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

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44

45 **Abstract**

46 **Background** : DYT1 dystonia is a neurological movement disorder characterized
47 by painful sustained muscle contractions resulting in abnormal twisting and
48 postures. In a subset of patients, it is caused by a loss-of-function mutation
49 ($\Delta E302/303$; or ΔE) in the luminal ATPases associated with various cellular activities
50 (AAA+) protein torsinA encoded by the *TOR1A* gene. The low penetrance of the ΔE
51 mutation (~30-40%) suggests the existence of unknown genetic modifiers of DYT1
52 dystonia.

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

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

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

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

67 **Introduction**

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

75

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

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

91

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

99

100 Consistent with this hypothesis, recent research shows that genetic background
101 modulates the phenotype of a mouse model of DYT1 dystonia [16]. In addition,
102 expression profiling in peripheral blood harvested from human DYT1 dystonia
103 patients harboring the ΔE mutation and asymptomatic carriers revealed a genetic
104 signature that could correctly predict disease state [17]. The functional classification
105 of transcripts that were differentially regulated in DYT1 dystonia patients relative to
106 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.

116

117 **Materials and methods**

118 **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.

125

126 **Purification of genomic DNA and RNA from Isolated Human Blood**

127 **Leukocytes**

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

134

135 **Sanger Sequencing of the TOR1A gene**

136 The DNA encoding portions of the *TOR1A* gene was PCR products amplified from
137 the genomic DNA using the following primer pairs: 1) *TOR1A* (c.646G>C, D216H)-

138 F: TAATTCAGGATCAGTTACAGTTGTG and -R:

139 TGCAGGATTAGGAACCAGAT; and 2) *TOR1A* (c.904_906/907_909ΔGAG,

140 Δ302/303E)- F: GTGTGGCATGGATAGGTGACCC and -R:

141 GGGTGAAGTGTGGAAGGAC. From the cDNA using the following primer pairs:

142 Transcriptome(873bp) -F: ATCTACCCGCGTCTCTAC and -R:

143 ATAATCTAACTTGGTGAACA; The resulting PCR products were purified using

144 QIAquick PCR Purification Kit (Qiagen[®]) and then undergoing Sanger sequencing

145 (Genomics[®], Taipei, Taiwan).

146

147 **Whole exome sequencing**

148 Purified human genomic DNA was sheared into ~150-200 base-pair fragments
 149 using the S220 Focused-Ultrasonicator (Covaris, Woburn, Massachusetts)
 150 according to the instructions provided by the manufacturer. SureSelectXT Human
 151 All Exon V6 +UTR (Agilent Technologies, Santa Clara, CA) was then used to
 152 perform exome capture and library preparations The library were then sequenced
 153 using a NovaSeq 6000 System (Illumina, San Diego, CA) with 150 base-pair reads
 154 and output data up to 10 Gb per sample. After sequencing, Genome Analysis
 155 Toolkit (GATK) best practices workflows of germline short variant discovery
 156 (<https://software.broadinstitute.org/gatk>) was used to perform variant calling with
 157 default parameters [21]. Briefly, the Burrows-Wheeler Aligner was first used to align
 158 the sequenced exomes with the most up-to-date human genome reference build
 159 (hg38) ("GRCh38 - hg38 - Genome - Assembly - NCBI". ncbi.nlm.nih.gov). Next,
 160 duplicate reads were removed using Picard after which the GATK was used to
 161 perform local realignment of the sequenced exomes with the reference genome and
 162 base quality recalibration. Then, GATK-HaplotypeCaller was used to call germline
 163 SNPs (single-nucleotide polymorphism) and indels. After variant calling, ANNOVAR
 164 was used to variant annotation [22] with database, include refGene,
 165 clinvar_20170905 (<https://www.ncbi.nlm.nih.gov/clinvar/>), avsnp150, dbnsfp33a,
 166 gnomad_genome, dbcsnv11. Annotated variants were selected with the following
 167 criteria: (1) filtering with exonic region, (2) removing synonymous mutation, (3) read
 168 depth ≥ 20 . Next, we categorized these filtered variants according to the principle of

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

173

174 **Results**

175 **Clinical Observations**

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

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

195 **Examination of Known DYT1 Dystonia-Associated Mutations**

196 To determine if either Patient 1 (subject 1) or Patient 2 (subject 11) harbored known
197 DYT1 dystonia-associated mutations in their genomes, we used Sanger sequencing
198 to screen their *TOR1A* genes for the presence of the ΔE mutation. Our results show
199 that both patients are heterozygous for the ΔE mutation (Fig 1. Two patients with
200 their family pedigrees and Sanger sequencing data. Family pedigree (A,E) and
201 Sanger sequencing data (B,F)). We also sequenced the *TOR1A* genes of nine other
202 family members of Patient 1. No ΔE mutation was found in the genomes of his
203 unaffected mother (subject 2), male sibling (subject 4) or other relatives (subject 5, 7,
204 8,10), while his father (subject 3), paternal aunt (subject 6), and cousin of Patient 1
205 (subject 9) were asymptomatic heterozygotic ΔE mutation carriers. Then, we asked
206 if the previously described protective modifier mutation D216H was present within
207 the *TOR1A* gene of the core family of patient 1, including subject 1,2,3,4 [25].
208 However, none of the family members examined were positive for the D216H
209 mutation (Fig1. C,D). These results suggest that Patient 1 inherited the ΔE mutation
210 from the paternal side of his family and that the absence of DYT1 dystonia in his

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

213

214 **Examination of potential misregulation of alternative splicing by mutations** 215 **that affect splicing signals/machinery**

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

224

225 **Identification of DYT1 Dystonia-Associated Genome Variants in the *TOR1A*** 226 **gene**

227 To begin to identify potential genetic modifiers of the penetrance of the ΔE mutation,
228 we performed WES on genomic DNA purified from blood leukocytes isolated from
229 Patient 1 and Patient 2 as well as three asymptomatic ΔE mutation carriers from the
230 first family (i.e. the father, paternal aunt, and cousin) and the mother of patient 1 who
231 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.

244

245 **Identification of DYT1 dystonia-associated Genome Variants (DYT1 variants)**

246 **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

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

263
 264 Of the 264 variants identified by our WES-based screen, 11 variants were predicted
 265 to be loss-of-function mutations (Table 2) by using the bioinformatics tools sorting
 266 intolerant from tolerant (SIFT) and polymorphism phenotyping (PolyPhen) [29].
 267 Furthermore, gene ontology analysis performed using the Database for Annotation,
 268 Visualization and Integrated Discovery (DAVID) [30] identified clustered annotations
 269 of genes in which the DYT1 variants were identified by our WES-based approach.
 270 There are total 30 annotation clusters generated by this tool as listed in Table S1.
 271 Next, we filtered these categories with enrichment score >1 and *p* value <0.05 and the
 272 results were enriched for those that encode proteins that contain the epidermal
 273 growth factor-like domain (ten genes), have dioxygenase (four genes) or Rho

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

291

292 **Discussion**

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

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

308 In this work, we describe the identification of 264 variants in 195 genes that are
 309 associated with DYT1 dystonia. Below, we will discuss the potential implications of
 310 our results on our understanding of the pathogenesis and pathophysiology of DYT1
 311 dystonia. Specifically, we will explore the connections between the DYT1 variants
 312 identified here and the following established cellular functions of torsinA:
 313 cytoskeletal regulation, endoplasmic reticulum stress, and lipid metabolism. In
 314 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].

335 More recently, torsinA was identified as a key regulator of the mechanical
 336 integration of the nucleus and the cytoskeleton via the conserved nuclear
 337 envelope-spanning linker of nucleoskeleton and cytoskeleton (LINC) complex
 338 [31-33]. The core of LINC complexes is formed by the transluminal interaction
 339 between the outer and inner nuclear membrane Klarischt/ANC-1/SYNE homology
 340 (KASH) and Sad1/UNC-84 (SUN) proteins, respectively [44]. KASH proteins
 341 interact with the cytoskeleton and signaling proteins within the cytoplasm [45],
 342 whereas SUN proteins interact with chromatin, other inner nuclear membrane
 343 proteins, and the nuclear lamina within the nucleoplasm [46].

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

350

351 DYT1 dystonia patient-derived fibroblasts were also shown to have increased
 352 susceptibility to damage by mechanical forces [48] strongly suggests that cellular
 353 mechanics may impact the pathogenesis and/or pathophysiology of DYT1 dystonia.
 354 All cells, including neurons, adapt their mechanical properties by converting
 355 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].

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

385

386 **DYT1 variants in association with protein synthesis and transport and ER** 387 **homeostasis**

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

396

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

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

422

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

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

487

488 **Conclusions**

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

501

502 **Supporting information**

503 Table S1 and Figure S1.

504

505 **Acknowledgments**

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

514

515 **Author contributions**

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

520

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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 <i>TOR1A</i>	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	3.23×10 ⁻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	3.23×10 ⁻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	3.23×10 ⁻⁵	Pathogenic (Ozelius 2016)
(9)	Asymptomatic Carrier	rs80358233	Exon 5	Nonframeshift deletion	904_906/ 907_909ΔGAG	ΔE302/ 303	0.5	Unknown	3.23×10 ⁻⁵	Pathogenic (Ozelius 2016)
(11)	Patient	rs80358233	Exon 5	Nonframeshift deletion	904_906/ 907_909ΔGAG	ΔE302/ 303	0.5	Unknown	3.23×10 ⁻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_9insG GGGTAA GCA	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	<i>FAM135B</i>	C1445T	P482L	0.5081		T	B	Spinal and bulbar muscular atrophy
rs448012	<i>FLT4</i>	C2670G	H890Q	0.4763		D	B	Lymphatic malformation; hemangioma
rs2229519	<i>GBE1</i>	A568G	R190G	0.4615		D	B	Glycogen storage disease iv
rs2792751	<i>GPAM</i>	A127G	I43V	0.7206		T	B	Unknown
rs3732215	<i>HJURP</i>	C629G	S210C	0.3879		T	B	Unknown
rs2286430		G226A	E76K	0.3926		T	B	
rs2072597	<i>KLF1</i>	T304C	S102P	0.6002		T	B	Congenital dyserythropoietic anemia, type iv
rs2429051	<i>KLF17</i>	G170C	S57T	0.676		T	B	Unknown
rs2485652		A467G	N156S	0.4608		T	B	
rs1064608	<i>MTCH2</i>	C841G	P281A	0.5837		D	P	Unknown
rs7995033	<i>MTMR6</i>	A955G	I319V	0.6064		T	B	Autosomal dominant polycystic kidney disease
rs11556093	<i>NUP58</i>	G100A	A34T	0.6098		T	B	Unknown
rs6951485	<i>OR2A25</i>	G224A	S75N	0.6752		T	B	Unknown
rs2961135		G625C	A209P	0.6588		T	B	
rs1466684	<i>P2RY13</i>	C536	T179M	0.842		T	B	Unknown
rs35385129	<i>PVR</i>	C1171A	R391S	0.3621		T	B	Paralytic poliomyelitis
rs4795690	<i>RHBDL3</i>	G739A	V247M	0.3973		T	B	Unknown
rs1022478	<i>RIBC2</i>	C804G	F268L	0.5551		N/A	B	Unknown
rs1506418	<i>SERPINB11</i>	G16A	A6T	0.617		N/A	P	Unknown
rs1506419		T37A	W13R	0.6165		N/A	D	
rs12729295	<i>SLC35E2B</i>	G934A	V312I	0.4782		N/A	B	Unknown
rs2042791	<i>SPAG16</i>	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	<i>ARHGEF37</i>	C1756A	P586T	0.2975		T	B	Unknown
rs8066889	<i>ARL16</i>	A28C	S10R	0.1959		T	B	Unknown
rs33995001	<i>ATP10D</i>	C128T	T43I	0.1446		T	B	Unknown
rs3750690	<i>ATRNL1</i>	A2822T	Q941L	0.0092		T	D	Unknown
rs115476782	<i>BTNL10</i>	G376T	A126S	0.0334		N/A	N/A	Unknown
rs2274067	<i>C1orf131</i>	C82G	L28V	0.0854		D	D	Unknown
rs3813728	<i>C1R</i>	G781A	G261R	0.0932		T	D	Ehlers-danlos syndrome
rs1801046		C455T	S152L	0.3511		T	B	
rs10951942	<i>C7orf57</i>	G220T	A74S	0.2769		D	D	Unknown
rs12657663	<i>CAMLG</i>	G232A	V78I	0.2357		T	B	Congenital diaphragmatic hernia
rs6886	<i>CAPG</i>	A911G	H304R	0.4653		T	B	Breast cancer biomarker for bone metastasis
rs1801449	<i>CAPN3</i>	G706A	A236T	0.1279		T	B	Limb-girdle muscular dystrophy
rs76069883	<i>CCDC74A</i>	G688A	G230S	0.0067		T	B	Unknown
rs3177472	<i>CCDC74B</i>	G839A	R280H	0.4827		T	B	Unknown
rs2259332		T728C	V243A	0.4602		T	B	
rs7226091	<i>CCDC137</i>	C381G	H127Q	0.1965		T	B	Unknown
rs11546630		G686A	R229Q	0.1952		T	B	
rs11546631		C844T	R282W	0.1955		T	B	
rs6740879	<i>CCDC138</i>	G329A	R110K	0.0458		T	B	Unknown
rs12606658	<i>CCDC178</i>	G124A	A42T	0.0297		D	B	Unknown
rs2230552	<i>CCT6B</i>	T143C	V48A	0.5422		D	D	Pelvic varices
rs3809727	<i>CDRT1</i>	C734G	A245G	0.4412		T	B	Charcot-Marie-Tooth disease
rs16959164	<i>CEACAM20</i>	C1064T	S355L	0.0721		T	B	Unknown
rs3734381	<i>CEP85L</i>	A409G	S137G	0.3075		T	B	Myeloproliferative neoplasm
rs6081901	<i>CFAP61</i>	G1105A	V369I	0.2128		T	B	Unknown

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

							disorder with microcephaly, cataracts, and renal abnormalities
rs696217	<i>GHRL</i>	C178A	L60M	0.1922	T	D	Eating disorder
rs75027378	<i>GLOD4</i>	C842A	A281E	0.0589	T	B	Unknown
rs3796130	<i>GP9</i>	G466A	A156T	0.2373	T	B	Bernard-Soulier syndrome; gray platelet syndrome
rs1042303	<i>GPLD1</i>	A2080G	M694V	0.2627	T	B	Autism spectrum disorders
rs3841128	<i>GRIA1</i>	31dupC	P10fs	0.0309	N/A	N/A	Depression; Status epilepticus
rs56058441	<i>HGS</i>	G2197T	A733S	0.198	T	B	Unknown
rs2295778	<i>HIF1AN</i>	C121G	P41A	0.2571	T	B	Hypoxia; nephronophthisis
rs397814627	<i>HOXD9</i>	794_795i nsGCA	P265del insPQ	0.3988	N/A	N/A	Brachydactyly-syndactyly syndrome
rs2296436	<i>HPS1</i>	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	<i>HRNR</i>	G2296A	G766S	0.042	T	B	Unknown
rs12094334	<i>IGFN1</i>	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	<i>INPP5B</i>	G16A	G6S	0.3246	D	B	Low oculocerebrorenal syndrome
rs7251	<i>IRF3</i>	G461C	S154T	0.3288	T	B	Acute encephalopathy, infection-induced
rs75304543	<i>JADE2</i>	G42T	L14F	0.3554	N/A	N/A	Unknown

rs17618244	<i>KLB</i>	G2183A	R728Q	0.1819		T	B	Unknown
rs198977	<i>KLK2</i>	C442T	R148W	0.2004		T	B	Prostate cancer
rs201142403	<i>KRT6A</i>	T745C	F249L	0.1457		T	B	Pachyonychia congenita
rs199613662		G722A	G241D	0.1127		D	B	
rs201663666		G721A	G241S	0.1097		T	B	
rs652423	<i>KRT6B</i>	A680G	N227S	0.3876		T	B	Pachyonychia congenita
rs61745883		G332A	G111D	0.3331		D	D	
rs61914500		G262A	G88R	0.2388		T	B	
rs2226548	<i>KRTAP13-4</i>	G175A	A59T	0.2884		T	B	Unknown
rs2832873	<i>KRTAP15-1</i>	C127A	L43M	0.2884		D	P	Unknown
rs2298437	<i>KRTAP19-4</i>	A143G	Y48C	0.3241		T	B	Unknown
rs28622470	<i>LIMCH1</i>	C1238T	T413M	0.0972		D	D	Unknown
rs73135482		C1429A	L477I	0.0969		T	B	
rs60455691	<i>LIPT2</i>	C158T	A53V	0.1792	0.5,1	T	B	Mitochondrial lipoylation defect associated with severe neonatal encephalopathy
rs3816614	<i>LRP4</i>	G4937A	R1646Q	0.3798		T	B	Cenani-Lenz syndactyly syndrome; congenital myasthenic syndrome
rs2306029		A4660G	S1554G	0.2342		D	B	
rs6485702		A3256G	I1086V	0.2827		T	B	
rs17286758	<i>LRRC2</i>	A247G	T83A	0.0999	0.5,0.5	T	B	Unknown
rs2042919	<i>LRRC8E</i>	A155G	E52G	0.2382		T	B	Unknown
rs2115108		T182C	M61T	0.2654		T	B	
rs2302607	<i>METTL22</i>	G655A	A219T	0.3574		T	B	Unknown
rs78616323	<i>MICAL3</i>	C3827T	T1276I	0.1596		D	B	Joubert syndrome
rs75658007	<i>MRM3</i>	G427A	V143I	0.0537		D	B	Unknown
rs80220493		T428A	V143D	0.0537		D	P	
rs11546280	<i>MRPL12</i>	T313C	S105P	0.1969		T	B	Brain glioblastoma multiforme; brain cancer
rs2216662	<i>MUC16</i>	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	<i>MUC17</i>	G12998A	R4333Q	0.1058		T	B	Biliary papillomatosis; colorectal cancer
rs3817552	<i>MYBPC1</i>	C1365G	H455Q	0.2979		D	D	Arthrogryposis; lethal congenital contracture syndrome
rs6421985		A487C	M163L	0.2435		T	B	
rs7482965	<i>NLRP6</i>	A1082T	Y361F	0.254		T	B	Unknown
rs56128139	<i>NME8</i>	T1478C	I493T	0.1158	0.5,0.5	D	B	Primary ciliary dyskinesia
rs2270182		A1451T	N484I	0.1755		T	P	
rs2275799	<i>NRAP</i>	G844A	A282T	0.1998		T	B	Myopathy, myofibrillar
rs75155858	<i>NRG1</i>	G1376T	G459V	0.3453		D	P	Schizophrenia; mucinous lung adenocarcinoma
rs200668592	<i>NRG2</i>	1779_17 84del	593_59 5del	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	0.5,0.5	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_299 del	0.0718		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	<i>RAET1L</i>	C79G	R27G	0.0296		T	B	Unknown
rs3744872	<i>RBFA</i>	A733C	N245H	0.4944		T	B	Unknown
rs112636230	<i>RBMXL1</i>	A120G	I40M	0.2333		T	B	Unknown
rs10963	<i>RBP5</i>	G55A	D19N	0.4646	0.5,1	T	B	Total anomalous pulmonary venous return
rs78143373	<i>RHBDF1</i>	A2017G	I673V	0.0867		T	B	Palmoplantar keratoderma
rs148731719	<i>RNF213</i>	G13195A	A4399T	0.0524		T	B	Moyamoya disease; anaplastic large cell lymphoma
rs200943820	<i>RSPH10B</i>	C443T	T148M	0.0975	0.5,0.5	T	D	Unknown
rs2295769	<i>SERPINB6</i>	A310G	M104V	0.2679		T	B	Autosomal recessive non-syndromic sensorineural deafness
rs6092	<i>SERPINE1</i>	G43A	A15T	0.0885		T	B	Plasminogen activator inhibitor-1 deficiency
rs1136287		C215T	T72M	0.5702	0.5,1	T	B	
rs10409962	<i>SIGLEC8</i>	T508C	S170P	0.11		T	B	Unknown
rs11150813	<i>SLC25A10</i>	G892A	V298I	0.3398		N/A	N/A	Unknown
rs13259978	<i>SLC7A2</i>	G82C	D28H	0.0957		T	B	Lysinuric protein intolerance
rs576516	<i>SMAP1</i>	T1247C	M416T	0.2882		T	B	Retinitis pigmentosa
rs758896527	<i>SPATA31C1</i>	211_221 del	H71fs	0.0269	0.5,0.5	N/A	N/A	Unknown
rs61759822	<i>STPG3</i>	C652T	L218F	0.3067		T	P	Unknown
rs10883859	<i>TAF5</i>	T388G	S130A	0.4215		T	B	Unknown
rs1052692	<i>TCF3</i>	G1291A	G431S	0.218		T	B	Agammaglobulinemia
rs12498609	<i>TET2</i>	C86G	P29R	0.2077		D	P	Refractory anemia; myelodysplastic syndrome
rs1025806	<i>TEX38</i>	C596T	A199V	0.3389	0.5,1	T	B	Unknown
rs3827816	<i>TNC</i>	G1813A	V605I	0.3891	0.5,0.5	T	B	Deafness;

								Bullous keratopathy
rs2269495	<i>TNIP2</i>	C938T	A313V	0.3585		T	B	Unknown
rs16847812	<i>TNN</i>	G865A	D289N	0.241	0.5,1	T	B	Dysplastic nevus
rs6694078		A2575G	M859V	0.3444		T	B	syndrome
rs12369033	<i>TNS2</i>	G29C	R10T	0.1079		D	B	Unknown
rs371929937	<i>TPSAB1</i>	A8G	N3S	0.169		T	B	Systemic mastocytosis
rs1131877	<i>TRAF3</i>	T386C	M129T	0.4163		T	B	Acute encephalopathy, infection-induced
Chr19: 6751057 ^s	<i>TRIP10</i>	T1513A	S505T	NA		N/A	B	Wiskott-aldrich syndrome
rs3762735	<i>TRMT10C</i>	C167G	P56R	0.1462	0.5,0.5	T	B	Combined oxidative phosphorylation deficiency; mitochondrial metabolism disease
rs2072394	<i>TRUB2</i>	G145C	V49L	0.0605		T	B	Dyskeratosis congenita
rs33970858	<i>TSNARE1</i>	G164C	R55P	0.3317		T	B	Schizophrenia
rs7814359		T52C	F18L	0.3292		T	B	
rs34379910	<i>TSPAN10</i>	T652C	Y218H	0.1994	0.5,1	N/A	D	Unknown
rs1052422	<i>TUBA3E</i>	T1204C	W402R	0.4738		N/A	B	Microlissencephaly
rs13000249		C661A	R221S	0.4252		N/A	P	
rs13000721		C377T	A126V	0.4655	0.5,0.5	N/A	B	
rs3863907		G302A	S101N	0.4698		N/A	B	
rs307658	<i>UBAP2</i>	A1016G	N339S	0.1675		T	B	Unknown
rs2072767	<i>UNC93A</i>	G748A	V250I	0.2382		T	B	Unknown
rs9459921		G1083A	M361I	0.2591		D	B	
rs3744793	<i>USP36</i>	G811A	V271I	0.5318	0.5,1	T	B	Unknown
rs15818	<i>VPS11</i>	A2636G	K879R	0.1788		N/A	B	Hypomyelinating leukodystrophy
rs597371	<i>VWA2</i>	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	<i>ATXN7</i>	Ataxin-7	1,1	PMID: 22100762
rs150150392	<i>CCDC66</i>	Coiled-coil domain containing 66	1,1	PMID: 28235840
rs76069883	<i>CCDC74A</i>	Coiled-coil domain-containing protein 74A	0.5,0.5	PMID: 31521166
rs3177472 rs2259332	<i>CCDC74B</i>	Coiled-coil domain-containing protein 74B	0.5,0.5	
rs6740879	<i>CCDC138</i>	Coiled-coil domain-containing protein 138	0.5,0.5	PMID: 31304627
rs3734381	<i>CEP85L</i>	Centrosomal protein of 85 kDa-like	0.5,0.5	PMID: 21399614
rs6081901	<i>CFAP61</i>	Cilia- and flagella-associated protein 61	0.5,0.5	PMID: 30122541
rs3738952	<i>CUL3</i>	Cullin-3	0.5,1	PMID: 19995937
rs869801	<i>DOCK1</i>	Dedicator of cytokinesis protein 1	0.5,0.5	PMID: 24637113
rs688906 rs589623	<i>DYNC2H1</i>	Dynein cytoplasmic 2 Heavy Chain 1	1,1	PMID: 25470043
rs78616323	<i>MICAL3</i>	Microtubule-associated monooxygenase, calponin, and LIM domain-containing 3	0.5,0.5	PMID: 27528609
rs56128139	<i>NME8</i>	Thioredoxin domain-containing protein 3	0.5,0.5	PMID: 17360648
rs35385129	<i>PVR</i>	Poliovirus receptor	1,1	PMID: 20964795
rs2042791	<i>SPAG16</i>	Sperm-associated antigen 16	1,1	PMID:

		protein		21655194
Chr19: 6751057	<i>TRIP10</i>	Cdc42-interacting protein 4	0.5,0.5	PMID: 11069762
rs2274791	<i>TTLL10</i>	Inactive polyglycyclase	1,1	PMID: 19427864
rs1052422 rs13000249 rs13000721 rs3863907	<i>TUBA3E</i>	Tubulin alpha-3E chain	0.5,0.5	PMID: 17543498
rs4838865	<i>TUBGCP6</i>	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	<i>AKAP13</i>	A-kinase anchor protein 13	0.5,0.5	PMID: 24183240
rs6886	<i>CAPG</i>	Macrophage-capping protein	0.5,0.5	PMID: 18266911
rs1801449	<i>CAPN3</i>	Calpain-3	0.5,0.5	PMID: 11950589
rs2230552	<i>CCT6B</i>	Chaperonin containing TCP1 Subunit 6B	0.5,0.5	PMID: 9013858
rs2544809	<i>DBN1</i>	Drebrin	0.5,0.5	PMID: 20215400
rs869801	<i>DOCK1</i>	Dedicator of cytokinesis protein 1	0.5,0.5	PMID: 25452388
rs28622470 rs73135482	<i>LIMCH1</i>	LIM and calponin homology domains-containing protein 1	0.5,0.5	PMID: 28228547
rs3817552	<i>MYBPC1</i>	Myosin-binding protein C, slow- type	0.5,0.5	PMID: 8375400
rs2270182	<i>NRAP</i>	Nebulin-related-anchoring protein	0.5,0.5	PMID:

rs2275799				19233165
rs10151658	<i>SYNE2</i>	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	<i>KRT6A</i>	Keratin, type II cytoskeletal 6A	0.5,0.5	PMID: 7543104
rs652423 rs61745883 rs61914500	<i>KRT6B</i>	Keratin, type II cytoskeletal 6B	0.5,0.5	PMID: 9618173
Chr11: 71527323§	<i>KRTAP5-7</i>	Keratin-associated protein 5-7	0.5,0.5	PMID: 31691815
rs2226548	<i>KRTAP13-4</i>	Keratin-associated protein 13-4	0.5,0.5	
rs2832873	<i>KRTAP15-1</i>	Keratin-associated protein 15-1	0.5,0.5	
rs2298437	<i>KRTAP19-4</i>	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	<i>CHGB</i>	Chromogranin B	1,1 1,1	PMID: 31691815
rs3746866	<i>DOP1B</i>	DOP1 leucine zipper-like protein B	0.5,0.5	PMID: 16301316
rs7995033	<i>MTMR6</i>	Myotubularin-related protein 6	1,1	PMID: 19038970
rs1466684	<i>P2RY13</i>	P2Y purinoceptor 13	1,1	PMID: 30576484
rs3786734	<i>PPP1R15A</i>	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	<i>ALOXE3</i>	Hydroperoxide isomerase ALOXE3	0.5,0.5	PMID: 21558561
rs1042034	<i>APOB</i>	Apolipoprotein B-100	0.5,0.5	PMID: 15797858
rs1056827 rs10012	<i>CYP1B1</i>	Cytochrome P450 1B1	0.5,1	PMID: 15258110
rs2261144	<i>CYP2A7</i>	Cytochrome P450 2A7	0.5,0.5	PMID: 21873635
rs13280444	<i>FAM135B</i>	Protein FAM135B	1,1	PMID: 21873635
rs2267161	<i>GAL3ST1</i>	Galactosylceramide sulfotransferase	0.5,0.5	PMID: 25151383

rs2792751	<i>GPAM</i>	Glycerol-3phosphate acyltransferase 1, mitochondrial	1,1	PMID: 18238778
rs7995033	<i>MTMR6</i>	Myotubularin-related protein 6	1,1	PMID: 22647598
rs73403546	<i>PLA2G4F</i>	Cytosolic phospholipase A2 zeta	0.5,0.5	PMID: 21873635
rs1064213	<i>PLCL1</i>	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	<i>AHNAK2</i>	E1048D	0.382	Charcot-Marie-Tooth disease, Demyelinating, Type 4F	PMID: 31011849
rs28362581	<i>AMPD2</i>	A82T	0.3146	Pontocerebellar hypoplasia, Type 9	PMID: 29463858
rs6755527	<i>ARHGEF3</i>	L341V	0.462	Charcot-Marie-Tooth disease, Type 4H	PMID: 14508709
rs3774729	<i>ATXN7</i>	V717M	0.4981	Spinocerebellar ataxia 7	PMID: 30473770
rs1801449	<i>CAPN3</i>	A236T	0.1279	Limb-girdle Muscular dystrophy	PMID: 31540302
rs200227742	<i>CDK11B</i>	G113R	0.0758	Neuroblastoma	PMID: 7777541
rs3809727	<i>CDRT1</i>	A245G	0.4412	Charcot-Marie-Tooth disease, Type 1a	PMID: 11381029
rs2544809	<i>DBN1</i>	I446V	0.2506	Alzheimer disease	PMID: 28597477
rs3746866	<i>DOP1B</i>	P1149H	0.2389	Down syndrome	PMID: 16303751
rs13280444	<i>FAM135B</i>	P482L	0.5081	Spinal and bulbar muscular atrophy, X-Linked	PMID: 30391288
rs2229519	<i>GBE1</i>	R190G	0.4615	Polyglucosan body neuropathy, adult form	PMID: 25544507
rs61753060	<i>GEMIN4</i>	Q258R	0.058	Neurodevelopmental disorder with microcephaly, cataracts, renal abnormalities, and	PMID: 25558065

				microcephaly	
rs1042303	<i>GPLD1</i>	M694V	0.2627	Autism spectrum disorders	PMID: 25448322
rs3841128	<i>GRIA1</i>	P10fs	0.0309	Depression; Status epilepticus	PMID: 22057216
rs7251	<i>IRF3</i>	S154T	0.3288	Acute encephalopathy, Infection-induced	PMID: 26216125
rs60455691	<i>LIPT2</i>	A53V	0.1792	Neonatal severe encephalopathy with lactic acidosis and brain abnormalities and lipoic acid biosynthesis defect	PMID: 28757203
rs3816614 rs2300629 rs6485702	<i>LRP4</i>	R1646Q S1554G I1086V	0.3798 0.2342 0.2827	Congenital myasthenic syndrome	PMID: 28825343
rs78616323	<i>MICAL3</i>	T1276I	0.1596	Joubert syndrome (Cerebelloparenchymal disorder)	PMID: 26485645
rs11546280	<i>MRPL12</i>	S105P	0.1969	Brain glioblastoma multiforme and Brain cancer	PMID: 26781422
Chr2: 15940678 15940685	<i>MYCN</i>	R31P G34fs	NA	Cerebral primitive neuroectodermal tumor	PMID: 28453467
rs75155858	<i>NRG1</i>	G459V	0.3453	Schizophrenia	PMID: 30500411
rs2270182 rs2275799	<i>NRAP</i>	N484I A282T	0.1755 0.1998	Myopathy, myofibrillar	PMID: 30986853
rs200668592	<i>NRG2</i>	593_59 5del	0.0883	Charcot-Marie-Tooth disease, demyelinating form	PMID: 10369162
rs148731719	<i>RNF213</i>	A4399T	0.0524	Moyamoya disease	PMID: 29387438

rs35385129	<i>PVR</i>	R391S	0.3621	Paralytic poliomyelitis	PMID: 11597452
rs10151658	<i>SYNE2</i>	L5186M	0.6646	Emery-Dreifuss Muscular dystrophy	PMID: 21496632
rs1131877	<i>TRAF3</i>	M129T	0.4163	Acute encephalopathy, Infection-induced	PMID: 20832341
rs33970858 rs7814359	<i>TSNARE1</i>	R55P F18L	0.3317 0.3292	Schizophrenia	PMID: 27668389
rs1052422 rs13000249 rs13000721 rs3863907	<i>TUBA3E</i>	W402R R221S A126V S101N	0.4738 0.4252 0.4655 0.4698	Microlissencephaly	PMID: 17571022
rs4838865	<i>TUBGCP6</i>	L567S	0.8241	Microcephaly with chorioretinopathy	PMID: 31077665
rs15818	<i>VPS11</i>	K879R	0.1788	Hypomyelinating leukodystrophy	PMID: 27473128
rs76337191	<i>ZIC5</i>	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 ($\Delta E302/E303$) 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).