

Case Report

Novel *SLC16A2* mutations in Chinese patients with Allan-Herndon-Dudley Syndrome

Jiaping Wang^a, Qingping Zhang^a, Shujie Yu^b, Xiru Wu^a and Xinhua Bao^{a,*}

^a*Department of Pediatrics, Peking University First Hospital, Beijing, China*

^b*Department of Neurology, Harbin Children's Hospital, Harbin, Heilongjiang Province, China*

Abstract. We aimed to delineate the clinical profiles of Chinese patients with Allan-Herndon-Dudley Syndrome (AHDS), an X-linked intellectual disability caused by *SLC16A2* mutation and affecting males only. Clinical features of five males from four Chinese families, who manifested with severe cognition impairment, developmental delay, hypotonia accompanied by dystonia, were described and summarized. All of them displayed a distinctive thyroid hormone change, with increased FT₃, reduced FT₄, and normal TSH. Three of them had neuroimaging tests and all presented with hypomyelination. Two of them demonstrated some facial anomalies, including long face, narrow forehead and tent mouth. Targeted next-generation sequencing associated with intellectual disability was performed. Four *SLC16A2* mutations (c.916C>T, p.Gln306*; c.61G>T, p.Glu21*; c.695_699delATGGT, p.Asn232Sfs*7; c.42delC, p.Trp15Glyfs*69) were identified, of which three *SLC16A2* mutations were novel. Our findings expand mutational spectrum of *SLC16A2* and provide support for delineating the clinical features of Chinese patients with AHDS. In addition, this is the first report of AHDS in Chinese cohort. We recommend that male patients with intellectual disability, developmental delay and severe hypotonia, especially those with distinctive thyroid hormone change should be tested for *SLC16A2* mutations.

Keywords: Allan-herndon-dudley syndrome, X-linked intellectual disability, thyroid hormone, *SLC16A2*

1. Introduction

It is generally known that thyroid hormone (TH) is important for the growth and development of different tissues, especially the brain [1, 2]. It is established that TH is involved in the regulation of expression of hundreds of genes in primary astrocytes [2]. Thyroid hormone has two forms: T₄ (3,3',5,5'-tetraiodothyronine) and T₃ (3,3',5-triiodothyronine). TH activity is mediated through the binding of T₃ to nuclear thyroid hormone receptors (TRs). The active sites of the TRs are located intracellularly. Consequently, TH has to cross multiple membranes in order to reach the nuclear receptors. It was originally assumed that TH enters target cells by passive diffusion because of its lipophilic structures. However, it has become increasingly clear that cellular influx and efflux of TH are facilitated by transmembrane protein transporters [3, 4]. Several transporter families have been identified, of which monocarboxylate transporter 8 (MCT8) deserves special attention because of its role in disease [5].

*Corresponding author: Xinhua Bao, M.D., Ph.D., Department of Pediatric Neurology, Peking University First Hospital, No 1, Xi'anmen, Dis Xicheng, Beijing, China. Tel./Fax: +86 010 83573238; E-mail: zwhang@pku.edu.cn.

MCT8 protein is encoded by the *SLC16A2* gene, which is located on chromosome Xq13.2. The protein has a wide tissue distribution, such as liver, kidney, pituitary and thyroid gland, as well as brain [5]. *SLC16A2* mutations have been associated with Allan-Herndon-Dudley syndrome (AHDS) (OMIM #300523), an X-linked disease affecting males only, characterized by abnormal thyroid hormone level and severe neurological deficits, with axial hypotonia, spastic quadriplegia, impaired or absent speech, and muscle hypoplasia [5]. Here we describe five males from four Chinese families with *SLC16A2* gene mutations.

2. Case presentation

All five patients were male, of whom the age ranged from 8 months and 19 days to 8 years and 9 months. Case 1 and Case 2 were siblings from one Chinese family. The pregnancy was uneventful for all of them. They all presented with severe psychomotor retardation, and never achieved head control, sitting, crawling, standing, or verbal communication. They lacked autonomic activities, had no speech and showed poor response to the outside. Hypotonia accompanied by dystonia was another marked feature observed in all patients. The parents described that when excited or crying, they always became rigid like a stick or developed a twisted posture, but became floppy when relaxed or sleeping. Two of them displayed long face, narrow forehead and cheeks, opened mouth, thick lips, tented upper lips and large ears (Fig. 1). There was no significant regression during the course of the disease in our patients.

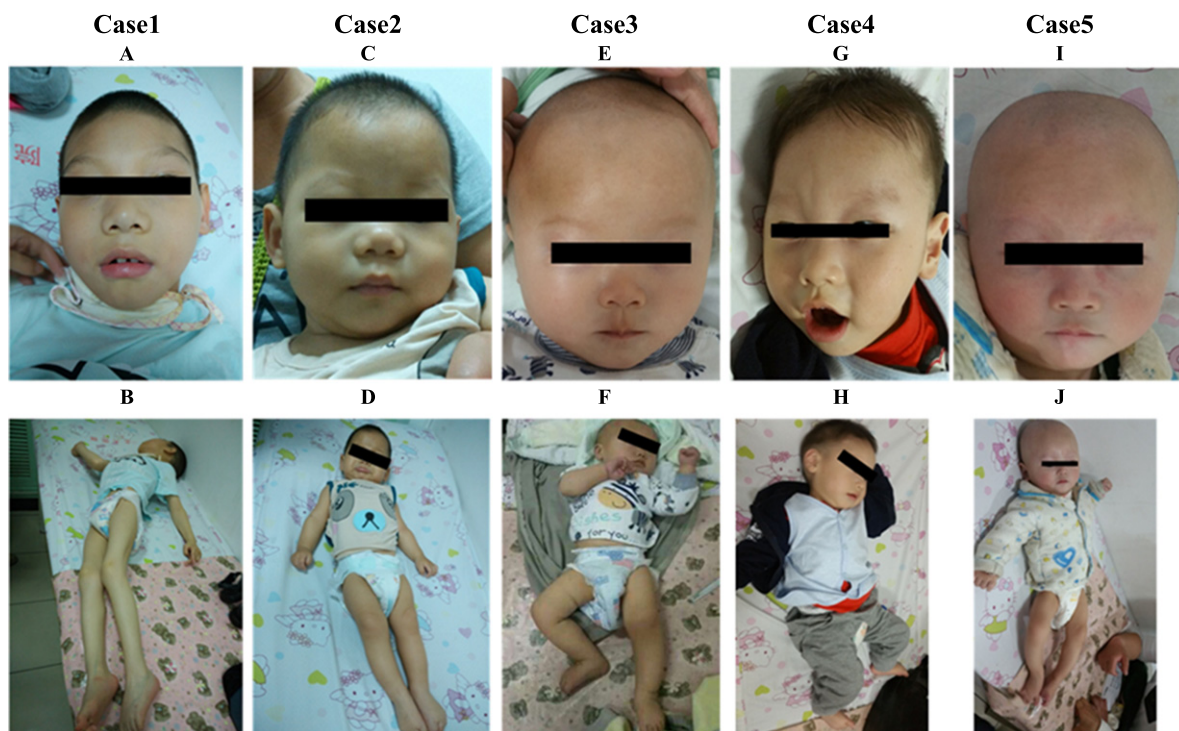


Fig. 1. Pictures of 5 AHDS males in our study. A and G show that patients (Case 1 and 4) had facial appearance, including elongated and myopathic face, prominent eyes, narrow forehead, narrow cheeks, bigger ears, tented upper lip. C, E and I show that the patients (Case 2, 3 and 5) had normal appearance. B and J showed patients (Case 1 and 4) displaying twisted posture. Muscle hypoplasia was also obvious in Case 1.

Table 1
Thyroid hormone levels of five patients and two heterozygous carrier in our study

Patient	Case 1 (8 y, 9 mo)	Case 2 (9 mo)	Case 3 (8 mo)	Case 4 (8 mo)	Case 5 (7 mo)	Het 1	Het 2	Reference range
TT ₃ (nmol/L)	3.32	3.32	4.64↑	5.34↑	5.17↑	2.10	1.71	1.6–4.3
FT ₃ (pmol/L)	7.77 ↑	8.61↑	7.01↑	13.75↑	10.31↑	5.13	5.76	3.50–6.50
TT ₄ (nmol/L)	54.20↓	31.30↓	58.80↓	43.52↓	48.6↓	101.6	84.9	82.4–171.2
FT ₄ (pmol/l)	10.67↓	8.45↓	10.13↓	7.12↓	7.99↓	13.68	14.99	11.48–22.70
TSH (μIU/ml)	3.56	3.38	3.38	4.96	4.86	2.79	1.27	0.64–6.27

y, years; mo, months. Het 1, mother of Case 1 and Case 2. Het 2, mother of Case 5.

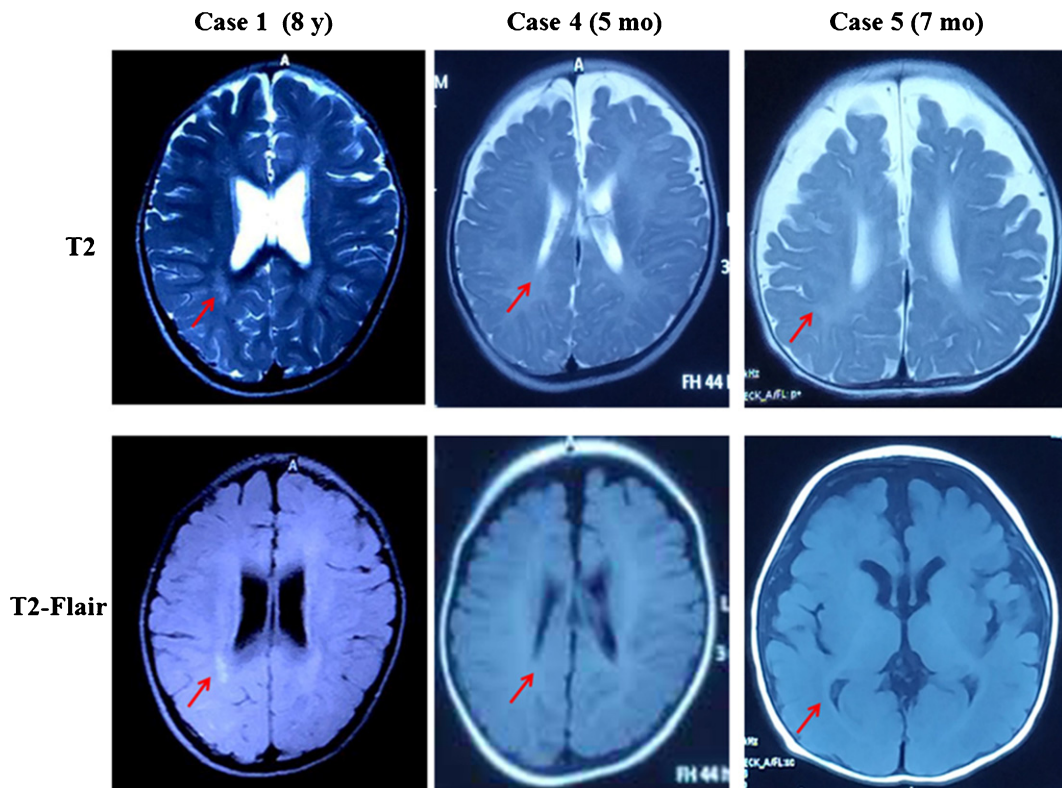


Fig. 2. MRI of Case 1, 4 and 5. The red arrow points to delayed myelination of white matter.

Laboratory tests including liver and renal function, lactic acid, creatine kinase, and ammonia were normal. Metabolic screening including plasma amino acids, acylcarnitine profile and urine organic acids were unremarkable. Thyroid hormone test revealed a distinct set of changes in all 5 patients, including increased FT₃, decreased FT₄, and normal TSH (Table 1). Thyroid hormone level was normal in two female carriers in this study. All three patients who had neuroimaging demonstrated delayed myelination (Fig. 2). Three out of five patients had sinus tachycardia on electrocardiography (ECG).

Through targeted next-generation gene sequencing panel consisting 264 genes (supplemental material 1) associated with intellectual disability, four mutations were identified in *SLC16A2* gene in four families; three of these mutations were novel. These *SLC16A2* mutations (c.916C>T, p.Gln306*; c.61G>T, p.Glu21*; c.695_699delATGGT, p.Asn232Sfs*7; c.42delC, p.Trp15Glyfs*69) included two

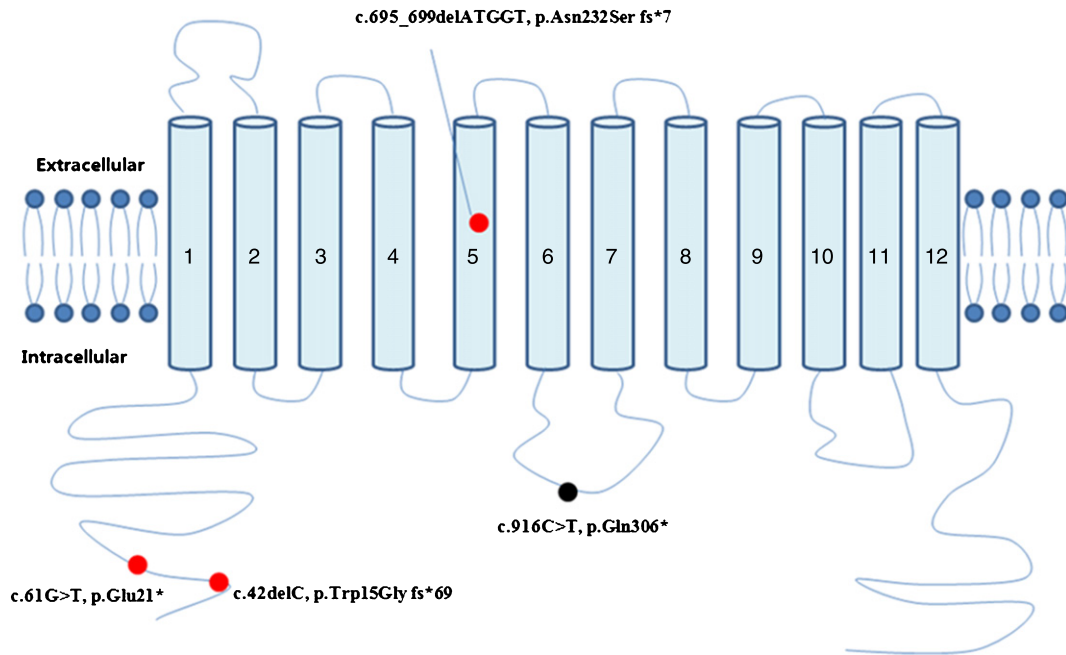


Fig. 3. Schematic representation of the MCT8 protein and the locations of mutations found in this study. Red dot: novel mutations. Black dot: previously reported mutations.

nonsense mutations and two micro-deletions. Two of these four *SLC16A2* mutations were *de novo*, and the other two were of maternal origin. A schematic representation of the MCT8 protein, including the mutations identified in this study is shown in Fig. 3.

The detailed clinical information and mutations of five patients were summarized in Table 2.

3. Discussion

In this study, four *SLC16A2* mutations were identified, including three novel mutations, which provided support for delineating the clinical features of Chinese patients with *SLC16A2* mutations. In addition, this is the first report of AHDS in Chinese cohort.

Due to the deficiency of MCT8, caused by *SLC16A2* mutations, T_3 cannot enter the neurons. The reduced uptake of T_3 into neurons results in T_3 accumulation in serum. TH has two forms: T_3 and T_4 . T_3 is the active form of TH. In liver, T_4 is converted to T_3 by type 1 iodothyronine deiodinase (D1) [6]. The elevated serum T_3 level causes liver hyperthyroidism and positively regulates *DIO1* gene expression, which consequently increases D1 activity. This results in increased production of T_3 at the expense of T_4 , which further enhances the serum T_3 accumulation and causes the consumption of serum T_4 [7]. In addition, reduced TH entry into the paraventricular nucleus of hypothalamus and the pituitary gland impairs the negative feedback loop of the hypothalamic-pituitary axis [8]. As a result, AHDS patients display a characteristic thyroid hormone profile with increased FT_3 , decreased FT_4 and normal TSH. Thyroid hormone changes in our patients were in accordance with this characterization. As AHDS is an X-linked disorder, which affects males only, female carriers are usually asymptomatic, but increased serum FT_3 was described in 25% of female carriers [8]. In our study, two female carriers had normal thyroid hormone level.

Thyroid hormone is vital for normal brain development, especially within the first years of life. MCT8 deficiency results in thyroid hormone deprivation in the brain. Hypothyroidism in the central

Table 2
Clinical information of 5 patients with AHDS

Patients	Case 1	Case 2	Case 3	Case 4	Case 5
Age	8 y, 9 mo	9 mo	8 mo, 19 d	1 y, 3 mo	9 mo
Hypotonia	+	+	+	+	+
Dystonia	+	+	+	+	+
Motor development	Severe delayed	Severe delayed	Severe delayed	Severe delayed	Severe delayed
Cognition	Severe delayed	Severe delayed	Severe delayed	Severe delayed	Severe delayed
Speech	No speech	No speech	No speech	No speech	No speech
Weight	17.2 kg (<-3SD)	7.6 kg (-1SD~-2SD)	9 kg (Normal)	9.5 kg (-1SD)	8 kg (-1SD~-2SD)
MRI	demyelination (8 y, 9 mo)	-	-	demyelination (5 mo)	demyelination (7 mo)
ECG	sinus tachycardia (161//min, Ref: 80-100/min)	sinus tachycardia, (142/min, Ref: 110-130/min)	Normal (115/min, Ref: 110-130/min)	Normal (106/min, Ref: 110-130/min)	sinus tachycardia (164/min, Ref: 110-130/min)
BMD	Normal	Normal	-	Normal	-
Hyperreflexia	+	+	+	+	+
Babinski	+	+	+	+	+
<i>SLC16A2</i> mutation	c.916C>T, p.Gln306*	c.916C>T, p.Gln306*	c.61G>T, p.Glu21*	c.695_699delATGGT, p.Asn232Ser fs*7	c.42delC, p.Trp15Gly fs*69
Origin	Maternal	Maternal	<i>De novo</i>	<i>De novo</i>	Maternal
Reported/Novel	R	R	N	N	N

y, years; mo, months; SD, standard deviation; MRI, magnetic resonance imaging; Ref, reference; ECG, Electrocardiograph; BMD, bone mineral density; N, novel; R, reported.

nervous system leads to severe neurological symptoms, including psychomotor retardation, hypotonia, dystonia, and lack of speech [5]. However, other tissues such as liver, kidney, and muscle, which do not depend exclusively on MCT8, are exposed to toxic levels of T₃. The hyperthyroidism in the peripheral tissue leads to low body weight, tachycardia, sleeplessness, and reduced muscle mass [5, 9]. All five patients of our study presented with severe psychomotor stagnation, axial hypotonia and dystonia. Hyperthyroidism as well as muscle weakness, muscle hypoplasia and low weight were more obvious in the older patients (Case 1 and Case 4). Sinus tachycardia, which is attributed to the peripheral hypermetabolism was detected in three of five patients.

Besides, it has been reported that affected males usually have distinct facial features including elongated and myopathic face with prominence of the eyes, synophrys and prominence of the lower lip [5]. In our study, Case 1 and Case 4 (aged 8 years and 9 months, 1 year and 3 months, respectively) had the similar facial characteristics, while the facial features of the other three younger patients were unremarkable, suggesting facial features change as patients get older.

Hypomyelination on brain MRI is common in AHDS. Markedly delayed myelination had been reported in some patients [10-12]. Three patients in our study displayed hypomyelination on MRI. Therefore, AHDS should be considered in males with severe psychomotor retardation, increased T₃ and decreased T₄ and hypomyelination on brain MRI.

To date, more than 80 pathogenic mutations in *SLC16A2* have been registered in the Human Gene Mutation Database (HGMD) and some genotype-phenotype correlations are recognized [13]. Patients with Ser194Phe, Lys568Pro, Lys434Trp mutations had milder psychomotor retardation in comparison to patients with other mutations. They had fairly well-preserved fine motor skills and were able to

walk with some support. Some of them even developed some dysarthric speech. *In vitro* function analysis demonstrated that the mutated protein possessed residual function of T₃ uptake [14]. Other mutations (for example, Val235Met, Ser448*, Phe229del, Lys471Pro and Lys512Pro) have previously been reported to result in complete loss of MCT8 function. *In vitro* functional analysis showed that these mutations almost had no functions, they either lost the capability of trafficking to the plasma membrane, or lost the affinity to substrate. Meanwhile, the expression of mutant protein was profoundly reduced. Therefore, compared with mutations with some residual functions, these mutations lead to more severe clinical outcome [13]. The four *SLC16A2* mutations found in our study lead to a premature termination of MCT8 protein, losing the last several TMDs and the cytoplasmic region at the carboxy end of the protein. The truncated MCT8 protein might lose most of its functions, leading to the severe phenotype of our patients.

So far, there are no effective therapies for AHDS. Some patients were treated with propylthiouracil (PTU) in combination with Levothyroxine (LT4). The thyrotoxic condition in peripheral organs could be improved. However, there was no benefit on the severe cognition impairment [15–17]. The key in the treatment of AHDS is to supply appropriate amount of intracellular thyroid hormone, bypassing MCT8 during the brain developmental period. Some patients were treated with Diiodothyropropionic Acid (DITPA), a TH analog, which could enter the neurons independent of MCT8 transporter activity. However, no intellectual improvement was seen in these patients [18]. A recent study found that 3,3',5-triiodothyroacetic acid (TRIAC) can restore neuronal differentiation in the hypothyroid brain of *Mct8/Oatp1c1*-double knockout mice, which replicates the clinical picture of human MCT8 deficiency [19]. TRIAC has been used in patients with thyroid cancer and with resistance to TH [20]. In Erasmus Medical Center in Rotterdam, Netherlands there is an ongoing clinical trial treating AHDS patients with TRIAC (<https://clinicaltrials.gov/ct2/results?cond=ALLAN-HERNDON-DUDLEY%20SYNDROME>). No study results have been reported so far. The clinical use of TRIAC in AHDS patients needs further studies.

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