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Diagnosis of spinal xanthomatosis by next generation sequencing: identifying a rare, treatable and mimic of hereditary spastic paraparesis.

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Abstract

Cerebrotendinous xanthomatosis is an autosomal recessive disorder of bile acid metabolism causing a range of progressive neurological symptoms. Even in the presence of the classical triad of neurological dysfunction, tendon xanthoma, and early onset cataracts, the diagnosis is often missed. It can mimic more common conditions such as hereditary spastic paraparesis or multiple sclerosis, particularly when the phenotype is spinal xanthomatosis where the disease causes a spastic paraplegia. Early recognition and initiation of chenodeoxycholic acid therapy may prevent irreversible neurological damage. The introduction of next generation sequencing to screen for a large number of genetic disorders associated with progressive spastic paraparesis will allow earlier identification and initiation of treatment of these patients and their families, and will be particularly helpful in atypical cases such as the individual described in this case report.

Introduction

Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive lipid storage disease caused by mutations in the *CYP27A1* gene which leads to a deficiency of the enzyme sterol 27-hydroxylase.[1] Subsequent dysfunction of chenodeoxycholic acid synthesis results in a build-up of precursor molecules: cholesterol and cholestanol. These become deposited in the central nervous system causing dementia, psychiatric disturbances, epilepsy, cerebellar syndromes, peripheral neuropathy and spastic paraparesis. Deposition in other tissues causes early-onset cataracts, tendon xanthomas, premature arteriosclerosis and osteoporosis. Chronic diarrhoea can occur, probably due to impaired bile acid production.[1] Identification is vital as treatment can potentially reverse the manifestations. The introduction of genetic screening using next generation sequencing has the potential to identify previously unrecognised cases of CTX and other rare genetic diseases providing the opportunity for diagnosis and treatment.

Case History

A 27-year-old man presented with a 3-year history of spontaneous clonus, leg stiffness and falls. He had suffered from chronic diarrhoea from early childhood, the cause of which had remained uncertain despite extensive investigation. He also had a history of depression and had suffered from urinary frequency for several years. He reported a normal childhood development and average school performance. His paternal aunt who lived in another part of the country was reported to suffer from multiple sclerosis and his paternal cousin was described as having an abnormal gait. Neither parent suffered from any neurological disorder.

Neurological examination demonstrated a few beats of unsustained gaze evoked nystagmus on lateral gaze to both sides and a brisk jaw jerk. Fundoscopy was normal and there was no evidence of cataracts. His upper limbs were normal except for symmetrically brisk reflexes.

Examination of the lower limbs revealed pes cavus, considerably increased tone, sustained ankle clonus, diffuse hyper-reflexia and extensor plantar responses. There was no evidence of tendon xanthomas and no other cerebellar signs. Examination of his father also revealed pes cavus, but no other neurological abnormality. Neurological examination of the patient's mother was normal.

Investigations and disease course

Routine blood tests and magnetic resonance imaging of the brain and whole spine were normal. Cerebrospinal fluid examination showed an elevated protein level of 954mg/L but a normal white cell count and oligoclonal bands were absent.

The patient was given a diagnosis of hereditary spastic paraparesis (HSP). Genetic testing for the dominant HSP genes *SPAST* (SPG4), *ATL1* (SPG3A) and *REEP1* (SPG31) revealed no mutation. He was followed up in clinic over the next 13 years during which time his symptoms gradually progressed and he became wheelchair dependent. He also developed reduced sensation to pinprick, joint position and vibration peripherally with depression of the ankle jerks, which had previously been brisk. He suffered from two episodes of depression requiring hospitalisation.

In the process of establishing next generation sequencing equipment, the Sheffield Diagnostic Genetics Service (SDGS) screened stored samples from a group of patients with presumed HSP in whom a genetic diagnosis had not been established, for a panel of genes associated with motor system disorders. Analysis of a subpanel of 40 genes associated with spastic paraplegia revealed this patient to be homozygous for a previously reported mutation in exon 6 of the *CYP27A1* gene, c.1183C>T, predicted to result in the p.Arg395Trp (R395W) protein change.[2]

This mutation was confirmed by Sanger sequencing. No significant variants were identified in any of the other 39 genes analysed. The serum cholestanol level was then tested and found to be raised (112μmol/L, normal range <16) which confirmed the diagnosis of cerebrotendinous xanthomatosis.

Discussion

Hereditary spastic paraparesis (HSP) is a genetically heterogeneous syndrome characterised by progressive lower extremity spasticity, often disproportionate to any degree of weakness.[3] A large number of genetic mutations have now been identified with a highly variable phenotype amongst carriers but *de novo* mutations are also possible and therefore the absence of a family history should not exclude the diagnosis. As in our patient's case, HSP is often associated with urinary disturbance and can be associated with cerebellar signs, pes cavus and sensory symptoms due to dorsal column involvement or neuropathy.[3] However, whilst a number of other atypical symptoms such as mental retardation, dementia, epilepsy, and cataracts may be seen in some types of HSP, chronic diarrhoea has not been reported and is a "red flag" feature prompting consideration of other causes for the neurological disorder.[3]

Typical cerebrotendinous xanthomatosis (CTX) can present with a variety of neurological features not dissimilar to those of HSP, with pyramidal signs (81%), cerebellar signs (56%), intellectual problems (57%), seizures (24%) and neuropathy (24%) in one case series, along with the classic signs of cataract (90%) and tendon xanthomas (45%).[4] Chronic diarrhoea was reported in 33-50% of cases.[4,5] Spinal xanthomatosis is a rarely described variant of CTX in which patients present with symptoms of corticospinal and dorsal column tract dysfunction without many of the additional classic features CTX and is typically milder in its disease course.[6,7] In a series of seven patients with spinal xanthomatosis, only one patient had tendon

xanthomas and four gave a history of chronic diarrhoea but (unlike our patient) all had juvenile cataracts.[6] Of these cases, three received incorrect initial diagnoses, including multiple sclerosis, cervical myelopathy and HSP. A further two cases of spinal xanthomatosis involving a progressive myelopathy without the classic features of CTX and normal MRI imaging have also been reported.[7,8] In CTX, typical MRI findings of symmetrical cerebral and cerebellar white matter lesions are expected, but in spinal xanthomatosis MRI may demonstrate specific T2 weighted post-gadolinium MRI changes of extensive white matter pathology in the lateral and dorsal tracts of the spinal cord.[6] The fact that these were seen using phased-array coils with sensitivity for intrinsic spinal cord lesions might explain why MRI appearances using standard technology may be normal.[6,8] As the case described in this report demonstrates, normal MRI appearances should therefore not detract from a possible diagnosis of spinal xanthomatosis. Diagnosis should be confirmed by measuring serum cholestanol and urinary bile alcohol levels and molecular genetic analysis for mutations in *CYP27A1*.

Treatment with chenodeoxycholic acid (CDCA) replacement reduces serum cholestanol levels in CTX patients.[9] In a series of 17 cases, after one year of treatment, there was significant clinical improvement with resolution of dementia (10 out of 13 cases), improvement or resolution of pyramidal and cerebellar signs (13 out of 17) and resolution in neuropathy (6 out of 7).[9] The addition of simvastatin to CDCA was associated with a further reduction in cholestanol compared to CDCA alone, whilst also reducing levels of total and LDL cholesterol and increasing levels of HDL cholesterol.[10] No additional clinical improvement were observed following a six-month course of the combination therapy compared to CDCA alone, but given that premature atherosclerosis and coronary artery disease have been reported, simvastatin

may convey a benefit in primary prevention of cardiovascular complications.[10,11] Patients should also undergo lifestyle counselling to further reduce their cardiovascular risk.

The diagnosis of the spinal form of CTX was made in this patient following next generation sequencing (NGS) of a panel of 41 genes associated with motor system disorders (Table 1). Validation data for the NGS technology used shows that both the test sensitivity and test specificity is >99%, with confidence intervals of 95% for both. Hence, it is expected to detect all mutations other than large deletions or duplications. However, work is currently underway to evaluate the use of NGS technology for dosage analysis and to increase the number of genes associated with motor system disorders that can be analysed. To date 72 spastic gait disease-loci and 55 spastic paraplegia genes (SPGs) have been identified, with all modes of inheritance described encompassing a very wide phenotype.[12] New developments in genetic sequencing technology, combined with a gradual reduction in the cost of screening, will, in future, allow a molecular diagnosis to be established more frequently in this group of patients.

Table 1. Next generation sequencing gene panel for screening of HSP-like disorders

Disease / Locus	Gene
SPG1	*L1CAM*
SPG2	*PLP1*
SPAX1	*VAMP1*
SPAX4	*MTPAP*
SPAX5 / SCA28	*AFG3L2*
SPG3A	*ATL1*
SPG4	*SPAST*
SPG5A	*CYP7B1*
SPG6	*NIPA1*
SPG7	*SPG7*
SPG8	*KIAA0196*
SPG10	*KIF5A*
SPG11	*SPG11*
SPG12	*RTN2*
SPG13	*HSPD1*
SPG15	*ZFYVE26*
SPG17	*BSCL2*
SPG20	*SPG20*
SPG21 (Mast syndrome)	*SPG21*
SPG26	*B4GALNT1*
SPG22	*SLC16A2*
SPG28	*DDHD1*
SPG30	*KIF1A*
SPG31	*REEP1*
SPG33	*ZFYVE27*
SPG35	*FA2H*
SPG46	*GBA2*
SPG48	*AP5Z1*
SPG53	*VPS37A*
SPG54	*DDHD2*
SPG55	*C12orf65*
SPG56	*CYP2U1*
IAHSP	*ALS2*
CTX	*CYP27A1*
ALS11	*FIG4*
DYT5	*GCH1*
FAD-SP	*PSEN1*
ARSACS	*SACS*
ALS16	*SIGMAR1*
DYT9	*SLC2A1*
NBIA5 / SENDA	*WDR45*

Key points

In patients with presumed HSP diagnosis is important to identify treatable causes, prevent complications and provide screening and genetic counselling for family members. It is therefore important to consider the wider differential diagnosis in the many patients with an HSP-like syndrome in whom screening for the most common genetic mutations is negative, particularly where there are additional or atypical symptoms. In the future, increasing identification of genetic mutations associated with HSP and improved access to next generation sequencing will increase the speed and likelihood of diagnosis and offer the opportunity for treatment in many more cases. This is particularly pertinent in CTX where treatment has the potential to arrest or even reverse the disease. Until genetic sequencing becomes standard practice however, it is important to emphasise that metabolic screening for cerebrotendinous xanthomatosis should be performed in all patients with two out of four of the following features: juvenile-onset cataracts, intractable diarrhoea, tendon xanthomas or progressive neurological symptoms, even when neuroimaging is normal.

Affiliations and competing interests

Esther Hobson is a National Institute for Health Research (NIHR) Doctoral Research Fellow.

Pamela Shaw is an NIHR Senior Investigator. The authors of this manuscript have no competing interests.

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