Parkinsonism and Related Disorders 134 (2025) 107781

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

TOR1AIP2 as a candidate gene for dystonia-hemichorea/hemiballism

Efthymia Kafantari [a,*], Victoria J. Hernandez [b], Ján Necpál [c,d], Marina Leonidou [a], Regina Baureder [e], Carola Hedberg-Oldfors [f], Robert Jech [g], Michael Zech [h,i,j], Thomas U. Schwartz [b], Andreas Puschmann [a,k]

[a] Lund University, Skåne University Hospital, Department of Clinical Sciences Lund, Neurology, Lund, Sweden
[b] Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA
[c] 2nd Department of Neurology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[d] Department of Neurology, Zvolen Hospital, Zvolen, Slovakia
[e] Lund University, Skåne University Hospital, Department of Clinical Sciences Lund, Clinical Neurophysiology, Lund, Sweden
[f] Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[g] Department of Neurology, Charles University in Prague, 1st Faculty of Medicine and General University Hospital in Prague, 121 08, Prague, Czech Republic
[h] Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany
[i] Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany
[j] Institute of Advanced Study, Technical University of Munich, Garching, Germany
[k] SciLifeLab National Research Infrastructure, Sweden

ARTICLE INFO

Keywords:
Dystonia
Hemiballism
Hemichorea
Stereotypy
TOR1AIP2
DYT1
TorsinA
Nuclear envelope

ABSTRACT

Dystonia is a movement disorder characterized by genetic and clinical heterogeneity. A recurring p.(Glu303del)-deletion in *TOR1A* is a well-established cause for DYT-*TOR1A* (DYT1), an autosomal dominant early-onset isolated dystonia. *TOR1A* encodes TorsinA, an AAA + ATPase located in the nuclear envelope. By whole exome analyses of a family with a novel dystonia-hemichorea-/hemiballism phenotype, we identified a *TOR1AIP2* NM_001199260.2 c.1234A > G p.(Arg412Gly) variant. The variant is very rare in databases and was absent from whole exome data from >1000 dystonia patients. *TOR1AIP2* encodes LULL1, a transmembrane protein that activates TorsinA, and correct interaction between TorsinA and LULL1 is essential for proper nuclear envelope architecture. The p.(Arg412Gly) variant disrupts the binding interface between TorsinA and LULL1 around p. Arg412; this same interface is also impaired in DYT1. Functional analyses via a co-purification assay revealed that interaction between TorsinA-LULL1^{Arg412Gly} is weaker than the wild-type interaction, and that it resembles the situation in DYT1 (TorsinA$^{\Delta E303}$-LULL1). A second family with milder dystonia, hemichorea, and stereotypic leg flexion during gait and a *TOR1AIP2* p.(Gln338His) variant was identified. The clinical phenotype of both families shared proximal arm movements, and flutter in facial musculature. Expressivity of the movement disorder symptoms was variable. Several proteins in the nuclear envelope have been implicated in various forms of neurodevelopmental disorders with dystonia. Taken together, our findings suggest *TOR1AIP2* as a new candidate gene implicated in a complex hereditary movement disorder with dystonia and hemichorea/hemiballism.

1. Introduction

Dystonia is characterized by clinical and genetic heterogeneity. The etiology of dystonia remains largely unknown, but a proportion of patients have a monogenic form; many known monogenic forms include combinations of dystonia with additional movement disorder signs and symptoms [1,2].

In this work we describe two families with relatively unusual clinical presentations of combined dystonia, hemichorea, hemiballism and stereotypy who both carried candidate variants in the *TOR1AIP2* gene co-segregating with the clinical phenotype. Experimental studies showed that the molecular effect of the initial family's variant is very similar to that of the most common *TOR1A* variant that causes classical Oppenheim disease (DYT-*TOR1A*, DYT1). The protein encoded by *TOR1AIP2,* LULL1, resides in the endoplasmic reticulum, including the outer membrane of the nuclear envelope [3]. A number of additional neurological disorders with dystonia are caused by mutations in proteins in the nuclear envelope or nuclear pore complexes [2]. Our findings

* Corresponding author. Department for Neurology, Skåne University Hospital, Entrégatan 7, 222 42, Lund, Sweden.
E-mail address: Efthymia.Kafantari@med.lu.se (E. Kafantari).

https://doi.org/10.1016/j.parkreldis.2025.107781
Received 19 August 2024; Received in revised form 7 March 2025; Accepted 9 March 2025
Available online 11 March 2025

suggest *TOR1AIP2* as an additional candidate gene implicated in combined dystonia.

2. Methods

All studied individuals in Family 1, from Sweden, were included in a research study on rare neurological diseases and provided their informed consent to participate. The study was approved of the regional ethics review board.

The Swedish index patient was repeatedly referred to the movement disorders clinic at our tertiary center. Her mildly affected son was also examined at our clinic. Her unaffected daughter as well as her two unaffected half-siblings were interviewed, observed and examined via high-quality video connection and provided blood samples (Fig. 1A). The index patient was examined clinically by the authors at her age 53, 60 and 63 years. Video material from these visits as well as a video from a visit at 47 years to her local neurologist were obtained and analyzed.

Genomic DNA was extracted from peripheral blood obtained from 2 affected individuals and 3 clinically unaffected family members; whole exome sequencing (WES) was performed, and data mapped to human genome build hg19/GRCh37. An exome-wide search was performed first whereby rare variants present in the two affected and absent in the three unaffected family members were identified. Then, a list of 535 protein-coding genes implicated in dystonia or encoding proteins with a known interaction with proteins involved in the pathogenesis of monogenic dystonia, 5 RNA genes, and 7 dystonia loci was compiled from publicly available databases and from published review articles (Supplemental Table). Rare variants in genes from this list were filtered according to co-segregation with the phenotype in the family and *in silico* parameters. Detailed information on genetic and bioinformatic methodology is provided in the Supplement.

Rare *TOR1AIP2* variants were then searched for in exome and genome data available at Skåne University Hospital, Sweden (15 exomes from dystonia patients, over 280 exomes from patients with other movement disorders), and the Munich Exome Server (Technical University of Munich and Helmholtz Center Munich, Germany) containing over 1000 exomes from dystonia patients, and more than 25,000 exomes from individuals who were tested for various clinical conditions or as relatives of affected subjects.

This led to the identification of a second family (Family 2), from Slovakia, with a movement disorder and another candidate variant in *TOR1AIP2*, in the index patient. The index patient and her son were re-examined clinically within the present study, a video was obtained from the index patient's affected brother who was not available for examination in person due to geographical distance from our centers. DNA was analyzed from 4 family members as shown in Fig. 1. Members of Family 2 were part of a genomic sequencing project focusing on heterogeneous dystonia syndromes; written informed consent was obtained under ethics review board-approved protocols [4].

All persons seen on the images and videos in/accompanying this article provided consent to the print and online publication of this material.

Binding of p.(Arg412Gly)-mutated LULL1 (LULL1^{R412G}) to TorsinA was examined *in silico* and tested in functional experiments, and compared to the interaction of wild type proteins, as well as to the situation in DYT1 with p.(delGlu303)-mutated TorsinA (TorsinA$^{\Delta E303}$). Detailed biochemical methods are provided in the Supplementary material.

3. Results

3.1. Family 1

The index patient of **Family 1 (P746**) was a 63-year-old female. At the age of 37 years, she started to experience episodes of stereotyped, involuntary and fast horizontal head rotation. From the age of 45, some of these were combined with inward rotation of her right forearm. Subsequently, dystonic torsion of the upper body with protrusion of the right shoulder and a fast ballistic elevation of the right arm occurred during some of the episodes. Later, similar episodes occurred also affecting the left shoulder and left arm, and from age 59 in the lower extremities including fast thigh flexion in the hip joint when lying down and plantar flexion of the feet when standing. During our examinations and in videotapes, rapid involuntary rotation of the head to the left with a subsequent, almost simultaneous ballistic movement of the ipsilateral arm was triggered when the patient was lying on her back (video). The patient was able to prevent the involuntary torsion of her head by placing her left hand on the left side of her neck, or by the touch of a structure supporting the back of her head when seated. The patient had difficulty sleeping and was treated with melatonin and propiomazine. No psychiatric or neuropsychiatric symptoms or symptoms associated with dysfunction of the autonomic nervous system were noted. Repeated neurological examination revealed mild lordosis in the cervical and thoracic spine. On examination of biceps and ankle reflex, the reflexes

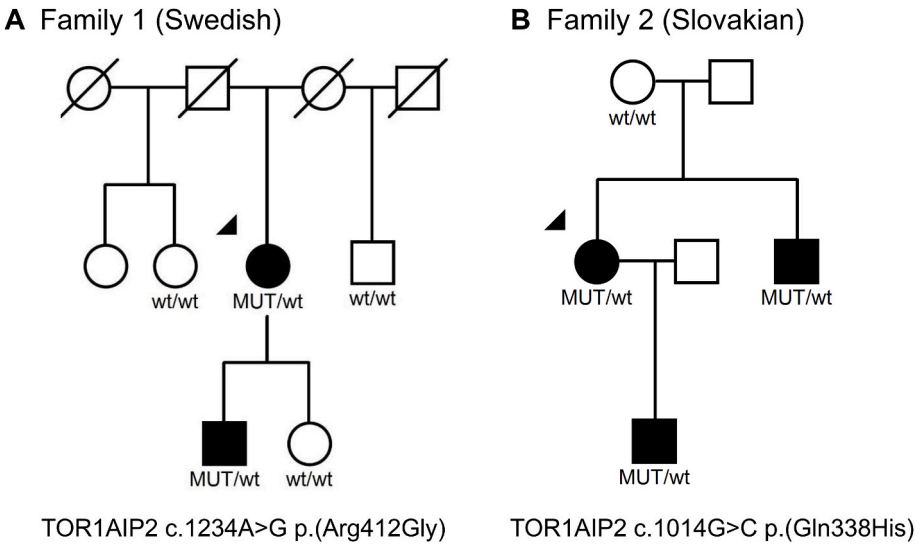

Fig. 1. Pedigree drawings of two families with dystonia and rare variants in *TOR1AIP2*. Black symbols denote symptomatic individuals. MUT: variant; wt wild type.

were accompanied by myoclonic movements of both arms or the head and neck. In her gait, a mild asymmetry of arm movements was noted where her right arm moved less than her left; this was interpreted either as dystonia in the right arm and/or mild chorea in the left. There was no muscle weakness, abnormal muscle tone or ataxia. Cranial nerve and sensory examination were normal. The patient received treatment with clonazepam 2 mg daily and botulinum toxin injections in the trapezius muscle every 12th week, which partially alleviated some of the symptoms. Frame-by-frame evaluation of videos from stereotypical episodes at 47, 53 and 60 years of age showed that the exact sequence and temporal course of involuntary movements remained unchanged over these 14 years (Video, Fig. 2, Supplemental Fig. 1). Her brain MRI was normal (Supplemental Fig. 2). Surface electromyography was performed of sternocleidomastoid, trapezius and deltoid muscles bilaterally and with simultaneous EEG recording from the right motor cortex. During the stereotyped attacks of head torsion to the left and hemiballistic upward movement of the left arm, the recordings revealed bursts of EMG activity between 0.41 and 0.70 s in duration (Supplemental Fig. 3). Some of these rapid episodes were followed by a more tonic activation of the muscles of up to a few seconds duration. Our technical equipment did not allow for back-averaging nor for analysis of Bereitschaftspotential. Sensory evoked potentials were normal.

The patient's son (**P747**) had noticed involuntary muscle activity around his mouth when attempting to smile that had started in middle school age (13–15 years). When he was 27 years old, he first experienced episodic dystonic movements of his head turning upwards and to one side, lasting for about 20 s. On examination at age 29 there was mild head tremor, involuntary task-specific jerky movements in the perioral musculature (Video Family 1). When re-examined by the authors at age 38 years of age, involuntary perioral movements had remained unchanged and were still visible, occurring only when the patient smiled. He had mild laterocollis to the right side and held his hand to the right

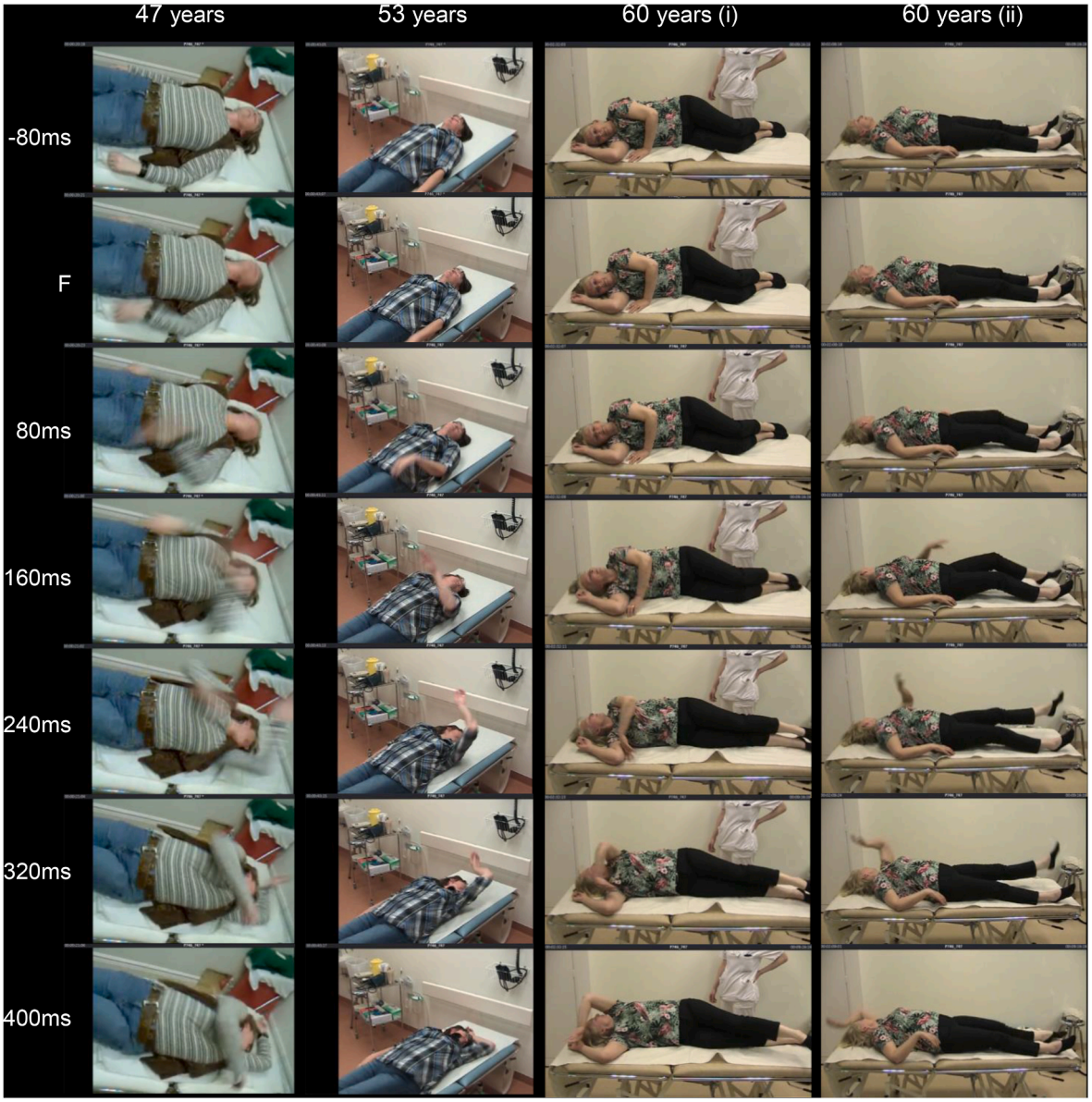

Fig. 2. Serial images of four episodes of the index patient's stereotypical attacks with torsion dystonia and hemiballism. The index patient was videotaped at 47, 53 and 60 years of age as indicated. The patient was asked to lie on her back, which triggered attacks of head turn to the left, a fast ballistic movement of the left arm upwards, torsion of the upper body to the left, and flexion of the left hip. Episodes remained highly stereotypical over the 13-year observation period. The examination table was placed away from walls to avoid injuries. F: First discernible body movement, as determined in full-size still images. See Supplemental Fig. 1 for the complete series of all frames from the videos recorded at 25 frames/s, and Video Supplement Family 1 for videos.

cheek; and the right trapezius muscle was tense. When supine, he had involuntary visible muscular flutter in his chin. When walking and especially when changing gait direction, there were episodes with exaggerated rapid elbow flexion, lifting one hand about 10 cm higher than the contralateral hand. It was impossible to classify these rapid movements as dystonic or choreatic. He reported that the dystonic head movements had had a fluctuating course, occurring at times of increased stress or after more intense physical work with his arms.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2025.107781

Interviews, observation and telemedicine examination of the three unaffected family members revealed that they did not have any similar symptoms or signs.

3.2. Family 2

The index patient of Family 2 (Fig. 1B) was a Slovakian female who was evaluated for subjective symptoms affecting her speech, that were investigated without pathological findings and that resolved spontaneously. On examination at age of 38 years, she displayed involuntary choreatic movements in her body and especially her face, which she was not aware of. She had mild difficulty with saccade initiation, positive tongue protrusion sign, and patellar areflexia. During gait, there was reduced right arm swing with posturing and dystonic stiffness of the right leg, as well as an unusual stereotypic movement of the right leg with intermittent pronounced knee flexion, interspersed among normal gait movements. She denied any compulsive character of these stereotypic movements. On the left side, there was mild hemichorea. MRI of the head was unremarkable. Nerve conduction studies revealed mixed but predominantly sensory axonal polyneuropathy. There was no cognitive impairment (MoCA 28/30). She subsequently developed ovarian carcinoma with peritoneal carcinomatosis but agreed to re-examination (Video Family 2). At this renewed examination at age 40, her facial chorea had increased and the leg affection visible during gait had persisted. An extensive workup for alternative diagnoses including paraneoplastic movement disorders was negative. Whole exome sequencing was performed within a research study on unresolved dystonia patients, as detailed elsewhere [4].

Supplementary video related to this article can be found at https://doi.org/10.1016/j.ceramint.2025.02.045

Her 10-year-old son was born by normal delivery and achieved pediatric psychomotor milestones normally. Since his infancy, he had mild abnormal posturing of his right upper limb and slightly clumsy gait. On examination there were subtle hyperkinesias of the fingers and clear posturing of the little finger on the right, enhancing by repetitive movements of the opposite site, consistent with dystonia. There was patellar hyporeflexia contrasting with bilaterally positive extensor plantar responses. Clonus and velocity-dependent hypertonus were not present. When he walked, reduced right arm swing and dystonic stiffness of the right leg as well as mild hyperkinesias of the left arm and hand were noted (Video Family 2). MRI of the head was normal, whereas nerve conduction studies showed incipient sensory axonal polyneuropathy.

Her 42-year-old brother was not examined by the authors because of geographical distance. He had received clinical a diagnosis of "cerebral palsy"; however, he reported progressive hypertonia of his right leg and right arm. Review of videos provided by the patient showed a probable combination of dystonic posturing with distal weakness in his right arm and plantar flexion of the foot with a tendency to shuffling gait mimicking steppage gait (Video Family 2). No neurophysiological or radiological studies were performed.

3.3. Genetic analyses

Our exome-wide search in Family 1 revealed 41 rare variants with a CADD-phred score above 20 that were present in the affected mother-son pair and absent in the three unaffected family members. Of the 41 variants, seven missense variants were in genes associated with neurological phenotypes, but the genotype and/or our patients' clinical phenotype were not compatible with these conditions. WES analysis of variants in genes in our expanded dystonia gene list revealed 9 rare variants shared between mother and son, and 4 of these were also absent from the unaffected members. From these, two were synonymous with very low pathogenicity prediction scores and another one was consistently considered benign in several prediction tools.

Both these analyses identified a heterozygous variant c.1234A > G p.(Arg412Gly) in *TOR1AIP2* (NM_001199260.2, ENST00000609928.6; rs753337635, R412G; Supplemental Fig. 4). This variant was absent from gnomAD genomes and 1000 Genomes and present in 7 of 1,180,028 Non-Finnish European alleles in the gnomAD exome database (v4.0.1, allele frequency 0.000005932). It was absent from alleles from other ethnic groups in gnomAD, and absent from 25,000 examined in-house exomes (Munich, Germany). It had a CADD-phred score of 23.8 [5,6].The gene and phenotype were entered into GeneMatcher but this failed to identify any matches.

No variants in *TOR1AIP2* were found in 15 exomes of patients with dystonia and rare movement disorders at Lund University. A search among more than 1000 exomes from dystonia patients (Munich Exome Server) revealed 2 rare variants in *TOR1AIP2* (NM_145034.5), each in one patient: c.1314T > A p.(Met438Leu), that was considered irrelevant as the cause of a very rare clinical phenotype because of its allele frequency in gnomAD among East Asians of 0.007, and c.1014G > C p.(Gln338His; Q338H), that was only present in 20 of 1,614,092 alleles in gnomAD (0.00001239; CADD-phred 17.50). All three affected members of Family 2 were heterozygous for the c.1014G > C variant in *TOR1AIP2* (Fig. 1) whereas the unaffected mother of the index patient did not carry this variant. *In silico* molecular models show that this residue is located on the surface of the luminal domain of LULL1 but not involved in binding to TorsinA (Fig. 4). We found no potentially disease-causing *TOR1AIP2* variants in WES data from individuals without dystonia.

3.4. Functional assays

In the encoded protein LULL1, the R412G variant is a substitution of arginine, a basic amino acid with a long side chain, by glycine, a neutral amino acid without side chain. Interestingly, this particular R412 residue of LULL1 had previously been reported to form hydrogen bonds to the neighboring E302 and E303 residues of TorsinA, the very residue that is deleted in the recurring p.(delGlu303) 3bp in-frame deletion (ΔE303) which is by far the most common cause of DYT1 [7]. This suggested that the variant likely affects TorsinA activation by disrupting or weakening LULL1 binding. The variant's effect on its binding to TorsinA was investigated experimentally. The semiquantitative pull down assay revealed that TorsinA^{wt}-LULL1^{R412G} (reflecting the situation in Family 1) molecularly phenocopies TorsinA$^{\Delta E303}$-LULL1^{wt} (reflecting the situation in DYT1). As shown in Fig. 3, the TorsinA^{wt}-LULL1^{R412G} interaction is weakened significantly compared to TorsinA^{wt}-LULL1^{wt}, and almost as severely as in TorsinA$^{\Delta E303}$-LULL1^{wt} interaction, which had been identified as the crucial molecular mechanism for classic DYT1 dystonia [7]. This implies that the R412G variant affects TorsinA activation by disrupting LULL1 binding, but also that the biological effect of TorsinA$^{\Delta E303}$ is stronger than that of LULL1^{R412G}.

4. Discussion

Our results suggest *TOR1AIP2* as a new candidate gene implied in a combined movement disorder with features of dystonia, asymmetric arm movements with dystonic and/or hemichoreatic components, as well as fluttering movements in perioral and lower facial muscles. One patient also had hemiballism and involuntary upper body torsion and neck extension. *TOR1AIP2* encodes the luminal domain like LAP1 (LULL1) protein that interacts with TorsinA, the protein involved in

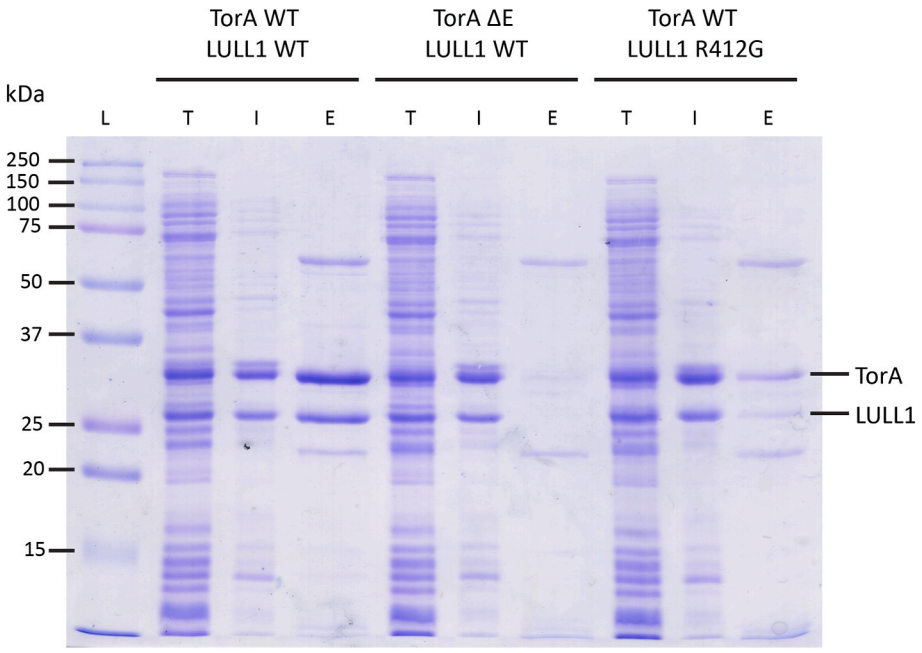

Fig. 3. Binding between LULL1 and TorsinA was examined using a Ni-affinity co-purification with recombinant, bacterially expressed protein. TorsinA is polyhistidine-tagged whereas LULL1 is not. Complex formation is observed by an approximate 1:1 ratio of TorsinA and LULL1 in Ni eluates as visualized on SDS-PAGE. Whereas wild type TorsinA (TorA WT) complexes wild type LULL1 (LULL1 WT) in the eluate, E303-deleted TorsinA (TorA ΔE) abolishes binding with LULL1 WT, as shown previously [7]. LULL1 R412G results in markedly reduced complex formation, phenocopying the TorA ΔE/LULL1 WT results. L, protein marker; T, total lysate; I, insoluble fraction; E, Ni eluate.

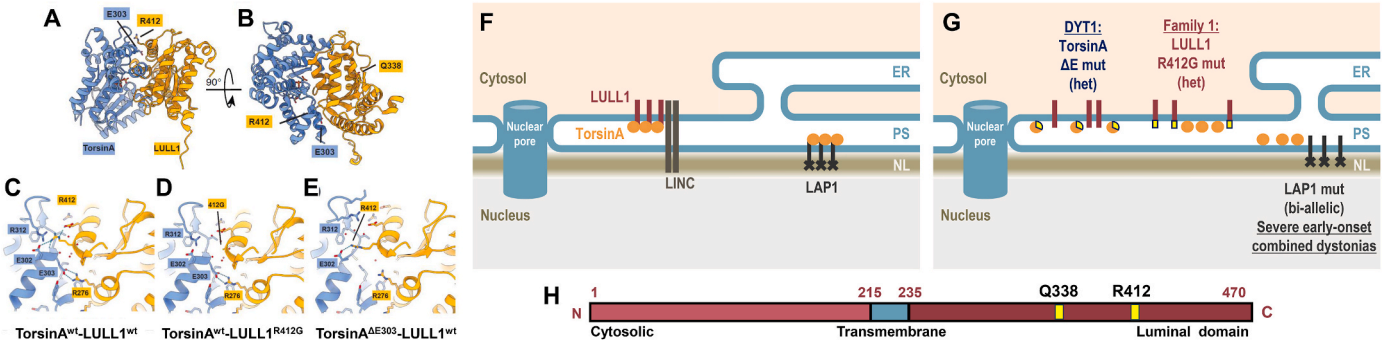

Fig. 4. Models of R412G-mutated LULL1 and its suggested effect. **A-E** The binding site of TorsinA to LULL1. **A and B:** Overview of the TorsinA:LULL1 complex (Protein Data Bank code: 5J1S). TorsinA in blue, LULL1 in orange. The positions of the LULL1 variants in the two families described here (Family 1 LULL1-R412G, Family 2: LULL1 Q338H) and of the recurrent TorsinAΔE303 mutation causing DYT1 are indicated. **C:** Close-up of the wild type (wt) TorsinA:LULL1 interface, showing that the R412 residue of LULL1 forms several hydrogen bonds with TorsinA. **D:** The R412G mutation in LULL1 (Family 1) disrupts hydrogen bonding at R412. **E:** In DYT1, lack of the E303 residue (ΔE303 deletion) disturbs hydrogen bonds with LULL1 residues in the vicinity of LULL1 R412. Therefore, LULL1^{R412G} likely phenocopies TorsinA$^{\Delta E303}$, affecting the binding of TorsinA to LULL1. The LULL1 Q338H mutation is not expected to interfere with TorsinA binding and awaits further molecular analysis. **F:** LULL1 is located in the outer lipid bilayer of the nuclear envelope and in endoplasmic reticulum (ER) membranes. It binds to and activates TorsinA, with which it forms heterooligomers. LULL1-TorsinA complexes are thought to be important for the proper architecture of the nuclear envelope, possibly by regulatory interaction with the Linker of nucleoskeleton and cytoskeleton (LINC) complex or other nuclear envelope proteins. LAP1 similarly interacts with TorsinA but is located in the inner bilayer of the nuclear envelope and is anchored to the nuclear lamina. **G:** In DYT1 dystonia, the deletion of one of two neighboring glutamic acid residues (ΔE) in TorsinA, impairs binding of TorsinA to LULL1 and LAP1; this has been shown to cause different alterations in the nuclear envelope, most notably protrusions of the inner nuclear membrane into the outer nuclear membrane (nuclear envelope blebbing, not shown). In Family 1 presented here, TorsinA binding to LULL1 is similarly impaired because of the R412G mutation in LULL1. Biallelic missense variants in LAP1 have been identified in a severe neurodevelopmental syndrome with myopathy, cardiomyopathy and, in some patients, dystonia (see article main text). **H:** Diagram of LULL1. Cytosolic, transmembrane and luminal domains are displayed. The localization of the identified variants is also shown. The LULL1 mutations in both Family 1 and Family 2 are located within the protein's luminal domain. Figure is based on data from these references: [7,12,29,30]. PS, perinuclear space; NL, nuclear lamina. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

early onset primary dystonia (DYT1). Importantly, the genetic variant revealed by whole exome sequencing of 2 affected and 3 unaffected members of Family 1 involves the R412 residue of LULL1, which is known to bind to TorsinA and to participate in hydrogen bonding between LULL1 and TorsinA. DYT1 caused by the recurrent TorsinA$^{\Delta E303}$ deletion weakens the same binding region. Our functional studies confirm that the LULL1 variant of Family 1 markedly disturbs binding to TorsinA, but not quite as severely as in DYT1. This is compatible with the later-onset and milder clinical phenotype of Family 1 compared to early onset DYT1. We identified a second family, Family 2, where another rare

variant in *TOR1AIP2* co-segregated with clinically milder symptoms than in Family 1 in three affected members and was absent in the proband's unaffected mother. The *TOR1AIP2* variant in Family 1 was identified both in an open exome-wide approach and in an independent approach using a newly compiled gene list with known genes implicated in dystonia and with genes for proteins known to interact with proteins involved in the molecular pathogenesis of dystonia.

TorsinA is activated by two type II transmembrane proteins residing in the nuclear envelope, lamina-associated polypeptide 1 (LAP1) and LULL1, encoded respectively by *TOR1AIP1* and *TOR1AIP2* [8–10]. Crystallographic and biochemical evidence suggested that LAP1 and LULL1 activate ATP hydrolysis in TorsinA by inserting a conserved arginine residue, termed the arginine finger, into the ATP binding site of TorsinA [7,8]. Comparison of the high-resolution structures of wild-type TorsinA-LULL1 and mutant TorsinA-LULL1 suggested that the cause of DYT1 is improper TorsinA activation, as known mutations cause protein misfolding or weaken or abolish binding to LAP1 or LULL1. Specifically in the case of the recurrent TorsinA$^{\Delta E303}$ deletion, the TorsinA^{E303}–LULL1^{R276} interaction is lost and the hydrogen-bonding network involving three TorsinA (E302, F306 and R312) and two LULL1 (R412 and E416) residues is disrupted [7] (Fig. 4). The LULL1^{R412G} mutation of Family 1 introduces a major structural change to this binding site by changing the long side chain of arginine to a hydrogen atom of glycine. LULL1^{R412G} likely interrupts the TorsinA binding site similarly to TorsinA$^{\Delta E303}$ at the above-mentioned binding site, consistent with our *in vitro* binding data (Fig. 3). TorsinA-LULL1 complexes are considered essential components of the structural architecture of the two adjacent double-lipid bilayers that form the nuclear envelope (Fig. 4).

TOR1AIP2 has not previously been implicated in human disease, but biallelic pathogenic variants in *TOR1AIP1* cause a severe recessive infantile/childhood onset disease with various abnormalities, including severe dystonia [11–14]. A patient homozygous for *TOR1AIP1* NM_015602 c.1448A > C p.(Glu482Ala) developed appendicular-onset dystonia at age 5 that soon generalized, was present permanently, extremely painful and refractory to treatment; the patient's cognitive development was not impaired [12]. A case series of seven patients with biallelic *TOR1AIP1* variants identified truncal hypotonia and limb hypertonia as a common feature in this severe neuropediatric disorder with high lethality [13]. Located in the inner nuclear membrane, LAP1 anchors TorsinA to the nuclear lamina and activates TorsinA's ATPase activity [15] (Fig. 4). Besides *TOR1A* and *TOR1AIP1*, variants in other genes encoding nuclear pore or nuclear envelope proteins have been linked to dystonia (reviewed in Ref. [2]): Biallelic pathogenic variants in *NUP62* and *NUP54* cause dystonia and choreoathetoid limb movements with infantile or childhood onset and striatal lesions [16,17]. Dystonia has also been described in patients with Allgrove syndrome or recessive spinocerebellar ataxia 8, caused by variants in *AAAS* or *SYNE1* [2].

The clinical phenotype of the index patient of Family 1 was unusual and to our knowledge the combination of stereotyped head rotation with fast ballistic arm movements, fast involuntary head extension and small fluttering of facial or perioral musculature has not been previously described. In fact, a diagnosis of functional movement disorder had been entertained for this patient for many years. However, the patient's history failed to fulfill most of the criteria that are considered to indicate functional origin [18]: The symptomatology started to develop gradually, rather than suddenly, and the main components had remained constant for over 13 years as shown in the video and still images. The patient had no history of psychiatric disease. However, the movements only occurred when the patient was lying down or sitting and appeared relatively quickly when they were observed or filmed, which has been described as a sign indicating that movement disorder may be functional [18,19]. The clinical phenotype of Family 2 included with asymmetric arm movements in all three members during gait, and, in the index patient, facial muscular movements as well as stereotypical intermittent leg flexion when walking. Both families shared dystonia, involuntary facial movements and proximal arm movements. We note that the most severely affected patient, the proband of Family 1, is also the oldest of all examined members, but had had milder movement disorder symptoms since age 37.

Many patients with DYT1 display a specific clinical phenotype that differs from other forms of dystonia: Torsion spasms, defined as irregular, fast spasmodic movements have been described repeatedly in DYT1 [20]. DYT1 typically involves dystonic movements rather than dystonic posturing, and typically has limb onset [21]. Dystonia in upper extremity is a common sign in DYT1 [22]. Involuntary and relatively rapid large amplitude arm movements in the shoulder joint are seen in patients with DYT1 caused by the classical 3bp deletion (for example case nr 2 in Ref. [23]), as are opisthotonus or similar fast extension of the head and trunk caused by paraspinal musculature [23]. Four of five patients in the two families with *TOR1AIP2* variants described here had fast intermittent (phasic) involuntary movements in the shoulder joint (videos), albeit of greatly varied amplitude and vehemence, which we suggest may be a feature common to patients with pathogenic variants in *TOR1A* or *TOR1AIP2* [24].

TOR1AIP2 c.1234A > G p.(Arg412Gly) fulfills the following criteria for variant pathogenicity published in 2015 by the American Collage of Medical Genetics and Genomics (ACMG) [25]: It is very rare in population databases (PM2 supporting). On the other hand, lines of computational evidence suggest no impact on gene product (BP4 moderate). However, CADD-phred score was more than 20 (23.8) and Polyphen-2 predicted a probably damaging effect. Our functional studies added clear evidence for a damaging effect of the variant. The co-purification assay revealed that the TorsinA^{wt}-LULL1^{R412G} interaction is weakened by presumably affecting LULL1 binding. This fulfills the ACMG guideline's PS3 criterium, providing *in vitro* functional evidence supportive for a damaging effect on gene or gene product, with a strong degree of evidence. Moreover, co-segregation was perfect and reached an N of 9/64 (supporting pathogenic segregation data) in Family 1 [26]. By adding these criteria, we formally classify the variant as of unknown significance. No alternative explanation for the dystonia in Family 1 was found. We thus consider *TOR1AIP2* c.1234A > G p.(Arg412Gly) probably disease-causing in this family.

TOR1AIP2 c.1014G > C p.(Gln338His) is classified as likely benign by Varsome but as a variant of uncertain significance by Franklin by genoox. It was very rare in population databases (PM2 supporting evidence). Co-segregation was perfect with N = 1/8 (PP1 supporting). Computational evidence suggests no impact on gene or gene product (BP4 strong evidence) although the variant's CADD-phred score was 17.5, indicating it belongs to the 1.78 % most deleterious variants. Importantly, the family with this variant was identified among >1000 exomes of patients with dystonias and the clinical phenotype of the particular (Slovakian) family in several regards was very similar to the one in the Swedish family. We thus consider it a probable cause of the disease in that family.

Taken together, formally, our data provides a "limited" degree of evidence for the association of the *TOR1AIP2* gene with the neurological disease described here [27]. Observations of additional patients and families with the same symptomatology and deleterious variants would be able to confirm the association. The disease phenomenology was mild on most but one individual and has not previously been described to our knowledge, which could explain why there were no entries on *TOR1AIP2* in GeneMatcher.

Phenotypic heterogeneity within families with TorsinA$^{\Delta E303}$ deletion is well known [28], and given the shared clinical and pathobiological features with the disorder in the two families presented here, it is quite possible that similar phenotypic heterogeneity is part of this disorder. Further limitations of our study include the use of exome sequencing data, rather than whole genome data, and we acknowledge the possibility that a true causative variant lies in a genomic region that was not captured. Also, we currently cannot explain the exact disease mechanism of the Q338H variant; we hypothesize that this mutation on the LULL1 surface may specifically affect binding to one of the candidate

TorsinA interactors, but this awaits further exploration [29].

5. Conclusions

Based on clinical, genetic and functional data and high biological plausibility we consider our work to provide sufficient evidence to nominate *TOR1AIP2* as a new candidate gene for a not previously described phenotype with dystonia, hemichorea and hemiballism. We note that the dystonias associated with mutations of nuclear envelope proteins share certain phenotypical features that may distinguish them from other forms of dystonia; the dystonia in these forms is often generalized and may involve fast and vehement limb movements in proximal joints, and choreoathetotic movements seem a recurring disease manifestation.

CRediT authorship contribution statement

Efthymia Kafantari: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Victoria J. Hernandez:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Ján Necpál:** Writing – review & editing, Writing – original draft, Investigation. **Marina Leonidou:** Writing – original draft, Investigation. **Regina Baureder:** Writing – review & editing, Investigation. **Carola Hedberg-Oldfors:** Writing – review & editing, Validation. **Robert Jech:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition. **Michael Zech:** Writing – review & editing, Validation, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation. **Thomas U. Schwartz:** Writing – review & editing, Project administration, Methodology, Funding acquisition. **Andreas Puschmann:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Funding

This study was funded by research grants from Region Skåne, Skåne University Hospital, the Swedish government (via ALF, avtal för läkarutbildning och forskning), and infrastructure support from MultiPark-a strategic research environment at Lund University and The Swedish Parkinson Academy, all in Sweden, to A.P. M.Z. receives research support from the German Research Foundation (DFG458949627; ZE 1213/2–1). M.Z. acknowledges grant support from the EJP RD (EJP RD Joint Transnational Call 2022) and the German Federal Ministry of Education and Research (BMBF, Bonn, Germany), awarded to the project PreDYT (PREdictive biomarkers in DYsTonia, 01GM2302), and from the Federal Ministry of Education and Research (BMBF) and the Free State of Bavaria under the Excellence Strategy of the Federal Government and the Länder, as well as by the Technical University of Munich — Institute for Advanced Study, all in Germany. M.Z.'s research is supported by a "Schlüsselprojekt" grant from the Else Kröner-Fresenius-Stiftung (2022_EKSE.185). R.J.'s research is supported by the Czech Ministry of Health – grant AZV NW24-04-00067 and the project National Institute for Neurological Research, Czech Republic (Programme EXCELES, ID Project No. LX22NPO5107) and sunded by the European Union – Next Generation EU. This work was supported by NIH grants R01-AR065484 and R35-GM141834 to T.U.S., U.S. Department of Defense grant W81XWH-18-1-0515, and by NIH Pre-Doctoral Training Grant T32GM007287 (to V.J.H.).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Co-author is member of the Editorial Board of Parkinsonism and Related Disorders - M.Z. Co-author was Associate Editor for Parkinsonism and Related Disorders until Dec 2023 - A.P.If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the members of both families for their participation in this research. We thank Prof. Håkan Widner, Skåne University Hospital, Sweden, for reviewing the videos and Dr. Martin Daniš, Trnava, Slovakia, for providing additional blood samples and videos from Family 2, as well as Dr. Gert Andersson and Dr. Erik Westhall, Dept. for Clinical Neurophysiology, Lund, Sweden, for their support with the neurophysiological studies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2025.107781.

References

[1] A. Albanese, K. Bhatia, S.B. Bressman, et al., Phenomenology and classification of dystonia: a consensus update, Mov. Disord. 28 (2013) 863–873.
[2] Fonzo A. Di, H.A. Jinnah, M. Zech, Dystonia genes and their biological pathways, Int. Rev. Neurobiol. 169 (2023) 61–103.
[3] R.E. Goodchild, W.T. Dauer, The AAA+ protein torsinA interacts with a conserved domain present in LAP1 and a novel ER protein, J. Cell Biol. 168 (2005) 855–862.
[4] M. Zech, R. Jech, S. Boesch, et al., Monogenic variants in dystonia: an exome-wide sequencing study, Lancet Neurol. 19 (2020) 908–918.
[5] N.J. Risch, S.B. Bressman, G. Senthil, et al., Intragenic Cis and Trans modification of genetic susceptibility in DYT1 torsion dystonia, Am. J. Hum. Genet. 80 (2007) 1188–1193.
[6] V. Siokas, A.M. Aloizou, Z. Tsouris, et al., Risk factor genes in patients with dystonia: a comprehensive review, Tremor Other Hyperkinet Mov (N Y) 8 (2018) 559.
[7] F.E. Demircioglu, B.A. Sosa, J. Ingram, et al., Structures of TorsinA and its disease-mutant complexed with an activator reveal the molecular basis for primary dystonia, Elife 5 (2016).
[8] R.S. Brown, C. Zhao, A.R. Chase, et al., The mechanism of Torsin ATPase activation, Proc. Natl. Acad. Sci. U. S. A 111 (2014) E4822–E4831.
[9] C. Zhao, R.S. Brown, A.R. Chase, et al., Regulation of torsin ATPases by LAP1 and LULL1, Proc. Natl. Acad. Sci. U. S. A 110 (2013) E1545–E1554.
[10] B.A. Sosa, F.E. Demircioglu, J.Z. Chen, et al., How lamina-associated polypeptide 1 (LAP1) activates Torsin, Elife 3 (2014) e03239.
[11] Y. Ben-Haim, L. Armon, B. Fichtman, et al., Generation and characterization of iPSC lines from two nuclear envelopathy patients with a homozygous nonsense mutation in the TOR1AIP1 gene, Stem Cell Res. 56 (2021) 102539.
[12] I. Dorboz, M. Coutelier, A.T. Bertrand, et al., Severe dystonia, cerebellar atrophy, and cardiomyopathy likely caused by a missense mutation in TOR1AIP1, Orphanet J. Rare Dis. 9 (2014) 174.
[13] B. Fichtman, F. Zagairy, N. Biran, et al., Combined loss of LAP1B and LAP1C results in an early onset multisystemic nuclear envelopathy, Nat. Commun. 10 (2019) 605.
[14] G. Kayman-Kurekci, B. Talim, P. Korkusuz, et al., Mutation in TOR1AIP1 encoding LAP1B in a form of muscular dystrophy: a novel gene related to nuclear envelopathies, Neuromuscul. Disord. 24 (2014) 624–633.
[15] A.J. Rampello, S.M. Prophet, C. Schlieker, The role of torsin AAA+ proteins in preserving nuclear envelope integrity and safeguarding against disease, Biomolecules 10 (2020).
[16] L. Basel-Vanagaite, L. Muncher, R. Straussberg, et al., Mutated nup62 causes autosomal recessive infantile bilateral striatal necrosis, Ann. Neurol. 60 (2006) 214–222.
[17] P. Harrer, A. Schalk, M. Shimura, et al., Recessive NUP54 variants underlie early-onset dystonia with striatal lesions, Ann. Neurol. 93 (2023) 330–335.
[18] M. Hallett, Functional (psychogenic) movement disorders - clinical presentations, Park. Relat. Disord. 22 (Suppl 1) (2016) S149–S152.
[19] G.S. Gilmour, G. Nielsen, T. Teodoro, et al., Management of functional neurological disorder, J. Neurol. 267 (2020) 2164–2172.
[20] B.J. Robottom, W.J. Weiner, C.L. Comella, Early-onset primary dystonia, Handb. Clin. Neurol. 100 (2011) 465–479.
[21] L. Ozelius, N. Lubarr, DYT1 early-onset isolated dystonia. I, in: M.P. Adam, J. Feldman, G.M. Mirzaa, et al. (Eds.), GeneReviews((R)), 1993. Seattle (WA).
[22] S.B. Bressman, C. Sabatti, D. Raymond, et al., The DYT1 phenotype and guidelines for diagnostic testing, Neurology 54 (2000) 1746–1752.
[23] P. Pavelekova, R. Jech, M. Zech, et al., Atypical presentations of DYT1 dystonia with acute craniocervical onset, Park. Relat. Disord. 83 (2021) 54–55.
[24] L. di Biase, A. Di Santo, M.L. Caminiti, et al., Classification of dystonia, Life 12 (2022).

[25] S. Richards, N. Aziz, S. Bale, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (2015) 405–424.
[26] G.P. Jarvik, B.L. Browning, Consideration of cosegregation in the pathogenicity classification of genomic variants, Am. J. Hum. Genet. 98 (2016) 1077–1081.
[27] N.T. Strande, E.R. Riggs, A.H. Buchanan, et al., Evaluating the clinical validity of gene-disease associations: an evidence-based framework developed by the clinical genome resource, Am. J. Hum. Genet. 100 (2017) 895–906.
[28] V.S. Kostic, M. Svetel, K. Kabakci, et al., Intrafamilial phenotypic and genetic heterogeneity of dystonia, J. Neurol. Sci. 250 (2006) 92–96.
[29] P. Gonzalez-Alegre, Advances in molecular and cell biology of dystonia: focus on torsinA, Neurobiol. Dis. 127 (2019) 233–241.
[30] C. Ostlund, A. Hernandez-Ono, J.Y. Shin, The nuclear envelope in lipid metabolism and pathogenesis of NAFLD, Biology 9 (2020).