Case Report

# Neuronal Ceroid Lipofuscinoses: Report of Five Cases in Kuwait

Maliha Askar Soud Al-Bloushi[1], Jehoram T Anim[2], Yousif Kasim Habeeb[1]
[1]Department of Pediatrics, Neurology Unit, Mubarak Al-Kabeer Hospital, Kuwait
[2]Department of Pathology, Faculty of Medicine, Kuwait University, Kuwait

## ABSTRACT

We report upon five children with neuronal ceroid lipofuscinoses diagnosed over an eight year period (1996-2004) in the pediatric neurology unit of a tertiary service hospital in Kuwait. Three cases were diagnosed by lysosomal enzymes while the two other cases were confirmed by skin biopsy. Although molecular genetic studies, when available, allow for definitive diagnosis, ultrastructural studies of skin biopsy material are simple available alternative. We feel that this disorder is under diagnosed and skin biopsies should be utilized when this disorder is considered.

KEYWORDS: neuronal ceroid lipofuscinoses, neurodegenerative disease, skin biopsy

## INTRODUCTION

Neuronal ceroid lipofuscinoses (NCLs) constitute the most common group of progressive neurodegenerative diseases in children with an autosomal recessive inheritance[1,2]. Clinicopathologic and genetic studies have proved that NCLs constitute a group of highly heterogeneous disorders[3,4]. The precise cause remains undetermined but all available evidence suggest an inborn error of metabolism[5]. The NCLs include eight forms that result from genetic deficiency on genes CLN (1) to CLN (8)[6]. Four classic forms which include infantile (INCL), late infantile (LINCL), juvenile (JNCL) in addition to adult (ANCL) form and four variants of LINCL[6]. The disease is characterized by visual impairment, progressive myoclonic epilepsy, decline in cognitive and motor skills resulting in premature death[1,7,8]. The course reflects progressive neurodegeneration. The diagnosis is usually made based on demonstration of autoflourescent lysosomal lipopigment in rectal biopsies and skin biopsies[9,10]. With recent advances in molecular genetics, diagnosis of certain subtypes is possible, without the need for invasive procedures like rectal biopsies. Thus, prenatal diagnosis became an option.

## CASE REPORTS
### Case 1

BA was the first born male child of a young Kuwaiti couple who are distant relatives. He had uneventful pre and perinatal periods. He was born at term weighing 3 kg. He was seen at nine months and was considered entirely normal although his parents thought he had poor eye contact and appeared socially indifferent. There was some concern regarding his development; although he sat independently at eight months and walked unassisted at 16 months, he did not respond appropriately to verbal stimuli. He was first seen by a neurologist at the age of 22 months. By this time he was showing signs of regression as he had lost some of his acquired milestones: stopped walking independently, lost speech (he used to say up to three words), stopped laughing when tickled and lost some manual skills such as waving good bye.

Examination revealed no dysmorphic features. He was socially indifferent with no eye contact and an expressionless child. His weight was on the 20th centile, length on the 40th centile but he had microcephaly (below the 3rd centile). Few myoclonic jerks were noted during examination. Ophthalmologic examination was normal including both fundi. Cranial nerve examination was normal. He could sit unsupported with good head control. He had normal power in all muscle groups but hypotonia with brisk tendon reflexes and negative Babinski sign. The rest of his examination was negative.

All his laboratory investigations were normal, including blood and cerebrospinal fluid lactate, liver function, calcium, magnesium, peroxisomal

address correspondence to:
Dr Maliha A. AL-Bloushi, Department of Pediatrics, Mubarak Al-Kabeer Hospital, Kuwait. Tel: 5318502, Pager: 9174029, E-mail: dr_askar@hotmail.com
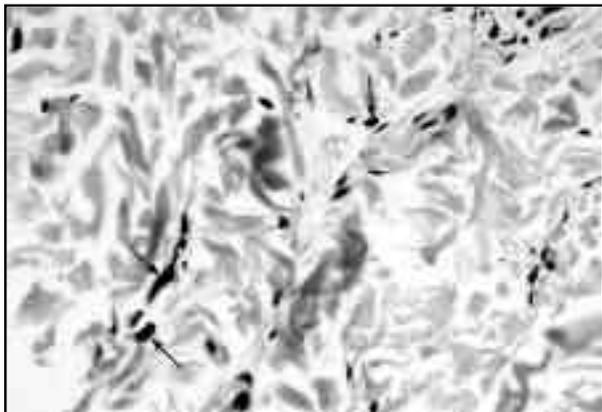
**Fig. 1:** H&E stained section shows brownish pigment granules in dermal fibroblasts (arrows).
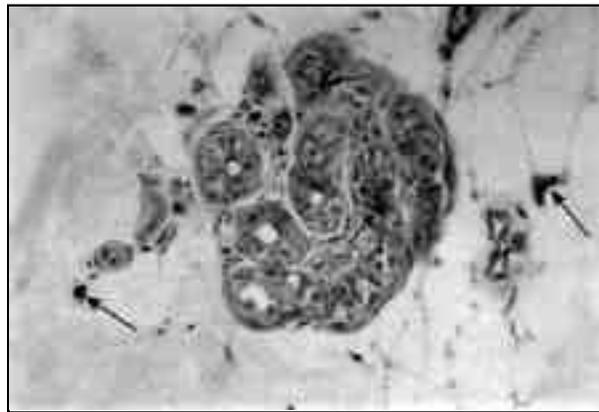


**Fig. 2:** The granules stained positively with sudan black, suggesting a ceroid-lipofuscin nature (arrows).

function tests, lysosomal enzymes, urine organic acid and aminoacid chromatography and karyotype. EEG was moderately abnormal with abnormal background activity. Electroretinogram (ERG) suggested a marked disturbance with almost loss of function in the visual pathways to the cerebral cortex. Visual evoked potential (VEP) was normal. Brain MRI showed severe cerebral atrophy with hypointense thalami and incomplete myelination of the white matter. His hearing threshold was equivocal in both ears. The diagnosis of INCL and LINCL were considered which are caused by deficiency of palmitoyl protein thioesterase (PPT) and tripeptidyl amino peptidase I (TPP) respectively. PPT assay showed low levels of 0.6 nmol/hr/mg ptn (normal range of 17-139) consistent with the diagnosis of INCL with normal TPP assay.

He was treated with sodium valproate for his myoclonic seizures with relatively good control. The clinical course was of progressive deterioration and regression. When last seen he was eleven years old with severe spastic quadriplegia and contractures at all joints, decorticate posturing and pendular nystagmus.

### Case 2 and 3

The second and third cases are siblings of case one, a girl (ten year old now) and a boy (eight year old) both of whom had similar presentation and course. The PPT assay was also low in both: 1.9 nmol/hr/mg ptn and 1.1 nmol/hr/mg ptn respectively. Both mother and father had the enzyme assay. It was 10.3 nmol/hr/mg ptn in the former and 20 nmol/hr/mg ptn in the latter suggestive of carrier state in both parents. All had normal TPP levels.

The mother became pregnant for the fourth time in 2002 and chorionic villous sampling (CVS) of her fetus revealed PPT level of 44 nmol/hr/mg ptn. She gave birth to a healthy boy who is now two year old.

### Case 4

The fourth case is RS, the product of a full term delivery. She had uneventful pre, peri and neonatal periods. Her parents were related and she had one healthy brother. She was developmentally normal with no problems till the age of three years when she, according to the parents, started to lose milestones: lost speech at three years had deterioration in motor skills with frequent falls and was unable to climb stairs at four years. From the age of 4.4 years, she had frequent myoclonic seizures.

We saw her for the first time at the age of five years. Examination revealed a friendly girl, interested in her surroundings. There was no dysmorphism although she had microcephaly, with weight and height on the 50th centile. Her gait was spastic. Cranial nerve examination was normal including both fundi. She had generalized hypertonia with clonus at the ankles, brisk deep tendon reflexes and positive Babinski sign. There was no weakness in any muscle groups, no neurocutaneous stigmata, normal liver size and no palpable spleen.

All her laboratory investigations were normal including blood lactate, blood ammonia, liver function tests, calcium, magnesium, bicarbonate, peroxisomal function, lysosomal enzymes, urine organic acid and aminoacid chromatography karyotype. EEG was markedly abnormal. Brain MRI showed cerebral atrophy with hypointense thalami with incomplete myelination of the white matter in the occipital region.

Full thickness skin biopsy confirmed the diagnosis of neuronal ceroid lipofuscinoses. Skin biopsy specimen was divided into three portions. One portion was fixed in 3% glutaraldehyde and processed for electron microscopy. A second portion was fixed in 10% buffered formalin and processed routinely for light microscopy. The third portion was sectioned fresh on a cryostat, mounted in saline and examined under fluorescence microscope. H&E stained sections showed
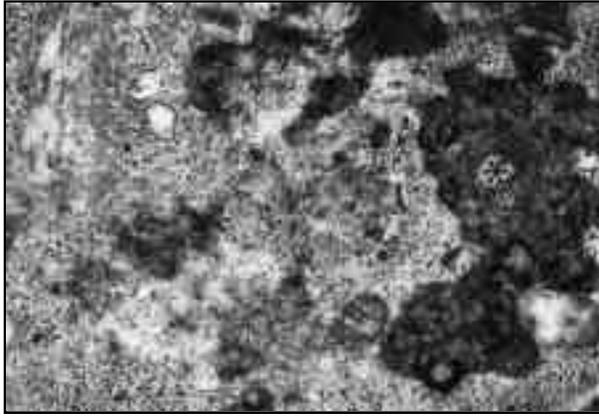
**Fig. 3:** The granules demonstrate whitish autofluorescence under the fluorescence microscope (asterisk).

brownish pigment granules in dermal fibroblasts (Fig. 1) as well as within sweat gland epithelium. The granules were negative with stains for melanin and haemosiderin but stained positively with sudan black, suggesting a ceroid-lipofuscin nature (Fig. 2). Electron microscopy showed amorphous densities and residual bodies (Fig. 3). The granules also demonstrated whitish autofluorescence under the fluorescence microscope (Fig. 4). She was treated with sodium valproate for her epilepsy with good control of her seizures but there was progressive deterioration in her motor skills leading to loss of ability to walk independently.

### Case 5

MF is a girl who was born after normal pregnancy at term to distantly related parents. She had three healthy siblings. She had acquired normal skills till the age of seven years when she had few attacks of generalized tonic clonic seizures for which she was treated with carbamazepine, with good control for two years. She presented to us at nine years of age with progressive speech loss, change in behavior and interactions and loss of sphincter control. She became withdrawn and stopped going to school because of her condition and worsening seizures.

Examination revealed a wasted child, not interactive and not interested in her surroundings. Her head circumference was on the 25th centile, weight on the 10th centile and height below the third centile. There was no opthalmoplegia but she was not fixing or following. Her fundi were normal. She was unable to sit still. She had rigidity in the lower limbs more than the upper limbs. There was ankle contracture and she demonstrated intention tremor with myoclonia.

She had no neurocutaneous stigmata or organomegaly. She followed a relentlessly progressive course involving loss of motor, visual and cognitive functions. She had difficulty with swallowing and
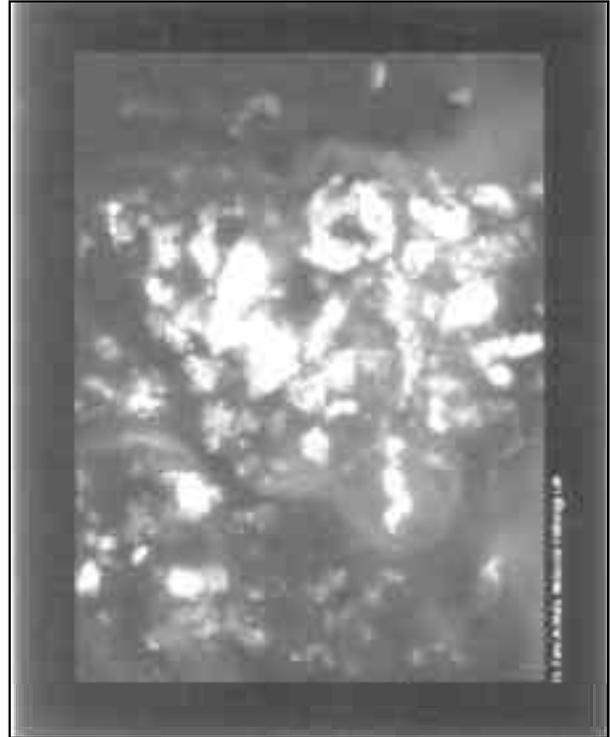


**Fig. 4:** Electron microscopy showing amorphous densities and residual bodies.

was kept on nasogastric feeding. She also developed recurrent cough probably secondary to aspiration. She was treated with sodium valproate for her epilepsy.

All her investigations were normal including blood and cerebrospinal fluid (CSF) lactate, CSF routine and titers for chronic infections including measles, liver function, calcium, magnesium, blood biotinidase activity, peroxisomal function, lysosomal enzymes, karyotype, urine organic acid and aminoacid chromatography and nerve conduction study. EEG was markedly abnormal but not suggestive of subacute sclerosing panencephalitis (SSPE). Brain MRI showed hypointense thalami with increased signal intensity in the occipital white matter affecting right more than left. The diagnosis was confirmed by skin biopsy demonstrating autoflourescent intracellular material.

### DISCUSSION

NCLs are relatively frequent group of inherited disorders. They occur world-wide with varying incidence[11]. In Europe the incidence is 1.2-1.6 / 100,000 live births[12]. Cumulative incidence was 1.61 / 100,000 live births but 0.87 / 100,000 for JNCL and 0.73 for the infantile NCL[12]. The highest reported incidence is in Finland (1:20,000 live birth)[11].

Common to all NCLs is lysosomal accumulation of autoflourescent ceroid lipopigment material in neural and extraneural cells[1,9]. The autoflourescent

**Table 1:** Characterestics of the major neuronal ceroid lipofuscinoses[5]

| | Range Onset (Yr) | Mean Onset (Yr) | First Symptom | Course | Onset to Death | Incidence (% Neuronal Ceroid-Lipofucinoses) | EM Predominant Findings | Genetics | Chromosome |
|---|---|---|---|---|---|---|---|---|---|
| Juvenile | 4 - 9 | 5.93 ± 1.35 | Visual, behavioral | Chronic | 16 | 49 | FP | AR | D16 |
| Late infantile/ early childhood | 1 - 3 | 2.54 ± 0.71 | Seizures | Acute | 6 | 21 | CL | AR | Not D16 |
| Infantile | 0 - 1.5 | 0.96 ± 0.50 | Deterioration myoclonus | Acute | 4 | 9 | GR | AR | DI |
| Adult | 27 - 28 | 29.67 ± 4.80 | Seizures/ motor | Chronic | > 20 | 9 | GR | AR/AD | UNK |
| Atypical | Variable | Variable | Variable | Variable | > 20 | 12 | Variable | Unknown | Unknown |

FP = Fingerprint cytosomes; CL = curvilinear cytosomes; GR = granular cytosomes; AR = autosomal recessive; AD = autosomal dominant; UNK = unknown genetics; D = chromosome

intracellular lipopigment tend to distend the cytoplasm of affected cells. Despite the multisystem distribution, only brain tissue shows severe dysfunction and cell death[4,5]. It is now known that ceroid and lipofuscin are composed of many different substances, including lipids, waxy pigments and a variety of proteins[5].

The cytosomes consists of mixtures of four distinct and characteristic membrane-bound osmiophilic profiles; classic lipofuscin, fingerprint profiles which predominate in chronic juvenile form, curvilinear inclusion bodies in infantile forms and pure granular profiles which predominate in some infantile and in late adult type[5].

The disorder is characterized by visual impairment, progressive myoclonic epilepsy, cognitive decline and premature death[1,7, 8]. The course reflects progressive neurodegeneration. Eventually every patient shows psychomotor deterioration. The outcome is always lethal within a few years[13]. Retinal degeneration is an early consequence[14,15]. The electroretinogram (ERG) is abnormal early in all three types of childhood NCL and eventually is totally ablated[14,16].

The NCLs are subdivided into several subtypes (Table 1) according to age of onset, clinical course and ultrastructural features of the storage material. The three main childhood varieties include infantile NCL (INCL) or (CLN1), late-infantile NCL ( LINCL) or (CLN2) and juvenile NCL (JNCL) or (CLN3)[8,17]. Molecular genetics of these three subtypes was recently clarified.

In the first subtype, INCL, or Santavuori - Haltia - Hagberg disease, there are mutations in genes encoding a lysosomal enzyme, palmitoyl protein thioesterase 1(PPT1) with locus CLN1 on chromosome 1p32[18]. This type is associated with predominance of granular inclusions in biopsied material. Cases 1-3 in this study represent this subtype based on demonstration of low PPT levels

in the subjects and borderline levels in both parents making them obligate carriers. Mutations in CLN1 gene also result in different childhood phenotypes (LINCL and JNCL) with granular osmiophilic deposits[18,19]. In addition to childhood forms, mutations in this gene also result in NCL in adults with onset in the fourth decade [20]. All CLN1 patients are deficient in PPT1. Similar to childhood forms with granular osmiophilic deposits, few cases were reported in a ANCL with autosomal dominant inheritance (Parry type) but interestingly with normal PPT1 enzyme level probably indicating gene distinct from CLN1-CLN8 genes [21].

The second subtype, LINCL, or Jansky-Bielschowsky disease, is caused by mutations in CLN2 gene which encodes a lysosomal enzyme tripeptidyl peptidase 1 (TPP1) with locus on chromosome 11p15[2,18]. The fourth case is an example of this subtype based on the age of presentation and characteristic inclusions seen in the skin biopsy.

The third subtype, JNCL or Batten disease, is caused by mutations in gene encoding a 438-aminoacid membrane protein (CLN3 on chromosome 16p12.1) commonly related to 1.02kb deletion[5,18]. There is predominance of curvilinear inclusion bodies in biopsied material. This is represented by our fifth case. As in this case, it is characterized clinically by visual and behavioral problems with slow progression over a few years [5]. The mean age of onset is 5.93 ± 1.35 years with onset between four and nine years. In contrast to our case, seizures occur later in life. Usually, the initial symptom is visual deterioration but in our case speech loss was the initial concern. It is possible that visual problems were not noted initially or were overshadowed by more obvious symptoms of speech loss and impaired interaction.

Other childhood forms include Finnish variant LINCL (CLN5) with locus on chromosome 15q21-

23[18] and Northern epilepsy or progressive epilepsy with mental retardation (CLN8)[22]. A total of 114 mutations have been described in the five human genes which cause NCL[23].

Early diagnosis is mandatory for avoiding further cases in families with hereditary metabolic brain disorders[24]. Diagnosis is made on the basis of clinical, electrophysiological, radiological and pathologic examination including electron microscopy[6]. Although only biochemical and molecular gentetic studies allow for definitive diagnosis, ultrastructural studies of biopsy material are still very useful[2].

Numerous tissues and organs are available for biopsy, among them brain (historical), rectum, skeletal muscle and peripheral nerve (largely by coincidence), skin and conjunctiva (the latter inferior to former in diagnostic yield) [10]. Unfortunately, the most convenient diagnostic method of examining circulating lymphocytes was not done. Treatment includes supportive measures and anticonvulsant medication.

Neuropathologic and neuroradiologic explanation of clinical symptomatology correlates best with neuronal loss and not neuronal storage[25]. This neuronal loss especially of dopaminergic neurons makes patients with JNCL and ANCL more susceptible to develop a life-threatening condition, neuroleptic malignant syndrome, after exposure to antipsychotic therapy. It should be considered when these patients develop hyperthermia, autonomic instability, extrapyramidal symptoms with or without altered level of consciousness. A high index of suspicion with early intervention is vital to avoid lethal outcome of neuroleptic malignant syndrome[26-28].

Early diagnosis is important for genetic counseling. Delay in diagnosis could be avoided by high index of suspicion in patients with unexplained visual loss, careful examination of lymphocytes for vacuolation and inclusion bodies together with molecular genetics studies. If our first case was diagnosed early in the course, genetic counseling and perinatal diagnosis would have helped the family to avoid having further cases. Whether this is a true statement or not, is to be debated as abortion is generally prohibited in Islamic culture with only few exceptions. However, the first family in this study did resort to this option.

## REFERENCES

1. Weimer JM, Kriscenski-Perry E, Elshatory Y, Pearce DA. The Neuronal ceroid lipofuscinoses: mutations in different proteins result in similar disease. Neuromolecular Med 2002; 1:111-124.
2. Wisniewski KE , Kida E, Golabek AA, Kaczmarski W , Connel F, Zhong N. Neuronal ceroid lipofuscinoses: classification and diagnosis. Adv Genet 2001; 45:1-34.
3. Mitchison HM, Mole SE. Neurodegenerative disease: The neuronal ceroid lipofuscinoses (Batten disease). Curr Opin Neurol 2001; 14:795-803.
4. Kida E, Golabek AA, Wisniewski KE. Cellular pathology and pathogenic aspects of Neuronal ceroid lipofuscinoses . Adv Genet 2001; 45:35-68.
5. Dyken PR. Neuronal ceroid-lipofuscinoses. Pediatrics Neurology, Principles and Practice, 1999:839-846.
6. Zong N. Neuronal ceroid lipofuscinoses and possible pathogenic mechanism. Mol Genet Metab 2000; 71:195-206.
7. Peltonen L, Savukoski M, Vesa J. Genetics of the neuronal ceroid lipofuscinoses. Curr Opin Genet Dev 2000; 10:299-305.
8. Goebel HH. The neuronal ceroid-lipofuscinoses. Semin Pediatr Neurol 1996; 3:270-278.
9. Sondhi D, Hackett NR, Apblett RL, Kaminsky SM, Pergolizzi RG, Crystal RG. Feasiblilty of gene therapy for late neuronal ceroid lipofuscinosis. Arch Neurol 2001; 58:1793-1798.
10. Goebel HH. Morphologic diagnosis in Neuronal ceroid lipofuscinosis. Neuropediatrics 1997; 28:67-69.
11. Santavuori P, Lauronen L, Kirveskari E , Aberg L, Sainio K, Autti T. Neuronal ceroid lipofuscinoses in childhood. Neurol Sci 2000; 21:35-41.
12. Crow YJ, Tolmie JL, Howatson AG , Patrick WJ, Stepenson JB. Batten disease in the west of Scotland 1974-1995 including five cases of the juvenile form with granular osmiophilic deposits. Neuropediatrics 1997; 28:140-144.
13. Jongen PJ, Gabreels FJ, Stekhoven JH, Renier WO, Le Coultere R, Begeer JH. Early infantile form of neuronal ceroid lipofuscinosis. Four Dutch cases and review of the literature. Clin Neurol Neurosurg 1987; 89:161-167.
14. Wilkinson ME. Ceroid lipofuscinosis, neuronal 3, Juvenile-Batten disease: case report and literature review. Optometry 2001; 72:724-728.
15. Birch DG. Retinal degeneration in retinitis pigmentosa and neuronal ceroid lipofuscinosis: An overview. Mol Genet Metab 1999; 66:356-366.
16. Weleber RG. The dystrophic retina in multisystem disorders:the electroretinogram in neuronal ceroid lipofuscinoses. Eye 1998; 12:580-590.
17. Gardiner RM. Genetic analysis of Batten disease. J Inherit Metab Dis 1993; 16:787-790.
18. Mole S, Gardiner M. Molecular genetics of the Neuronal ceroid lipofuscinoses. Epilepsia 1999; 40:29-32.
19. Wisniewski KE, Kida E, Connel F, Zhong N. Neuronal ceroid lipofuscinoses research update. Neurol Sci 2000; 21:S49-56.
20. Van Digglen OP, Thobois S, Tilikete C, et al. Adult Neuronal Ceroid Lipofuscinosis with Palmitoyl-Protein Thioesterase Deficiency: First adult-onset patients of a childhood disesase. Ann Neurol 2001; 50:269-272.
21. Nijssen PC, Ceuterick C, van Diggelen OP, et al. Autosomal dominant adult neuronal ceroid lipofuscinosis: a novel form of NCL with granular osmiophilic deposits without palmitoyl protein thioesterase 1 deficiency. Brain Pathol 2003; 13:574-581.
22. Ranta S, Lehesjoki AE. Northern epilepsy, a new member of the NCLfamily. Neurol Sci 2000; 21:S43-47.
23. Mole SE, Zhong NA, Sarpong A, et al. New mutations in the neuronal ceroid lipofuscinosis genes. Eur J Paediatr Neurol 2001; 5:7-10.
24. Santavuori P, Vanhanen SL, Autti T. Clinical and neuroradiological diagnostic aspects of neuronal ceroid lipofuscinoses disorders. Eur J Paediatr Neurol 2001; 5:157-161.

25. Boustany RM. Neurology of neuronal ceroid-lipofuscinoses: late infantile and juvenile types. Am J Med Genet 1992; 42:533-535.

26. Vercammen L, Buyse GM, Proost JE, *et al.* Neuroleptic malignant syndrome in juvenile neuronal ceroid lipofuscinosis associated with low-dose risperidone therapy. J Inherit Metab Dis 2003; 26:611-612.

27. Refi A, Schneider MF, Hoyer A, *et al.* Neuroleptic malignant syndrome in Kufs' disease. J Neurol Neurosurg Psychiatry 2003; 74:385-387.

28. Gupta S, Nihalani ND. Neuroleptic Malignant Syndrome. Prima Care Companion J Clin Psychiatry 2004; 6:191-104.