ARTICLE

The rare rs769301934 variant in *NHLRC1* is a common cause of Lafora disease in Turkey

Garen Haryanyan[1,2,11], Ozkan Ozdemir[1,2,3,4,11], Kemal Tutkavul[5], Aysin Dervent[6], Semih Ayta[7], Cigdem Ozkara[8], Baris Salman[1,2], Emrah Yucesan[9], Yesim Kesim[1,2], Seda Susgun[1,2,9], Ugur Ozbek[1,3,4], Betul Baykan[10], Sibel A. Ugur Iseri[1]✉ and Nerses Bebek[1,10]

© The Author(s), under exclusive licence to The Japan Society of Human Genetics 2021

Lafora disease (LD) is a severe form of progressive myoclonus epilepsy inherited in an autosomal recessive fashion. It is associated with biallelic pathogenic variations in *EPM2A* or *NHLRC1*, which encode laforin and malin, respectively. The disease usually starts with adolescent onset seizures followed by progressive dementia, refractory status epilepticus and eventually death within 10 years of onset. LD is generally accepted as having a homogenous clinical course with no considerable differences between *EPM2A* or *NHLRC1* associated forms. Nevertheless, late-onset and slow progressing forms of the disease have also been reported. Herein, we have performed clinical and genetic analyses of 14 LD patients from 12 different families and identified 8 distinct biallelic variations in these patients. Five of these variations were novel and/or associated with the LD phenotype for the first time. Interestingly, almost half of the cases were homozygous for the rare rs769301934 (NM_198586.3(*NHLRC1*): c.436 G > A; p.(Asp146Asn)) allele in *NHLRC1*. A less severe phenotype with an onset at a later age may be the reason for the biased inflation of this variant, which is already present in the human gene pool and can hence arise in the homozygous form in populations with increased parental consanguinity.

Journal of Human Genetics (2021) 66:1145–1151; https://doi.org/10.1038/s10038-021-00944-8

INTRODUCTION

Lafora's disease (LD), also known as progressive myoclonic epilepsy type 2 (EPM2; MIM: 254780) is a rare neurodegenerative disorder with an adolescent onset. Symptoms usually start with stimuli sensitive myoclonic, tonic-clonic, absence, atonic and visual seizures. At this point, the disease may be misdiagnosed as juvenile myoclonic epilepsy (JME), which is a more common and benign type of epilepsy. However, in LD the seizures tend to get increasingly intractable with slowing of background activity in electroencephalogram (EEG) recordings, unlike the pattern in JME. Other neurological findings including progressive dementia, psychosis, cerebellar ataxia and dysarthria are added to the clinical picture of LD in the succeeding years. The patients may be lost within 10 years of onset due to status epilepticus or causes related to neurological deterioration [1].

LD is caused by biallelic pathogenic variations in either *EPM2A* or *NHLRC1* (*EPM2B*) genes, which respectively encode laforin; a glycogen phosphatase that dephosphorylates glycogen and malin; a ubiquitin E3 ligase. Laforin dephosphorylates glycogen, while the role of malin remains elusive, yet it has been shown as a strong interacting partner of laforin. Either laforin or malin deficient LD has the same pathology; poorly branched and highly phosphorylated insoluble glycogen deposits (polyglucosan) accumulated in neural and other tissues [1]. These round polyglucosan aggregates, namely Lafora bodies (LB) are the morphological hallmarks of LD and used for differential diagnosis of LD from an axillary skin biopsy [2]. Although LBs are accepted as diagnostic markers, distinguishing LB from regular polysaccharide contents of apocrine sweat glands requires expertize and may lead to false-positive interpretation [3]. LBs have also been reported to be absent from a significant proportion of cases with genetically confirmed LD [4]. Therefore, it is important to identify genetic defects in *EPM2A* or *NHLRC1* genes to confirm the diagnosis. Genetic analysis may also be important in identifying pre-onset carriers and the expected clinical course progression in these individuals associated with distinct variations. It may eventually be important for these pre-onset carriers in LD families for newly emerging treatment options in accordance with genetic counselling guidelines.

Herein, we have analyzed *EPM2A* and *NHLRC1* genes using Sanger sequencing and/or whole exome sequencing in a case series of 14 individuals in order to confirm their clinical diagnosis of LD on the molecular level and also to understand the extent of genetic variation associated with this disease. We have identified

[1]Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey. [2]Graduate School of Health Sciences, Istanbul University, Istanbul, Turkey. [3]Department of Medical Genetics, Faculty of Medicine, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey. [4]Genome Studies Program, Institute of Health Sciences, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey. [5]Department of Neurology, Istanbul Haydarpasa Numune Education and Research Hospital, University of Health Sciences, Istanbul, Turkey. [6]Department of Neurology, Pediatric Neurology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey. [7]Department of Pediatrics, Child Neurology Unit, Haseki Training and Research Hospital, Istanbul, Turkey. [8]Department of Neurology and Clinical Neurophysiology, Cerrahpasa Medical Faculty, Istanbul University Cerrahpasa, Istanbul, Turkey. [9]Department of Medical Biology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey. [10]Department of Neurology and Clinical Neurophysiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey. [11]These authors contributed equally: Garen Haryanyan, Ozkan Ozdemir. ✉email: sibel.ugur@istanbul.edu.tr

Received: 13 March 2021 Revised: 27 May 2021 Accepted: 28 May 2021
Published online: 11 June 2021

eight distinct variants, including the rare *NHLRC1* variant; rs769301934 (NM_198586.3: c.436 G > A; p.(Asp146Asn)) as the most prominent cause of LD, at least in this case series.

MATERIALS AND METHODS

Patients and clinical assessments

Fourteen patients from 12 distinct families with a possibility of LD diagnosis were recruited to this study in order to resolve the underlying genetic defect (Table 1, Supplementary Material). Physical and neurological examinations were performed for all available family members and detailed information on family history was collected. Magnetic resonance imaging of the brain and EEG recordings were performed on the affected siblings, whenever possible. DNA was extracted from peripheral blood samples of both affected individuals and their available family members using QIAamp DNA Blood Maxi Kit (Qiagen GmbH, Hilden, Germany). The study protocol was approved by the Istanbul University, Faculty of Medicine Ethics Committee. Informed consents were obtained from or for all patients and available unaffected family members.

Analyzing *NHLRC1* and *EPM2A* for sequence variants

The MANE Select transcripts of *NHLRC1* (NM_198586.3; ENST00000340650.6) and *EPM2A* (NM_005670.4; ENST00000367519.9) have been used for all variant annotations. The single coding exon of *NHLRC1* was PCR-amplified in three overlapping fragments, while the coding region of multi exonic *EPM2A* was amplified using primer pairs located in introns and/or untranslated regions (for primer sequences and conditions, see Supplementary Material). PCR products were Sanger sequenced using standard techniques. Sequence data were analyzed using CLC Main Workbench v6.5. (CLC Bio, Aarhus, Denmark). The same primers were used for PCR amplification and DNA sequencing. Nucleotide and codon numbering refer to the cDNA sequence starting at the translation initiation site, with +1 as the A of the ATG initiation codon.

Whole exome sequencing

Whole exome sequencing was performed for four affected individuals (Cases 6, 7, 10 and 11) through the service provided by Macrogen Inc. (Seoul, Korea). Exonic DNA was captured with the SureSelect Human All Exon V4 Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced with the HiSeq2000 instrument (Illumina, San Diego, CA, USA). Exome data analysis was performed by our in-house pipeline. Briefly, reads were aligned to the hg19 reference genome using Burrows–Wheeler aligner [5] and later bam manipulations were conducted using Picard tools (http://broadinstitute.github.io/picard/). Variants were called with GATK HaplotypeCaller and annotated using Ensembl Variant Effect Predictor API [6, 7]. The data were filtered for variants in *EPM2A* and *NHLRC1*. The sequencing reads covering *NHLRC1* and *EPM2A* were also investigated manually by Integrative Genomics Viewer to detect any low coverage regions and Sanger sequenced accordingly [8].

RESULTS

Combined analysis of Sanger and exome sequencing together with segregation analysis whenever possible has led us to identify 8 distinct biallelic variations of *NHLRC1* and *EPM2A* in 12 unrelated families (Tables 1 and 2 and Figs. 1 and 2). All variants were classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) criteria adapted herein for LD [9] and has been prepared for submission to the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/). We have specifically used the PP4 evidence of ACMG/AMP criteria for variants identified in individuals with LB, as LBs are accepted as useful diagnostic tools for LD.

We have identified four novel variations in genes *NHLRC1* (NM_198586.3:c.77 G > A and NM_198586.3:c.1144_1147delACAGinsC) and *EPM2A* (NM_005670.4:c.259 A > G, and NM_005670.4: c.292 T > C) in the homozygous state. The *NHLRC1* variant c.77 G > A changes the cysteine residue at position 26 into a tyrosine. This amino acid position has previously been shown to be altered in LD patients into a serine residue and a stop codon by c.76 T > A (rs28940575) and c.78 C > A (CM050720), respectively. Therefore, it may be a significant hotspot position. Two brothers (Sib Pair #2: cases 10 and 11) were found to be compound heterozygous for a missense variant (rs104893955; NM_005670.4(EPM2A):c.94 T > G) and an essential splice donor variant (rs1361221383; NM_005670.4 (EPM2A): c.301 + 1 G > T) in *EPM2A*, respectively. Although the splice region variant has been reported in the gnomAD database once in the heterozygous state, it is the first time that this variant is associated with LD. We have also identified three variants (rs769301934; NM_198586.3(*NHLRC1*): c.436 G > A, rs104893955; NM_005670.4(*EPM2A*): c.94 T > G and rs104893950; NM_005670.4 (*EPM2A*): c.721 C > T) that had already been reported as pathogenic or likely pathogenic in the ClinVar database. Interestingly, we have observed the particular *NHLRC1* variant, namely rs769301934 (NM_198586.3: c.436 G > A; p.(Asp146Asn)) in six unrelated individuals in the homozygous state comprising the genetic defect exceeding half of the case series.

The proposed amino acid alterations of all variations presented herein except for the splice site variation (rs1361221383; NM_005670.4(*EPM2A*): c.301 + 1 G > T) are mapped to NHLRC1 and EPM2A protein diagrams (Fig. 3).

The mean age at onset in LD patients with p.Asp146Asn is 18 years as in our case series (mean, 18 ± 3 years of age). Interestingly, past medical history of case 5 based on outpatient clinic records and depiction of her family members was suggestive of 4–5 generalized tonic-clonic seizures between 6 and 11 years of age. She was seizure-free till the age of 15 years, when she started to have generalized tonic-clonic seizures again. For the last 4 years, she has started having absence seizures and myoclonic jerks with increased severity and frequency. At her last visit at 21 years of age, she was dysarthric and had tandem gait difficulty. Her intelligence quotient was 60 on Alexander's performance scale. She reported having difficulty in daily activities mainly due to memory lapses. The parents note that the paternal uncle of case 5 had epilepsy who was seizure-free with valproate. As progressive neurological features related to LD have started at 15 years of age for case 5, we have accepted her age of onset for LD as 15 years. The few seizures before this age may be related to another epileptic phenomenon.

DISCUSSION

Herein, we report analysis of *EPM2A* and *NHLRC1* genes in 14 patients from 12 LD families from Turkey. Of the 8 distinct variants, 5 variants were novel and/or associated with the LD phenotype for the first time. The rs769301934 (NM_198586.3:c.436 G > A; p.(Asp146Asn)) variant in *NHLRC1* was observed in six cases constituting the most frequent cause of LD among these cases.

Laforin protein encoded by *EPM2A* consists of 331 amino acids (UniProt ID: O95278) with a carbohydrate-binding module (CBM20) at the N-terminus (residues 1–124) and a tyrosine-protein phosphatase domain at the C-terminus (residues 243–311). The CBM20 domain allows laforin to bind glycogen and complex carbohydrates [10]. All missense variations identified in this study lies within this CBM20 domain, which may affect the affinity of laforin to carbohydrates. Interestingly, the compound heterozygote sibs (sib pair #2) have a missense variant in CBM20 domain (NM_005670.4: c.94 T > G; p.Trp32Gly) and an essential splice donor variant (NM_005670.4:c.301 + 1 G > T). The splicing variant c.301 + 1 G > T lies at the very beginning of the first intron and it probably alters the wild type donor site resulting in a null allele. Therefore, the LD phenotype in these sibs can mainly be attributed to the pathogenic missense variant residing in the CBM20 domain and the lack of a functional allele compensating for this variant. It has previously been shown that the CBM20 domain mutant p.Trp32Gly disrupts the targeting of laforin to intracellular glycogen particles along with an abnormal cytoplasmic distribution [11]. The variant rs104893950 (NM_005670.4 (*EPM2A*): c.721 C > T; p.(Arg241*)) also interferes with the open

Table 1. Review of clinical features of individuals with *NHLRC1* or *EPM2A* variants

Case	Sex	Gene	Skin biopsy	Parental consanguinity	Age of onset	Seizure type	Clinical evaluation at last visit
1 (Sib pair #1)	M	*NHLRC1*	LB	Y	13	GTCS, Myo, SP,CP, SE	Bed-dependent, limited cooperation at the age of 30
2 (Sib pair #1)	M	*NHLRC1*	NP	Y	14	Myo, Visual Aura	Similar to Case 1
3	M	*NHLRC1*	LB	Y	18	GTCS, Myo, AbS, NCSE	Ataxia, difficulty to walk, disarthria, Moderate cognitive impairment
4	F	*NHLRC1*	NP	Y	22	GTCS	Difficulty at school, anomic aphasia, need to help at daily activities
5	F	*NHLRC1*	LB	N	15	Myo, GTCS, AbS	Disarthria, ataxia, IQ-60, need to help in daily activities
6	F	*NHLRC1*	NP	Y	15	Myo, AbS	Mild cognitive impairment and generally independent in daily activities at the age of 19
7	F	*NHLRC1*	NP	Y	18	GTCS, Myo, AbS	Mild cognitive impairment and generally independent in daily activities
8	F	*NHLRC1*	LB	N	20	Myo	Generally independent in daily activities except for dysarthria
9	M	*NHRLC1*	LB	Y	13	Occ; Myo; GTCS	21 y: Frequent seizures, status epileptics episodes, Unable to walk, talk or swallow. Frequent hospitalizations in ICU due to infections ad worsening of seizures
10 (Sib pair #2)	M	*EPM2A*	NP	N	11	Visual auras and GTCS	Absence seizures, myoclonia and tonic clonic seizures
11 (Sib pair #2)	M	*EPM2A*	NP	N	13	Myo, GTCS	Clinical diagnosis of Lafora disease at the age of 16. Series of myoclonic-tonic seizures when awake
12	F	*EPM2A*	LB	Y	14	GTCS	15 y: SP-dec and Myo; 20 y: SWwS due to AMyo at axial muscles and all extremities, TMyo and severe cognitive impairment
13	F	*EPM2A*	LB	N	12	Myo, AbS, Visual Aura	Wheelchair dependent since high school, excitus following status epilepticus
14	M	*EPM2A*	LB	Y	6	Myo, GTCS	Ataxia, dysarthria, incoordination, tremor, moderate cognitive impairment at the age of 15; exitus at the age of 18

AMyo Action myoclonus, *AbS* Absence Seizures, *CP* complex partial, *GTCS* Generalized Tonic Clonic Seizure, *ICU* Intensive care unit, *LB* Lafora body positive, *Myo* Myoclonic seizures, *NCSE* Non convulsive status epilepticus, *NP* Not performed, *Occ* Occipital seizures, *SE* Status epilepticus, *SP* simple partial, *SP-dec* School performance decline, *SWwS* Sitting and walking with support, *TMyo* Myoclonus provoked by tactile stimulus, *WWS* Walking with support

Table 2. List of LD associated variants identified in this study

Patient ID(s)	hg19 and hg38 Positions	cDNA	Protein (Domain)	Zyg	Exon	Variant ID (Frequency)	Neighboring Variant	Clin Var	ACMG Evidence	Class
NHLRC1 (NM_198586.3; ENST00000340650.6)										
Sib Pair #1 (Cases 1–2)	6-18122761-C-T 6-18122530-C-T	c.77 G > A	p.(Cys26Tyr) (Ring)	hom	1/1	Novel	rs28940575 (c.76 T > A; p.C26S) and CM050720 (c.78 C > A; p.Cys26*)	NA	PM1-Mod, PM2-Mod, PM5-Mod*, PP1-Sup*, PP3-Sup, PP4-Str*	LP
Cases 3–8	6-18122402-C-T 6-18122171-C-T	c.436 G > A	p.(Asp146Asn) (NHL1)	hom	1/1	rs769301934 (T: 6.37592e-05)	–	P(3)	PS4-Str*, PM1-Mod, PM2-Mod, PP4-Str*, PP5-Sup	P
Case 9	6-18121691_18121694 delinsG 6-18121460_18121463 delinsG	c.1144_1147 delinsC	p.(Thr382_Ala383 delinsPro) (NHL6)	hom	1/1	Novel	–	NA	PM1-Mod*, PM2-Mod*, PP4-Str*	LP
EPM2A (NM_005670.4; ENST00000367519.9)										
Sib Pair #2 (Cases 10-11)	6-146056541-A-C 6-145735405-A-C	c.94 T > G	p.(Trp32Gly) (CBM20)	het	1/4	rs104893955 (C: 7.96400e-06)	–	LP(1); P(1)	PM1-Mod, PM2-Mod, PP1-Sup*, PP3-Sup, PP5-Sup	LP
Case 12	6-146056376-T-C 6-145735240-T-C	c.259 A > G	p.(Lys87Glu) (CBM20)	hom	1/4	Novel	CM105523 (c.259 A > T; p.Lys87*)	NA	PM1-Mod, PM2-Mod, PP3-Sup, PP4-Str*	LP
Case 13	6-146056343-A-G 6-145735207-A-G	c.292 T > C	p.(Ser98Pro) (CBM20)	hom	1/4	Novel	–	NA	PM1-Mod, PM2-Mod, PP4-Str*, BP4-Sup	LP
Sib Pair #2 (Cases 10-11)	6-146056333-C-A 6-145735197-C-A	c.301 + 1 G > T	–	het	Int 1/3	rs1361221383 (A: 3.255e-05)	–	NA	PVS1-Mod*, PM2-Mod*, PM3-Sup*, PP1-Sup*, PP3-Sup*	P
Case 14	6-145948827-G-A 6-145627691-G-A	c.721 C > T	p.(Arg241*)	hom	4/4	rs104893950 (A: 7.66494e-05)	–	P(6)	PM2-Mod, PP3-Sup, PP4-Str*, PP5-Sup	LP

Variants are listed according to their cDNA positions in their relevant genes. For novel variants, the 'neighboring variant' column shows previously reported pathogenic LD variants that affect the same amino acid. For the frequency column, the highest allele frequency has been retrieved from the population genetics tab of Ensembl variant browser, which may represent frequency data from gnomAD exomes/genomes or TOPmed. Sib Pair #2 has been shown to be compound heterozygous for the two variants in *EPM2A*. All variants mentioned in this table are classified according to the ACMG/AMP guideline using Intervar (http://wintervar.wglab.org). Any additional ACMG/AMP evidence is marked with an asterisk

Ben benign, *Dlt* deleterious, *Int* intron, *LP* likely pathogenic, *Mod* Moderate, *NA* not available, *P* pathogenic, *PosD* possibly damaging, *PrD* probably damaging, *Str* Strong, *Sup* Supporting, *Tol* tolerated, *VUS* Variant of unknown significance, *Zyg* zygosity

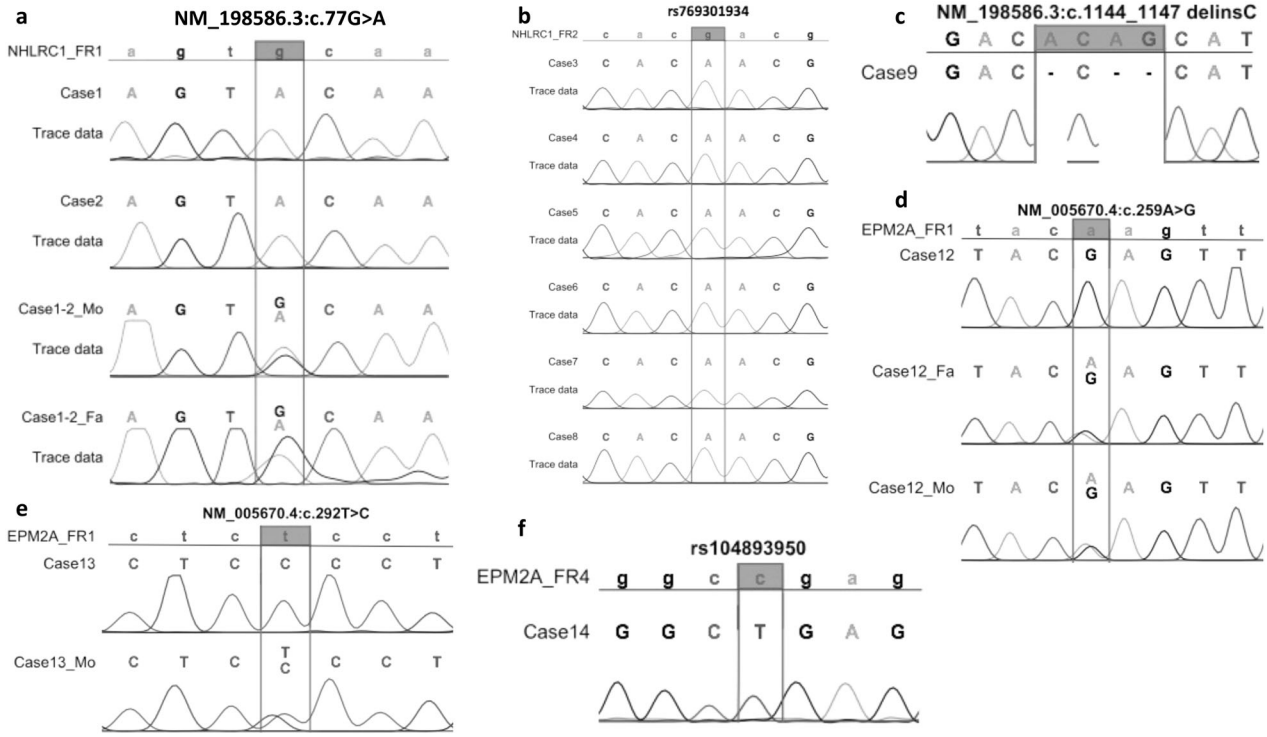

Fig. 1 Sanger sequencing results of homozygous variations detected in *NHLRC1* and *EPM2A* (**a**–**f**). Segregation analysis is also included whenever possible. Human Genome Variation Society (HGVS) nomenclature is only used for novel variations. Fa: Father, Mo: mother

reading frame of *EPM2A*. As the variant resides in the last exon, the encoded transcript is not eligible for the non-sense mediated RNA decay (NMD) mechanism [12]. Therefore, this variation in the homozygous state most likely results in a truncated form of laforin lacking the essential protein-phosphatase domain. This may explain the dramatic phenotypic severity of case 14 with young onset and his unfortunate loss at 18 years old. The other *EPM2A* related cases (10–13) are all associated with missense variations in the CBM20 domain, which may alter the affinity of laforin to carbohydrates.

NHLRC1 encoded malin is an E3-ubiquitin ligase consisting of 395 amino acids (UniProt ID: Q6VVB1). Malin has a RING type zinc finger domain at the N-terminus (residues 26–72) and six NHL repeats in the C-terminus (residues 113–157, 161–204, 205–245, 248–300, 301–349 and 350–393). RING domain interacts with specific E2-conjugating enzymes, while NHLs mediate other protein-protein interactions, including binding to laforin [13]. The two distinct variants in *NHLRC1* identified herein lies within the RING domain (NM_198586.3: c.77 G > A; p.Cys26Tyr) and NHL1 repeat (rs769301934; NM_198586.3: c.436 G > A; p.Asp146Asn), respectively.

The present study also underlies the contribution of the particular *NHLRC1* variant in the NHL1 repeat, namely rs769301934 (NM_198586.3: c.436 G > A; p.(Asp146Asn)) as a common cause of LD in this case series. rs769301934 is a rare variant observed in Non-Finnish European, Ashkenazi Jews and other populations as reported by the gnomAD database. It may be suggested that this rare variant already present in the human genetic pool may be a leading cause of LD in populations with high parental consanguinity rate, including Turkey. Accordingly, two other studies report identification of rs769301934 in a total of three patients from two families from Turkey [14, 15]. The increased prevalence of rs769301934 has prompted us to include the PS4 evidence for ACMG/AMP criteria based pathogenicity classification (Table 2). A less severe phenotype with an onset at a later age (an average of 18 years in our cases) reported for this variant may also be the reason for the biased inflation of this variant in screened cases [16, 17]. Nevertheless, we cannot rule out an ascertainment bias toward relatively older patients with relatively better progression as our case series had been collected mostly from adult neurology clinics.

The slower disease course attributed to *NHLRC1* is possibly due to the relatively high frequency of rs769301934 (NM_198586.3: c.436 G > A; p.(Asp146Asn)) in LD. This variant leads to a partial loss of malin function; the interaction between laforin and malin is disrupted although E3 ubiquitin ligase activity is conserved [18].

Several types of variations have been detected in *NHLRC1* and *EPM2A* genes including stop gain, frameshift, missense variants and large deletions (http://projects.tcag.ca/lafora/). As the condition is recessive, the variations are biallelic and especially homozygous in populations with an increased consanguinity rate [19]. Whenever compound heterozygous, it is much harder to elaborate on genotype-phenotype correlations. The potential involvement of modifier genes that may modulate the disease course, including the age of onset and degree of mental deterioration, has also complicated this picture [20]. Therefore, it is important to genetically diagnose and clinically correlate the phenotype in well-defined cases. This may eventually lead to personalized clinical management and treatment options in affected individuals and their pre-onset family members along with genetic counseling.

Five of the eight distinct variations reported herein are associated with the LD phenotype for the first time. Two of these variations reside within *NHLRC1* and the remaining three in *EPM2A* genes, respectively. Interestingly, all *NHLRC1* variations in this study have a missense or inframe insertion and deletion consequence on the protein level without altering the open reading frame. Obviously, there is a single isoform for the malin E3 ubiquitin ligase encoded by *NHLRC1* due to the lack of alternative transcripts for this intronless gene. The ring domain comprising amino acids 26–72 of malin is accepted to be responsible for its ubiquitin ligase function (UniProt ID: Q6VVB1).

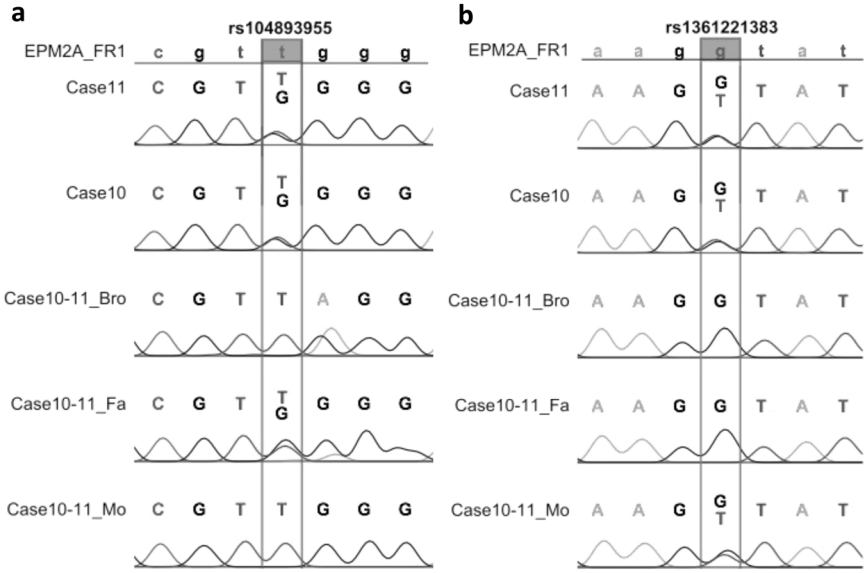

Fig. 2 Sanger sequencing results of two heterozygous variations in *EPM2A* detected in sib pair #2 (cases 10 and 11) and validation of these variants for compound heterozygosity in the parents (a, b). Bro: Brother, Fa: Father, Mo: mother

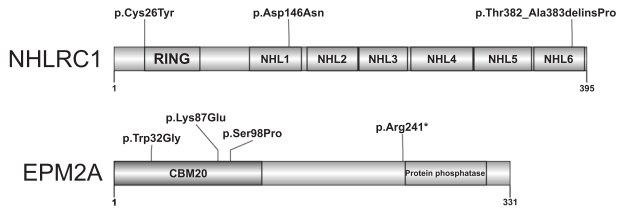

Fig. 3 The proposed amino acid alterations of all variations presented herein except for the splice site variation (rs1361221383) mapped to NHLRC1 and EPM2A protein diagrams

In order to obtain a better genotype to phenotype resolution of *NHLRC1*, The Lafora Progressive Myoclonus Epilepsy Mutation and Polymorphism Database (http://projects.tcag.ca/lafora/) has been investigated. There are 79 variations presented in this database that are classified as 'mutation associated with disease'. 49 of these variations are amino acid substitutions that do not alter the open reading frame. The remaining 30 nonsense or frameshift variations shoud be considered by keeping the intronless nature of *NHLRC1* in mind. Intronless genes are known to be NMD-insensitive [21], so nonsense or frameshift variations probably result in truncation of malin; instead of causing null alleles *via* NMD as in eukaryotic multiple-exon genes. Most of these truncating variations in the aforementioned database reside 3′ to the gene region encoding the essential ring domain and hence only cause loss of one or more of the repetitive NHL domains. Therefore, it can be speculated that most pathogenic *NHLRC1* variations irrespective of the variant consequence may interfere with the activity of malin in varying degrees. The alternating residual activity of malin may then be correlated with the severity of the phenotype. This can be reason why missense variations in cases 1 and 9 (c.77 G > A; p.(Cys26Tyr) and c.1144_1147delinsC; p.(Thr382_Ala383delinsPro), respectively) are more severe with younger age of onsets and limited motor activities, while another missense variation rs769301934 (NM_198586.3: c.436 G > A; p.(Asp146Asn)) is associated with a less severe phenotype with a later age of onset as in cases 3–8. Nevertheless, it must be noted that even with the same pathogenic variation in the homozygous state, there is variable expressivity.

The management for LD basically utilizes anti-epileptic drugs-especially valproate, which modulates the severity and frequency of seizures and myoclonus. Currently, strategies that focus on reducing brain glycogen synthesis are accepted as promising candidates for LD treatment. In 2016, the use of metformin that had long been used in the treatment of type 2 diabetes has been approved by the European Commission as an orphan drug to use in the treatment of LD (EU/3/16/1803). This drug activates AMP-protein kinase, which in turn can inhibit glycogen synthesis. Alternatively, glycogen synthesis may be suppressed by targeting glycogen synthase (GS) or protein phosphatase 1 (PP1) subunit R5 via genome editing, at RNA level using antisense oligonucleotides or RNA interference or at the protein level using small tailored molecules [22]. For all these potential approaches, a distinct diagnosis of LD clinically and genetically remains the initial step.

In conclusion, we have presented the spectrum of clinical and genetic variations in 13 individuals with LD. We have also adapted ACMG criteria for LD to classify the associated variants. LD is a severe form of autosomal recessive progressive myoclonus epilepsy. Although rare, the progressive, devastating and usually fatal clinical picture of LD manifesting in previously unaffected adolescents or adults makes it important to follow a correct and efficient diagnostic route and apply clinical management and therapeutic strategies based on genotype-phenotype correlations.

REFERENCES

1. Verhalen B, Arnold S, Minassian BA. Lafora Disease: a Review of Molecular Mechanisms and Pathology. Neuropediatrics. 2018;49:357–62.
2. Malek N, Stewart W, Greene J. The progressive myoclonic epilepsies. Pr Neurol. 2015;15:164–71.
3. Turnbull J, Tiberia E, Striano P, Genton P, Carpenter S, Ackerley CA, et al. Lafora disease. Epileptic Disord. 2016;18:38–62.
4. Lesca G, Boutry-Kryza N, De Toffol B, Milh M, Steschenko D, Lemesle-Martin M, et al. Novel mutations in EPM2A and NHLRC1 widen the spectrum of Lafora disease. Epilepsia. 2010;51:1691–8.
5. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754–60.
6. Van Der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinforma. 2013;43:11.10.1–33.
7. Mclaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, et al. The Ensembl Variant Effect Predictor. Genome Biol. 2016;17:122.
8. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nat Biotechnol. 2011;29:24–6.

9. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
10. Kuchtová A, Gentry MS, Janeček Š. The unique evolution of the carbohydrate-binding module CBM20 in laforin. FEBS Lett. 2018;592:586–98.
11. Wang J, Stuckey JA, Wishart MJ, Dixon JE. A unique carbohydrate binding domain targets the lafora disease phosphatase to glycogen. J Biol Chem. 2002;277:2377–80.
12. Coban-Akdemir Z, White JJ, Song X, Jhangiani SN, Fatih JM, Gambin T, et al. Identifying genes whose mutant transcripts cause dominant disease traits by potential gain-of-function alleles. Am J Hum Genet. 2018;103:171–7.
13. García-Gimeno MA, Knecht E, Sanz P, Lafora. Disease: a Ubiquitination-Related Pathology. Cells. 2018;7:87.
14. Baykan B, Striano P, Gianotti S, Bebek N, Gennaro E, Gurses C, et al. Late-onset and slow-progressing Lafora disease in four siblings with EPM2B mutation. Epilepsia. 2005;46:1695–7.
15. Salar S, Yeni N, Gündüz A, Güler A, Gökçay A, Velioğlu S, et al. Four novel and two recurrent NHLRC1 (EPM2B) and EPM2A gene mutations leading to Lafora disease in six Turkish families. Epilepsy Res. 2012;98:273–6.
16. Lanoiselée HM, Genton P, Lesca G, Brault F, De Toffol B. Are c.436G > A mutations less severe forms of Lafora disease? A case report. Epilepsy Behav Case Rep. 2014;2:19–21.
17. Franceschetti S, Gambardella A, Canafoglia L, Striano P, Lohi H, Gennaro E, et al. Clinical and genetic findings in 26 Italian patients with Lafora disease. Epilepsia. 2006;47:640–3.
18. Vilchez D, Ros S, Cifuentes D, Pujadas L, Vallès J, García-Fojeda B, et al. Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. Nat Neurosci. 2007;10:1407–13.
19. Singh S, Ganesh S. Lafora progressive myoclonus epilepsy: a meta-analysis of reported mutations in the first decade following the discovery of the EPM2A and NHLRC1 genes. Hum Mutat. 2009;30:715–23.
20. Singh S, Ganesh S. Phenotype variations in Lafora progressive myoclonus epilepsy: possible involvement of genetic modifiers? J Hum Genet. 2012;57:283–5.
21. Grzybowska EA. Human intronless genes: functional groups, associated diseases, evolution, and mRNA processing in absence of splicing. Biochem Biophys Res Commun. 2012;20:1–6.
22. Nitschke F, Ahonen SJ, Nitschke S, Mitra S, Minassian BA. Lafora disease - from pathogenesis to treatment strategies. Nat Rev Neurol. 2018;14:606–17.

ACKNOWLEDGEMENTS

The authors are grateful to the patients and their relatives for their participation in this study. This work was supported by the grants of Scientific Research Projects Coordination Unit of Istanbul University (grant reference numbers TDP-2017-25510 and 25323). EY & YK and OO & BS have been fellows of The Scientific and Technology Research Council of Turkey (TUBITAK) projects with number: 113S331 and 214S222, respectively. We also thank to Turkish Academy of Sciences for the 2019 Distinguished Young Scientist Award to SAUI.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s10038-021-00944-8.

Correspondence and requests for materials should be addressed to S.A.U.I.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.