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CASE REPORT

A first case of adrenomyeloneuropathy with mutation Y174S of the adrenoleukodystrophy gene

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Abstract

The patient first noticed spasticity and weakness in his legs. He was diagnosed with chronic myelogenous leukemia (CML); the symptoms were attributed to neuropathy associated with CML. By treatment with dasatinib, he achieved complete hematological remission, but his difficulty in walking was not improved. His neurological symptom worsened together with an increase in body temperature and then disappeared together with a normalized body temperature, which may be attributed to the Uhthoff's phenomenon often observed in multiple sclerosis. He later developed acute fever, vomiting and a high adrenocorticotropic hormone (ACTH) level, which was diagnosed as adrenal insufficiency. Eventually, he was diagnosed with a milder form of adrenoleukodystrophy (ALD), adrenomyeloneuropathy (AMN) by increased levels of Very Long Chain Fatty Acids (VLCFAs) and genetic testing of the ATP binding cassette subfamily D member 1 (ABCD1) gene. A missense mutation (c.521A>C, p.Tyr174Ser), previously reported to induce severe cerebral ALD, was detected in exon1. Thus, clinical manifestation of ALD is determined by interaction between the primary ABCD1 mutation and modifying genetic and environmental factors. Physicians should be aware of the differing symptoms of AMN and determine the level of VLCFAs in patients having primary adrenal insufficiency, especially those complicated with neurological dysfunction. This is the first report of an AMN patient complicated with CML.

INTRODUCTION

X-linked adrenoleukodystrophy (X-ALD; MIM 300100) is a disorder caused by defect in the ATP binding cassette subfamily D member 1 (ABCD1) gene. This results in accumulation of very-long-chain fatty acids (VLCFAs) in the central and peripheral nervous systems, adrenal glands and testis, which leads to demyelination of the nervous system and dysfunction of the affected organs. From the distinct phenotypes, there are several categories of ALD, childhood cerebral ALD and adult adrenomyeloneuropathy (AMN) being the most common (Kemp *et al.* 2016).

AMN, a milder form of ALD, begins in adolescence or early adulthood and the myelopathy progresses slowly. AMN accounts for about 25% of all ALD patients (Steinberg *et al.* 2015). Early diagnosis of AMN is important not only for the proband, but also for genetic testing of predisposed family members. However, AMN patients presenting with chronic progressive spasticity of the legs are very difficult to diagnose because various neurological disorders must be considered. In addition to slowly progressing myelopathy, certain other characteristics complicate early diagnosis of AMN, including its rarity, multisystem symptomatology, and ambiguous pattern of inheritance. The first manifestation of ALD in male patients is usually adrenal insufficiency, which is typically observed in childhood. The penetrance of adrenal insufficiency in ALD has been reported to be 50–100%, although this has not been fully investigated (Kaltsas *et al.* 2000). In adulthood, signs of myelopathy invariably develop; progressive cerebral demyelination can occur both in childhood and adulthood. The lack of a straightforward genotype–phenotype correlation in ALD is exemplified in patients with the same mutation, both within and between families, who nevertheless present distinctive phenotypes.

We learned from the present case that it is important not to overlook nonspecific symptoms other than neurologic manifestation in relation to AMN. In addition to adrenal insufficiency, family history, testicular dysfunction, and bladder dysfunction are essential for early diagnosis of AMN.

The present case also developed chronic myelogenous leukemia in the same period of time; this is the first such report, although the causal relationships remain to be elucidated.

METHODS

Immunocytochemistry

The antibodies used in this study were rabbit anti-70kDa peroxisomal membrane protein (PMP70) (1:1000; a gift from Dr. Imanaka, Toyama University, Japan) and mouse anti-human ABCD1 (1:100; #-MAB2164, Merck Millipore, Darmstadt, Germany) as primary antibodies, and goat anti-rabbit immunoglobulin (IgG) fluorescein isothiocyanate (FITC) conjugate (1:500; BioSource international, #ALI440) and Cy™3 AffiniPure F(ab')$_2$ Fragment Donkey Anti-Mouse IgG (H+L) (1:500; Jackson ImmunoResearch Inc, #715-166-151), as second antibodies. Fibroblasts were cultured on a coverslip placed in a six-well plate. They were fixed with 4% paraformaldehyde, washed with phosphate-buffered saline (PBS), permeabilized with 0.1% Triton X-100, and then incubated with the primary antibody. Unbound antibody was washed out with PBS, and the specimens were incubated with the secondary antibody labeled with a fluorescent dye. The immunofluorescent signal was detected using LSM710 confocal laser scanning microscope (Carl-Zeiss, Oberkochen, Germany).

CASE REPORT

A 22-year old man was referred to our department of diabetes and endocrinology after two episodes of critical illness suggesting adrenal insufficiency during the previous two months. His condition had begun two years before: at age 20, he became aware of spasticity and weakness in the legs. At age 21, he had an abnormally high value of white blood cells and was then diagnosed as CML by detection of an abnormal arrangement of chromosomes, t (9; 22) (q34; q11.2) by G-band staining of bone marrow at the hospital in his hometown. His symptoms in lower extremities thus were attributed to neuropathy associated with CML and he began treatment with dasatinib in the department of hematology in our hospital. About two weeks later, he was in complete hematological remission, but his difficulty in walking was not improved and he consulted the department of neurology.

He was hospitalized and had a thorough checkup to investigate the cause of spasticity and weakness in the legs. An MRI of brain and spinal cord showed no particular findings. His neurological symptom worsened together with an increase in body temperature and then disappeared together with a normalized body temperature, which may be attributed to Uhthoff's phenomenon, which is often observed in multiple sclerosis. He also was suspected to have spinocerebellar degeneration and started to take baclofen for spasticity. He received an infusion of intrathecal baclofen (25 mg), which was slightly effective in reducing the Asworth score of lower limb spasticity. However, the improvement was not sufficient for the patient to decide to have an implanted pump for intrathecal baclofen infusion at discharge. One month after leaving the hospital, he had acute fever and vomiting of unknown origin for several days, but the condition resolved itself. The next month he had similar ill health and the neurologist in charge found a high adrenocorticotropic hormone (ACTH) level (239.8 pmol/L). He was then referred to our department.

At that time, he was a 22 year-old thin, young man. He was 174.8 cm tall and weighed 56.7 kg. He had noticed a 9 kg weight loss over the half year before

due to appetite loss. Increased skin pigmentation was not seen. His pubic and axillary hair was sparse. He went into hospital and took an adrenal screening test; his 8:00 ACTH and cortisol level was 97.9 pmol/L and 265 nmol/L, respectively. There were no changes in ACTH-cortisol circadian rhythm or responses in serum cortisol values after standard high-dose ACTH stimulation test. The plasma renin activity was slightly high, but serum aldosterone was normal. These laboratory tests confirmed the diagnosis of primary adrenal insufficiency (Table 1). Head MRI was performed to check for hypopituitary lesions, and showed a slightly increased signal in the posterior arm of the internal capsule in both contrast-enhanced T1 weighted and FLAIR images, which was not detected in the images taken before (Figure 1).

The combination of primary adrenal deficiency and spastic paraparesis suggested X linked ALD and we then explored a detailed family history (Figure 2). The patient's mother had a stroke at 35 years old and was prescribed a steroid hormone as she had been suspected of angitis. His uncle on his mother's side was diagnosed with spinocerebellar degeneration at twenty years old. His grand-uncle on his mother's side fell after being bedridden for a year, and died in his twenties. His family tree was consistent with X-linked recessive inheritance.

Eventually, he was diagnosed with AMN by the increased levels of the VLCFAs in his plasma (Table 2) and genetic testing of his ABCD1 gene. A missense mutation (c.521A>C, p.Tyr174Ser) was detected in exon1 (Figure 3). We also examined the location of the mutant ABCD1 by immunocytochemistry: ABCD1 was detected and superimposed with peroxisomes confirmed by PMP70 (70kDa peroxisomal membrane protein) staining in control fibroblasts, whereas only PMP70 was observed in the patient's fibroblasts, indicating that the mutant ABCD1 protein was not located at peroxisomes in fibroblasts of the patient (Supplemental Figure 1). The patient's mother had the same heterozygote mutation and proved to be the carrier. The level of her VLCFAs was slightly elevated (Table 2).

Tab. 1. Laboratory tests confirming primary adrenal insufficiency.

(early morning)	
Cortisol	264.9 nmol/L
ACTH	97.9 pmol/L
Plasma renin activity	4.9 μg/L/h
Aldosterone	440.4 pmol/L
LH	23.6 IU/L
FSH	17.7 IU/L
Testosterone	20.3 nmol/L
Free testosterone	37.1 pmol/L
Na	137 nmol/L
K	4.1 nmol/L
Creatinine	77.8 μmol/L
Blood urea nitrogen	4.3 mmol/L
(ACTH stimulation test)	**Cortisol**
Before stimulation	229.0 nmol/L
30 min	212.4 nmol/L
60 min	229.0 nmol/L

Tab. 2. Levels of the VLCFAs in the proband and his mother.

(VLCFA)	Proband	Mother	Average ± SD
C24:0/C22:0	1.83	0.85	1.05±0.16
C25:0/C22:0	0.103	0.025	0.024±0.006
C26:0/C22:0	0.054	0.018	0.012±0.005

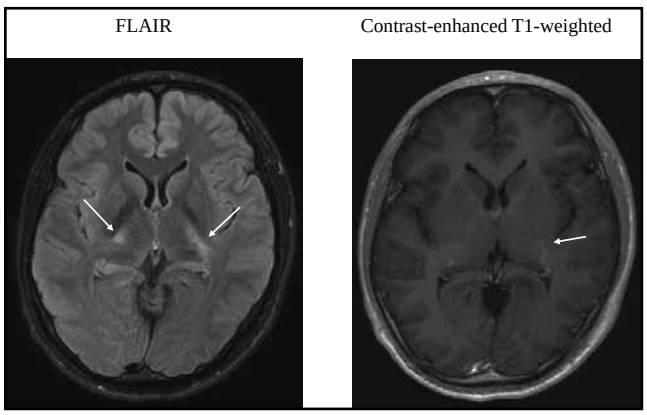


Fig. 1. Head MRI of the proband both in contrast-enhanced T1 weighted and in FLAIR (fluid attenuation inversion recovery) method at age of 24. Brain axial FLAIR MR image shows slightly high intensities in bilateral posterior limbs of internal capsules at age of 24 (left panel, arrow). Also, by contrast enhanced T1 weighted MR image at age of 24, the bilateral posterior limbs of the internal capsule (right panel, arrow) is intensified slightly. The gadolinium contrast medium was used at double dose for enhancement. No abnormal signal was detected in white matter of brain.

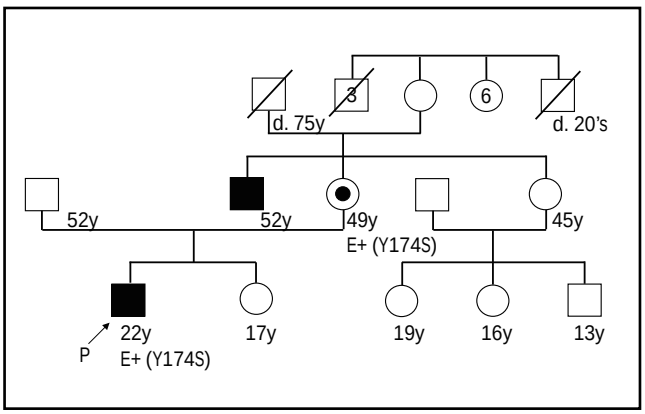


Fig. 2. Family pedigree of the present case. Circles represent females; squares, males. Individuals with neurological findings are indicated by black symbols. Arrow indicates the proband of this family. The individuals identified with the mutation of *ABCD1* are indicated as E+. The present age is also shown.

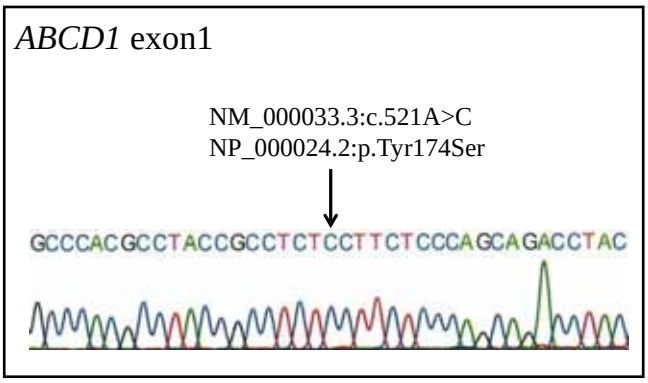


Fig. 3. The mutation identified in the present family. The GenBank accession number of gene and protein of ABCD1 is NM_000033.3 and NP_000024.2, respectively.

Our patient also was troubled with erectile dysfunction (ED) from age 21 years. Free testosterone and DHEA-S were lower but within normal range and gonadotropin was high (Table 1). His ED was therefore thought to result from testicular dysfunction due to AMN. For treatment of ED, an intramuscular injection of testosterone enanthate at dose of 250mg was administered, but was ineffective. He then took tadalafil, and the problem was resolved. In addition, he had mild symptoms of dysuria. But the result of uroflowmetry was within normal range and we decided to observe the progress with regard to neurologic bladder dysfunction.

For treatment of adrenal insufficiency, he started hydrocortisone 10mg/day, and plasma ACTH and cortisol improved to proper values. Three years after diagnosis of AMN, the patient's gait disturbance remains unchanged or is gradually improving. Haematopoietic stem cell transplantation (HCT) was not performed because the brain MRI showed no change in follow up study. The CML maintained major molecular responses (https://www.nccn.org/professionals/physician_gls/f_guidelines.asp). Genetic testing of family members is under consideration.

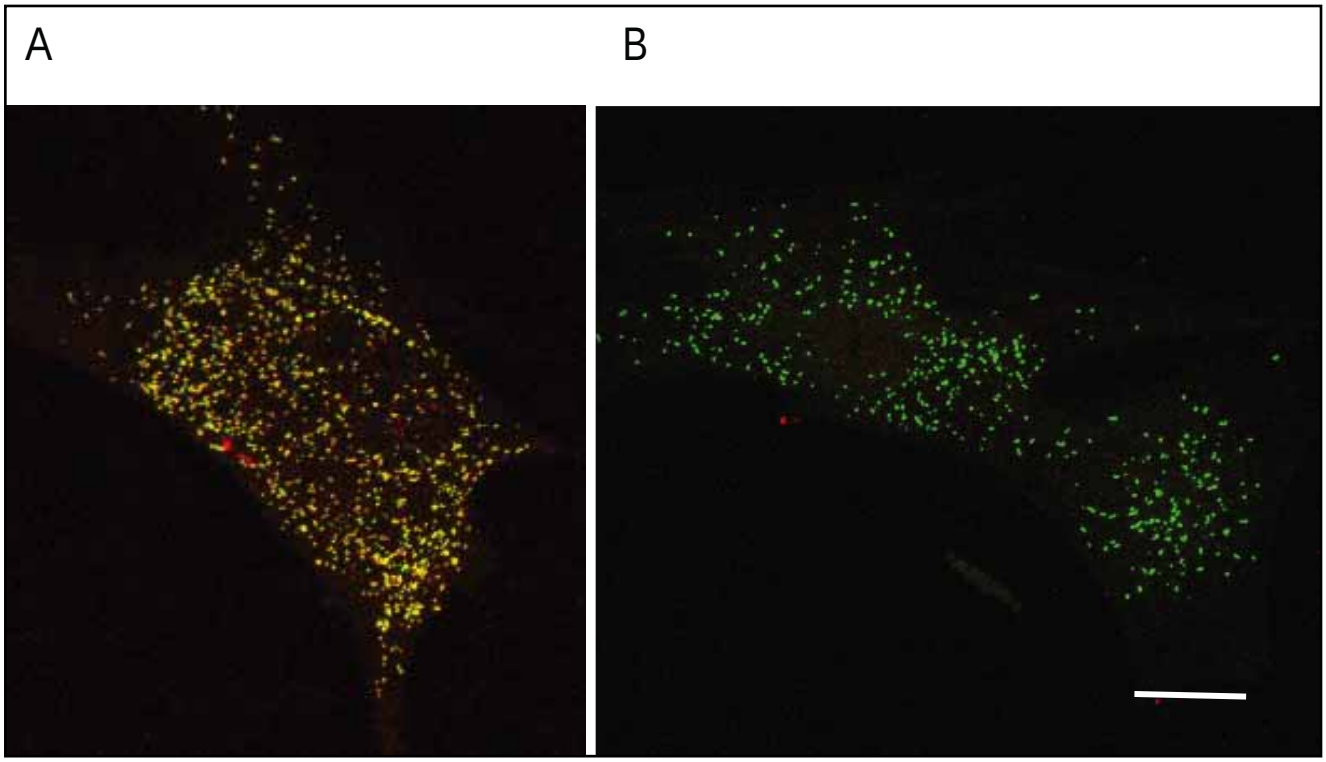
DISCUSSION

In this case, the initial feature of presentation was spasticity and weakness in the legs, which is insufficient for a diagnosis of AMN. Müller vom Hagen *et al.* reported that AMN is responsible for about 5% of cryptic, adult-onset, lower limb spasticity in a systematic screening approach (Müller vom Hagen *et al.* 2014), and is difficult to distinguish from myelopathy in clinical diagnosis. The adrenal insufficiency was the final clue to diagnosis of AMN. Dubey *et al.* (2005) found that 80% of asymptomatic patients with X-linked ALD had impaired adrenal function and that the youngest, a boy, was 5 months of age (Dubey *et al.* 2005). Thus, adrenal insufficiency could be one of the first, telling symptoms of AMN. On the other hand, penetrance of 50–100% has been reported for adrenal insufficiency in ALD (Kaltsas *et al.* 2000). Diagnosis of adrenal insufficiency tends to be delayed; patients often present with nonspecific symptoms such as fatigue, loss of appetite, weight loss, nausea, vomiting, and abdominal pain. Moreover, the loss of adrenal function is a gradual, progressive phenomenon, and the disease is difficult to suspect in early stages. Skin hyperpigmentation is a specific sign of adrenal insufficiency, as ACTH is a potent stimulator of melanogenesis in human melanocytes. Hsieh & White (2011) reported that hyperpigmentation was observed in 67% of primary adrenal-insufficient children; in our case, there was no hyperpigmentation. Hyperpigmentation is thought to increase with the advance of adrenal insufficiency; nonoccurrence of hyperpigmentation does not exclude diagnosis of adrenal insufficiency. It is therefore important to test for adrenal insufficiency by measuring the levels of ACTH and cortisol in repeated illnesses of unknown origin. If the plasma ACTH is higher than 22 pmol/L, there is a high possibility of primary adrenal insufficiency (Oelkers *et al.* 1992).

It is well known that ALD exhibits intrafamilial phenotypic variability; there is no correlation between *ABCD1* mutations and the clinical phenotypes (Kemp *et al.* 2001). So far, two pedigrees were reported to have the Y174S mutation of ABCD1. One Spanish patient with *de novo* Y174S mutation was reported to have childhood cerebral ALD. Japanese patients (brothers) with the mutation showed childhood cerebral ALD and adolescent cerebral ALD (Barcelo *et al.* 1995; Shimozawa *et al.* 2011). On the other hand, our case with the mutation presented a milder adult form of ALD, AMN. The reason for the heterogeneity remains unclear, but several genetic and environmental factors are likely involved. In the present case, family history was significant for suspicion of AMN. It is possible that information on affected family members is limited due to death in young children. Careful inquiry into the family's history is therefore important.

It has been reported that injection of intrathecal baclofen is effective for spasticity (Chu *et al.* 2001). If the spasticity increases in the future, implant of an infusion pump for intrathecal baclofen should be considered, even though its effect in preliminary treatment was limited. Regarding testicular dysfunction, there are few reports. It has especially significant implications on health and quality of life, as AMN patients develop the disease in their twenties. In this case, intramuscular injection of testosterone enanthate was ineffective, possibly due to the patient's normal range of serum free testosterone. Because there is a possibility that this might decrease later, careful follow up is required. Other case reports also find gynecomastia and balding from testicular dysfunction (Suryawanshi *et al.* 2015; Konig *et al.* 2000). In addition, the progress of dysuria imputable to bladder dysfunction has to be watched closely.

In this case, CML developed almost concurrently with AMN. However, there is no evidence of a relationship between AMN and CML; this is the first report of



Supplemental Fig. 1. Immunofluorescence analysis of ABCD1 expression in the fibroblasts. In control fibroblasts (A), ABCD1-positive dots were co-localized with PMP70-positive dots. In the patient's fibroblasts (B), ABCD1-positive dots were hardly detectable. Bar=20 μm.

the complication. This is interesting because HCT is effective in both cerebral ALD and CML. More accumulation of AMN cases is necessary to clarify whether the two diseases develop at the same time.

In conclusion, physicians should be aware of the varying symptoms of AMN and check the level of VLCFAs in patients with primary adrenal insufficiency, especially those complicated with neurological dysfunction. Successive MRIs of brain and spinal cord for the proband is necessary to monitor the development to cerebral ALD. Women who have borne carriers of the ABCD1 mutation may be especially affected, as more than 80% of them develop signs and symptoms of myelopathy, but without adrenal insufficiency or cerebral demyelination, by the age of 60 years (Engelen *et al.* 2014). As the level of the mother's VLCFAs is only slightly elevated, periodic neurological examination will make early detection of the onset of AMN possible and facilitate her medical treatment.

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Disclosure statement

The authors declare no conflict of interest relevant to this study.

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