Neuronal ceroid lipofuscinoses type 8: Expanding genotype/phenotype diversity-first report from Saudi Arabia

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ABSTRACT

يُعتبر داء الليبوفوسيني العصبي من أكثر أمراض الخثل العصبي شيوعاً في مرحلة الطفولة والتي تتسم بالتشنجات مع تدهور عصبي يتمثل بالخرف وفقدان التوازن واضطرابات بالرؤية مع خلل بالحركة. الداء الليبوفوسيني العصبي الرضيعي المتأخر هو الأكثر شيوعاً من هذا المرض والذي يكون مرتبط بالمورثات *(CLN2, CLN5, CLN6, CLN8)*. لقد سجلنا حصول 3 حالات من داء الليبوفوسيني النمط 8 لدى عائلتين مختلفتين و قد تم إثبات التشخيص بالتحليل الجزيئي في حالتين منها. كما أنه لوحظ في الحالات السابقة إجهاضات عفوية و موت وإعاقة حركية مبكرة ممايعكس احتمالية وجود ارتباط بين هذا الداء من النمط *CLN8* مع أمراض خثلية عصبية غير معروفة. هذه الدراسة تصف الداء الليبوفوسين الرضيعي المتأخر نمط *CLN8* في منطقة الشرق الأوسط وفي السعودية بشكل خاص. أن النتائج التي حصلنا عليها تعطي بُعداً جديداً للنمط المورثي و الظاهري لهذا المرض لدى العرق العربي.

Neuronal ceroid lipofuscinoses (NCLs) are the most common group of neurodegenerative diseases that presents in childhood and are characterized by seizures and progressive neurological deterioration, which results in dementia, ataxia, visual failure, and various forms of abnormal movement. The most common form of neuronal ceroid lipofuscinoses is late infantile (LI-NCL), in association with the genes *CLN2, CLN5, CLN6,* and *CLN8*. We report the cases of neuronal ceroid lipofuscinoses type 8 in 3 patients from 2 unrelated families, which was confirmed by molecular testing in 2 of them. Multiple spontaneous abortions, early death, and early onset of motor disability were observed in our cases, reflecting a possible association of NCL 8 with other unrecognized neurodegenerative diseases. Our results expand the genotypic/phenotypic background of variant late Infantile-NCL in Arabic ethnicity.

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Neuronal ceroid lipofuscinoses (NCLs) are a group of genetically inherited lysosomal neurodegenerative diseases that mainly presents in childhood.[1] The NCL is characterized by permanent cell damage and ultimately multi-organ dysfunction due to intracellular accumulation of abnormal autofluorescent lipopigment materials in multiple cells, including neurons, skin, and skeletal muscle.[2-3] Typically, NCL is clinically characterized by both motor and mental regression, seizures, visual impairment, and early death. Historically, NCL phenotypes were classified into 6 categories, based on both the clinical presentation of the patient and their age at onset (congenital, infantile, late infantile, variant late infantile, juvenile, and adult). To date, fourteen NCL genetic forms (*CLN1* to *CLN14*) have been identified, which has led to a new classification system based on the associated gene.[2,4] The NCL accounts for 5% of all neurodegenerative disorders in Saudi Arabia.[5] The worldwide prevalence of NCL varies among different geographic and ethnic regions, but ranges from 1: 100,000 to 1: 1,000,000 live births.[2] The most frequent form of NCL is late infantile NCL (LI-NCL), with a reported incidence of 0.78: 100,000 live births.[6] The NCL type 8 can cause two main phenotypes: epilepsy progressive with mental retardation (EPMR), and variant LI-NCL (vLI-NCL). A new congenital *CLN8* phenotype characterized by psychomotor retardation and epilepsy from birth was

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recently described by Pesola et al[7] from Argentina. Here, we report the cases of 3 patients from 2 unrelated families who demonstrating the LI- NCL phenotype, 2 of whom were diagnosed through molecular testing to have a homozygous mutation of the *CLN8* gene.

Case Report. ***Patient A. Patient information.*** This was the case of a 4.6-year-old Saudi boy, who was the 5th child from a 1st degree consanguineous marriage. He was born full term from an uneventful pregnancy. The child was developing completely normal, until the age of 2 years. Thereafter, he started to display myoclonic seizures that progressively increased in frequency during the subsequent 2 years. By the age of 3 years, he started regressing in cognition, speech, motor, and vision, respectively. Within the last year, he progressively became ataxic, incontinent, and developed spastic quadriplegia. The patient had 5 normal siblings; however, the mother revealed a history of having 3 spontaneous abortions. Furthermore, the patients' family demonstrated a history of similar phenotypes of neuroregression in four relatives, with the onset of disease ranging between 2 and 4 years of age, and lifespan ranging between 8 and10 years (**Figure 1**). Unfortunately, documentation for these patients was not available.

Clinical findings. Physical examination of the patient revealed spastic quadriplegia with ataxic movement. The patient had no dysmorphic features or neurocutaneous stigmata. Growth parameters were within normal ranges: height=100 cm (at 10th percentile), weight= 15 kg (at 25th percentile), and head circumference=49 cm (at 10th percentile). Ophthalmologic examination of both eyes revealed pale optic discs.

Diagnostic evaluation. By the age of 4 years, an electroencephalogram (EEG) showed a generalized slow background with polyspike epileptiform discharges; visual evoked potential (VEP) was reduced, and electroretinography (ERG) was normal. Brain magnetic resonance imaging (MRI) revealed signs of cerebellar atrophy. LI-NCL was suspected and confirmed by molecular testing, which showed a homozygous deletion in *CLN8* c.(?_-1)_(543+1_544-1)del, which spans the first exon.

Therapeutic intervention. Patient was initially started with valproic acid, and levetiracetam was subsequently added. Daily myoclonic seizures persisted despite maximizing both doses.

Follow up and outcome. Patient was assigned a multidisciplinary team for follow-up care. He was a spastic quadriplegic, and his condition continued to deteriorate rapidly; furthermore, he was not responding to neither the antiepileptic drugs nor physiotherapy.

Patient B. Patient information. Patient B was a 7-year-old Saudi female, and was the 5th child from a 1st degree consanguineous marriage. She was born at full-term from an uneventful pregnancy. The patient demonstrated global developmental delays, particularly

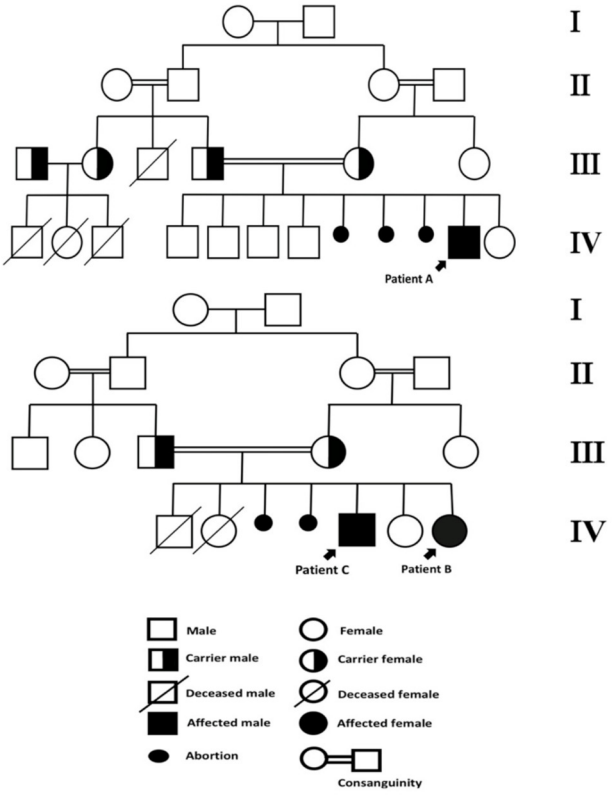

Figure 1 - Family pedigrees of patients A, B, and C.

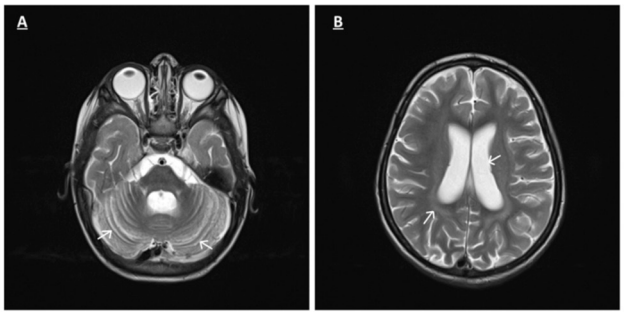

Figure 2 - Axial T2 weighted MRI images **A and B**) of patient B show enlarging of cerebellum folia , cortical sulci and ventricles with slight hyperintensity of cerebral white matter related to early cerebellar atrophy.

Table 1 - The clinical and molecular data of our cases and additional selected reported CLN8 cases with LI-NCL

Cases	Our patients			Gao et al[8] 2018	Allen et al[9] 2012	Reinhardt et al. 2009 patient 3 & 4[10]	Beesley et al[1] 2016 child B
	Patient A	Patient B	Patient C				
Country of origin	Saudi Arabia	Saudi Arabia	Saudi Arabia	China	Ireland	Turkey	UK
Consanguinity	Yes	Yes	Yes	No	No	Yes	No
Abortion history	Yes	Yes	Yes	Not reported	Not reported	Not reported	Not reported
Onset of disease	2 years	2 years	3 years	4 years	4 years	2.5 years	4 years
Age at diagnosis	4.6 years	3 years	-	Not mentioned	5 years	Not mentioned	Not mentioned
Initial symptoms	Myoclonic seizures	Unsteady gait and frequent falls	Speech delay Ataxic gait	Seizures	Increasing clumsiness	Delayed speech development	Motor regression + worsening gate
Clinical presentations	Myoclonic seizures Cognitive, speech, and motor regression Ataxic gait Visual impairment	Seizures Motor, speech, and cognitive regression Ataxic gait Visual impairment	Speech delay Motor and cognitive regression Seizures Visual impairment	Seizures Motor, cognitive and speech regression Ataxic gait Autism-like Visual impairment Dysphagia	Developmental delay Speech, psychomotor and cognitive regression Visual impairment Seizures	Speech delay seizures Motor and cognitive regression Visual failure Behavioral changes	Language delay Ataxic gait Motor regression Seizures Visual impairment Dysphagia
Type of seizures	Myoclonic Seizures	Myoclonic Seizures GTC	Myoclonic Seizures GTC	Refractory Seizures	Complex partial seizures	Myoclonic Seizures	Myoclonic Seizures Drop attack
Motor disability	Chair bound by age of 5 years	Chair bound by age of 4 years	Bedridden by age of 6 years	Bedridden by age of 8.6 years	Chair bound by age of 5.5 years	Bedridden by age of 8 years	Chair bound by age of 5.9 years
Neurophysiology (EEG, VEP, ERG)	EEG: diffuse slow background with generalized epileptiform discharges. ERG: normal VEP: reduced	EEG: diffuse slow background activity with generalized epileptiform discharges. ERG: absent VEP: reduced	EEG: Diffuse slow background activity with generalized epileptiform discharges. ERG & VEP: not done	EEG: irregular and slow background activity with generalized sequences of atypical spike-wave discharges. ERG & VEP: not mentioned	EEG: slow background, complex partial seizures ERG: absent VEP: reduced	EEG: spileptic discharges with generalized polyspike wave activity. ERG &VEP: not mentioned	EEG: not mentioned VEP: abnormal ERG: absent
Neuro imaging	MRI: cerebellar atrophy	MRI: cerebral and cerebellar atrophy	MRI: cerebellar atrophy	MRI: cerebral and cerebellar atrophy	MRI: hyperintensity of white matter and cerebellar atrophy.	MRI: cerebral and cerebellar atrophy	Cerebellar atrophy, low signal change abnormality in thalami bilaterally
Genetic study	CLN8 c.(?_-1) (543+1_544-1) del spans exon 1	C.699-700delGT pPhe234Profs*12 del spans exon 2	Deceased before.	Two variants: c.298 C > T (p.Gln100Ter); c.551 G > A(p. Trp184Ter)	c.562_563delCT, p.(Leu188Valfs*58) (paternal); 8p23.3 terminal deletion, de novo	c.544-2566_590del2613	Hemizygous for a novel variant: 54 kb deletion (paternal); c.728T>C P.(Leu243Pro) (maternal)
location	Exon1	Exon2	-	Exon2 & Exon3	Exon 3	In3/Exon3	Not mentioned
Age of death	Alive	Alive	8 years	Alive at age of 8 years	Alive at age of 5.5 years	Both alive at 10 years	9 years

with speech and gross motor skills. She was able to sit without support by the age of 18 months, crawl by the age of 20 months, and walk by the age of 24 months. Additionally, she was able to say "mama" and "baba" by the age of 20 months of age, but was unable to speak any further words thereafter. By the age of 2 years, she developed an ataxic gait with frequent falls and myoclonic seizures. Subsequently, she started demonstrating regression in motor, cognition, speech, and vision, respectively. She developed

spastic quadriplegia by the age of 4 years. During the subsequent 2 years, she developed generalized tonic-clonic (GTC) seizures, which then became refractory, despite receiving high doses of three antiepileptic drugs. The patient's family history was significant (**Figure 1**), including having a brother (Patient C) with a similar phenotype. She also had two siblings who died early from unknown causes by the ages of 2 and 4 months. The patient's mother also revealed a history of having 2 spontaneous abortions with no cause identified.

Clinical findings. Patient B was spastic quadriplegic. She had no dysmorphic features or neurocutaneous stigmata. Growth parameters were within low normal ranges: height=115 cm (at 10th percentile), weight=17 kg (at 3rd percentile), and head circumference= 50 cm (at 25th percentile). Basic funduscopic examination was performed by an ophthalmologist, which revealed severely pale optic discs in both eyes.

Diagnostic evaluation: At 3 years-of-age, an EEG showed diffuse slow background activity with generalized epileptiform discharges; ERG was absent, and VEP was reduced. Brain MRI revealed early cerebral and cerebellar atrophy (**Figure 2**). NCL was suspected, and was subsequently confirmed by molecular testing, which showed a homozygous frameshift variant in the *CLN8* gene, c.699_700delGT p.Phe234Profs*12, which spans exon 2.

Therapeutic intervention. The patient's seizures were refractory, despite receiving high doses of three antiepileptic drugs including phenobarbitone, valproic acid, and levetiracetam.

Follow up and outcome. Follow-up-care was provided by a multidisciplinary team; however, her condition continued to deteriorate overtime, and she did not respond to antiepileptics. Patient was frequently admitted for recurrent respiratory infections associated with vomiting and weight loss. Ultimately, gastrostomy tube (GT) feeding was started.

Patient C. Patient information. Patient C was an older brother of patient B (**Figure 1**). He was the 3rd child from this marriage and was born at full-term from an uneventful pregnancy. He was brought to the hospital by his parents following the diagnosis of his sister's condition, patient B. The child was initially free of any symptoms, demonstrating a phenotype similar to that of patient B. By the age of 7 years, he displayed developmental regression, ataxia with frequent falls, along with myoclonic and GTC seizures.

Clinical findings. The patient was bedridden, severely spastic quadriplegic, and on GT feeding. Growth parameters were within normal ranges. The patient had no neurocutaneous stigmata.

Diagnostic evaluation. By age of 7 years, an EEG was conducted, which showed diffuse slow background activity with generalized epileptiform discharges. Brain MRI revealed cerebellar atrophy. Unfortunately, he died before genetic diagnostic study could be conducted.

Follow up and Outcome: The patient's condition was progressively deteriorating, and he was not responding to antiepileptics, until he died at the age of 8 years due to recurrent respiratory infections.

Discussion.

In this study, we expand the ethnic diversity of NCL type 8 patients, as we report the cases of 3 patients from two non-related families from Saudi Arabia. The patients presented with seizures, ataxia, and neuroregression in speech, cognitive, motor function, and vision. The patients presented here underwent an initial basic screening and a metabolic work-up including complete blood count, electrolyte levels, renal function tests, liver function test, ammonia levels, lactate levels, tandem mass spectroscopy, and urine for organic acid, all of which were negative. Combining clinical and MRI findings, NCL was suspected. Ultimately, molecular testing, for both patients A and B, was performed to confirm the diagnosis. Patient A's results revealed a homozygous deletion in *CLN8*. The observed deletion, *CLN8* c.(?_-1)_(543+1_544-1)del, spans the first exon, and its estimated genomic breakpoints were chr8:1719131-1719853. On the contrary, patient B's results revealed a homozygous *CLN8* pathogenic gene mutation, C.699-700delGTpPhe234Profs*12 del, which spans exon 2. According to the modified Variant Classification ACMG 2015 guidelines, both variants were classified as likely pathogenic, and a diagnosis was finally confirmed. Combining the clinical presentation and molecular testing, the clinical courses of both patients A and B were similar to vLI-NCL rather than EPMR, due to their age at onset, myoclonic epilepsy, and the rapid regression thereafter. Unfortunately, patient C died before genetic testing could be performed; however, considering the phenotype and disease course of patient B, we speculate that patient C had the same phenotype as his sister, patient B. Our cases showed similar clinical presentations similar to those of other reported vLI-NCL cases, as shown in **Table 1**, which ranged from cognitive, motor, and speech regression with seizure, ataxia, and visual symptoms, to spastic quadriplegia and the loss of ambulation. Seizures, mainly myoclonic seizures, were the most commonly reported symptom, and visual deterioration was the latest manifestation. Our cases are different from

the cases reported by Gao et al. and Beesley et al., in which their patients had dysphagia, while none of our patients did.[1,8] Furthermore, our cases, mainly patient B, reported an earlier presentation of clinical symptoms and onset of motor disabilities compared with patients in the other reported cases. Cerebellar atrophy was observed in the brain MRIs of all our patients, which was consistent with the previously reported cases of NCL type 8 (**Table 1**). Here, we highlight the importance of performing an early brain MRI and its findings for the early recognition and diagnosis of vLI-NCL caused by *CLN8* mutations. Our study is unique in that, upon reviewing the history of our patients, we observed that multiple spontaneous abortions during the 2nd and 3rd trimesters were reported by both families, and the family of patient B reported early deaths during the first few months of life with no obvious cause, which has not been previously reported to be associated with NCL type 8. With the current study, we raise multiple questions for further investigations and research, to elucidate a possible association between NCL type 8 with other unrecognized neurodegenerative diseases that can explain this phenomenon. In conclusion, these are the cases of vLI-NCL caused by *CLN8* reported in Saudi Arabia. Our study expands the genotypic/phenotypic background of vLI- NCL in Arabic ethnicity. We emphasize the importance of early recognition, diagnosis, and counseling in NCL patients, particularly in Arabic regions with a high rate of consanguineous marriages, especially with the recent advances in the use of antisense oligonucleotide medication as a possible treatment modality for NCL type8 in the future.

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