed in our cohort while it does not typically occur in the above disorders. This association when present may help clinicians to recognize LNPK-related disorder in the clinical setting. Main features of the

NDD disorders presenting with the "ear-of-the-lynx" sign and comparison with LNPK are displayed in the supplemental Table 2.

In addition, most patients (72.2%) had additional T2-FLAIR hyperintensity of the substantia nigra. Remarkably, loss of normal susceptibility signal drop-out of the substantia nigra is found in some neurodegenerative disorders such as Parkinson disease and related conditions[22] in which the nigrostriatal pathway is impaired. However, signal alterations of the substantia nigra are unusual in neurodevelopmental disorders and have never been described in the group of SPGs linked to ER protein dysfunction. Notably, *LNPK* is abundantly expressed in the human substantia nigra [nTPM (normalized protein-coding transcripts per million): 9.2 according to the Human Protein Atlas database], yet its role in the nigrostriatal dopaminergic circuit remains to be investigated. Neurological follow-up of affected individuals with *LNPK* pathogenic variants will be important to determine whether they may develop parkinsonism later in 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 *S. 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 harboring a homozygous truncating variant in *LNPK* 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[23]. Of note, autophagosomes form at the ER in mammals and ER membrane contacts are known to play a central role in regulating autophagosome formation[24]. 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 signaling 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 *LNPK* homologue (lnp-1) in *C. elegans* causes mislocalization of presynaptic proteins, suggesting a role of Lnp-1 in synaptogenesis through regulation of vesicular transport or localization[25]. 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 LNPK-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.

Data availability

All variants have been deposited into LOVD database:

https://databases.lovd.nl/shared/variants/KIAA1715/unique

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Competing Interests

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing completed at Baylor Genetics Laboratories.

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Figure Legend

Figure 1 Pedigrees of the families, photos, clinical summary of the affected individuals and schematic representation of the gene and protein with all the pathogenic variants

(A) Pedigrees of families 1-11. In the pedigrees, squares represent males, circles females, black shaded symbols denote patients harboring biallelic *LNPK* variants. Plus (+) and minus (−) signs indicate absence or the presence of the *LNPK* variants ([+/+] wild-type, [+/−] heterozygote and [−/−]

homozygote for the *LNPK* variant). Pedigrees of previously reported patients are at the bottom of panel A separated by a line. (B) Bar graph showing the distribution of the most relevant clinical and radiological features among the total patients (20) identified so far with biallelic *LNPK* variants. Red: number of patients out of 18 showing each feature. Blue: number of patients without each specific feature. Gray: brain MRI not available for 2 individuals. GDD, global developmental delay; ID, intellectual disability (C) Clinical features of patients with homozygous *LNPK* variants showing subtle and non-specific dysmorphic features such as medially flared eyebrows, long palpebral fissures, prominent philtrum, long chin in patient II:3 of family 1, bilateral infraorbital crease and thin upper lip vermilion in patient II:2 of family 2, almond shaped eyes, anteverted nares and thin upper lip vermilion in patient II:1 of family 4; uplifted earlobes in patient II:3 of family 8; smooth philtrum, thin upper lip vermilion and uplifted earlobes in patient II:2 of family10; deep set eyes, thin upper lip vermilion and uplifted earlobes in patient II:2 of family 11; low frontal hairline and thick earlobes in patients A-III-1 and A-III-2 previously published by Breuss et al. 2018. (D) Schematic depiction of transcript (ENST00000272748.9) and the full-length LNP protein (GenBank: NP_085153.1) showing two transmembrane domains (dark blue), a coiled coil region (green) and a $zinc^{2+}$ finger domain (orange). The variants identified in the current cohort are displayed in bold. Note that the variant c.726del p.(Pro243Leufs*2) was also identified in the original manuscript of Breuss et al. 2018.

Figure 2 neuroimaging features of LNPK-related disorder

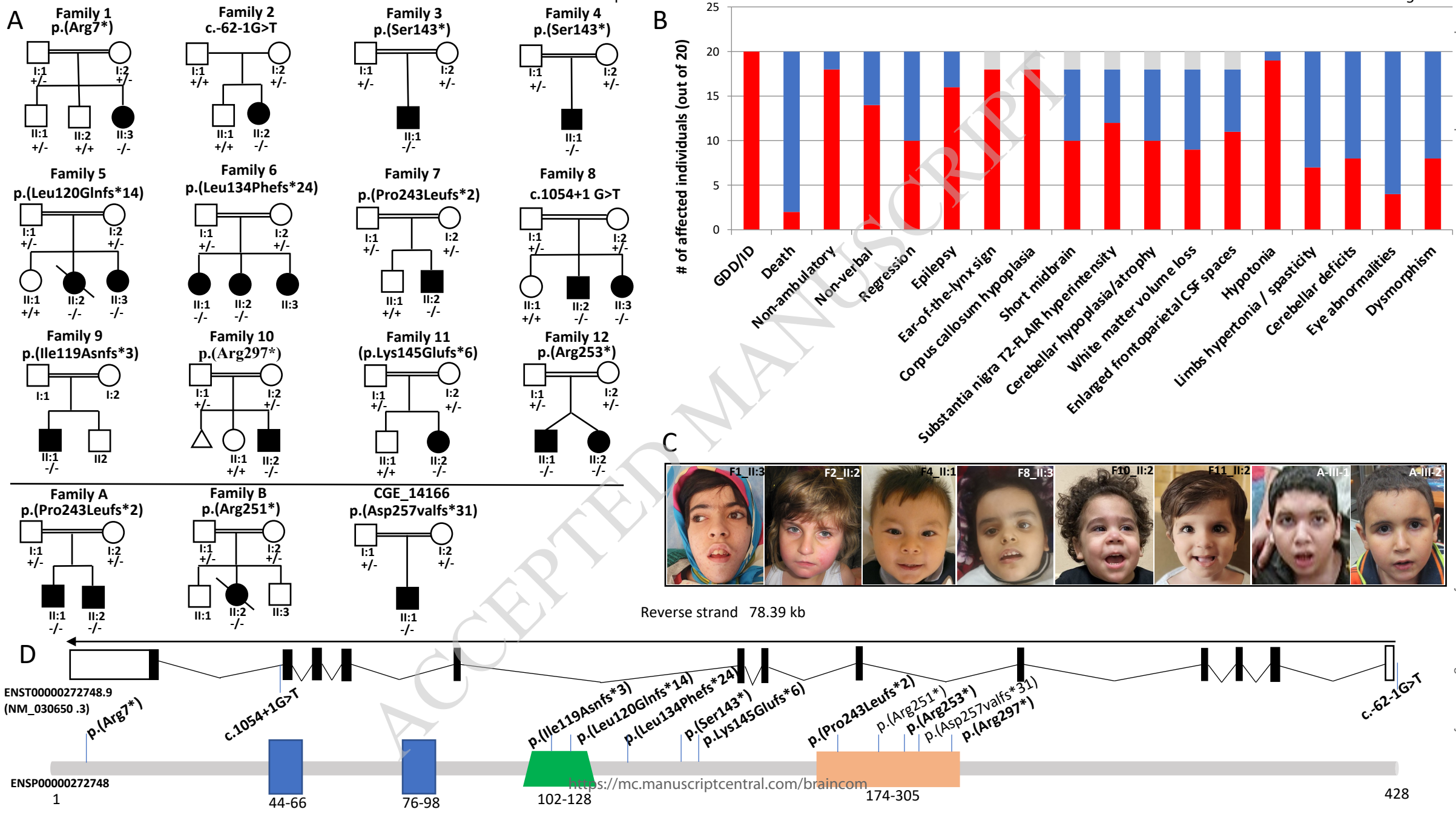
Brain MRI studies performed in patient II:1 from family 6 at 4 years of age (A-D) and in patient II:2 from family 7 at 2.5 years of age (E-H). Sagittal T1- (A) or T2-weighted (E) images demonstrate corpus callosum hypoplasia with prevalent involvement of the anterior portions (thick arrows). Coronal (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).

Table 1. **Genetic and phenotypic characteristics of patients with *LNPK* variants.**

Family ID	1	2	3	4	5		6		7	8		9	10	11	12		13			14
Patient	II:3	II:2	II:1	II:1	II:2	II:3	II:1	II:2	II:2	II:2	II:3	II:1	II:2	II:2	II:1	II:2	A-III-1	A-III-2	B-III-2	CGE_14
Age, sex	13y, F	16y, F	13y, M	3y5m, M	9y, F died	2.5y, F	19y, F	15y, F	7y, M	7y, M	3y, F	12y, M	3y, M	2y, F	3y,M	3y,F	15y, M	7y4m, M	16y, F died	9y, F
GDD/ID	++++	+++	++++	+++	+++	++	+++	++	++++	+++	++	+++	++	++	+++	+++	+++	+++	+++	+++
Non-ambulate	+	+*	+	+	+	+	+	+	+	+	+	+*	+	+	+	+	+	-	-	+*
Non-verbal	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+
Regression	+	-	+	-	+	-	+	-	+	-	+	+	-	-	-	-	+	-	+	+
Epilepsy	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+	+
Seizure-AOO	10m	6y	4y	2m	3y		2y	18m	2y	4y	2y	5y			2y3m		2y	2y	6y	7y
Seizure type	Myo, TC	Myo, TC	Myo	Focal, TC	Myo, TC		TC, atyp Abs.	NA	Myo	Focal TC	TC	Myo, TC			Myo, TC		Myo	Myo	TC	Myo, TC
Seizure frequency	Up to 100/day	3-4/week	4-5/day	1-2/month	20/day		20/day	NA	30-50/day	1/month	1-2/day	3-4/day			NA		Up to 10/day	NA	Up to 10/day	Up to 20/day
Response to ASM	-	+	-	+	-		-	NA	-	-	-	-			-		-	+	-	-
Age at brain MRI	8y3m	7y	8y	1y	1y1m	NA	4y10m	1y6m	2y5m	3y	4y	8m; 1y9m	3y	2y	NA	2y	6y	4y	14y	2y7m; 9y
CCH	+	+	+	+	+	NA	+	+	+	+	+	+	+	+	NA	+	+	+	+	+
Ears of lynx sign	+	+	+	+	+	NA	+	+	+	+	+	+	+	+	NA	+	+	+	+	+
WMVL	-	-	+++	-	-	NA	++	++	-	++	++	-	-	-	NA	-	+	+	+	++
Enlarged FP CSF spaces	+	-	+	+	+	NA	+	+	-	+	+	+	-	-	NA	-	-	-	+	+
Midbrain height	Short	Short	Normal	Short	Normal	NA	Short	Short	Normal	Short	Short	Normal	Normal	Short	NA	Normal	Normal	Normal	Short	Short
Substantia nigra SA	-	+	+	-	-	NA	+	+	+	+	+	-	+	+	NA	+	-	-	+	+
Cerebellum	Mild atrophy	Normal	Mild atrophy, IVH	Normal	Mild IVH	NA	Mild IVH	Mild IVH	Mild IVH	Normal	Normal	Mild IVH	Normal	Normal	NA	Normal	Normal	Mild IVH	Mild atrophy	Mild IVH
OFC (SDS)	-0.9	-3.2	+0.5	-1.2	+0.6	+0.2	+3.3	+2.5	-2.6	+1.1	+1	-1.1	-0.1	-2.4	NA	NA	-1.1	-1.0	-1.4	+1.14
Axial hypotonia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Limbs hypertonia	-	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+
Cerebellar signs	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+
Dysmorphism	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-
Eye features	-	Bil. cataracts	Nysta-gmus	Bil. ONA	Nystagmus esotropia	Esotro-pia	-	Esotro-pia	Nysta-gmus	-	-	Esotropia, Bil. cataracts	-	-	-	-	-	-	Bil. ONA	-

ASM, antiseizure medications; AOO, of onset; atyp, atypical; Bil., bilateral, CCH, corpus callosum hypoplasia; CSF, cerebrospinal fluid; GDD, global developmental delay; DTR, deep tendon reflexes; F, female; FP, frontoparietal; hom, homozygous; myo, myoclonic; TC, tonic clonic; ID, intellectual disability; IVH, inferior vermis hypoplasia; m, months; OFC, occipital frontal circumference; ONA, optic nerve atrophy; M, male; myo, myoclonic; NA, not available; SA, signal alterations; SDS, standard deviations; WMVL, white matter volume loss; y, years; * previously able to walk, unable to walk after regression; + and - present or absence of a specific feature respectively. Family 13 and 14 have been reported by Breuss et al. 2018 and Türkyılmaz et al. 2022, respectively.



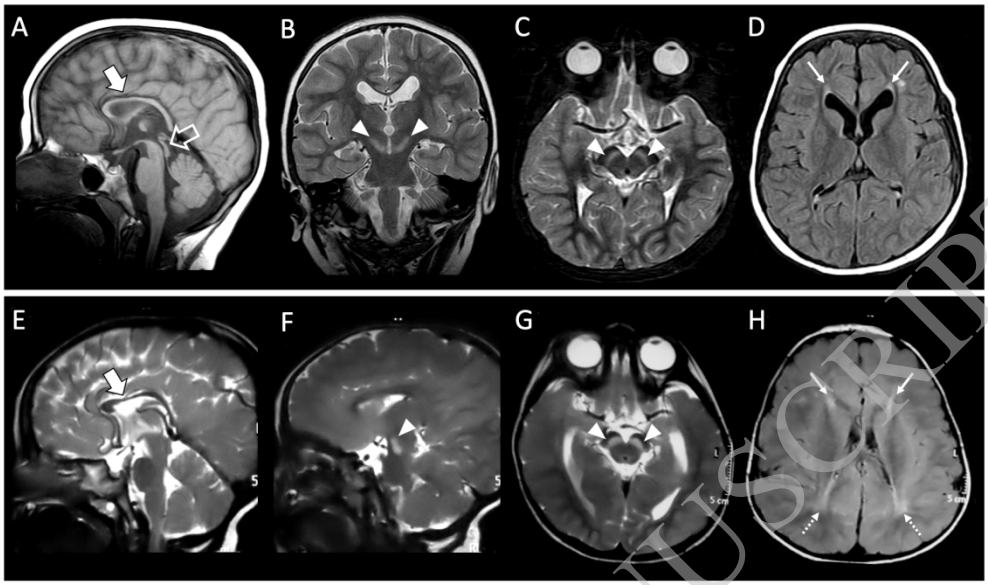


Figure 2

158x95mm (300 x 300 DPI)

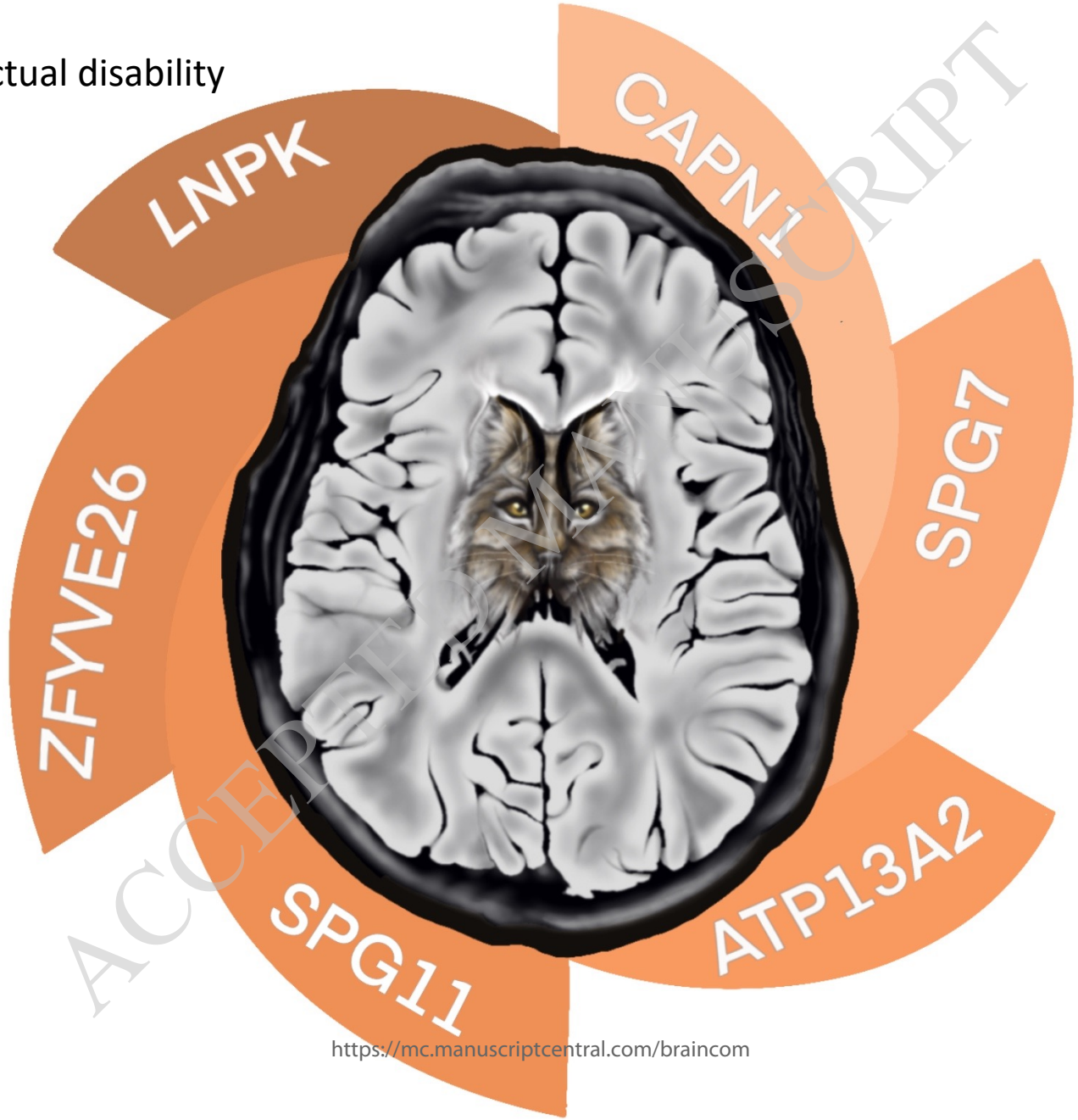
Recessive neurological disorders with the ear-of-the-lynx sign

LNPK
- Moderate – severe intellectual disability
- +/- regression
- Myoclonic seizure
- Age of onset: congenital

***ZFYVE26* (SPG15)**
- Mild intellectual disability
- Spasticity
- +/- dystonia/parkinsonism
- +/- neuropathy
- Age of onset: 5-61 years (Mean age 23 years)

SPG11
- Mild intellectual disability
- Spasticity
- +/- parkinsonism
- +/- neuropathy
- Age of onset: 1-31 years

SPG: Spastic paraplegia



CAPN1
- Spasticity
- Age of onset: adulthood (Mean age 19 years)

SPG7
- Spasticity
- Age of onset: 10-72 years

ATP13A2
- Normal early development - regression
- Parkinsonism
- Spasticity
- Psychiatric features
- Supranuclear gaze palsy
- +/- neuropathy
- Age of onset: adulthood (Mean age 32 years)