Whole exome sequencing identifies novel DYT1 dystonia-associated genome variants as potential disease modifiers

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Abstract

Background : DYT1 dystonia is a neurological movement disorder characterized by painful sustained muscle contractions resulting in abnormal twisting and postures. In a subset of patients, it is caused by a loss-of-function mutation (ΔE302/303; or ΔE) in the luminal ATPases associated with various cellular activities (AAA+) protein torsinA encoded by the *TOR1A* gene. The low penetrance of the ΔE mutation (~30-40%) suggests the existence of unknown genetic modifiers of DYT1 dystonia.

Methods : To identify these modifiers, we performed whole exome sequencing of blood leukocyte DNA isolated from two DYT1 dystonia patients, three asymptomatic carriers of the ΔE mutation, and an unaffected adult relative.

Results : A total of 264 DYT1 dystonia-associated variants (DYT1 variants) were identified in 195 genes. Consistent with the emerging view of torsinA as an important regulator of the cytoskeleton, endoplasmic reticulum homeostasis, and lipid metabolism, we found DYT1 variants in genes that encode proteins implicated in these processes. Moreover, 40 DYT1 variants were detected in 32 genes associated with neuromuscular and neuropsychiatric disorders.

Conclusion : The DYT1 variants described in this work represent exciting new targets for future studies designed to increase our understanding of the pathophysiology and pathogenesis of DYT1 dystonia.

Keywords: DYT1 dystonia; *TOR1A*; torsinA; neurogenetics; whole exome sequencing

Introduction

Dystonias are a heterogeneous collection of hyperkinetic neurological movement disorders that are characterized by involuntary muscle contractions resulting in abnormal repetitive movements and postures [1, 2]. Dystonias can be acquired as the result of environmental insults (i.e. central nervous system infection, toxins, and traumatic brain injury) [2, 3] as well as inherited due to genetic mutations [4]. While several causative genes are known, the mechanisms underlying their contribution to dystonia pathogenesis and/or pathophysiology remain unclear.

Early onset torsion dystonia, or DYT1 dystonia, is the common and severe inherited dystonia [5]. It is a primary torsion dystonia, as dystonia is the only clinical symptom present in patients and it is inherited in a monogenic fashion. The majority of DYT1 dystonia cases are caused by the autosomal dominantly inherited deletion of a GAG codon (c.904_906/907_909ΔGAG) from the *TOR1A* gene, which removes a glutamic acid residue (ΔE302/303; or ΔE) from the C-terminus of the encoded luminal ATPase torsinA [6, 7]. The ΔE mutation is considered a loss-of-function mutation because homozygous torsinA-knockout and homozygous torsinA$^{\Delta E}$-knockin mice both die perinatally and exhibit neurons with abnormal blebbing of the inner nuclear membrane into the perinuclear space of the nuclear

envelope [8]. In addition, the ΔE mutation impairs the ability of torsinA to interact with its major binding partners the inner nuclear membrane protein lamina-associated polypeptide 1 (LAP1) and the endoplasmic reticulum/outer nuclear membrane protein luminal domain-like LAP1 (LULL1) [9], which stimulates the ability of torsinA to hydrolyze ATP above negligible background levels *in vitro* [10].

Surprisingly, only ~30-40% of individuals heterozygous for the ΔE develop DYT1 dystonia despite the presence of abnormalities in brain metabolism and the cerebellothalamocortical pathway in all carriers [11-15]. Collectively, these clinical findings demonstrate that the presence of the ΔE mutation results in abnormal brain function regardless of whether or not an individual develops DYT1 dystonia. Moreover, they suggest the hypothesis that the penetrance of the ΔE mutation may be influenced by additional as-of-yet unknown genetic factors.

Consistent with this hypothesis, recent research shows that genetic background modulates the phenotype of a mouse model of DYT1 dystonia [16]. In addition, expression profiling in peripheral blood harvested from human DYT1 dystonia patients harboring the ΔE mutation and asymptomatic carriers revealed a genetic signature that could correctly predict disease state [17]. The functional classification of transcripts that were differentially regulated in DYT1 dystonia patients relative to unaffected carriers identified a variety of potentially impacted biological pathways,

including cell adhesion, cytoskeleton organization and biogenesis, development of the nervous system, G-protein receptor signaling, and vesicle-mediated pathway/protein transport. Since these biological pathways have all been previously associated with torsinA function [4, 18-20], we hypothesize that the penetrance of the ΔE mutation and therefore the development of DYT1 dystonia may depend upon the presence or absence of variants in genes that encode proteins that influence biological pathways associated with torsinA function. Below, we describe the use of whole exome sequencing (WES) to identify genetic variants in DYT1 dystonia patients but neither unaffected ΔE mutation carriers nor the unaffected control.

Materials and methods

Human Subjects

This study recruited 11 human subjects, including two patients from two separate families of Taiwanese ancestry. All subjects (or legal guardians) gave their written informed consent for participation and the study was approved by the Institutional Review Board of the Tri-Service General Hospital at the National Defense Medical Center in Taipei, Taiwan (IRB# 1-107-05-164). Detailed clinical information was obtained from corresponding clinicians and medical records.

Purification of genomic DNA and RNA from Isolated Human Blood Leukocytes

Genomic DNA was purified from human leukocytes using the MagPurix® Blood DNA Extraction Kit LV and run in the MagPurix 24® Nucleic Acid Extraction System (Labgene Scietific®, SA, Châtel-Saint-Denis, Switzerland) following the instructions provided by the manufacturer. Total RNA was purified using Tempus™ Spin RNA Isolation Kit. Reverse transcription and cDNA synthesis were performed by QuantiTect® Reverse Transcription Kit.

Sanger Sequencing of the TOR1A gene

The DNA encoding portions of the *TOR1A* gene was PCR products amplified from the genomic DNA using the following primer pairs: 1) *TOR1A* (c.646G>C, D216H)- F: TAATTCAGGATCAGTTACAGTTGTG and –R: TGCAGGATTAGGAACCAGAT; and 2) *TOR1A* (c.904_906/907_909ΔGAG, Δ302/303E)- F: GTGTGGCATGGATAGGTGACCC and –R: GGGTGGAAGTGTGGAAGGAC. From the cDNA using the following primer pairs: Transcriptome(873bp) -F: ATCTACCCGCGTCTCTAC and –R: ATAATCTAACTTGGTGAACA; The resulting PCR products were purified using QIAquick PCR Purification Kit (Qiagen®) and then undergoing Sanger sequencing (Genomics®, Taipei, Taiwan).

Whole exome sequencing

Purified human genomic DNA was sheared into ~150-200 base-pair fragments using the S220 Focused-Ultrasonicator (Covaris, Woburn, Massachusetts) according to the instructions provided by the manufacturer. SureSelectXT Human All Exon V6 +UTR (Agilent Technologies, Santa Clara, CA) was then used to perform exome capture and library preparations The library were then sequenced using a NovaSeq 6000 System (Illumina, San Diego, CA) with 150 base-pair reads and output data up to 10 Gb per sample. After sequencing, Genome Analysis Toolkit (GATK) best practices workflows of germline short variant discovery (https://software.broadinstitute.org/gatk) was used to perform variant calling with default parameters [21]. Briefly, the Burrows-Wheeler Aligner was first used to align the sequenced exomes with the most up-to-date human genome reference build (hg38) ("GRCh38 - hg38 - Genome - Assembly - NCBI". ncbi.nlm.nih.gov). Next, duplicate reads were removed using Picard after which the GATK was used to perform local realignment of the sequenced exomes with the reference genome and base quality recalibration. Then, GATK-HaplotypeCaller was used to call germline SNPs (single-nucleotide polymorphism) and indels. After variant calling, ANNOVAR was used to variant annotation [22] with database, include refGene, clinvar_20170905 (https://www.ncbi.nlm.nih.gov/clinvar/), avsnp150, dbnsfp33a, gnomad_genome, dbscsnv11. Annotated variants were selected with the following criteria: (1) filtering with exonic region, (2) removing synonymous mutation, (3) read depth ≥20. Next, we categorized these filtered variants according to the principle of

inheritance. Finally, the variants of interest were validated by manually viewing them in the Integrative Genomics Viewer. All of the whole exome sequencing data generated in this study are deposited online at GenBank (https://www.ncbi.nlm.nih.gov/sra/PRJNA523662).

Results

Clinical Observations

Patient 1 was a 12-year-old male of Taiwanese descent who initially presented with waddling gait at seven years of age, which progressed to upper limb tremor and pronation within a few months. Over time, the patient sequentially displayed head tilt, scoliosis, kyphosis, repetitive and active twisting of his limbs. Five years after the onset of his symptoms, the patient showed generalized and profound muscle twisting and contraction, including dysarthria and dysphagia. The patient now presents with a sustained opitoshtonous-like posture and needs full assistance with executing his daily routines. Unfortunately, the patient did not benefit greatly from medical treatment and he refused deep brain stimulation due to the risks associated with the necessary surgical procedure. Neither he nor his family had a prior history of dystonia-related neurological movement disorders. Medical records from the hospital where Patient 1 received care prior to this study indicate that the patient lacks any mutations in his *FXN* or *THAP1* loci, which are both differential diagnoses of genetic, progressive, and neurodegenerative movement disorders [23, 24].

Patient 2 was a 40-year-old male of Taiwanese descent who initially presented with mild foot dystonia followed by cervical dystonia in his early twenties. He is able to execute his daily routines as the result of medical treatment. The patient had no prior history of dystonia-related neurological movement disorders and clinical information regarding the medical history of his family is unavailable.

Examination of Known DYT1 Dystonia-Associated Mutations

To determine if either Patient 1 (subject 1) or Patient 2 (subject 11) harbored known DYT1 dystonia-associated mutations in their genomes, we used Sanger sequencing to screen their *TOR1A* genes for the presence of the ΔE mutation. Our results show that both patients are heterozygous for the ΔE mutation (Fig 1. Two patients with their family pedigrees and Sanger sequencing data. Family pedigree (A,E) and Sanger sequencing data (B,F)). We also sequenced the *TOR1A* genes of nine other family members of Patient 1. No ΔE mutation was found in the genomes of his unaffected mother (subject 2), male sibling (subject 4) or other relatives (subject 5, 7, 8,10), while his father (subject 3), paternal aunt (subject 6), and cousin of Patient 1 (subject 9) were asymptomatic heterozygotic ΔE mutation carriers. Then, we asked if the previously described protective modifier mutation D216H was present within the *TOR1A* gene of the core family of patient 1, including subject 1,2,3,4 [25]. However, none of the family members examined were positive for the D216H mutation (Fig1. C,D). These results suggest that Patient 1 inherited the ΔE mutation from the paternal side of his family and that the absence of DYT1 dystonia in his

father, paternal aunt, and cousin cannot be attributed to the presence of the protective D216H mutation.

Examination of potential misregulation of alternative splicing by mutations that affect splicing signals/machinery

In addition to investigate the common genomic DNA mutation in *TOR1A* gene, mRNA extracted from leukocytes of the first patient and his parents were measured to evaluate any aberrant alternative splicing which may cause disease status [26]. There are five splicing variants and two protein production according the reference from Ensembl (human (GRCh38).p13). The results demonstrated that mRNA of *TOR1A* gene not only revealed similar gene expression level among the patient and his parents, but also showed the same GAG deletion between the patient and his father (Fig S1).

Identification of DYT1 Dystonia-Associated Genome Variants in the *TOR1A* gene

To begin to identify potential genetic modifiers of the penetrance of the ΔE mutation, we performed WES on genomic DNA purified from blood leukocytes isolated from Patient 1 and Patient 2 as well as three asymptomatic ΔE mutation carriers from the first family (i.e. the father, paternal aunt, and cousin) and the mother of patient 1 who did not harbor the ΔE mutation in her genome. Consistent with the Sanger

sequencing results described above, WES confirmed the presence of a single copy of the ΔE mutation in the exomes of Patient 1, his father, paternal aunt, and cousin as well as Patient 2, while demonstrating its absence from the mother of Patient 1 (Table 1). In addition, WES demonstrated that the D216H mutation was absent from all six exomes examined. Interestingly, three additional previously reported *TOR1A* variants, rs13300897, rs2296793 and rs1182 [27, 28], were found in the exomes of two of the asymptomatic ΔE mutation carriers and the mother from the family of Patient 1 (Table 1). However, the absence of these variants from the exomes of either Patient 1 or Patient 2 diminishes the likelihood that they are genetic modifiers of the penetrance of the ΔE mutation. Collectively, these findings motivated us to search for genome variants outside of the *TOR1A* gene that might influence the penetrance of the ΔE mutation.

Identification of DYT1 dystonia-associated Genome Variants (DYT1 variants) Outside of the TOR1A Gene

We hypothesized that candidate modifiers of the penetrance of the ΔE mutation would be those genome variants that were present in the exomes of both symptomatic patients and absent from the exomes of the asymptomatic ΔE mutation carriers and mother of the Patient 1. To begin to test this hypothesis, we examined the results of our WES for genome variants that fit this criterion and identified a total of DYT1 variants 264 variants in 195 genes. Based on their

respective allele frequencies (AFs), we further classified these variants into three inheritance groups: 1) Autosomal recessive (AR); 2) Autosomal dominant (AD); and 3) *De novo* (DN) mutation (Table 2). The 53 genome variants found in 43 genes classified as AR had AFs of 1 for both patients, an AF of 0.5 or 1 for the mother of Patient 1, an AF of 0.5 for the father of Patient 1, and an AF of 0 or 0.5 for the paternal aunt and cousin of Patient 1. The 201 variants found in 149 genes classified as AD had AFs of 0.5 or 1 for both patients, and AF of 0.5 or 1 for the mother of Patient 1, and an AF of 0 for the rest of the family members of Patient 1. Finally, the 10 variants in 5 genes classified as DN had AFs of 0.5 or 1 for both patients, and were not present in any of the other exomes examined.

Of the 264 variants identified by our WES-based screen, 11 variants were predicted to be loss-of-function mutations (Table 2) by using the bioinformatics tools sorting intolerant from tolerant (SIFT) and polymorphism phenotyping (PolyPhen) [29]. Furthermore, gene ontology analysis performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) [30] identified clustered annotations of genes in which the DYT1 variants were identified by our WES-based approach. There are total 30 annotation clusters generated by this tool as listed in Table S1. Next, we filtered these categories with enrichment score>1 and p value<0.05 and the results were enriched for those that encode proteins that contain the epidermal growth factor-like domain (ten genes), have dioxygenase (four genes) or Rho

guanyl-nucleotide exchange factor activity (four genes), or exhibit the ability to interact with the actin cytoskeleton (seven genes) (Table S1). Notably, genes for endoplasmic reticulum stress and lipid metabolism, which are linked to DYT1 functions (discussed below), also shown a trend of enrichment (Table S1). The enrichment of cytoskeleton-related genes and the known function of TOR1A in regulation of the mechanical integration of the nucleus and the cytoskeleton prompted us to look closer on the genes that harbor DYT1 variants via literature search [31-33]. There are 45 DYT1 variants in 34 genes that are associated with cytoskeleton (Table 3). In addition, 17 DYT1 variants in 16 genes are found to be linked to endoplasmic reticulum and protein and lipid metabolism (Table 4), which TOR1A is known to have functional indications at [18, 34]. Lastly, further reviewing previous studies identified 40 DYT1 variants in 32 genes that have disease associated with human neuropsychiatric disorders or neuromuscular diseases (Table 5). Taken together, our results suggest that potential regulators of the ΔE mutation may participate in the regulation of the following established cellular functions performed by torsinA: cytoskeletal organization, endoplasmic reticulum homeostasis, and protein and lipid metabolism.

Discussion

The underlying cause of phenotype variation from the same allele remains largely unknown in most cases when a particular genotype is inherited. Emerging evidence

indicate that modifier genes may contribute to phenotypic variations [35]. For example, patients with thalassemia, a disorder caused by defective β-globin synthesis, have diverse clinical characteristics and variable expressivity. A number of factors underlie this phenotypic diversity, including the involvement of numerous modifier genes at other genetic loci that affect the production of β-globin [36]. Similarly, DYT1 dystonia patients have a wide spectrum of symptom severity, which reflects the incomplete penetrance of the pathogenic ΔE mutation and the variable expressivity of the disease. For most diseases, variable expressivity of the disease phenotype is the norm among individuals who carry the same disease-causing allele or alleles [37], despite the causes are not always being clear. Since the alternative splicing defects in the regulatory process may affect cellular functions and are the cause of many human diseases [26], this is not likely the potential mechanism in our cases.

In this work, we describe the identification of 264 variants in 195 genes that are associated with DYT1 dystonia. Below, we will discuss the potential implications of our results on our understanding of the pathogenesis and pathophysiology of DYT1 dystonia. Specifically, we will explore the connections between the DYT1 variants identified here and the following established cellular functions of torsinA: cytoskeletal regulation, endoplasmic reticulum stress, and lipid metabolism. In addition, we will examine the relationship revealed between DYT1 dystonia and the

neuromuscular and neuropsychiatric disorders linked with the genes in which we identified DYT1 dystonia-associated genomic variants.

DYT1 variants and the cytoskeleton

Of the 195 genes that we identified as harboring 264 DYT1 variants, 45 variants in 34 genes encode proteins that constitute or associate with the cytoskeleton (Table 3). The identification of DYT1 variants in genes encoding proteins related to cytoskeletal function is consistent with the emerging view of torsinA as a critical regulator of cellular mechanics. The first evidence to suggest that torsinA might be involved in cytoskeletal regulation was the finding that the nematode torsinA protein OOC-5 was required for the rotation of the nuclear-centrosome complex during early embryogenesis [38, 39]. In addition, the fruit fly torsinA protein torp4a/dTorsin was implicated in the regulation of the actin cytoskeleton [40]. Furthermore, the over-expression of a torsinA construct containing the ΔE mutation was shown to inhibit neurite extension in human neuroblastoma cells and to increase the density of vimentin intermediate filaments around the nucleus [41]. The relationship between torsinA and the cytoskeleton is further strengthened by reports of the impaired migration of dorsal forebrain neurons and fibroblasts from torsinA-knockout mice as well as DYT1 dystonia patient-derived fibroblasts [32, 42, 43].

More recently, torsinA was identified as a key regulator of the mechanical integration of the nucleus and the cytoskeleton via the conserved nuclear envelope-spanning linker of nucleoskeleton and cytoskeleton (LINC) complex [31-33]. The core of LINC complexes is formed by the transluminal interaction between the outer and inner nuclear membrane Klarischt/ANC-1/SYNE homology (KASH) and Sad1/UNC-84 (SUN) proteins, respectively [44]. KASH proteins interact with the cytoskeleton and signaling proteins within the cytoplasm [45], whereas SUN proteins interact with chromatin, other inner nuclear membrane proteins, and the nuclear lamina within the nucleoplasm [46].

While the precise mechanism of torsinA-mediated LINC complex regulation remains unclear, the hypothesis is supported by the finding that torsinA loss elevates LINC complex levels in the mouse brain, which impairs brain morphogenesis [47]. More recently, fibroblasts isolated from DYT1 dystonia patients were shown to have increased deformability similar to that of fibroblasts harvested from mice lacking the two major SUN proteins SUN1 and SUN2 [48].

DYT1 dystonia patient-derived fibroblasts were also shown to have increased susceptibility to damage by mechanical forces [48] strongly suggests that cellular mechanics may impact the pathogenesis and/or pathophysiology of DYT1 dystonia. All cells, including neurons, adapt their mechanical properties by converting extracellular mechanical stimuli into biochemical signals and altered gene

expression through the process of mechanotransduction [49, 50]. Since mechanotransduction instructs neuronal differentiation, proliferation, and survival [51, 52], it is possible that defective mechanotransduction of neurons in the developing brain may contribute to the pathogenesis and/or pathophysiology of DYT1 dystonia. Based on the information provided above, it is intriguing that we identified DYT1 variants in the KASH protein nesprin-2-encoding *SYNE2* gene and the *NUP58* gene, which encodes the nuclear pore complex protein nup58 (Table 3 and Fig2). In the future, it will be interesting to test if the DYT1 variants found in *SYNE2* and *NUP58* negatively impact LINC complex-dependent nuclear-cytoskeletal coupling and/or mechanotransduction.

It is tempting to speculate that the impairment of the microtubule cytoskeleton is particularly relevant to dystonia pathogenesis given the enrichment of DYT variants that we found in genes that encode microtubule-associated proteins. Microtubules are fundamentally important for the structure and function of neurons, which are some of the most highly polarized cells in the human body [53]. Microtubules establish the polarized architecture of neurons and serve as tracks for microtubule motor proteins as they carry proteins and lipids to where they are needed for proper neuronal function. Thus, defects in microtubule dynamics and organization underly a wide array of neurological and neuropsychiatric disorders [54-56].

Consistent with our identification of 4 DYT1 variants in the *TUBA3E*, which encodes the protein α-tubulin-3E, mutations in the β-tubulin-4A-encoding *TUBB4A* gene cause another hereditary dystonia, Whispering dysphonia or DYT4 dystonia [57, 58]. These mutations result in the formation of disorganized microtubule networks and the impaired growth of neuronal processes similar to the clinical phenotypes observed in DYT4 dystonia patients [59, 60]. Future experiments designed to test the impact of the DYT1 variants in *TUBA3E* on the organization and function of neuronal microtubules will help elucidate the role of the microtubule cytoskeleton to the manifestation of DYT1 dystonia.

DYT1 variants in association with protein synthesis and transport and ER homeostasis

Accumulating evidence indicate a role of TOR1A in the cellular protein quality control system in which TOR1A could be both substrate and effector [18]. In the 264 genome variants, we observed six variants in five genes, *CHGB, DOP1B, MTMR6, P2RY13* and *PPP1R15A*, that are annotated with protein synthesis and transport functions (Table 4). Notably, CHGB and PPP1R15A has also been linked to endoplasmic reticulum stress [61-63]. These findings support the previously proposed hypothesis that elevated levels of endoplasmic reticulum stress contributes to DYT1 dystonia pathogenesis [64-72].

TorsinA functions to protect against insults from protein aggregates in the neural system [65]. Protein aggregates are products of protein misfolding commonly seen in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and prion disease, which triggers endoplasmic reticulum stress response [73, 74]. In the TOR1A ΔE mutation background, we identified six candidate modifier genome variants in five genes that have known functions in endoplasmic reticulum for protein post translational modification, protein translocation and endoplasmic reticulum stress response (Table 4). Among them, DOP1B has neurological roles in both human and mice [75, 76]. Whether DOP1B's endoplasmic reticulum cellular function has a causal effect on its neurological role remains to be investigated. Collectively, our data provide clinical indications of candidate genes and genome variants for further investigation on the underlying mechanisms of TOR1A dependent ER dysfunction in DYT1 dystonia.

DYT1 variants and lipid metabolism

TOR1A also has a pivotal role in lipid metabolism as demonstrated by the hepatic steatosis of liver-specific torsinA-knockout mouse model [34] and the requirement for the Drosophila torsinA homologue for proper lipid metabolism in adipose tissue [77]. Because of its functional indication in lipid metabolism, TorsinA is thought to promote membrane biogenesis [19] and synaptic physiology [78]. There are 11 DYT1 dystonia associated genome variants identified in ten lipid metabolism genes

ALOXE3, APOB, CYP1B1, CYP2A7, FAM135B, GAL3ST1, GPAM, MTMR6, PLA2G4F and *PLCL1* (Table 4), which suggest potential genetic interactions between the ΔE mutation and genome variants that might change membrane homeostasis.

TOR1A regulates lipid metabolism in both fruit flies and mammals [34, 77]. TOR1A facilitates cell growth, raises lipid content of cellular membrane and is involved in membrane expansion [77]. The linkage between the TOR1A ΔE mutation and 10 lipid metabolic genes suggest the impact on lipid metabolism associated cellular functions could be amplified by clustered mutations and genome variants. Two genes in this category have known functions in the neural system. The *GAL3ST1* gene encodes galactose-3-O-sulfotransferase 1 that involves in the synthesis of a major lipid component of the myelin sheath galactosylceramide sulfate [79]. *Gal3st1* deficient mice develop tremor, progressive ataxia, hind limb weakness, aberrant limb posture and impaired limb coordination with morphological defects in the neural system [80]. PLCL1 Involves in an inositol phospholipid-based intracellular signaling cascade. PLCL1 is phospholipase C like protein lacking the catalytic activity. PLCL1 binds and sequesters inositol triphosphates to blunt the downstream calcium signaling [81]. PLCL1 has been linked to the trafficking and turnover of GABAA receptors in neurons [82, 83]. Physiologically, loss of PLCL1 increases the incidence of chemically induced seizure in mice [84]. These findings indicate an

essential role of PLCL1 in controlling the neural signaling transduction. While the functional impact of the genome variants on GAL3ST1 and PLCL1 awaits further investigation, their association with the TOR1A ΔE mutation suggests potential functional interactions between these molecules in DYT1 dystonia.

Connections between the genes harboring DYT1 variants and their implicated neuromuscular and neuropsychiatric disorders

The loss of torsinA function in either the cerebral cortex or cerebellum result in motor dysfunction [85-87], indicating a neuronal component of TOR1A's function in dystonia. Based on these observations, we examined the 195 genes that carry candidate ΔE mutation modifiers for their association with neuropsychiatric and neuromuscular disorders. Such link was identified in 32 genes with 40 genome variants (Table 5). These include the *AHNAK2*, *ARHGEF3*, *CDRT1*, *GBE1* and *NRG2* genes in associated with peripheral neuropathy (Charcot-Marie-Tooth disease and Polyglucosan body neuropathy, adult form). The *AMPD2*, *ATXN7* and *MICAL3* genes are linked to cerebellar diseases (Pontocerebellar Hypoplasia, type 9 and spastic paraplegia 63, autosomal recessive; Spinocerebellar ataxia 7; Joubert syndrome (cerebelloparenchymal disorder)). Lastly, the *IRF3*, *TRAF3* and *LIPT2* genes are associated with encephalopathy (acute, infection-induced; encephalopathy, neonatal severe, with lactic acidosis and brain abnormalities and lipoic acid biosynthesis defects. Overall, more than 16% of the identified 195 genes

are in association with neuropsychiatric and neuromuscular diseases related disorders, demonstrating the significance of the linkage between DYT1 dystonia and these diseases.

Study Limitations

The present study examined five individuals who have the TOR1A ΔE mutation. Among them, two have disease presentation and three are asymptomatic carriers. Furthermore, one affected patient and the three asymptomatic carriers are in the same family, which is an advantage to have a relatively close genetic background for modifier screening. Data from this family identified 1725 of genome variants as candidate modifiers. With the addition of the second affected patient, the number of candidate modifier variants were further narrowed down to 264. This number could have been reduced if data from more affected patients or asymptomatic carriers are available. Unfortunately, family members of the second affected patient declined to participate in the study. Due to the rareness of DYT1 dystonia in Taiwan, it is difficult to increase sample size within the Taiwanese population in foreseeable future. Alternatively, meta-analysis of our dataset with WES results from other populations across the world, once publicly available, may help to identify the common modifiers in the general population [88, 89].

The WES data allows identification of candidate modifiers in the coding genome. However, majority of the GWAS signals are mapped to the noncoding regions of the genome and accumulating evidence point to disease associations with the noncoding genome [90]. Mutations in the noncoding genome may impact cis-acting element functions and chromatin conformations that direct gene expression. Future inclusion of the whole genome sequencing assay may help to identify additional modifiers for the DYT1 dystonia.

Conclusions

In summary, we propose that genome variants within nuclear-cytoskeletal coupling network may constitute potential modifier variants, which could synergistically reduce the threshold of disease onset of DYT1 dystonia and accelerates the clinical symptoms and signs of dystonia. We believe that this study provided a path to unravel candidate genome variants as modifiers. Our findings not only echo the previous research highlighting the defect of mechanosensing and mechanotransduction regulated by TOR1A [48], but provide knowledge for further understanding the disease origin of the DYT1 dystonia as well. We will recommend the physicians to test these variants once the TOR1A ΔE mutation patient show normal alleles within other *TOR1A* locus and other major binding proteins in their study. We also provide a list of candidate genes and genome variants for future mechanistic studies on DYT1 dystonia.

Supporting information

Table S1 and Figure S1.

Acknowledgments

We would like to thank the first patient and his family members who provided the DNAs, RNAs, and clinical information necessary for this research study. We would also like to thank Dr. Chin-Hsien Lin (Department of Neurology, National Taiwan University Hospital, Taipei, Taiwan) who kindly provided the DNA sample and clinical information of the second patient. This research was funded by Tri-Service General Hospital, grant number TSGH-C108-021 (C.F.H.), TSGH-C108-022 (C.F.H.) and National Institutes of Health GM129374 (G.W.G.L.), Z99-ES999999 (S.P.W.).

Author contributions

CFH: Conceptualization, Writing-original draft, Resources. GWGL: Visualization, Writing-review & editing. FCL: Methodology, Investigation. CSH: Data curation, Visualization. SMH: Supervision, Writing - review &editing. JSH: Data curation, Supervision. SPW: Supervision, Writing-review & editing, Resources.

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Table 1. Genomic Variants in the Exons, Promoter Regions and 3'-UTR of the *TOR1A* Gene between the Patients and the Other Family Members.

Subject Number†	Human Subject†	Variants in *TOR1A*	Site of Variant	Exonic Function	DNA Change	AA‡ Change	AF‡ in Person	AF in Taiwan§	AF in Dataset§	Interpretation
(1)	Patient	rs80358233	Exon 5	Nonframeshift deletion	904_906/ 907_909ΔGAG	ΔE302/ 303	0.5	Unknown	$3.23^{\wedge}10^{-5}$	Pathogenic (Ozelius 2016)
(2)	Healthy Control	rs13300897	Promoter	-	C>T	-	0.5	0.174	0.1683	Polymorphism (Vulinovic 2014)
		rs2296793	Exon 2	Synonymous SNV	246G>A	A82A	0.5	0.1943	0.2253	Polymorphism (Vulinovic 2014)
		rs1182	3'-UTR	-	C>A	-	0.5	0.178	0.1666	Possible modifier (Siokas 2017)
(3)	Asymptomatic Carrier	rs13300897	Promoter	-	C>T	-	0.5	0.174	0.1683	Polymorphism (Vulinovic 2014)
		rs2296793	Exon 2	Synonymous SNV	246G>A	A82A	0.5	0.1943	0.2253	Polymorphism (Vulinovic 2014)
		rs80358233	Exon 5	Nonframeshift deletion	904_906/ 907_909ΔGAG	ΔE302/ 303	0.5	Unknown	$3.23^{\wedge}10^{-5}$	Pathogenic (Ozelius 2016)
		rs1182	3'-UTR	-	C>A	-	0.5	0.178	0.1666	Possible modifier (Siokas 2017)
(6)	Asymptomatic Carrier	rs13300897	Promoter	-	C>T	-	0.5	0.174	0.1683	Polymorphism (Vulinovic 2014)
		rs2296793	Exon 2	Synonymous SNV	246G>A	A82A	0.5	0.1943	0.2253	Polymorphism (Vulinovic 2014)
		rs80358233	Exon 5	Nonframeshift deletion	904_906/ 907_909ΔGAG	ΔE302/ 303	0.5	Unknown	$3.23^{\wedge}10^{-5}$	Pathogenic (Ozelius 2016)
(9)	Asymptomatic Carrier	rs80358233	Exon 5	Nonframeshift deletion	904_906/ 907_909ΔGAG	ΔE302/ 303	0.5	Unknown	$3.23^{\wedge}10^{-5}$	Pathogenic (Ozelius 2016)
(11)	Patient	rs80358233	Exon 5	Nonframeshift deletion	904_906/ 907_909ΔGAG	ΔE302/ 303	0.5	Unknown	$3.23^{\wedge}10^{-5}$	Pathogenic (Ozelius 2016)

† Subject 1: first patient, subject 2: the mother of the first patient, subject 3: the father of the first patient, subject 6: the aunt of the first patient, subject 9: the first son of the aunt of the first patient, subject 11: second patient.
‡ AA: amino acid, AF: allele frequency.
§Taiwan biobank, https://taiwanview.twbiobank.org.tw ; gnomAD (genome aggregation database), https://gnomad.broadinstitute.org.

Table 2. WES-identified DYT1 variants.

Autosomal Recessive Inheritance								
Variants	**Gene Symbol**	**DNA Change**	**AA Change**	**AF in Asia**	**AF in Patients**	**SIFT†**	**Poly-Phen2‡**	**Disease Association with Gene (PubMed)**
rs36000545	*AATK*	T3488C	F1163S	0.5378	1,1	T	B	Unknown
rs55791176	*AHNAK2*	A3144C	E1048D	0.382		T	B	Charcot-Marie-Tooth disease
rs3772219	*ARHGEF3*	T1021G	L341V	0.462		D	B	Charcot-Marie-Tooth disease; osteoporosis
rs11689281	*ASIC4*	G1847T	R616L	0.6178		T	B	Unknown
rs11695248		T1856C	V619A	0.6191		T	B	
rs3774729	*ATXN7*	G2149A	V717M	0.4981		T	B	Spinocerebellar ataxia 7
rs61561984	*C1orf195*	A191T	Y64F	0.5359		N/A	N/A	Unknown
rs10804166	*C2orf80*	A454G	S152G	0.8426		T	B	46 xy gonadal dysgenesis
rs12269028	*ZNF22-AS1*	T188A	I63N	0.3994		N/A	N/A	Unknown
rs150150392	*CCDC66*	8_9insGGGTAAGCA	L3_G4 delins LGX	0.5544		N/A	N/A	Leber congenital amaurosis
rs866149312	*CDK11A*	T1973C	V658A	0.2041		T	B	Neuroblastoma
rs200982240		G1177A	D393N	0.2278		T	B	
rs6658335		G1108A	G370R	0.1702		D	B	
rs910122	*CHGB*	G533A	R178Q	0.5617		T	B	Pheochromocytoma; glucagonoma
rs236152		C1058G	A353G	0.5614		T	B	
rs2230804	*CHUK*	G802A	V268I	0.4657		T	B	Cocoon syndrome
rs11715522	*CX3CR1*	T24G	F8L	0.6658		T	N/A	Age-related macular degeneration
rs4813043	*DEFB128*	A81T	K27N	0.4956		T	B	Unknown
rs1898883	*DISP2*	C139G	P47A	0.8517		T	B	Unknown
rs1898882		G167C	C56S	0.8383		D	B	
rs688906	*DYNC2H1*	A4238G	K1413R	0.7816		T	B	Short-rib thoracic

rs589623		G8612A	R2871Q	0.7876		T	B	dysplasia with or without polydactyly
rs13280444	*FAM135B*	C1445T	P482L	0.5081		T	B	Spinal and bulbar muscular atrophy
rs448012	*FLT4*	C2670G	H890Q	0.4763		D	B	Lymphatic malformation; hemangioma
rs2229519	*GBE1*	A568G	R190G	0.4615		D	B	Glycogen storage disease iv
rs2792751	*GPAM*	A127G	I43V	0.7206		T	B	Unknown
rs3732215	*HJURP*	C629G	S210C	0.3879		T	B	Unknown
rs2286430		G226A	E76K	0.3926		T	B	
rs2072597	*KLF1*	T304C	S102P	0.6002		T	B	Congenital dyserythropoietic anemia, type iv
rs2429051	*KLF17*	G170C	S57T	0.676		T	B	Unknown
rs2485652		A467G	N156S	0.4608		T	B	
rs1064608	*MTCH2*	C841G	P281A	0.5837		D	P	Unknown
rs7995033	*MTMR6*	A955G	I319V	0.6064		T	B	Autosomal dominant polycystic kidney disease
rs11556093	*NUP58*	G100A	A34T	0.6098		T	B	Unknown
rs6951485	*OR2A25*	G224A	S75N	0.6752		T	B	Unknown
rs2961135		G625C	A209P	0.6588		T	B	
rs1466684	*P2RY13*	C536	T179M	0.842		T	B	Unknown
rs35385129	*PVR*	C1171A	R391S	0.3621		T	B	Paralytic poliomyelitis
rs4795690	*RHBDL3*	G739A	V247M	0.3973		T	B	Unknown
rs1022478	*RIBC2*	C804G	F268L	0.5551		N/A	B	Unknown
rs1506418	*SERPINB11*	G16A	A6T	0.617		N/A	P	Unknown
rs1506419		T37A	W13R	0.6165		N/A	D	
rs12729295	*SLC35E2B*	G934A	V312I	0.4782		N/A	B	Unknown
rs2042791	*SPAG16*	A1083C	Q361H	0.4176		T	B	Rheumatoid arthritis

rs10151658	*SYNE2*	C15556A	L5186M	0.6646		T	B	Emery-dreifuss muscular dystrophy
rs3998860	*TET1*	A3369G	I1123M	0.8407		T	B	Acute myocardial infarction
rs614486	*TEX38*	T312G	D104E	0.7637		T	B	Unknown
rs2274791	*TTLL10*	G1733A	G578D	0.5856		D	B	Unknown
rs4838865	*TUBGCP6*	T1700C	L567S	0.8241		T	B	Chorioretinopathy-microcephaly syndrome
rs1801195	*WRN*	G3222T	L1074F	0.6186		T	B	Werner syndrome; medulloblastoma
rs7258088	*ZFP28*	C128G	A43G	0.6304		T	B	Unknown
rs8100431	*ZNF414*	A194G	Q65R	0.5379		D	D	Unknown
rs4801177	*ZNF470*	C1253T	T418I	0.6776		T	B	Unknown
53 variants/43 genes								
Autosomal dominant inheritance								
Variants	**Gene Symbol**	**DNA Change**	**AA Change**	**AF in Asia**	**AF in Patients**	**SIFT**	**Poly-Phen2**	**Disease Association with Gene (PubMed)**
rs34400049	*AGGF1*	C2092A	P698T	0.2625	0.5,0.5	T	B	Klippel-Trenaunay-Weber syndrome
rs377198190	*AHNAK2*	T3424C	F1142L	0.0049		T	B	Charcot-Marie-Tooth disease
rs11845640	*AKAP6*	C4475T	A1492V	0.2563		T	B	Unknown
rs2614668	*AKAP13*	G4264A	A1422T	0.3056		T	B	Familial breast cancer
rs3027232	*ALOXE3*	C32T	P11L	0.4412		T	B	Ichthyosis
rs28362581	*AMPD2*	G244A	A82T	0.3146		D	B	Pontocerebellar hypoplasia
rs1465582	*ANKLE1*	T1933G	L645V	NA		N/A	N/A	Unknown
rs1042034	*APOB*	G13013A	S4338N	0.264		T	B	Hypobetalipoproteinemia
rs6668968	*AQP10*	G44A	R15Q	0.2246		T	B	Unknown
rs6685323		C367T	H123Y	0.2265		T	B	

rs3733662	*ARHGEF37*	C1756A	P586T	0.2975		T	B	Unknown
rs8066889	*ARL16*	A28C	S10R	0.1959		T	B	Unknown
rs33995001	*ATP10D*	C128T	T43I	0.1446		T	B	Unknown
rs3750690	*ATRNL1*	A2822T	Q941L	0.0092		T	D	Unknown
rs115476782	*BTNL10*	G376T	A126S	0.0334		N/A	N/A	Unknown
rs2274067	*C1orf131*	C82G	L28V	0.0854		D	D	Unknown
rs3813728	*C1R*	G781A	G261R	0.0932		T	D	Ehlers-danlos syndrome
rs1801046		C455T	S152L	0.3511		T	B	
rs10951942	*C7orf57*	G220T	A74S	0.2769		D	D	Unknown
rs12657663	*CAMLG*	G232A	V78I	0.2357		T	B	Congenital diaphragmatic hernia
rs6886	*CAPG*	A911G	H304R	0.4653		T	B	Breast cancer biomarker for bone metastasis
rs1801449	*CAPN3*	G706A	A236T	0.1279		T	B	Limb-girdle muscular dystrophy
rs76069883	*CCDC74A*	G688A	G230S	0.0067		T	B	Unknown
rs3177472	*CCDC74B*	G839A	R280H	0.4827		T	B	Unknown
rs2259332		T728C	V243A	0.4602		T	B	
rs7226091	*CCDC137*	C381G	H127Q	0.1965		T	B	Unknown
rs11546630		G686A	R229Q	0.1952		T	B	
rs11546631		C844T	R282W	0.1955		T	B	
rs6740879	*CCDC138*	G329A	R110K	0.0458		T	B	Unknown
rs12606658	*CCDC178*	G124A	A42T	0.0297		D	B	Unknown
rs2230552	*CCT6B*	T143C	V48A	0.5422		D	D	Pelvic varices
rs3809727	*CDRT1*	C734G	A245G	0.4412		T	B	Charcot-Marie-Tooth disease
rs16959164	*CEACAM20*	C1064T	S355L	0.0721		T	B	Unknown
rs3734381	*CEP85L*	A409G	S137G	0.3075		T	B	Myeloproliferative neoplasm
rs6081901	*CFAP61*	G1105A	V369I	0.2128		T	B	Unknown

rs16858780	*CHRD*	A1888C	M630L	0.3296		T	B	Cornelia de lange syndrome
rs3740129	*CHST3*	G1070A	R 357Q	0.087		T	B	Spondyloepiphyseal dysplasia with congenital joint dislocations
rs3743193	*CHSY1*	C1075T	P359S	0.2051		T	B	Brachydactyly
rs34964084	*CPZ*	C17T	P6L	0.1768	0.5,1	T	B	Unknown
rs3738952	*CUL3*	G1501A	V501I	0.2714		T	B	Pseudohypoaldosteronism
rs1056827	*CYP1B1*	G355T	A119S	0.2131		N/A	B	Primary congenita glaucoma
rs10012		C142G	R48G	0.2135		N/A	B	
rs2261144	*CYP2A7*	T950C	M317T	0.2643	0.5,0.5	T	B	Unknown
rs2544809	*DBN1*	A1336G	I446V	0.2506		T	B	Alzheimer disease
rs368539076	*DHRS4L2*	203_204insA	L68fs	0.2159		N/A	N/A	Unknown
rs869801	*DOCK1*	G5440A	A1814T	0.1191		T	B	Chromosome 10q26 deletions syndrome
rs3746866	*DOP1B*	C3446A	P1149H	0.2389		D	P	Down syndrome
rs7813708	*FAM83A*	G541A	A181T	0.2311	0.5,1	T	B	Pancreatic cancer
rs17429619	*FAM114A1*	G706A	V236I	0.1045	0.5,0.5	T	B	Unknown
rs16858529	*FCRLB*	G812T	R271L	0.448	0.5,1	D	B	IgA nephropathy
rs1042229	*FPR1*	T576G	N192K	0.2784		T	B	Periodontitis
rs35870000	*FREM1*	G3634T	A1212S	0.1957	0.5,0.5	T	B	Bifid nose renal agenesis; anorectal malformations; manitoba oculotrichoanal syndrome
rs778805	*FUT6*	C370T	P124S	0.5813		D	B	Fucosyltransferase 6 deficiency; gastrointestinal carcinoma
rs2267161	*GAL3ST1*	G85A	V29M	0.3309		T	B	Unknown
rs61753060	*GEMIN4*	A773G	Q258R	0.058		T	B	Neurodevelopmental

								disorder with microcephaly, cataracts, and renal abnormalities
rs696217	*GHRL*	C178A	L60M	0.1922		T	D	Eating disorder
rs75027378	*GLOD4*	C842A	A281E	0.0589		T	B	Unknown
rs3796130	*GP9*	G466A	A156T	0.2373		T	B	Bernard-Soulier syndrome; gray platelet syndrome
rs1042303	*GPLD1*	A2080G	M694V	0.2627		T	B	Autism spectrum disorders
rs3841128	*GRIA1*	31dupC	P10fs	0.0309		N/A	N/A	Depression; Status epilepticus
rs56058441	*HGS*	G2197T	A733S	0.198		T	B	Unknown
rs2295778	*HIF1AN*	C121G	P41A	0.2571		T	B	Hypoxia; nephronophthisis
rs397814627	*HOXD9*	794_795insGCA	P265delinsPQ	0.3988		N/A	N/A	Brachydactyly-syndactyly syndrome
rs2296436	*HPS1*	A1448G	Q483R	0.154		T	B	Hermansky-Pudlak syndrome
rs2296434		C1112G	P371R	0.1908		T	D	
rs34533614		C808T	P270S	0.1912		N/A	N/A	
rs142594836	*HRNR*	G2296A	G766S	0.042		T	B	Unknown
rs12094334	*IGFN1*	G2087A	G696D	0.3513		D	N/A	Polypoidal choroidal vasculopathy
rs12063867		A2947G	M983V	0.3511		D	N/A	
rs7551098		C3257T	A1086V	0.3503		T	N/A	
rs7551538		G3517T	A1173S	0.3449		D	N/A	
rs12070918		A7196G	D2399G	0.3499		T	N/A	
rs35267671	*INPP5B*	G16A	G6S	0.3246		D	B	Lowe oculocerebrorenal syndrome
rs7251	*IRF3*	G461C	S154T	0.3288		T	B	Acute encephalopathy, infection-induced
rs75304543	*JADE2*	G42T	L14F	0.3554		N/A	N/A	Unknown

rs17618244	*KLB*	G2183A	R728Q	0.1819		T	B	Unknown
rs198977	*KLK2*	C442T	R148W	0.2004		T	B	Prostate cancer
rs201142403	*KRT6A*	T745C	F249L	0.1457		T	B	Pachyonychia congenita
rs199613662		G722A	G241D	0.1127		D	B	
rs201663666		G721A	G241S	0.1097		T	B	
rs652423	*KRT6B*	A680G	N227S	0.3876		T	B	Pachyonychia congenita
rs61745883		G332A	G111D	0.3331		D	D	
rs61914500		G262A	G88R	0.2388		T	B	
rs2226548	*KRTAP13-4*	G175A	A59T	0.2884		T	B	Unknown
rs2832873	*KRTAP15-1*	C127A	L43M	0.2884		D	P	Unknown
rs2298437	*KRTAP19-4*	A143G	Y48C	0.3241		T	B	Unknown
rs28622470	*LIMCH1*	C1238T	T413M	0.0972		D	D	Unknown
rs73135482		C1429A	L477I	0.0969		T	B	
rs60455691	*LIPT2*	C158T	A53V	0.1792		T	B	Mitochondrial lipoylation defect associated with severe neonatal encephalopathy
rs3816614	*LRP4*	G4937A	R1646Q	0.3798	0.5,1	T	B	Cenani-Lenz syndactyly syndrome; congenital myasthenic syndrome
rs2306029		A4660G	S1554G	0.2342		D	B	
rs6485702		A3256G	I1086V	0.2827		T	B	
rs17286758	*LRRC2*	A247G	T83A	0.0999	0.5,0.5	T	B	Unknown
rs2042919	*LRRC8E*	A155G	E52G	0.2382		T	B	Unknown
rs2115108		T182C	M61T	0.2654		T	B	
rs2302607	*METTL22*	G655A	A219T	0.3574		T	B	Unknown
rs78616323	*MICAL3*	C3827T	T1276I	0.1596		D	B	Joubert syndrome
rs75658007	*MRM3*	G427A	V143I	0.0537		D	B	Unknown
rs80220493		T428A	V143D	0.0537		D	P	
rs11546280	*MRPL12*	T313C	S105P	0.1969		T	B	Brain glioblastoma multiforme; brain cancer
rs2216662	*MUC16*	G29725A	V9909I	0.5808		D	B	Ovarian cancer

rs1833778		G28504A	A9502T	0.5753		T	B	
rs2547064		A23768C	D7923A	0.2805		T	B	
rs1867691		A21814G	I7272V	0.2801		T	B	
rs2121133		T15133C	S5045P	0.2799		T	B	
rs2591590		A14704G	I4902V	0.2782		D	B	
rs2591591		C12496A	H4166N	0.2803		D	B	
rs2547068		A12229T	T4077S	0.2803		T	B	
rs2591592		A12100T	I4034F	0.2855		T	P	
rs2591593		G11477A	G3826E	0.28	0.5,1	D	B	
rs2547072		C11363T	T3788I	0.2793		D	B	
rs2591594		G10718A	R3573H	0.282		T	B	
rs2547074		G10588A	V3530I	0.2802		D	B	
rs2547075		A10517C	K3506T	0.279		T	B	
rs2547076		G10496T	R3499M	0.2798		T	B	
rs1862462		C10010T	S3337L	0.2817		D	B	
rs2591597		A9643G	S3215G	0.2818		T	B	
rs73168398	*MUC17*	G12998A	R4333Q	0.1058		T	B	Biliary papillomatosis; colorectal cancer
rs3817552	*MYBPC1*	C1365G	H455Q	0.2979		D	D	Arthrogryposis; lethal congenital contracture syndrome
rs6421985	*NLRP6*	A487C	M163L	0.2435		T	B	Unknown
rs7482965		A1082T	Y361F	0.254	0.5,0.5	T	B	
rs56128139	*NME8*	T1478C	I493T	0.1158		D	B	Primary ciliary dyskinesia
rs2270182	*NRAP*	A1451T	N484I	0.1755		T	P	Myopathy, myofibrillar
rs2275799		G844A	A282T	0.1998		T	B	
rs75155858	*NRG1*	G1376T	G459V	0.3453		D	P	Schizophrenia; mucinous lung adenocarcinoma
rs200668592	*NRG2*	1779_1784del	593_595del	0.0883		N/A	N/A	Charcot-Marie-Tooth disease

rs769427	*OR1A1*	C853T	P285S	0.1247		D	P	Unknown
rs4836891	*OR1J2*	G494A	R165Q	0.2283		T	B	Unknown
rs142107755	*OR2T33*	G661T	A221S	0.0383		T	B	Unknown
rs200877558		T479C	V160A	0.0149		T	B	
rs227787	*OR3A3*	A949G	K317E	0.3538		D	B	Unknown
rs12885778	*OR4K1*	G266A	R89H	0.2635		T	B	Unknown
rs34394400		C910T	R304C	0.1892		D	B	
rs2318279	*OR4N2*	C397T	P133S	0.1125		T	B	Unknown
rs80295194	*OR10G3*	G875A	R292Q	0.0309		D	P	Unknown
rs7114672	*OR56A5*	G310A	V104M	0.2921		N/A	B	Unknown
rs208294	*P2RX7*	T463C	Y155H	0.3897		N/A	N/A	Extrapulmonary tuberculosis; tularemia
rs726684	*PCDHGA8*	T47G	L16R	0.1383		D	D	Unknown
rs2074912	*PCDHGC5*	A1709G	D570G	0.1293		T	B	Unknown
rs17208425		A2618G	E873G	0.1289		N/A	B	
rs7234309	*PIEZO2*	G4060A	V1354I	0.4988		T	N/A	Marden-Walker syndrome; distal arthrogryposis
rs73403546	*PLA2G4F*	C754G	L252V	0.1179		T	B	Unknown
rs1064213	*PLCL1*	G1999A	V667I	0.1865	0.5,1	D	D	Unknown
rs7424029	*POTEE*	G2601T	E867D	0.1611		T	B	Unknown
rs62178369		G2918A	G973D	0.3833		D	D	
rs2599794	*POTEF*	G2601T	E867D	0.4239		T	B	Unknown
rs2897665		A337G	S113G	0.5779		T	B	
rs143023559	*PPFIBP2*	893_895 del	298_299del	0.0718	0.5,0.5	N/A	N/A	Unknown
rs3786734	*PPP1R15A*	G94A	A32T	0.1741		D	D	Unknown
rs1769774	*PRAMEF1*	C652T	P218S	0.3745		T	B	Unknown
rs1052908		A423C	R141S	0.4245		D	B	
rs72819488	*PROM2*	G1537A	G513S	0.2172		T	P	Unknown
rs7260222	*PTOV1*	C74T	S25L	0.4574		N/A	B	A marker of aggressive

								diseases in carcinomas
rs117766916	*RAET1L*	C79G	R27G	0.0296		T	B	Unknown
rs3744872	*RBFA*	A733C	N245H	0.4944		T	B	Unknown
rs112636230	*RBMXL1*	A120G	I40M	0.2333		T	B	Unknown
rs10963	*RBP5*	G55A	D19N	0.4646	0.5,1	T	B	Total anomalous pulmonary venous return
rs78143373	*RHBDF1*	A2017G	I673V	0.0867		T	B	Palmoplantar keratoderma
rs148731719	*RNF213*	G13195A	A4399T	0.0524		T	B	Moyamoya disease; anaplastic large cell lymphoma
rs200943820	*RSPH10B*	C443T	T148M	0.0975	0.5,0.5	T	D	Unknown
rs2295769	*SERPINB6*	A310G	M104V	0.2679		T	B	Autosomal recessive non-syndromic sensorineural deafness
rs6092	*SERPINE1*	G43A	A15T	0.0885		T	B	Plasminogen activator inhibitor-1 deficiency
rs1136287		C215T	T72M	0.5702	0.5,1	T	B	
rs10409962	*SIGLEC8*	T508C	S170P	0.11		T	B	Unknown
rs11150813	*SLC25A10*	G892A	V298I	0.3398		N/A	N/A	Unknown
rs13259978	*SLC7A2*	G82C	D28H	0.0957		T	B	Lysinuric protein intolerance
rs576516	*SMAP1*	T1247C	M416T	0.2882		T	B	Retinitis pigmentosa
rs758896527	*SPATA31C1*	211_221 del	H71fs	0.0269	0.5,0.5	N/A	N/A	Unknown
rs61759822	*STPG3*	C652T	L218F	0.3067		T	P	Unknown
rs10883859	*TAF5*	T388G	S130A	0.4215		T	B	Unknown
rs1052692	*TCF3*	G1291A	G431S	0.218		T	B	Agammaglobulinemia
rs12498609	*TET2*	C86G	P29R	0.2077		D	P	Refractory anemia; myelodysplastic syndrome
rs1025806	*TEX38*	C596T	A199V	0.3389	0.5,1	T	B	Unknown
rs3827816	*TNC*	G1813A	V605I	0.3891	0.5,0.5	T	B	Deafness;

								Bullous keratopathy
rs2269495	*TNIP2*	C938T	A313V	0.3585		T	B	Unknown
rs16847812	*TNN*	G865A	D289N	0.241	0.5,1	T	B	Dysplastic nevus syndrome
rs6694078		A2575G	M859V	0.3444	0.5,0.5	T	B	
rs12369033	*TNS2*	G29C	R10T	0.1079		D	B	Unknown
rs371929937	*TPSAB1*	A8G	N3S	0.169		T	B	Systemic mastocytosis
rs1131877	*TRAF3*	T386C	M129T	0.4163		T	B	Acute encephalopathy, infection-induced
Chr19: 6751057[§]	*TRIP10*	T1513A	S505T	NA		N/A	B	Wiskott-aldrich syndrome
rs3762735	*TRMT10C*	C167G	P56R	0.1462		T	B	Combined oxidative phosphorylation deficiency; mitochondrial metabolism disease
rs2072394	*TRUB2*	G145C	V49L	0.0605		T	B	Dyskeratosis congenita
rs33970858	*TSNARE1*	G164C	R55P	0.3317		T	B	Schizophrenia
rs7814359		T52C	F18L	0.3292		T	B	
rs34379910	*TSPAN10*	T652C	Y218H	0.1994	0.5,1	N/A	D	Unknown
rs1052422	*TUBA3E*	T1204C	W402R	0.4738	0.5,0.5	N/A	B	Microlissencephaly
rs13000249		C661A	R221S	0.4252		N/A	P	
rs13000721		C377T	A126V	0.4655		N/A	B	
rs3863907		G302A	S101N	0.4698		N/A	B	
rs307658	*UBAP2*	A1016G	N339S	0.1675		T	B	Unknown
rs2072767	*UNC93A*	G748A	V250I	0.2382	0.5,1	T	B	Unknown
rs9459921		G1083A	M361I	0.2591		D	B	
rs3744793	*USP36*	G811A	V271I	0.5318		T	B	Unknown
rs15818	*VPS11*	A2636G	K879R	0.1788		N/A	B	Hypomyelinating leukodystrophy
rs597371	*VWA2*	A392G	E131G	0.3567	0.5,0.5	T	B	Type 1 diabetes mellitus; A marker for colon cancer

rs6942733	*ZAN*	T3035G	L1012R	0.2833	0.5,1	N/A	B	Deafness
rs76337191	*ZIC5*	C329A	A110E	0.1409	0.5,0.5	T	B	Holoprosencephaly
rs61734609	*ZNF221*	T1598C	L533P	0.1292		T	B	Unknown
rs6509138	*ZNF223*	C412A	L138I	0.3185		T	B	Ovarian carcinomas
rs2249769	*ZSCAN30*	A152C	Q51P	0.2609	0.5,1	T	B	Unknown
201 variants/149 genes								
***De novo* mutation**								
Variants	**Gene Symbol**	**DNA Change**	**AA Change**	**AF in Asia**	**AF in Patients**	**SIFT**	**Poly-Phen2**	**Disease Association with Gene (PubMed)**
rs200227742	*CDK11B*	G337A	G113R	0.0758	0.5,0.5	N/A	B	Neuroblastoma
rs779250776	*GOLGA6L2*	1531_1533del	511_511del	0		N/A	N/A	Unknown
rs68177477		G1509C	Q503H	0		T	B	
rs76062343		C1465G	Q489E	0.0054		T	B	
rs75486959		A1460T	E487V	0.001		T	B	
rs74565846		G1459A	E487K	0		D	B	
Chr11: 71527323§	*KRTAP5-7*	A23G	E8G	0.0008		D	B	Unknown
Chr2: 15940678§	*MYCN*	G92C	R31P	N/A		N/A	N/A	Cerebral primitive neuroectodermal tumor
Chr2: 15940685§		100_101del	G34fs	N/A		N/A	N/A	
rs774139549	*VEGFC*	C571G	P191A	N/A		N/A	P	Lymphedema
10 variants/5 genes								

†. SIFT: T: tolerable, D: deleterious, N/A: not applicable.
‡. Poly-Phen2: B: benign, P: possibly damaging, D: damaging, N/A: not applicable.
§. Genomic position without registered SNP ID available.

Table 3. DYT1 variant-harboring genes that encode cytoskeleton-associated proteins.

Microtubules				
Variant(s)	**Gene Symbol**	**Protein**	**AF in the two patients**	**References**
rs3774729	*ATXN7*	Ataxin-7	1,1	PMID: 22100762
rs150150392	*CCDC66*	Coiled-coil domain containing 66	1,1	PMID: 28235840
rs76069883	*CCDC74A*	Coiled-coil domain-containing protein 74A	0.5,0.5	PMID: 31521166
rs3177472 rs2259332	*CCDC74B*	Coiled-coil domain-containing protein 74B	0.5,0.5	
rs6740879	*CCDC138*	Coiled-coil domain-containing protein 138	0.5,0.5	PMID: 31304627
rs3734381	*CEP85L*	Centrosomal protein of 85 kDa-like	0.5,0.5	PMID: 21399614
rs6081901	*CFAP61*	Cilia- and flagella-associated protein 61	0.5,0.5	PMID: 30122541
rs3738952	*CUL3*	Cullin-3	0.5,1	PMID: 19995937
rs869801	*DOCK1*	Dedicator of cytokinesis protein 1	0.5,0.5	PMID: 24637113
rs688906 rs589623	*DYNC2H1*	Dynein cytoplasmic 2 Heavy Chain 1	1,1	PMID: 25470043
rs78616323	*MICAL3*	Microtubule-associated monoxygenase, calponin, and LIM domain-containg 3	0.5,0.5	PMID: 27528609
rs56128139	*NME8*	Thioredoxin domain-containing protein 3	0.5,0.5	PMID: 17360648
rs35385129	*PVR*	Poliovirus receptor	1,1	PMID: 20964795
rs2042791	*SPAG16*	Sperm-associated antigen 16	1,1	PMID:

		protein		21655194
Chr19: 6751057	*TRIP10*	Cdc42-interacting protein 4	0.5,0.5	PMID: 11069762
rs2274791	*TTLL10*	Inactive polyglycylase	1,1	PMID: 19427864
rs1052422 rs13000249 rs13000721 rs3863907	*TUBA3E*	Tubulin alpha-3E chain	0.5,0.5	PMID: 17543498
rs4838865	*TUBGCP6*	γ-tubulin complex component 6	1,1	PMID: 11694571
23 variants/18 genes				
Actin Filaments				
Variant(s)	**Gene Symbol**	**Protein**	**AF in the two patients**	**References**
rs2614668	*AKAP13*	A-kinase anchor protein 13	0.5,0.5	PMID: 24183240
rs6886	*CAPG*	Macrophage-capping protein	0.5,0.5	PMID: 18266911
rs1801449	*CAPN3*	Calpain-3	0.5,0.5	PMID: 11950589
rs2230552	*CCT6B*	Chaperonin containing TCP1 Subunit 6B	0.5,0.5	PMID: 9013858
rs2544809	*DBN1*	Drebrin	0.5,0.5	PMID: 20215400
rs869801	*DOCK1*	Dedicator of cytokinesis protein 1	0.5,0.5	PMID: 25452388
rs28622470 rs73135482	*LIMCH1*	LIM and calponin homology domains-containing protein 1	0.5,0.5	PMID: 28228547
rs3817552	*MYBPC1*	Myosin-binding protein C, slow-type	0.5,0.5	PMID: 8375400
rs2270182	*NRAP*	Nebulin-related-anchoring protein	0.5,0.5	PMID:

rs2275799				19233165
rs10151658	*SYNE2*	Nesprin-2	1,1	PMID: 22945352
12variants/10 genes				
Intermediate Filaments				
Variant(s)	**Gene Symbol**	**Protein**	**AF in the two patients**	**References**
rs201142403 rs199613662 rs201663666	*KRT6A*	Keratin, type II cytoskeletal 6A	0.5,0.5	PMID: 7543104
rs652423 rs61745883 rs61914500	*KRT6B*	Keratin, type II cytoskeletal 6B	0.5,0.5	PMID: 9618173
Chr11: 71527323§	*KRTAP5-7*	Keratin-associated protein 5-7	0.5,0.5	PMID: 31691815
rs2226548	*KRTAP13-4*	Keratin-associated protein 13-4	0.5,0.5	
rs2832873	*KRTAP15-1*	Keratin-associated protein 15-1	0.5,0.5	
rs2298437	*KRTAP19-4*	Keratin-associated protein 19-4	0.5,0.5	
10 variants/6 genes				
Total 45 variants/34 genes				

Table 4. DYT1 variant-harboring genes that encode proteins involved in protein and lipid metabolism and endoplasmic reticulum function.

Endoplasmic Reticulum function and protein metabolism				
Variant(s)	**Gene Symbol**	**Protein**	**AF in the two patients**	**References**
rs910122 rs236152	*CHGB*	Chromogranin B	1,1 1,1	PMID: 31691815
rs3746866	*DOP1B*	DOP1 leucine zipper-like protein B	0.5,0.5	PMID: 16301316
rs7995033	*MTMR6*	Myotubularin-related protein 6	1,1	PMID: 19038970
rs1466684	*P2RY13*	P2Y purinoceptor 13	1,1	PMID: 30576484
rs3786734	*PPP1R15A*	Protein phosphatase 1 regulatory subunit 15A	0.5,0.5	PMID: 21518769
6 variants/5 genes				
Lipid Metabolism				
Variant(s)	**Gene Symbol**	**Protein**	**AF in the two patients**	**References**
rs3027232	*ALOXE3*	Hydroperoxide isomerase ALOXE3	0.5,0.5	PMID: 21558561
rs1042034	*APOB*	Apolipoprotein B-100	0.5,0.5	PMID: 15797858
rs1056827 rs10012	*CYP1B1*	Cytochrome P450 1B1	0.5,1	PMID: 15258110
rs2261144	*CYP2A7*	Cytochrome P450 2A7	0.5,0.5	PMID: 21873635
rs13280444	*FAM135B*	Protein FAM135B	1,1	PMID: 21873635
rs2267161	*GAL3ST1*	Galactosylceramide sulfotransferase	0.5,0.5	PMID: 25151383

rs2792751	*GPAM*	Glycerol-3phosphate acyltransferase 1, mitochondrial	1,1	PMID: 18238778
rs7995033	*MTMR6*	Myotubularin-related protein 6	1,1	PMID: 22647598
rs73403546	*PLA2G4F*	Cytosolic phospholipase A2 zeta	0.5,0.5	PMID: 21873635
rs1064213	*PLCL1*	Inactive phospholipase C-like protein 1	0.5,1	PMID: 17254016
11 variants/10 genes				

Table 5. DYT1 variant-harboring genes that encode proteins associated with human neuropsychiatric disorders or neuromuscular diseases

Variant(s)	Gene Symbol	AA change	AF in Asia	Disease association	References
rs55791176	*AHNAK2*	E1048D	0.382	Charcot-Marie-Tooth disease, Demyelinating, Type 4F	PMID: 31011849
rs28362581	*AMPD2*	A82T	0.3146	Pontocerebellar hypoplasia, Type 9	PMID: 29463858
rs6755527	*ARHGEF3*	L341V	0.462	Charcot-Marie-Tooth disease, Type 4H	PMID: 14508709
rs3774729	*ATXN7*	V717M	0.4981	Spinocerebellar ataxia 7	PMID: 30473770
rs1801449	*CAPN3*	A236T	0.1279	Limb-girdle Muscular dystrophy	PMID: 31540302
rs200227742	*CDK11B*	G113R	0.0758	Neurobloastoma	PMID: 7777541
rs3809727	*CDRT1*	A245G	0.4412	Charcot-Marie-Tooth disease, Type Ia	PMID: 11381029
rs2544809	*DBN1*	I446V	0.2506	Alzheimer disease	PMID: 28597477
rs3746866	*DOP1B*	P1149H	0.2389	Down syndrome	PMID: 16303751
rs13280444	*FAM135B*	P482L	0.5081	Spinal and bulbar muscular atrophy, X-Linked	PMID: 30391288
rs2229519	*GBE1*	R190G	0.4615	Polyglucosan body neuropathy, adult form	PMID: 25544507
rs61753060	*GEMIN4*	Q258R	0.058	Neurodevelopmental disorder with microcephaly, cataracts, renal abnormalities, and	PMID: 25558065

				microcephaly	
rs1042303	*GPLD1*	M694V	0.2627	Autism spectrum disorders	PMID: 25448322
rs3841128	*GRIA1*	P10fs	0.0309	Depression; Status epilepticus	PMID: 22057216
rs7251	*IRF3*	S154T	0.3288	Acute encephalopathy, Infection-induced	PMID: 26216125
rs60455691	*LIPT2*	A53V	0.1792	Neonatal severe encephalopathy with lactic acidosis and brain abnormalities and lipoic acid biosynthesis defect	PMID: 28757203
rs3816614 rs2300629 rs6485702	*LRP4*	R1646Q S1554G I1086V	0.3798 0.2342 0.2827	Congenital myasthenic syndrome	PMID: 28825343
rs78616323	*MICAL3*	T1276I	0.1596	Joubert syndrome (Cerebelloparenchymal disorder)	PMID: 26485645
rs11546280	*MRPL12*	S105P	0.1969	Brain glioblastoma multiforme and Brain cancer	PMID: 26781422
Chr2: 15940678 15940685	*MYCN*	R31P G34fs	NA	Cerebral primitive neuroectodermal tumor	PMID: 28453467
rs75155858	*NRG1*	G459V	0.3453	Schizophrenia	PMID: 30500411
rs2270182 rs2275799	*NRAP*	N484I A282T	0.1755 0.1998	Myopathy, myofibrillar	PMID: 30986853
rs200668592	*NRG2*	593_595del	0.0883	Charcot-Marie-Tooth disease, demyelinating form	PMID: 10369162
rs148731719	*RNF213*	A4399T	0.0524	Moyamoya disease	PMID: 29387438

rs35385129	*PVR*	R391S	0.3621	Paralytic poliomyelitis	PMID: 11597452
rs10151658	*SYNE2*	L5186M	0.6646	Emery-Dreifuss Muscular dystrophy	PMID: 21496632
rs1131877	*TRAF3*	M129T	0.4163	Acute encephalopathy, Infection-induced	PMID: 20832341
rs33970858 rs7814359	*TSNARE1*	R55P F18L	0.3317 0.3292	Schizophrenia	PMID: 27668389
rs1052422 rs13000249 rs13000721 rs3863907	*TUBA3E*	W402R R221S A126V S101N	0.4738 0.4252 0.4655 0.4698	Microlissencephaly	PMID: 17571022
rs4838865	*TUBGCP6*	L567S	0.8241	Microcephaly with chorioretinopathy	PMID: 31077665
rs15818	*VPS11*	K879R	0.1788	Hypomyelinating leukodystrophy	PMID: 27473128
rs76337191	*ZIC5*	A110E	0.1409	Holoprosencephaly	PMID: 20531442
Total 40 variants/32 genes					

FIGURE 1

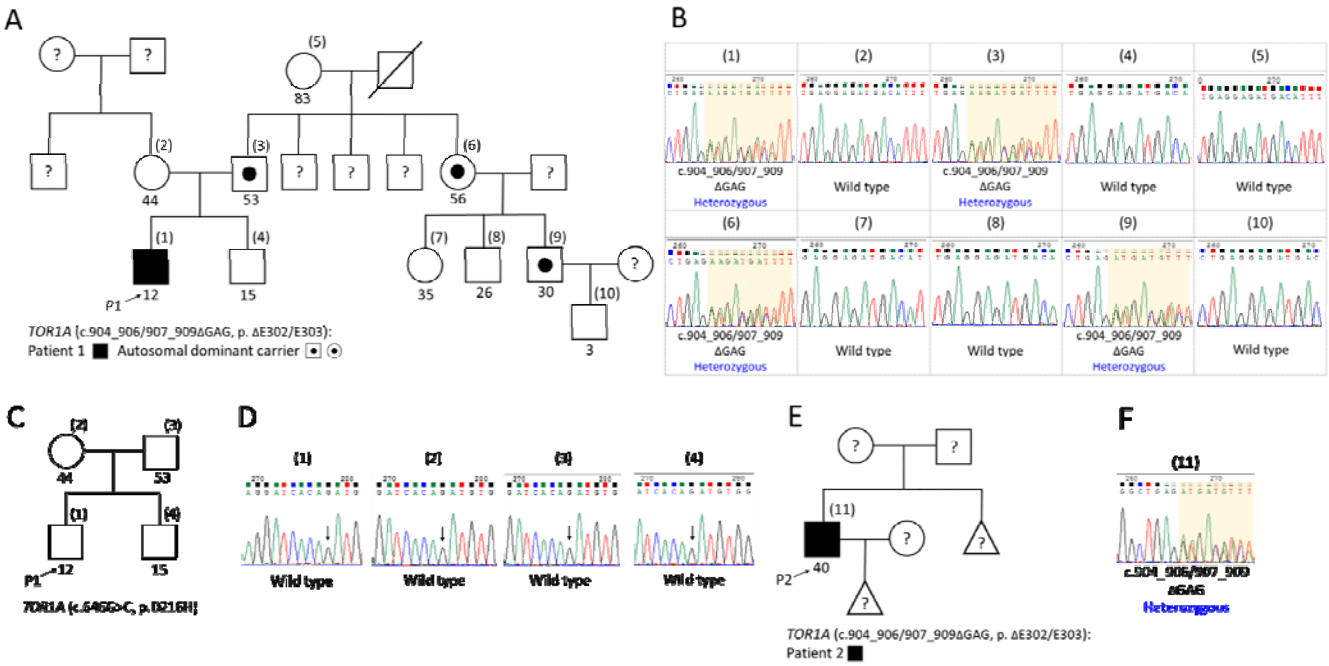

Figure 1. Two patients with their family pedigrees and Sanger sequencing data. Family pedigree (A,C,E) and Sanger sequencing data (B,D,F) of (A,B) 10 family members of TOR1A gene (c.904_906/907_909ΔGAG, p. ΔE302/E303), (C,D) 4 family (core family of the first family) members of TOR1A gene (c.646G>C, p.D216H) and (E,F) second patient of TOR1A gene (c.904_906/907_909ΔGAG, p. ΔE302/E303). (A,C,E) The arrows point out the two probands. The numbers within parentheses are the order of Sanger sequencing data and the numbers under the box/circle show the age (years old). The question marks within the box/circle indicate the unknown status because we don't have the DNAs sample for study. Triangles denote lack of gender information.

FIGURE 2

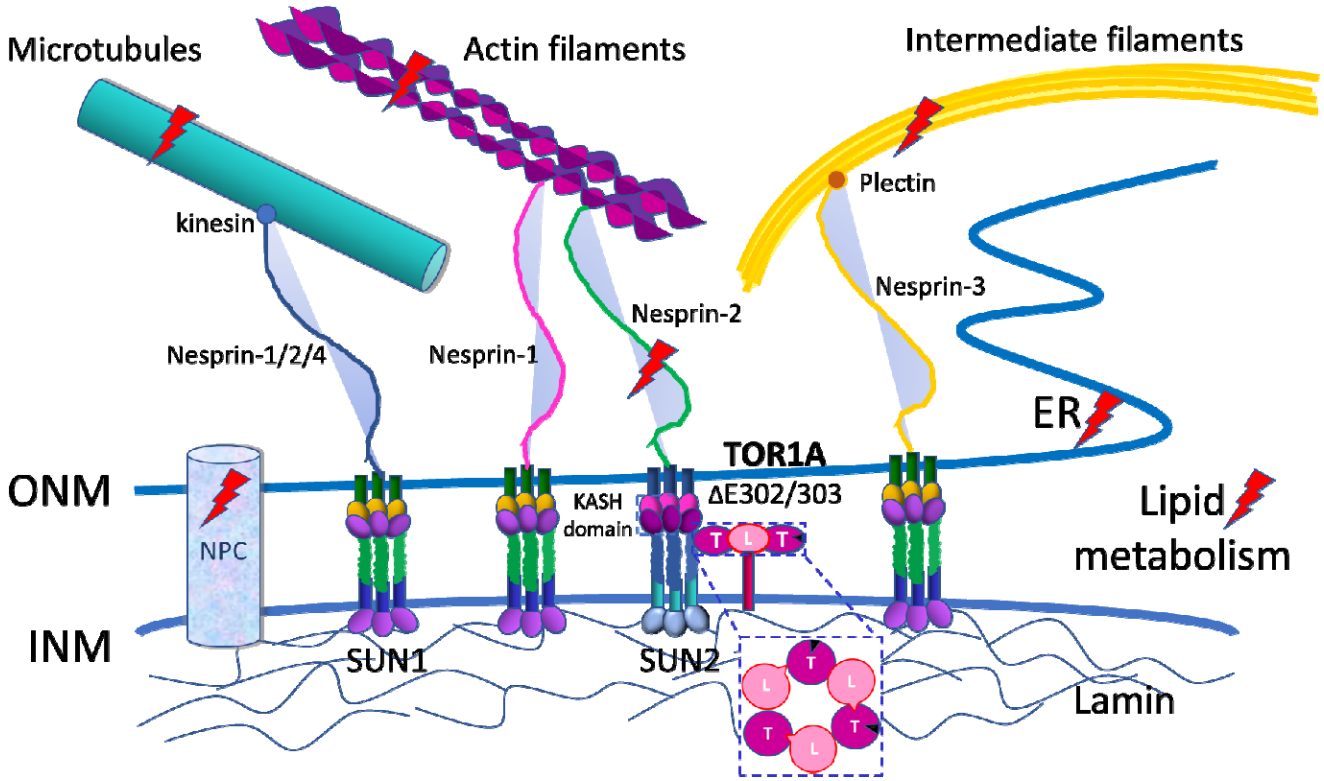

Figure 2. Multiple hits (variants) in the nuclear-cytoskeletal coupling network. TOR1A-LAP1 (or LULL1) heterohexamer regulates the assembly and function of LINC complex. The location of the defects at TOR1A (ΔE302/E303) and variants found in microtubules, actin filaments, intermediate filaments, nesprin-2, nuclear pore complex (NPC), endoplasmic reticulum (ER), and lipid metabolism. LINC complex (the linker of nucleoskeleton and cytoskeleton, consisting of KASH domain and SUN proteins), T (TOR1A), L (LAP1 or LULL1), KASH domain (Klarsicht, ANC-1, and Syne homology domain), SUN 1 and SUN 2 (SUN (Sad1, UNC-84) domain-containing protein 1 and 2), ONM (outer nuclear membrane), INM (inner nuclear membrane).