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A clinical and DNA study on patients with Neuronal ceroid lipofuscinosis in Eastern Province, Saudi Arabia.

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Abstract

Neuronal ceroid lipofuscinoses (NCLs) are a large group of autosomal recessive lysosomal storage disorders. Patients present with distinct clinical features. Confirmation of diagnosis is a challenge and involves complex biochemical, and enzymatic tests which may not be available locally. The aims of this study were to describe the clinical features and DNA results of patients attend Saad Specialist Hospital, Eastern Province, Saudi Arabia with clinical diagnosis of NCL. Seven children (age 6 months to 11 years) with common clinical features (microcephaly, developmental delay, neuroregression, behavior changes seizures, EEG changes and MRI changes of cerebral atrophy) - all suggestive of NCLs –were included in this study. Biochemical, metabolic, and DNA tests were performed in all patients. DNA results showed that one patient carries a homozygous mutation in CLN2 exon 12 (G514R5837A>T. Another patient carries a compound heterozygous mutation in CLN3 gene (IVSII-3C>T) and in CLN2 gene (G514R 5837 A>T in exon 12). A third patient carries a heterozygous mutation in CLN1 gene (IVSIII-18). The other 4 patients carry no mutation.

Key words: Neuronal Ceroid-Lipofuscinosis, mutation, DNA

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Introduction

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited, neurodegenerative, lysosomal-storage disorders characterized by progressive mental and motor deterioration, seizures, and early death. Visual loss is a feature of most forms [1]. Worldwide incidence of NCL ranges from 0.2 to 7 per 100 000 live births [2]. NCL account for 5 % of neurodegenerative disorders in Saudi Arabia [3].

NCLs show a characteristic, progressive accumulation of autofluorescent hydrophobic material, the so-called ceroid-lipofuscin, in the cytoplasm of neurons and to a lesser extent in many other types of cells [4, 5]. Phenotypes have been characterized clinically by age of onset and order of appearance of the clinical features such as epilepsy, developmental delay, and visual loss [6-8]. Definitive diagnosis of NCL is a challenge and involves complex biochemical tests including measuring enzyme levels of Palmitoyl protein thioesterase (PPT) and Tripeptidyl peptidase 1 (TTP1) in leukocytes, cultured fibroblasts, dried blood spots, and DNA. The aims of this study were to describe the clinical features and the molecular characteristics of patients with clinical diagnosis of NCL who attend the metabolic clinic at Saad Specialist Hospital, Eastern Province, Saudi Arabia.

Methods

Seven children (4 boys and 3girls) attend metabolic clinic at our institute with clinical diagnosis of NCL were included in this study. Patients have clinical features consistent with NCL. All patients were reviewed by the metabolic physician in charge of this clinic. Detailed history and comprehensive examinations were performed. All patients had detailed metabolic work up including serum amino acid, lactate, pyruvate, ammonia, acylcarnitine profile, urine organic acid and white cell lysosomal enzyme assay. EEG and MRI brain studies were performed in all patients.

Mutation screening

Patients with NCL were subjected to detailed DNA study. The exons and exon–intron boundaries of the *CLN1*, *CLN2*, and *CLN3* genes were screened for mutations by

genomic sequencing. Five ml of peripheral blood were collected in EDTA tubes from all patients. DNA was extracted from whole blood samples using Quiagen minikit (QIAamp^R DNA mini kit, Qiagen CA, USA)

Polymerase Chain reaction-Capillary Electrophoresis performed. Polymerase chain reaction (PCR) products were purified using a Qiagen purification kit and then assessed with a capillary electrophoresis bio-analyzer using the DNA 7500 chip. The purified PCR products were sequenced on an ABI 3130xI Genetic Analyzer using forward and reverse primers. DNA sequences of the entire coding sequence and exon –intron boundaries of the 3 genes CLN1, 2, and 3 were sequenced and compared to the Palmitoyl–protein thioesterase (CLN1) accession# NT_032977.8, CLN3 accesion #NT_010393.15 and CLN2 accesion # NT_009237.17. Homozygosity was evaluated over the *CLN1*, *CLN2*, and *CLN3* loci using fluorescently labelled microsatellite markers as previously described [9]

Results

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Seven patients with clinical diagnosis of NCL were included in this study. The clinical characteristics of our patients include intractable seizures, global developmental delay and central hypotonia as shown on table 1. EEG was abnormal in 5 patients. MRI brain showed abnormalities in all patients ranging from demylination to cerebral atrophy as shown in Table 1.

All biochemical investigations including serum amino acid, lactate, pruvate, ammonia, acylcarnitine profile, urine organic acid and white cell lysosomal enzyme assay, revealed normal results.

Of the 7 patients that underwent DNA testing 3 confirmed to carry mutations in the CLN genes. Patient number 6 (table 1) carries a homozygous mutation in CLN2 exon 12 (G514R5837A>T). Another patient (number 3) carries a compound heterozygous mutation in CLN3 gene (IVSII-3C>T) and in CLN2 gene (G514R 5837 A>T in exon 12). A third patient (number 4) carries a heterozygous mutation in CLN1 gene (IVSIII-18). The other 4 patients carry no mutation

Table 1: Characteristics of patients with neuronal ceroid-lipofuscinoses

Case	Sex	Age at Diagnosis	Presenting symptoms	Subsequent symptoms	EEG	MRI
1	Male	5 years	Global Developmental delay	Regression of milestones, microcephaly, extrapyramidal symptoms	Normal	Diffuse atrophy
2	Female	11 years	Seizures	Hyperactivity, aggressive, regression of milestones	Abnormal	Diffuse mild atrophy
3	Femal	1 year	Global developmental delay	Regression of milestones, seizures	Abnormal	Demyelination
4	Male	3 years	Global developmental delay	hypotonia, Brachymicrocephaly, hyper excitability	Normal	Diffuse atrophy
5	Female	6 months	Seizures	Global, developmental delay, visual impairment, extrapyramidal symptoms	Abnormal	Fronto Temporal atrophy
6	Male	6 months	Seizures	Global developmental delay, microcephaly	Abnormal	Cerebellar and vermian atrophy
7	Male	18 months	Seizures	Global developmental delay, retinitis pigmentosa	Abnormal	Diffuse atrophy

Discussion

Neuronal Ceroid Lipofuscinosis represent a relatively common cause of inherited neurometabolic regression disorders [1]. The diagnosis of NCL is often challenging and sometimes delayed. The age of diagnosis in our patients ranged from 6 month to 11 years. The major clinical manifestations of patients in this study were intractable seizures, global developmental delay and regression. Our results agree with previous reports studied the genotype-phenotype associations in subjects clinically affected by neuronal ceroid lipofuscinosis (NCL) [6, 10]. Previous studies reported that the most common initial symptoms of patients with NCL were developmental delay (30%) in

NCL1, seizures (42.4%) in NCL2, and vision problems (53.5%) in NCL3 [6,10]. Intractable seizures were the initial presentation symptom in 6 of our patients that ranged from massive myoclonic seizures to generalized tonic clonic and focal motor seizures. EEG changes showed continuous spike wave discharge and periodic lateralizing epileptogenic discharges in most of our patients. Generalized slowing and burst suppression pattern was also reported. Caraballo et al [11]. Described the clinical characteristics, and particularly the epileptic seizures and electroencephalographic findings, in a group of patients with neuronal ceroid lipofuscinosis (NCL). The initial symptom was epilepsy in all cases. Massive myoclonic and myoclonic-atonic seizures were the most frequent kinds of attacks. The epileptic seizures in most of our patients were resistant to therapy.
MRI brain study for most of our patients showed brain atrophy which was more severe in infantile than in juvenile forms.

The diagnostic strategy for NCL depends on age of presentation. When Infantile or late infantile onset is suspected enzymatic testing for NCL1 & NCL2 is done first, a molecular genetic analysis is done if the enzyme is deficient [12,13]. In this study we selected patients with clinical diagnosis of NCL and performed extensive biochemical investigations including measurement of white cells enzymes for lysosomal disorders. Skin biopsy for electron microscopy and NCL enzymes was not done for logestic reasons. Despite all these investigations, no definitive diagnosis was reached. DNA study for NCL was then performed for all patients.

Molecular technology has revolutionized the diagnosis of NCLs. Molecular genetic testing makes it possible not only to confirm clinical and pathological diagnoses but also to offer pre-symptom diagnosis and carrier screening for NCL families [7, 14, 15, 16, 17]. Molecular genetic testing makes it possible not only to confirm clinical and pathological diagnoses but also to offer pre-symptom diagnosis and carrier screening for NCL families [7, 14, 15, 16, 17]. Six genes have been identified that cause human NCL (CLN1, CLN2, CLN3, CLN5, CLN6, CLN8), and approximately 150 mutations have been described. The majority of mutations result in a characteristic disease course for each gene [6,7,8].Selective genetic testing of patients with distinct clinical features of NCLs may represent a simpler and quicker means of diagnosis. In the current study 3 of our patients carry mutations in NCL gene. In the other 4 patients no mutation could be detected. Those patients with no mutation may carry other NCL gene mutations not tested in the present study, as we studied NCL I, 2 and 3 genes only.
The diagnosis of NCL is often difficult and involves laboratory tests including skin biopsy and enzyme studies, which are usually not available in the Middle East. The specimens have to be sent overseas and this presents logistical problems as well as being expensive. Moreover, specimen may not be suitable for enzyme assay when reaches the distant laboratory for different reasons including transportation conditions. All these reasons may explain that all white cell enzyme assays for our patients did not confirm NCL diagnosis. Moreover, white cell enzyme testing is known to have lower specificity and sensitivity compare to fibroblast enzyme assay which was not done in our patients. In selected cases with typical clinical features of NCLs we propose that DNA testing, where available, is a useful tool for diagnosis and may be utilized early in the process of diagnostic testing and not necessarily before exhausting tissue and enzyme studies. DNA study for NCL is more patient friendly and a reasonable alternative to complex enzymatic tests especially if these tests are not locally available.

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