Clinical and genetic characterization of Neuronal ceroid lipofuscinoses (NCLs) in 29 Iranian patients: Identification of 11 novel mutations

Samareh Panjeshahi (panjeshahisamareh@gmail.com)
Shahid Beheshti University of Medical Sciences School of Medicine https://orcid.org/0000-0002-4893-5644
Parvaneh Karimzadeh
Shahid Beheshti University of Medical Sciences School of Medicine
Abolfazl Movafagh
Shahid Beheshti University of Medical Sciences School of Medicine
Farzad Ahmadabadi
Shahid Beheshti University of Medical Sciences School of Medicine
Elham Rahimian
Shahid Beheshti University of Medical Sciences School of Medicine
Sahar Alijanpour
Shahid Beheshti University of Medical Sciences School of Medicine
Mohammad Miryounesi
Shahid Beheshti University of Medical Sciences School of Medicine https://orcid.org/0000-0003-4705-3794

Research Article

Keywords: Neuronal ceroid lipofuscinoses (NCLs), whole exome sequencing(WES), New variant, Rare disease

Posted Date: February 1st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2514013/v1

Version of Record: A version of this preprint was published at Human Genetics on April 19th, 2023. See the published version at https://doi.org/10.1007/s00439-023-02556-y.

Abstract

Neuronal ceroid lipofuscinoses (NCLs) are neurodegenerative lysosomal storage diseases witch considered among the most frequent cause of dementia in childhood worldwide This study aimed to identify the gene variants, molecular etiologies, and clinical features in 23 unrelated Iranian families with NCL. In total, 29 patients with Neuronal ceroid lipofuscinoses (NCLs), diagnosed based on clinical manifestations, MRI neuroimaging, and electroencephalography (EEG), were recruited for this study. through whole exome sequencing(WES), functional prediction, Sanger sequencing, and segregation analysis, we found that 12 patients (41.3%) with mutations in the CLN6 gene, 7 patients (24%) with the TPP1(CLN2) gene variants, and 4 patients (13.7%) with mutations in the MFSD8(CLN7) gene. Also, mutations in each of the CLN3 and CLN5 genes were detected in 2 cases and mutations of each PPT1(CLN1) and CLN8 gene were observed in only 1 separate patient. We identified 18 different mutations, 11 (61%) of which are novel, never have been reported before, and the others have been previously described. The gene variants identified in this study expand the number of published clinical cases and the variant frequency spectrum of the Neuronal ceroid lipofuscinoses (NCLs) genes; moreover, the identification of these variants supplies foundational clues for future NCL diagnosis and therapy.

Introduction

Neuronal ceroid lipofuscinoses (NCLs) are complex group disorders of inherited severe neurodegenerative lysosomal storage diseases that usually onset is most often in childhood, sometimes in adulthood (1–3). This disease has been identified as one of the most frequent childhood-onset neurodegenerative disorders. The prevalence of NCLs has been estimated at 1:1,000,000 to 1:14,000 worldwide however; this far varies between geographical regions and different races[4–6]. Fourteen NCL types (CLN1 to CLN14) have been described to date[7–10], although a new subtype of NCL has also recently been proposed as CLN15 (TBCK)[11]. almost all of them are inherited in an autosomal recessive manner except one adult-onset form caused by mutations in DNAJC5/CLN4 that have been reported in autosomal dominant inheritance [7, 12]. NCL has two major hallmarks: first accumulation of autofluorescent storage material (ceroid) in the lysosome in the neurons and other cells and second neurodegeneration that affects CNS neurons such as ganglionic neuronal cells of the retina [1, 13].

from clinical perspective, NCL is mainly presented by epileptic seizures, mental and motor deterioration, visual impairment leading to blindness, progressive ataxia, dementia, and reduced life expectancy [3, 14, 15]. Classically, NCL-affected individuals have been classified into four categories (infantile, late-infantile, juvenile, and adult) which have been mainly defined regarding the clinical onset of symptoms however, some patients cannot be easily included in a specific group because of the significant variation in the age of onset and disease progression. Therefore it is preferred to classify NCL based on the genetic etiology of known genes. now, with the advent of molecular diagnosis and identification of causative genes, a novel NCL classification has been suggested taking into consideration the gene and mutation first and then with clinical phenotype, biochemical aspect, ultrastructural features pathological features[2, 16–18]. Nowadays, 13 genes are related to NCLs disease, including PPT1, TPP1, CLN3, DNAJC5, CLN5, CLN6, MFSD8, CLN8, CSTD, GRN, ATP13A2, CTSF and KCTD7 are detected[4, 8, 19]. based on the UCL database, more than 430 NCL etiological mutations have been reported in these genes (https://www.ucl.ac.uk/ncl-disease). Hence, the increasing implementation of next-generation sequencing panels and exome sequencing as essential diagnostic tools will lead to more accurate and faster diagnoses in patients with NCL, including those that differ from the known phenotypes usually due to so-called milder mutations [4, 20]. otherwise, NCL can be grouped under two major groups, Batten disease, and Kufs disease. Batten disease refers to childhood NCLs, regardless of the age of onset, whereas the term Kufs disease is assigned to the two major phenotypes of adult-onset NCL [Kufs A and B][6].

We enrolled 29 patients from 23 separated families with Batten disease symptoms in this study. All probands were evaluated using NGS technology.

we found that 12 patients (41.3%) with mutations in the CLN6 gene, 7 patients (24%) with the TPP1(CLN2) gene variants, and 4 patients (13.7%) with mutations in the MFSD8(CLN7) gene. Also, mutations in each of the CLN3 and CLN5 genes were detected in 2 cases (6.8%) and mutations of each PPT1(CLN1) and CLN8 gene were observed in only 1 separate patient (3.4%). We identified 18 different mutations, 11 (61%) of which are novel, never have been reported before, and the others have been previously described.

Method And Material

2.1 Ethical compliance

The study was approved by the ethics committee of the Institutional Review Board (IRB), with the approval code of IR.SBMU.MSP.REC.1400. 250 and carried out following the tenets of the Declaration of Helsinki [21]. Written informed consent was obtained from the patient's parents for the publication of this report, data, and any accompanying images.

2.2 Patients

Patients with initial symptoms of seizures, myoclonus, cognitive and motor function decline, ataxia, behavioral problems, visual and speech impairment were investigated. Families found to be affected by environmental and nongenetic factors were excluded from the study. Clinical data provided by the referring Child neurologist were annotated in conformity with the Human Phenotype Ontology (HPO) nomenclature [22].

After choosing the candidate families, precise genetic counseling was accomplished with each family, any information about them, including the family history of diseases, was carefully recorded, and finally, a family pedigree was drawn. complementary medical and clinical examinations such as MRI neuroimaging, electroencephalography (EEG), and eye examination were performed for all probands and all data were recorded.

The present study included 23 families consisting of 29 affected individuals together with their healthy family members, suspected of NCL disease. Five families presented more than one affected patient one of them is a twin brother and the remaining 16 families presented with one affected case. All of them

were born into families with healthy parents and were normal at birth (Fig. 1).

2.3 Exome Capture, Sequencing, And Bioinformatics Analysis

Whole blood samples were taken from patients and their available family members. Genomic DNA was extracted using DNA extraction standard salting out protocols.

Whole-Exome Sequencing (WES) was carried out in all 23 probands using a custom-designed Nimblegen chip-capturing array. Nearly 60 Mb of the targeted region on consensus coding sequences enriched from fragmented genomic DNA of the proband by around 758,086 probes designed for the human genome (Agilent SureSelectXT2 V7 exome). Then sequencing was performed on the Illumina HiSeq4000 platform (Illumina, San Diego, CA, USA). Paired-end sequence reads were aligned to the human reference genome (UCSC hg19/ GRCh37) using Burrows-Wheeler Aligner (BWA). Variant calling was run using SAMTools and Genome Analysis Toolkit (GATK v 3.7)[23–25]. Moreover, ANNOVAR software annotated and filtered the variants.

2.4 Variant Confirmation And Co-segregation Analysis

To confirm the variant in the probands, their parents, and also available siblings, bi-directional Sanger sequencing was applied. Referenced and designed primer sequences of the NCL genes were extracted from the National Center for Biotechnology Information (NCBI) database. Primers (F and R primers for mutation PCR) designed by Primer3 (http://primer3.ut.ee/) (available upon request), any possible secondary structures of the designed primers were predicted using Gene Runner software version 3.01. Then, we conducted the polymerase chain reaction (PCR) under standard conditions. The samples were sequenced using ABI Prism3130 Genetic Analyzer (Applied Biosystems), and the obtained results were analyzed using Chromas 2.5 (chromas.software.informer.com) software.

2.5 In-silico Evaluation Of The Pathogenicity Of Candidate Mutations

To predict the pathogenicity of the variant, we used different bioinformatics software and tools. The raw reads were filtered as clean reads and then aligned to the GRCh37 (hg19) human reference sequence. At first, variants were preferentially selected for further analysis and validation with their minor allele frequency (MAF) < 0.01 in the ExAc browser (exac.broadinstitute.org),1000 genome databases (http://browser.1000genome.org), Iranome (http://www.iranome.ir) and gnomAD (https://gnomad.broadinstitute.org) database. Also, the variants had to be located in exons, and introns regions that affected RNA splicing or regulatory elements.

Then Single nucleotide polymorphism (SNP) and short indel candidates were identified, and

the data were extracted from the Ensemble.org, dbSNP (http://www.ncbi.hlm.nih.gov/SNP), Clinvar, Franklin, VarSome, OMIM database, HGMD (www.hgmd.cf.ac.uk) and recently published articles in PubMed.

to predict the pathogenicity of the variants The SIFT utilities were used to forecast the change in protein structure. Conservation of each amino acid change was calculated using PhyloP2 and MutationTaster (http://www.mutationtaster.org/) algorithms were used to predict the effects of variants on protein function.

In addition, BDGP(https://www.fruitfly.org/seq_tools/splice.html), MaxEntScan, and Human Splicing finder (http://www.umd.be/HSF3/) were used to predict possible effects of splice site mutations.

All novel variants were named according to the guidelines of the Human Genome Variation Society (http://www.hgvs.org/) and were described and classified based on the standard guidelines of ACMG/Association for Molecular Pathology, and are scored based on the evidence and strength of each criterion as described in the ACMG guidelines.

Table 1
Clinical features and medical examination data in patients with NCLs disease in this study.

NO.ID	SEX	Age of onset	Age of diagnosis	Consanguinity	First symptoms	Seizure type	Ataxia	Regression	Visual impairment	Speech defect	MRI	EEG
P1	M[1]	5y[3]	10	Y	Ataxia	Myoclonus seizure	Y	Y	Moderate	Y	Cerebellar atrophy	Ab[6]
P2	F[2]	2.5y	6	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Blindness	Y	Global atrophy	Ab
P3	F	3m[4]	14	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Severe	Y	Global atrophy	Ab
P4	F	7y	9	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Moderate	Y	Cerebellar atrophy	Ab
P5	F	6y	10	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Severe	Y	N/A[5]	N/A
P6	M	2y	12	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Moderate	Y	N/A	N/A
P7	M	3y	9	Y	Ataxia	Refractory seizure	Y	Y	Blindness	Speechless	Global atrophy	Ab
P8	M	3.5y	11	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Severe	Y	Global atrophy	Ab
P9	M	3y	7	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Severe	Y	Global atrophy	Ab
P10	F	1.3y	4	N	Myoclonus seizure	Myoclonus seizure	Y	Y	Severe	Speechless	Global atrophy	Ab
P11	M	4y	16	Y	Speech defects	Myoclonus seizure	Y	Y	Severe	Y	Global atrophy	Ab
P12	M	4.5y	9	N	Speech defects	Myoclonus seizure	Y	Y	Severe	Y	Cerebellar atrophy	Ab
P13	M	2y	4	Y	Ataxia	Myoclonus seizure	Y	Y	just started	Y	Cerebellar atrophy	Ab
P14	F	3.9y	4.5	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	just started	Y	Global atrophy	Ab
P15	F	8y	20	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Severe	Y	cerebellar signs	Ab
P16	F	8y	8	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	just started	Y	Normal	Ab

NO.ID	SEX	Age of onset	Age of diagnosis	Consanguinity	First symptoms	Seizure type	Ataxia	Regression	Visual impairment	Speech defect	MRI	EEG
P17	M	2y	7	Y	Speech defect	Myoclonus seizure	Y	Y	Moderate	Speechless	Cerebellar atrophy	Ab
P18	M	4.m	5	Y	Myoclonus seizure	Myoclonus seizure	Y	Y	Severe	Speechless	Cerebellar atrophy	Ab
P19	F	1y	5	Y	Ataxia	Myoclonus seizure	Y	Y	Moderate	Y	Cerebellar atrophy	Ab
P20	F	2y	5	Y	Speech defect	Myoclonus seizure	Y	Y	Mild	Y	Cerebellar signs	Ab
P21	F	5y	7	Y	Ataxia	Myoclonus seizure	Y	Y	Moderate	Y	Cerebellar atrophy	Ab
P22	M	5	12		Ataxia	Myoclonus seizure	Y	Y	Blindness	Speechless	Global atrophy	Ab
P23	F	7	13		Ataxia	Myoclonus seizure	Y	Y	Blindness	Speechless	Normal	Ab
P24	M	4	8		Seizure	Refractory seizure	Y	Y	Blindness	Y	Cerebellar atrophy	Ab
P25	M	4.5	8	Y	Seizure	Refractory seizure	Y	Y	Blindness	Y	Cerebellar atrophy	Ab
P26	M	7.5	8	Y	Speech defect	Myoclonus seizure	Y	Y	Mild	Y	Normal	Ab
P27	M	3	3.5	Y	Ataxia	Myoclonus seizure	Y	Y	just started	Y	Cerebellar atrophy	Ab

1 = male/ 2 = female/ 3 = year/ 4 = month/ 5 = not available/ 6 = abnormal

Table 1 Clinical features and medical examination data in patients with NCLs disease in this study.

2.6 Genotype-phenotype Associations

The novel candidate variant or previously reported mutation to cause NCL disease was scrutinized and adapted to the clinical symptoms of each patient. They were also determined by using several databases such as Human Gene Mutation Database, Leiden Open Variation Database, PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), and Online Mendelian Inheritance in Man (https://omim.org/).

Results

3.2 Clinical Data

This study includes 23 families with 29 patients suffering from NCL disease. we should emphasize that the clinical diagnosis of all the NCL cases is in line with the diagnostic criteria that have already been approved for this disease including seizure, myoclonic epilepsy, regression, ataxia, visual impairment, and behavioral problems. Clinical symptoms were presented for 29 cases and cataloged based on Human Phenotype Ontology (HPO) terms are summarized in Table 1[22, 26].

The patients were followed over months to years (six months to 4 years) in a neurology and genetic outpatient clinic in our teams. During this time five patients passed away of complications caused by the disease (P2, P3, P5, P18 and P22).

In this study, eighteen homozygous variants in 7 different genes, were detected in 23 families studied, the location of the mutations is depicted in Fig. 2. these variations included seven previously reported and eleven apparently novel variants. a summary of detected mutations is provided in Table 2.

3.2.1. Patients with Mutations in the CLN6 Gene

Mutation in the CLN6 (NM_017882.3) gene cause Ceroid lipofuscinosis, neuronal,6A (OMIM#601780) disorder with a variable age of onset from 1.5 to 5 years of life after normal early development [27] and Ceroid lipofuscinosis, neuronal, 6B (Kufs type OMIM# 606725) form of 'Kufs disease,' which refers in general to adult-onset neuronal ceroid lipofuscinosis without retinal involvement [28], with autosomal recessive manner.

Among the 12 patients from 10 families (F1, F3, F7, F8, F12, F13, F14, F16, F22, F23) with homozygous CLN6 mutations (Table 2), the median age of onset was 3.8 years, with a range from 4 months (infantile) to 7(juvenile) years. In 5 cases (P4, P5, P6, P18 and P29) the first symptom was myoclonus seizure, in 4 patients (P1, P19, P21 and P28) was ataxia and in the rest of them (P11, P12 and P17) speech defect was manifested. All of them showed myoclonus seizure, mental and developmental regression, visual impairment from moderate to severe, ataxia, and speech defect ; while P17, P18, P28 and P29 were speechless. cerebellar atrophy in P1, P4, P12, P17, P18, P19, P21, P28 and P29 and Cerebral atrophy in P11 in brain MRI were found.

In P1, P11 and P29 separately, NGS showed a missense mutation (c.476C > T), in exon 4 of the CLN6 gene. This mutation affects a highly conserved residue p.Pro159Leu. This pathogenic mutation has previously been reported in Turkey.

Family 3 has three affected children with very similar symptoms and natural history. P4, the proband, was homozygous for a missense mutation in exon 6 of the CLN6:c.662A > C. This Likely Pathogenic mutation; has been reported previously. Sanger sequencing confirmed this mutation in both P5 and P6 patients. F5 expired at age 10 due to respiratory failure.

NGS analysis individually in P12 and P17 samples revealed a novel VUS (PP3, PM2, PP2) missense mutation in exon7(c.758T > C, p.L253P), of the CLN6 gene. This mutation affects highly conserved residue forecasts this change will disrupt the function of the protein.

We identified another novel variant in the P18 patient. this missense Likely Pathogenic variant is located in exon7, of the CLN6 (c.710C > T, p.Thr237Ile) and may damage the protein structure and function according to the prediction tools

In P19 proband we found out a missense variant in exon 4, CLN6: c.407G > A. This Pathogenic mutation has been reported before and showed that it affects p.Arg136His.

also in index P21 we reported a Likely Pathogenic mutation in exon 3, CLN6: c.252C > G. this nonsense mutation affects protein features (might be) and introduces an NMD.

Finally, in patient P28, we detected one more novel splice site intronic mutation in intron3 of the CLN6(c.486 + 5G > A) gene. this variant was classified as a variant of uncertain significance (VUS), but it showed some pathogenic indicatives based on in silico prediction programs, including the Human Splicing Finder and MaxEntScan.

This VUS variant splice site change was predicted by in silico analysis to affect the downstream domain in the protein.

3.2.2. Patients with Mutations in the TPP1 Gene

Ceroid lipofuscinosis, neuronal, 2 (OMIM#204500) underlies the pathogenesis of late infantile NCL (LINCL) caused by a mutation in the TPP1 (NM_000391.4) gene (29). We identified homozygous disease-causing mutations in TPP1 gene in 5 studied families (F2, F5, F9, F10 and F21) (Table 2). In these 7 patients, the median age of onset was 2.5 (late-infantile) years. five cases (P2, P3, P8, P9, P13, P16and P27) showed the symptom of the disease with myoclonus seizure; and the rest of them manifested the disease by ataxia.

All patients had myoclonus seizure, mental and developmental regression, ataxia, severe speech defect, and visual impairment, patient P2 was blind. cerebellar atrophy in (P2, P3, P8, P9 and P14,) and Cerebral atrophy in (P13 and P27) patients were detected in their brain MRI.

In family (F2), indexes P2 and P3 correspond to two sisters that passed away respectively at 6.5 and 14 years old. The progression of the disease in P2 was much faster than in her sister. Unfortunately, both sisters died due to respiratory complications. WES was performed for P2 and show a novel missense mutation in exon 10 of the TPP1(c.1205A > G, p.E402G) gene. This Likely Pathogenic (PP3, PM2, PP2, PP5) mutation was never reported before. The presence of this variation in indexes P2 and P3 were validated by Sanger sequencing. Surprisingly this mutation was also found by WES in another family(F5).this family has two 11 and 7 years old brothers. In proband (P8) NGS analysis showed this likely pathogenic mutation. We checked this finding in his brother (P9) and appeared that he has the same variant.

WES in P13 showed a recurrent nonsense mutation exon6 of the TPP1(c.622C > T, p.R208X) gene. This variant turned the highly-conserved amino acid Arg208 into the termination codon, this can amino acid sequence, and protein features affected and also Induced NMD.

we found another novel missense variant in P16, this mutation (c.1568A > G, p.H523R) is detected in exon13 of the TPP1 gene and affecting the highly conserved His523 residue, and has not been reported previously. this variant is classified as VUS (PM2,PP3,PP2) was predicted by in silico analysis to have a detrimental effect on protein function.

The last mutation that we detected in TPP1 was in P27, which recently showed symptoms of the disease. this Pathogenic missense mutation (c.1551 + 1G > T) affected splice site has been reported twice before.

3.2.3. Patients with Mutations in the MFSD8 Gene

Mutation in MFSD8) NM_001371596.2(gene develops type 7 NCL disease; Ceroid lipofuscinosis, neuronal, 7(610951) which is inherited in an autosomal recessive manner.

Three families (F4, F17andF19) had homozygous mutations in the MFSD8 gene (Table 2). All four (P7, P22, P24 and P25) patients presented a typical form of CLN7 and their symptoms were started median age of 3.3 years old (late infantile) by ataxia (P7 and P22) and seizure (P24 and P25).

P7, P24 and P25 have refractory seizures and P22 had myoclonus seizures, he died at 13 due to disease complications. All of them had mental and developmental regression, ataxia, and blindness. speechless and cerebellar atrophy was observed in P7 and P22 whiles speech defect and cerebellum atrophy detected in P24 and P25.

A pathogenic novel mutation in exon 7, MFSD8(c.616C > T, p.Q206X) was detected with WES in two (P7 and P22) probands separately. It is the first time that this pathogenic (PVS1, PM2, PP5) mutation is reported. both patients have almost the same course of the disease. Unlike other patients with CLN7, they did not have delayed psychomotor development but suffered from a sleep disorder, dysphagia, and bladder and bowel incontinence. This nonsense mutation converts Gln206 into a stop codon which can affect protein features and introduce an NMD so this change entirely destroy the function and structure of MFSD8 protein.

Family 19, has a twin boy patient. WES was performed for P24 and manifested a novel missense variant (c.847T > G, p.F283V) in exon9. This VUS (PM2) mutation affects a highly conserved residue (Phe283) and predicting this conversion should interrupt the protein function. The presence of this mutation in F25 was accredited by Sanger sequencing.

3.2.4. Patients with Mutations in the CLN5 Gene

Homozygous mutation in the CLN5 (NM_006493.4) gene is observed in Ceroid lipofuscinosis, neuronal, 5 (OMIM#256731) patients. In both 2 cases from 2 families (F18 and F20) with CLN5 mutations (Table 2), the age of onset was around 7 years(juvenile).

Index P23 started the symptoms of the disease with ataxia and after that myoclonus seizure. The symptoms continued with continuous neurological and motor regression, speechlessness, and blindness. NGS showed that she has a novel missense mutation in exon 3 of NCL5(c.476G > A, p.C208Y). This VUS (PM2, PP3, PM1) variant changes a highly conserved residue Cys208.

proband P26 showed the disease with a speech defect. myoclonus seizure, ataxia, regression, and progressive visual loss were started. WES revealed another novel Likely Pathogenic mutation in exon1 of the CLN5(c.172A > T, p.K107X) gene. This Stop-gain mutation shifted Lys107 into the termination codon, which can shorten the amino acid sequence, and protein features affected and also induced NMD.

3.2.5. Patients with Mutations in the CLN3 Gene

Ceroid lipofuscinosis, neuronal, 3 (OMIM#204200) juvenile NCL (JNCL) is caused by a homozygous mutation in the CLN3 (NM_001042432.2) gene. In this study, we described a family(F11) with two sisters that presented the disease at 8 years old (Late-juvenile) by myoclonus seizure (Table 2). Both of them have regressing, ataxia, speech, and visual defects, but the elder sister P15 shows more severe and advanced symptoms of the disease.

WES was performed for P15 and show a novel truncating mutation deletion c.1274_1275delTC, in exon 10 of the CLN3. Although this frameshift mutation (p.L425Pfs*7) is classified as VUS(PM2, PVS1)according to the ACMG classification but causes truncated protein and NMD as predicted by prediction tools

3.2.6. Patients with Mutations in the PPT1 and CLN8 Genes.

Mutations in PPT1(NM_000310.4) are responsible to cause Ceroid lipofuscinosis, neuronal, 1(OMIM#256730) underlying infantile NCL (or INCL), also known as Santavuori-Haltia disease [30].

P10 is a 4-year-old girl who started myoclonus seizure at 1.3 years (Infantile) (Table 2). She has progressive postnatal microcephaly (Head circumference = 47cm) and regression. Now she has ataxia, spasticity, speechlessness, optic atrophy, severe MR, sleep disturbances, and hypotonia. Her brain MRI displays global atrophy. NGS analysis for her showed a Pathogenic homozygous frameshift mutation in Exon2(c.169dupA), in the PPT1 gene. This change (p.M57Nfs*45) can introduce a premature termination codon (PTC), 57 amino acids downstream in palmitoyl-protein thioesterase (ppt) protein.

Also, Ceroid lipofuscinosis, neuronal, 8(OMIM#600143) is caused by a homozygous mutation in the CLN8 (NM_018941.4) gene (Table 2). The symptom of illness in P20 started with speech defect, then she showed ataxia and refractory myoclonus seizure, visual impairment, and bladder-bowel incontinence. Brain MRI indicated cerebellar signs. WES analysis in her sample revealed a novel pathogenic (PP5,PM2,PM5,PP3,PM1) homozygous missense mutation in exon2 of the CLN6 (c.208C > T) gene. This mutation affects a highly conserved residue Arg70 and converts it to Cys.

Table 2
Variant information of disease-causing genes was detected in the study.

NO.ID	Gene and transcript	Variant	rs	ACMG	SIFT	CAAD SCORE	polyphen	gnomAD (Aggregated)	Iranome	Reference
P1	CLN6 NM_017882.3	Exon4 c.476C > T p.P159L	rs919850756	P	Damaging	26.6	1.303	N/A	N/A	Kousi, Maria, et a (200)[31]
P2	TPP1 NM_000391.4	Exon10 c.1205A > G p.E402G	rs1471156821	LP	Damaging	28.5	0.789	0.0008%	N/A	This study
P3	TPP1 NM_000391.4	Exon10 c.1205A > G p.E402G	rs1471156821	LP	Damaging	28.5	0.789	0.0008%	N/A	This study
P4	CLN6 NM_017882.3	Exon6 c.662A > C p.Y221S	rs764571295	LP	Damaging	25.6	1.187	0.0025%	N/A	Sharp, Jul D., et al(2003) [27]
P5	CLN6 NM_017882.3	Exon6 c.662A > C p.Y221S	rs764571295	LP	Damaging	25.6	1.187	0.0025%	N/A	Sharp, Jul D., et al(2003)
P6	CLN6 NM_017882.3	Exon6 c.662A > C p.Y221S	rs764571295	LP	Damaging	25.6	1.187	0.0025%	N/A	Sharp, Jul D., et al(2003)
P7	MFSD8 NM_001371596.2	Exon7 c.616C > T p.Q206X	rs1209722075	P	Damaging	35	1.112	0.0004%	0.0625%	This study
P8	TPP1 NM_000391.4	Exon10 c.1205A > G p.E402G	rs1471156821	LP	Damaging	28.5	0.789	0.0008%	N/A	This study
P9	TPP1 NM_000391.4	Exon10 c.1205A > G p.E402G	rs1471156821	LP	Damaging	28.5	0.789	0.0008%	N/A	This study
P10	PPT1 NM_000310.4	Exon2 c.169dupA p.M57Nfs*45	rs386833634	P	N/A	N/A	5.81	0.0032%	N/A	Santorelli, Filippo M., et al.(1998 [32]
P11	CLN6 NM_017882.3	Exon4 c.476C > T p.P159L	rs919850756	P	Damaging	26.6	1.303	N/A	N/A	Kousi, Maria, et a (2009)
P12	CLN6 NM_017882.3	Exon7 c.758T > C p.L253P	-	VUS	Damaging	29.3	0.52	N/A	N/A	This study
P13	TPP1 NM_000391.4	Exon6 c.622C > T p.R208X	rs119455955	P	damaging	36	4.685	0.0251%	N/A	Sleat, David E., e al.(1997) [33]

NO.ID	Gene and transcript	Variant	rs	ACMG	SIFT	CAAD SCORE	polyphen	gnomAD (Aggregated)	Iranome	Reference
P14	TPP1 NM_000391.4	Exon13 c.1568A > G p.H523R	-	VUS	damaging	23	1.323	N/A	N/A	This study
P15	CLN3 NM_001042432.2	Exon16 c.1274_1275delTC p.L425Pfs*7	-	VUS	N/A	N/A	0.162	N/A	N/A	This study
P16	CLN3 NM_001042432.2	Exon16 c.1274_1275delTC p.L425Pfs*7	-	VUS	N/A	N/A	0.162	N/A	N/A	This study
P17	CLN6 NM_017882.3	Exon7 c.758T > C p.L253P	-	VUS	Damaging	29.3	0.52	N/A	N/A	This study
P18	CLN6 NM_017882.3	Exon7 c.710C > T p.T237I	rs779750025	LP	Damaging	27.9	0.503	0.0004%	N/A	This study
P19	CLN6 NM_017882.3	Exon4 c.407G > A p.R136H	rs769701646	P	Damaging	30	0.495	0.0039%	N/A	Di Fruscio, Giuseppin et al. (2015)[34]
P20	CLN8 NM_018941.4	Exon2 c.208C > T p.R70C	rs765097897	P	Damaging	26.2	2.578	0.002%	N/A	This study
P21	CLN6 NM_017882.3	Exon3 c.252C > G p.Y84X	-	LP	N/A	37	3.077	N/A	N/A	Faruq, M., et al.(2014 [35]
P22	MFSD8 NM_001371596.2	Exon7 c.616C > T p.Q206X	rs1209722075	P	Damaging	35	1.112	0.0004%	0.0625%	This study
P23	CLN5 NM_006493.4	Exon3 c.476G > A p.C208Y	-	VUS	N/A	29.5	0.597	N/A	N/A	This study
P24	MFSD8 NM_001371596.2	Exon9 c.847T > G p.F283V	-	VUS	Damaging	24.9	2.747	N/A	N/A	This study
P25	MFSD8 NM_001371596.2	Exon9 c.847T > G p.F283V	-	VUS	Damaging	24.9	2.747	N/A	N/A	This study
P26	CLN5 NM_006493.4	Exon1 c.172A > T p.K107X	rs1280128886	LP	N/A	45	1.632	0.0006%	N/A	This study
P27	TPP1 NM_000310.4	Intron11 c.1551 + 1G > T	-	P	N/A	33	N/A	N/A	N/A	Yu, Feng, e al.(2015) [36]

NO.ID	Gene and transcript	Variant	rs	ACMG	SIFT	CAAD SCORE	polyphen	gnomAD (Aggregated)	Iranome	Reference
P28	CLN6 NM_017882.3	Intron3 c.486 + 5G > A	rs754600522	VUS	Benign	25.3	N/A	0.0008%	N/A	This study
P29	CLN6 NM_017882.3	Exon4 c.476C > T p.P159L	rs919850756	P	Damaging	26.6	1.303	N/A	N/A	Kousi, Maria, et a (2009)

Abbreviations: P: pathogenic, LP, Likely pathogenic, VUS: uncertain significance, N/A: not available.

Discussion

Neuronal ceroid lipofuscinoses (NCLs) are considered among the most frequent cause of dementia in childhood worldwide (4). They are a group of genetically and clinically heterogeneous disorders that are determined by the accumulation of abnormal storage material NCL-specific lipopigments in neurons and the other cells in the body. abnormal storage of ceroid, with similar biochemical properties to lipofuscin, the “aging pigment” led to neuronal death in all gray regions of the brain. damage to the white matter, rate of progress and extent of involvement varies and depends on the type and severity of disease progression [6,13,37,38]. The molecular mechanisms of link the formation of endo-lysosomal storage and death of the neuronal cells have not so far been clearly manifest. the primary defect of lysosomal proteolytic activity is present in only three NCL forms, including CLN1/PPT1(palmitoyl-protein thioesterase 1), CLN2/TPP1(tripeptidyl peptidase I) and CTSD/ CLN10(cathepsin D (CTSD)). Also, we knew that DNAJC5(CLN4) gene encodes a Hsc70 co-chaperone involved in exocytosis and endocytosis. whereas in the remaining NCLs, mostly encode membrane proteins with unknown functions [39-41].

NCLs have a degenerative disease course and are characterized by myoclonic epilepsy, progressive movement and developmental regression, ataxia, different forms of visual impairment such as optic atrophy, retinal degeneration, retinopathy evolving into blindness, and variable cerebellar and cerebellum atrophy. These features lead to the disease being recognized as a disorder with a poor prognosis, significantly reducing sufferers' life expectancy. The age at disease onset ranges from birth to adulthood and the severity and type of symptoms are different depending on the subtype of NCLs [2,42]. During the last decade, advances in understanding the underlying pathophysiology of NCLs, through the identification of new genes, mutations and learning about the pathways involved in the formation and development of the disease, have led to the synthesis of several helpful drugs. In April 2017, the FDA approved serliponase alfa recombinant human tripeptidyl peptidase (TPP)1 for the treatment of NCL2, an innovative treatment available for this rare disease [43-45].

the estimation of the prevalence of this disease varies from 2 to 4 in 1.000.000 in Western countries. In IRAN, a large country with an estimated population of over 85 million, we still do not have an exact estimate of the NCLs disorders prevalence. however, the high consanguinity marriage frequency in Iran might imply an expected high risk for inherited disorders, especially autosomal recessive traits (46,47).

The data presented here is the first study from Iran demonstrating the genetic and clinical data of NCL disease in a cohort. This study reveals 29 diagnostic cases (18 unrelated and 11 siblings) of NCLs. Diagnosis of NCLs was based primarily on clinical findings. The clinical manifestations of our cases such as seizure, myoclonus, progressive decline of cognitive and motor capacities, ataxia, progressive visual loss, speech defect, and also as well as then complementary medical and clinical examinations such as MRI neuroimaging (showing cerebral atrophy and cerebellar atrophy), electroencephalography (EEG) and eye examination were in concordance with the previously affirmative phenotypes in NCL patients. As expected, cerebral and cerebellum atrophy and cerebellar signs were observed in our probands, the results of the EEG also suggested brain damages in the proband and the eye examinations showed different forms of visual impairment (from mild to severe and blindness).

All of our patients proved to carry a homozygous variant in the NCL’s gene. consanguinity was noted in 21 out of the 23 pedigrees (91.3%)(Fig.1). this high rate represents high homogeneity because of the high consanguineous marriage rate among the Iranian population. As we detected an identical novel variant in the PPT1(c.1205A>G) gene in two completely separate families (F2 and F5), each with two affected children. as well as we presented a stop gain mutation for the first time in the MFSD8 (c.616C>T) in two different families (F4 and F17). Also, another not reported variant in the CLN6(c.758T>C) was found in this study in two unrelated families (F8 and F12).

The ages ranged of cases from 3.5 to 20 years, and positive family history is detected in 6 families (F5, F9, F10, F15, F17, and F20).

We found 18 distinct mutations, including 11 (61.1%) novel mutations in seven different genes CLN6, TPP1 (CLN1), MFSD8 (CLN7), PPT1 (CLN2), CLN3, CLN8, and CLN5 (Fig. 3). hence our study notably expands the published dataset about this disease.

Among our reported mutations, 10(55.5%) were missense, 4(22.2%) nonsense (stop-gain), 2 (11.1%) splice-site, 1(5.5%) small deletion and 1(5.5%) small duplication (fig.3). According to the standards developed by the American College of Medical Genetics and Genomics (ACMG) for the classification of these variants, seven variants were classified as pathogenic (P), six as a variant of uncertain significance (VUS), and five as likely pathogenic (LP). Novel variants comprised one P, seven LP, and three VUS.

These powerful gene-based strategies of diagnosis with WES provide a reliable tool, which helps clinicians to reduce the diagnostic problem commonly observed that exists due to the phenotypic variability and heterogeneity in most genetic NCL disease types.

Infantile and childhood forms of NCL are the most common, CLN3 disease (juvenile NCL) afterward CLN2 disease (classical late infantile), is the most common form of this disease in western countries. CLN2 also is the most frequent in Southern Europe and the Mediterranean region. the uncommon form of childhood NCL are CLN10, CLN12, and CLN14.

But in this study, the most mutations were in the CLN6 (41.3%) gene related to the CLN6 disease and after in the TPP1(24.1%) gene related to the CLN2 disease. This nonconcurrence with previous estimates worldwide may be attributed to different factors: (a) differences in the genetic pool of Iranians due to racial diversity, (b) the absence of sufficient information on other sub-types of this disease or missing the patients due to faults of differential diagnosis and (c) small sample size is a potential limitation which may have introduced bias. Furthermore, some incompatibility in symptoms and phenotypic of our cases can be justified with this point that the classical form of each type of NCLs are generally correlated with the most common mutations in any gene, whiles unusual phenotypes and symptoms may arise from specific mutations and milder and later phenotypes may be due to so-called milder mutations or can be associated with related to the race of the patient and genetic differences caused by it. For example mutation c.662C>T in the CLN6 gene was reported to associate with a more protracted progression of disease (48) but we found a family (F3) with 3 affected children with this mutation, and the progress of the disease in these patients was similar to other patients carrying other mutations in the CLN6 gene, we can explain this discrepancy with differences in the genetic pool.

Further detailed research with more patients and long-term follow-up of patients will lead to progress in our awareness of these diseases, which is necessary to further understand the pathogenic mechanisms, a better understanding of genotype-phenotype correlation to improve the efficacy of genetic counseling, planning reasonable medical services and promote create new treatments.

Despite the limitations, we successfully identified 17 disease-causing variants, including 10 novel mutations using WES in 21 independent families. Our findings expand the spectrum of known mutations and their related clinical phenotypes in Iranian NCL patients.

Conclusion

We report the largest cohort of NCL disease patients in Iran so far. we identified 11 novel mutations and 7 other previously reported mutations (other ethnicities) in 23 Iranian families. It is hoped that the findings of this study will raise awareness for NCL disease and reduce the time from the start of first symptoms to diagnosis, helping families to choose prognostic counseling, prenatal diagnosis, and the best clinical therapeutic approach. moreover, these data may further elucidate mechanisms underlying NCLs to help create more effective treatments.

Declarations

- Compliance with Ethical Standards:

This study had no funding.

- Ethical approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee of the Institutional Review Board (IRB), with the approval code of IR.SBMU.MSP.REC.1400. 250 and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

- Informed consent:

Informed consent was obtained from all individual participants included in the study.

References

1. Zeman W, Dyken P. Neuronal ceroid-lipofuscinosis (Batten's disease): relationship to amaurotic family idiocy?. Pediatrics. 1969 Oct;44(4):570-83.
2. Mole SE, Williams RE, Goebel HH. Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. Neurogenetics. 2005 Sep;6(3):107-26.
3. Mole S, Williams R, Goebel H, editors. The neuronal ceroid lipofuscinoses (Batten disease). Oxford University Press; 2011 Mar 10.
4. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. Neurology. 2012 Jul 10;79(2):183-91.
5. Sleat DE, Gedvilaite E, Zhang Y, Lobel P, Xing J. Analysis of large-scale whole exome sequencing data to determine the prevalence of genetically-distinct forms of neuronal ceroid lipofuscinosis. Gene. 2016 Nov 30;593(2):284-91.
6. Simonati A, Williams RE. Neuronal Ceroid Lipofuscinosis: the multifaceted approach to the clinical issues, an overview. Frontiers in neurology. 2022 Mar 11:87.
7. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2015 Oct 1;1852(10):2237-41.
8. Mink JW, Augustine EF, Adams HR, Marshall FJ, Kwon JM. Classification and natural history of the neuronal ceroid lipofuscinoses. Journal of child neurology. 2013 Sep;28(9):1101-5.

9. Gardner E, Mole SE. The genetic basis of phenotypic heterogeneity in the neuronal ceroid lipofuscinoses. Frontiers in Neurology. 2021 Oct 18;12:754045.
10. Warrier V, Vieira M, Mole SE. Genetic basis and phenotypic correlations of the neuronal ceroid lipofusinoses. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2013 Nov 1;1832(11):1827-30.
11. Beck-Wödl S, Harzer K, Sturm M, Buchert R, Rieß O, Mennel HD, Latta E, Pagenstecher A, Keber U. Homozygous TBC1 domain-containing kinase (TBCK) mutation causes a novel lysosomal storage disease–a new type of neuronal ceroid lipofuscinosis (CLN15)?. Acta neuropathologica communications. 2018 Dec;6(1):1-5.
12. Nosková L, Stránecký V, Hartmannová H, Přistoupilová A, Barešová V, Ivánek R, Hůlková H, Jahnová H, van der Zee J, Staropoli JF, Sims KB. Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. The American Journal of Human Genetics. 2011 Aug 12;89(2):241-52.
13. Seehafer SS, Pearce DA. You say lipofuscin, we say ceroid: defining autofluorescent storage material. Neurobiology of aging. 2006 Apr 1;27(4):576-88.
14. Haltia M. The neuronal ceroid-lipofuscinoses. Journal of Neuropathology and Experimental Neurology. 2003 Jan 1;62(1):1-3.
15. Haltia M, Goebel HH. The neuronal ceroid-lipofuscinoses: a historical introduction. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2013 Nov 1;1832(11):1795-800.
16. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, Sabers A, Thomas SV, Vajda F, EURAP Study Group. Dose-dependent teratogenicity of valproate in mono-and polytherapy: an observational study. Neurology. 2015 Sep 8;85(10):866-72.
17. Kousi M, Lehesjoki AE, Mole SE. Update of the mutation spectrum and clinical correlations of over 360 mutations in eight genes that underlie the neuronal ceroid lipofuscinoses. Human mutation. 2012 Jan;33(1):42-63.
18. Kaminiów K, Kozak S, Paprocka J. Recent Insight into the Genetic Basis, Clinical Features, and Diagnostic Methods for Neuronal Ceroid Lipofuscinosis. International Journal of Molecular Sciences. 2022 Jan;23(10):5729.
19. Brudvig JJ, Weimer JM. On the cusp of cures: breakthroughs in Batten disease research. Current Opinion in Neurobiology. 2022 Feb 1;72:48-54. Mole SE, Williams RE, Goebel HH. Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. Neurogenetics. 2005;6(3):107–26.
20. Srivastava S, Cohen JS, Vernon H, Barañano K, McClellan R, Jamal L, Naidu S, Fatemi A. Clinical whole exome sequencing in child neurology practice. Annals of neurology. 2014 Oct;76(4):473-83.
21. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization. 2001;79(4):373.
22. Köhler S, Gargano M, Matentzoglu N, Carmody LC, Lewis-Smith D, Vasilevsky NA, Danis D, Balagura G, Baynam G, Brower AM, Callahan TJ. The human phenotype ontology in 2021. Nucleic acids research. 2021 Jan 8;49(D1):D1207-17.
23. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26(5):589-95.
24. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16):2078-9.
25. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297-303.
26. Trujillano D, Oprea GE, Schmitz Y, Bertoli-Avella AM, Abou Jamra R, Rolfs A. A comprehensive global genotype–phenotype database for rare diseases. Molecular genetics and genomic medicine. 2017 Jan;5(1):66-75.
27. Sharp JD, Wheeler RB, Parker KA, Gardiner RM, Williams RE, Mole SE. Spectrum of CLN6 mutations in variant late infantile neuronal ceroid lipofuscinosis. Human mutation. 2003 Jul;22(1):35-42.
28. Arsov T, Smith KR, Damiano J, Franceschetti S, Canafoglia L, Bromhead CJ, Andermann E, Vears DF, Cossette P, Rajagopalan S, McDougall A. Kufs disease, the major adult form of neuronal ceroid lipofuscinosis, caused by mutations in CLN6. The American Journal of Human Genetics. 2011 May 13;88(5):566-73.
29. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, Dyke JP, Cahan H, Slasor P, Jacoby D, Kohlschütter A. Study of intraventricular cerliponase alfa for CLN2 disease. New England Journal of Medicine. 2018 May 17;378(20):1898-907.
30. Hawkins-Salsbury JA, Cooper JD, Sands MS. Pathogenesis and therapies for infantile neuronal ceroid lipofuscinosis (infantile CLN1 disease). Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2013 Nov 1;1832(11):1906-9.
31. Kousi M, Siintola E, Dvorakova L, Vlaskova H, Turnbull J, Topcu M, Yuksel D, Gokben S, Minassian BA, Elleder M, Mole SE. Mutations in CLN7/MFSD8 are a common cause of variant late-infantile neuronal ceroid lipofuscinosis. Brain. 2009 Mar 1;132(3):810-9.
32. Santorelli FM, Bertini E, Petruzzella V, Di Capua M, Calvieri S, Gasparini P, Zeviani M. A novel insertion mutation (A169i) in the CLN1 gene is associated with infantile neuronal ceroid lipofuscinosis in an Italian patient. Biochemical and biophysical research communications. 1998 Apr 17;245(2):519-22.
33. Sleat DE, Donnelly RJ, Lackland H, Liu CG, Sohar I, Pullarkat RK, Lobel P. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. Science. 1997 Sep 19;277(5333):1802-5.
34. Di Fruscio G, Schulz A, De Cegli R, Savarese M, Mutarelli M, Parenti G, Banfi S, Braulke T, Nigro V, Ballabio A. Lysoplex: An efficient toolkit to detect DNA sequence variations in the autophagy-lysosomal pathway. Autophagy. 2015 Jun 3;11(6):928-38.
35. Faruq M, Narang A, Kumari R, Pandey R, Garg A, Behari M, Dash D, Srivastava AK, Mukerji M. Novel mutations in typical and atypical genetic loci through exome sequencing in autosomal recessive cerebellar ataxia families. Clinical Genetics. 2014 Oct;86(4):335-41.
36. Yu F, Liu XM, Chen YH, Zhang SQ, Wang K. A novel CLN2/TPP1 mutation in a patient with late infantile neuronal ceroid lipofuscinosis. Neurological ScienceS. 2015 Oct;36(10):1917-9.

37. Santavuori P. Neuronal ceroid-lipofuscinoses in childhood. Brain and Development. 1988 Jan 1;10(2):80-3.
38. Roine T, Roine U, Tokola A, Balk MH, Mannerkoski M, Åberg L, Lönnqvist T, Autti T. Topological alterations of the structural brain connectivity network in children with juvenile neuronal ceroid lipofuscinosis. American Journal of Neuroradiology. 2019 Dec 1;40(12):2146-53.
39. Mole SE. The genetic spectrum of human neuronal ceroid-lipofuscinoses. Brain pathology. 2004 Jan;14(1):70-6.
40. Kollmann K, Uusi-Rauva K, Scifo E, Tyynelä J, Jalanko A, Braulke T. Cell biology and function of neuronal ceroid lipofuscinosis-related proteins. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2013 Nov 1;1832(11):1866-81.
41. Nelson CA. A neurobiological perspective on early human deprivation. Child development perspectives. 2007 Jul;1(1):13-8.
42. Nita DA, Mole SE, Minassian BA. Neuronal ceroid lipofuscinoses. Epileptic Disorders. 2016 Sep;18(s2):S73-88.
43. Lewis G, Morrill AM, Conway-Allen SL, Kim B. Review of cerliponase alfa: recombinant human enzyme replacement therapy for late-infantile neuronal ceroid lipofuscinosis type 2. Journal of Child Neurology. 2020 Apr;35(5):348-53.
44. Geraets RD, Hastings ML, Kielian T, Pearce DA, Weimer JM. Moving towards effective therapeutic strategies for Neuronal Ceroid Lipofuscinosis. Orphanet journal of rare diseases. 2016 Dec;11(1):1-3.
45. Markham A. Cerliponase alfa: first global approval. Drugs. 2017 Jul;77(11):1247-9.
46. Cotman SL, Karaa A, Staropoli JF, Sims KB. Neuronal ceroid lipofuscinosis: impact of recent genetic advances and expansion of the clinicopathologic spectrum. Current neurology and neuroscience reports. 2013 Aug;13(8):1-1.
47. Mukherjee AB, Appu AP, Sadhukhan T, Casey S, Mondal A, Zhang Z, Bagh MB. Emerging new roles of the lysosome and neuronal ceroid lipofuscinoses. Molecular neurodegeneration. 2019 Dec;14(1):1-23.
48. Sharp, Julie D., et al. "Spectrum of CLN6 mutations in variant late infantile neuronal ceroid lipofuscinosis." Human mutation 22.1 (2003): 35-42.

Figures

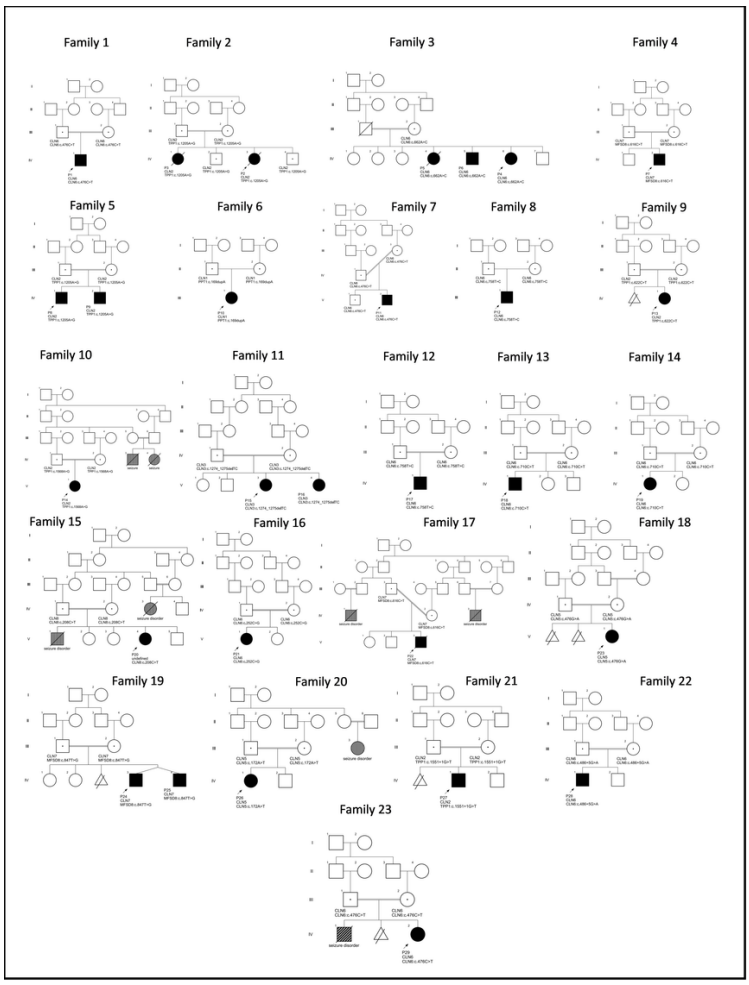

Figure 1

Pedigrees of 21 families with NCLs disease. Square and round symbols represent males and females, respectively. The slash indicates deceased individuals dot circle and square represented carrier.

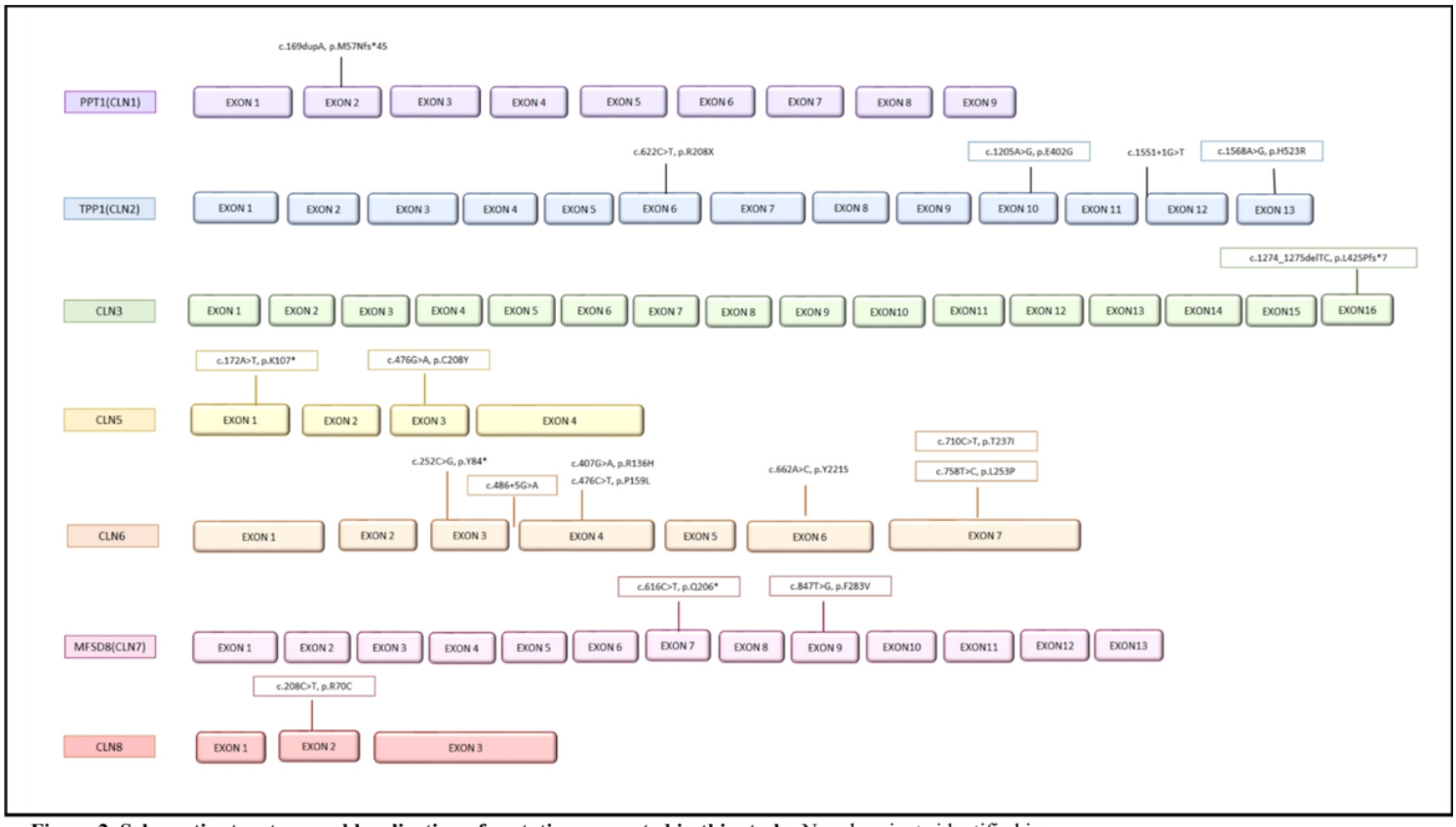

Figure.2 Schematic structure and localization of mutations reported in this study. Novel variants identified in this study are shown in the boxes.

Figure 2

See image above for figure legend

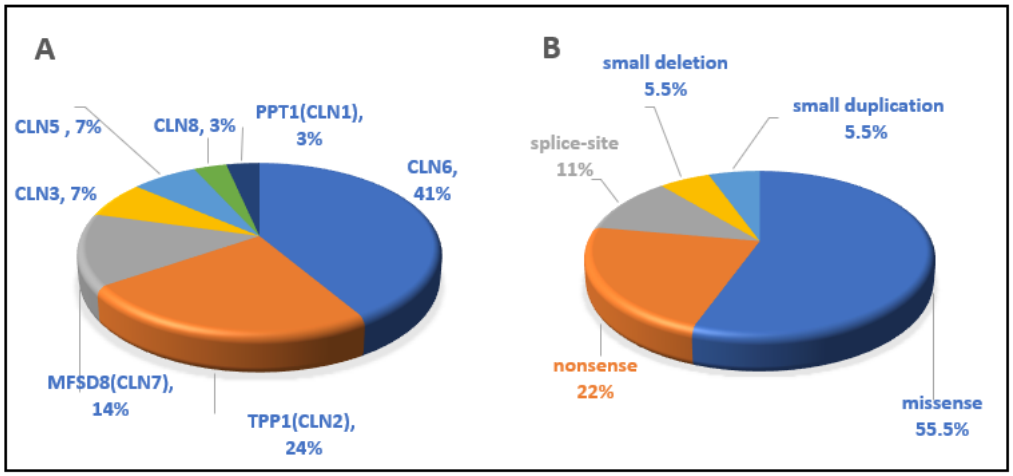

Figure 3

Graph representing the distribution of NCLs types (A) and mutation type (B)