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CASE REPORT

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Sudden death due to cerebrotendinous xanthomatosis confirmed by mutation analysis

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Abstract A case of sudden death of a 52-year-old mentally retarded Caucasian male is described where the rectal temperature was 43.4 °C 3 h postmortem. The autopsy revealed cerebrotendinous xanthomatosis (CTX), a rare hereditary metabolic disorder, as the primary disease. The diagnosis was confirmed by postmortem identification of two mutations (compound heterozygosity for R237X and IVS6+1G→A) in the sterol 27-hydroxylase (CYP27) gene. Both mutations have already been described in patients with CTX and can be considered the most likely cause of the disease. The pathomechanism of the excessive hyperthermia could not be completely elucidated.

Key words Cerebrotendinous xanthomatosis · CTX · Hyperthermia · Genotype · CYP27 gene

Introduction

Cerebrotendinous xanthomatosis (CTX) is a rare, autosomal recessive lipid storage disease first described in 1937 by Van Bogaert and coworkers [1]. Deficiency of the mitochondrial enzyme sterol 27-hydroxylase (CYP27) leads to abnormal bile acid synthesis with accumulation of cholesterol and cholestanol in various tissues. Clinical hallmarks of the disease are tendon xanthomas (particularly in the achilles tendon), juvenile cataracts and progressive neurological dysfunction. Biochemically, the disorder can be diagnosed by increased levels of α-cholestanol and bile alcohols in plasma. The sterol 27-hydroxylase gene maps to chromosome 2q33-qter and is defective in CTX. Various mutations of this gene have been identified in clinically diagnosed CTX patients, including missense, nonsense, and splice site point mutations, deletions and insertions [2].

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Case report

Clinical course

Medical data of the patient were only available from 1987 when he was 42 years old. At that time, low intelligence, slightly deviating gait and Babinski's sign on the left were noted. The patient underwent eye surgery in adolescence because of bilateral cataracts. In addition, behavioural disturbances since early childhood were reported by the father. In the years after 1987, progressive dysarthria and ataxia developed. In 1992, a cranial CT scan showed demyelination in the cerebellar white matter. Screening for metabolic disorders, however, failed to reveal the underlying disease which was thought to be untreatable. In recent years, the patient had been living in a home for disabled people. The day before his death, the patient was weak and apathetic. On the following morning, he was found in his bed dyspnoeic, sweating heavily and the body temperature measured axillary was 42.0 °C (it should, however, be mentioned that the thermometer used would not display values higher than 42.0 °C). A few minutes later, the patient died despite resuscitation attempts.

Autopsy findings

After admission of the body to the morgue 3 h postmortem, the rectal temperature was 43.4 °C. This finding was confirmed using a second thermometer. On external examination, bilateral hammertoes were the only remarkable findings. The autopsy, which was performed on behalf of the deceased's family, revealed extensive indurations in the cerebellar white matter. The plexus choroideus was enlarged and showed multiple yellowish deposits. The brain was edematous and showed moderate temporal and tonsillar herniation and small cristalline-like inclusions could be detected in all parts of the lungs. Additional findings were a cavernoma in the gyrus praecentralis of the left cerebral hemisphere, multiple hemangiomas of the liver and cholecystolithiasis. Toxicological analysis of blood and various tissues detected no traces of alcohol or drugs.

Histological findings

Extensive demyelination and gliosis were found in the cerebellar white matter, the pyramidal tracts and the thalamus. Xanthogranulomas were present within the demyelinated areas, consisting of

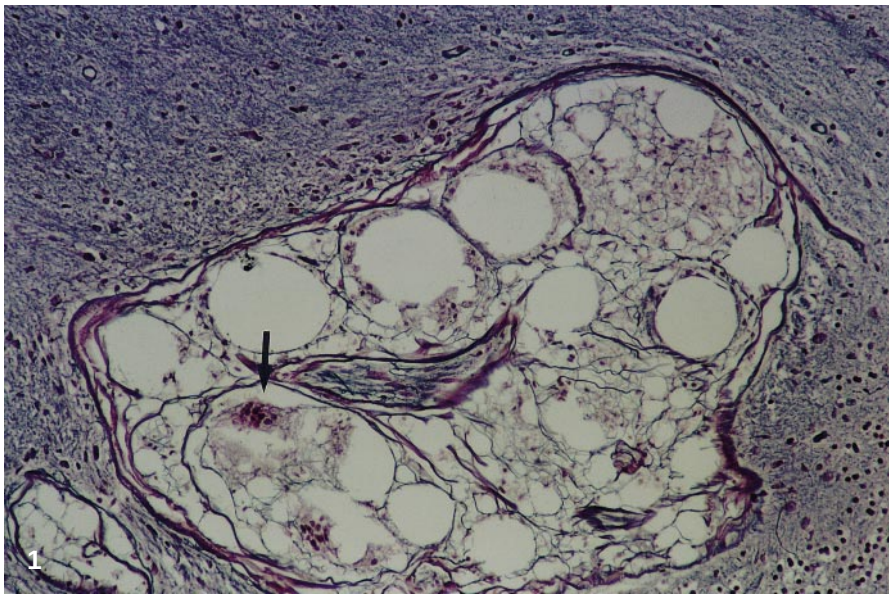

Fig. 1 Granulomatous lesion in the cerebellar white matter consisting predominantly of cystic spaces. → = multinucleated giant cell (Bodian × 390)

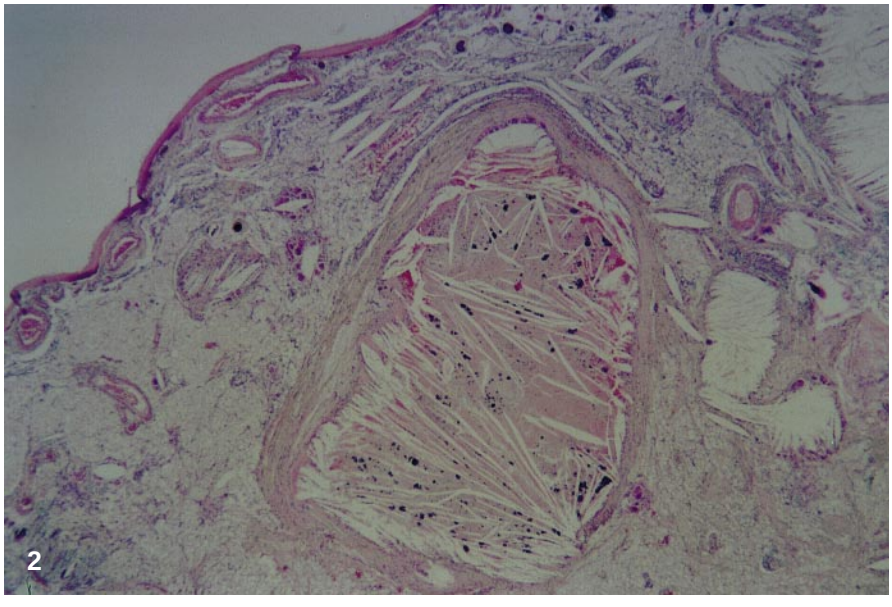

Fig. 2 Multiple xanthogranulomas in the plexus choroideus (HE × 43)

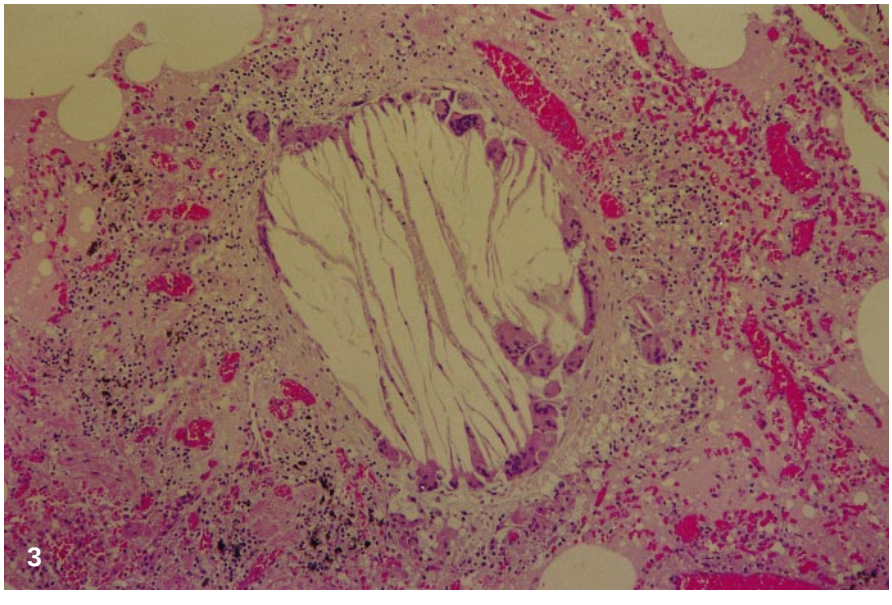

Fig. 3 Xanthogranuloma in the lungs. Note the wall of multinucleated giant cells (HE × 43)

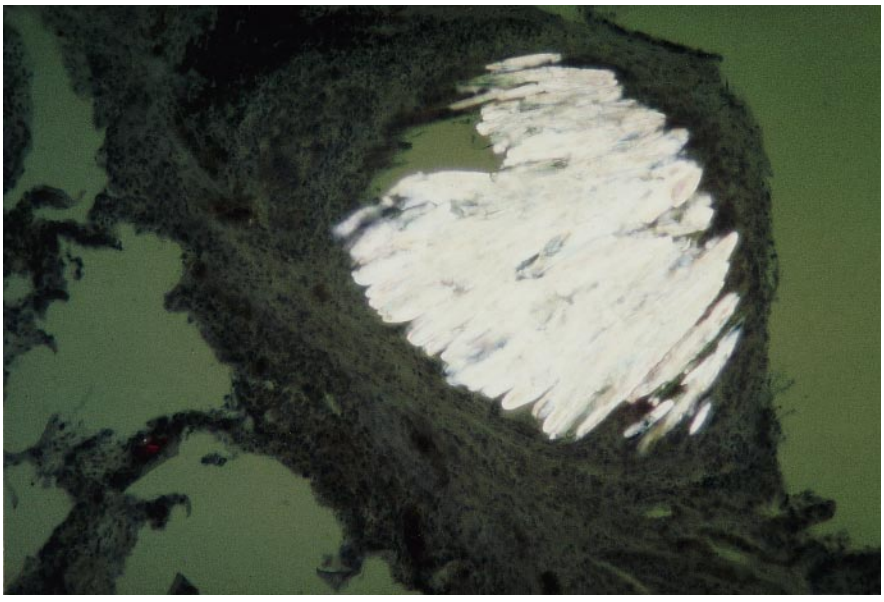

Fig. 4 Birefringance of the crystalline inclusions in polarized light (frozen section × 43)

cleft-like and cystic spaces surrounded by monocytes, macrophages and multinucleated giant cells (Fig. 1). No foci were found in the hypothalamus. Multiple xanthogranulomas were also found in the plexus choroideus and in the lungs (Figs. 2, 3). Birefringance of cristalline material within the clefts was observed in frozen sections (Fig. 4). The cause of death was diagnosed as cerebral dysregulation leading to excessive hyperthermia.

Materials and methods

DNA was isolated from paraffin-embedded tissue by the standard phenol/chloroform extraction method [3]. Nine exons and flanking intronic sequences of the CYP27 gene were amplified from the genomic DNA of the patient and unaffected controls by the polymerase chain reaction (PCR) using oligonucleotide primers designed according to the genomic sequence deposited in the Genebank (Seq ID: M62401) as well as by those given in Leitersdorf and coworkers [4]. Single strand conformational polymorphism (SSCP) analysis was performed as described elsewhere [5]. PCR fragments of the patient showing mobility shifts were purified and sequenced directly by using the ABI Prism Dye Terminator kit and an automatic ABI 310 sequencing apparatus.

Results

SSCP analysis detected mobility shifts for amplicons 4 (Fig. 5) and 6. Sequence analysis of exon 4 revealed a het-

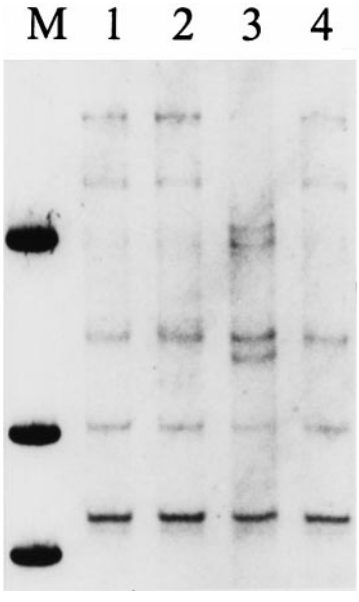

Fig. 5 Single stranded conformational polymorphism (SSCP) with mobility shift in exon 4. M = marker (ϕX HaeIII digest), 1, 2, 4 = control, 3 = patient

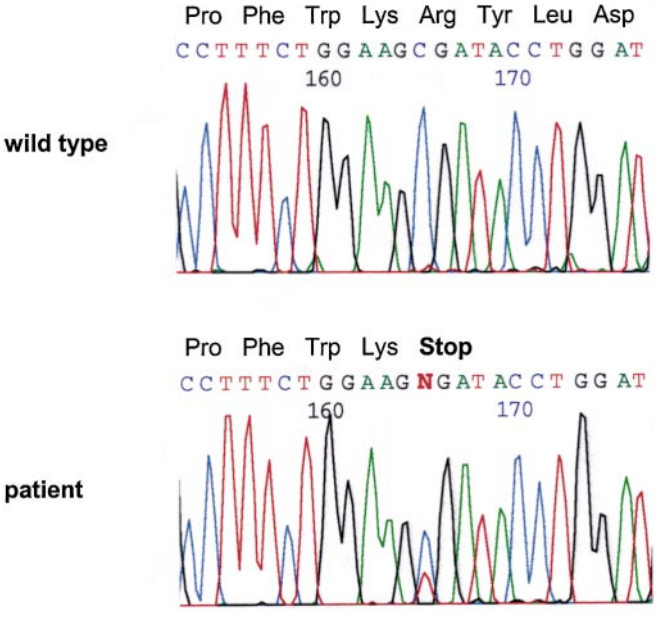

Fig. 6 Sequence analysis of exon 4 (forward strand): single base substitution of C to T, which changes the codon for arginine (CGA) to a stop codon (TGA)

erozygous single base substitution of C to T in codon 237 (Fig. 6), which changes the codon for arginine (CGA) to a stop codon (TGA), first described in 1997 by Ahmed and coworkers [6] in a Pakistani family. A second mutation, first described in 1997 by Garuti and coworkers [7] in an Italian family, was detected in intron 6. The heterozygous substitution of the evolutionary highly conserved G, the first nucleotide of the 5'splice junction consensus sequence to A is expected to severely affect the production of normally spliced mRNA.

Discussion

Cases of sudden death due to rare diseases are a matter of forensic interest [8–11]. Under certain circumstances, genetic analysis of formalin-fixed paraffin-embedded tissue is employed in forensic casework [12]. In the present case, the diagnosis of CTX was achieved postmortem by pathomorphological findings in correlation with the patient's history and molecular genetic analysis. Several typical features of CTX were present. The occurrence of hammertoes due to atrophic changes of the intrinsic musculature of the feet has been occasionally reported [13]. A higher incidence of benign vascular tumors (in this case cavernoma of the brain and hemangiomas of the liver) is not known to be associated with CTX. Despite the long lasting clinical course and the presence of typical symptoms, the diagnosis was not established ante mortem. This could be mainly due to the fact that CTX is an extraordinarily rare disorder. Furthermore, of the typical features of CTX, tendon xanthomas were not present or, at least, not obvious enough to be recognized. Reviewing the medical records, there was a note that 1 year before death, the measurement of α-cholestanol in the patient's serum was considered. Unfortunately, at that time, no laboratory could be found that was able to perform the analysis. Since the progression of CTX can be stopped pharmacologically, an early diagnosis is crucial. Patients who receive treatment with chenodeoxycholic acid show an arrest in the progression of CTX or even improvement of the symptoms [14].

It was not possible to elucidate the cause of the lethal hyperthermia morphologically. Presumably, perifocal edema in the thermoregulatory centers of the hypothalamus led to the extraordinarily high body temperature. Thyrotoxicosis due to previously undiagnosed hyperthyroidism is another possible cause of hyperthermia. Some authors reported endocrine abnormalities in cases of CTX, but they referred to hypothyroidism [15]. Moreover, in the present case, the thyroid gland was morphologically inconspicuous. Malignant hyperthermia syndrome and malignant neuroleptic syndrome were ruled out by toxicological analysis. In the absence of inflammatory foci, there was no indication for septicemia as the underlying cause of hyperthermia. To our knowledge, this is the first postmortem diagnosis of CTX including molecular analysis of the genetic defect. This case underlines the importance of a careful examination of all sudden deaths, especially if they occur outside hospital.

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