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Original Article

Genetic analysis of four cases of methylmalonic aciduria and homocystinuria, *cblC* type#

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Abstract: Methylmalonic aciduria and homocystinuria, *cblC* type, is the most common disorder of intracellular vitamin B12 (cobalamin, *cbl*) metabolism, which results in impaired biosynthesis of methylcobalamin and adenosylcobalamin. The gene MMACHC responsible for the *cblC* type had been identified, which enables molecular diagnostics. Here, we report four *cblC* type cases, which were identified by the typical manifestations, and a new approach of next-generation sequencing platform in pediatrics for genetic diseases, further confirmed by Sanger sequencing of the whole MMACHC gene. The article will replenish the mutational information of related genes to the cblC type, which makes for detecting of cblC disease through the newborn screening.

Keywords: Methylmalonic aciduria and homocystinuria, MMACHC, mutation, vitamin B12 (cobalamin, *Cb1*)

Introduction

Combined methylmalonic aciduria and homocystinuria, cblC type (MIM# 277400), is autosomal recessive mode of inheritance. It is claimed to be the most common inborn error of intracellular cobalamin metabolism, caused by mutations in the MMACHC gene located in chromosome region 1p34.1 with five exons [1]. Individuals with cblC deficiency do not synthesize AdoCbl and MeCbl, cofactors for the methylmalonyl-CoA mutase and methionine synthase enzymes, and display methylmalonic aciduria and homocystinuria. The patients with cblC disease display a wide spectrum of clinical manifestations including feeding difficulties, failure to thrive, hematologic, neurologic, metabolic (acidosis), ophthalmologic and dermatologic abnormalities. The cblC disease can develop severe complications despite treatment [2, 3]. The pathophysiology of cblC is not fully understood, but three important factors contribute to the disease-related complications. They are increased homocysteine concentrations, impaired methyl group metabolism, and oxidative stress [3, 4]; Characterization of the variation in the MMACHC gene can facilitate the prenatal and early diagnosis of cblC disease through expanded newborn screening, and increases the possibility to improve the outcome in patients with cblC disease. To date, the most prevalent mutation was c271dupA which account for the mutant alleles characterized in a crowd of cblC patients from around the world [1, 5, 6]. Here we report four cases with five mutations, including one novel change, and one case without any mutation in MMACHC gene. The full identification of mutations in the MMACHC gene is benefit for the detection of cblC disease.

Case reports

Case 1

A 4-month-old girl was admitted to hospital for light cognitive impairment and not be amused and vertical head instability. The patient presented aware consciousness, normal myodynamia but higher muscle tone by clinical examination. Pregnancy history described that it is the first child and first production. The new born has not fetal distress and asphyxia history. Related biochemical studies revealed that Lactic acid 5.4 mmol/L↑ (reference 4 mmol/L), blood ammonia 50 μmol/L (reference 20-60

Table 1. 48 genes related to organic acid metabolic disease

MUT	MMAA	MMAB	MCEE
MMACHC	MMADHC	LMBRD1	PCCA
PCCB	MTHFR	MTRR	CBS
MTR	PAH	PTS	GCH1
QDPR	PCBD1	GLDC	GCSH
MAT1A	HAL	IVD	HGD
PRODH	ALDH4A1	MCCC1	MCCC2
HIBCH	HMGCL	AUH	L2HGDH
DNAJC19	MVK	BTD	HLCS
D2HGDH	L2HGDH	GCDH	BCAT2
ETFA	FAH	TAT	HPD
ETFB	ETFDH	HMGCL	UGT1A1

μmol/L). Cranial MRI enhancement scanning displays the result of hydrocephalus. EEG showed atypical hypsarrhythmia. Plasma amino acids analysis by tandem mass spectrometry (MS/MS) showed higher homocysteine, citrulline/arginine, propionyl-L-carnitine, C3; propionyl-L-carnitine/free carnitine, C3/C0; propionyl-L-carnitine/acetyl-carnitine, C3/C2; and octanoyl-canitine/kawi-acyl-carnitine, C8/C12. Besides, organic acid test of urine by MS/MS showed an excessive urinary excretion of methylmalonic acid 18076 times of average level which supported methylmalonic aciduria (reference range < 2 mmol/mol). Besides, plasma homocysteine was 260.64 μmol/L, higher than the normal level (reference range 1-20 μmol/L in blood). Combined the two characteristic biochemical parameters and clinical manifestations, the patient was diagnosed as methylmalonic aciduria and homocystinuria.

Case 2

An 11-year-old boy was admitted to hospital because of "intermittent headache, spasm, somnolence, paralysis of facial features". The patient is with aware consciousness. The skull CT demonstrated that bilateral lateral ventricle triangle choroid plexus slightly punctuated with high density of shadow. Cranial MRI enhancement scanning was normal. EEG showed slow background activity. The results of plasma total homocysteine and urine organic acid analysis are homocysteine of 396.84 μmol/L and methylmalonic acidria of 2058 times of average level respectively, both elevated far more than the normal level. Plasma amino acids analysis showed higher Hcy, C3/C0, C3/C2, C8/C2 and C8/C12, similar to the status of case 1.

Case 3

A 9-month-old girl was admitted to hospital. She can't sit alone, pronounce summation tone and has poor eyesight. Cranial MRI enhancement scanning was cerebral dysplasia. EEG showed hypsarrhythmia. Blood screening test showed Lactic acid 5.4 mmol/L↑. Plasma homocysteine was 174.22 μmol/L. Urine screening test showed. Methylmalonic aciduria (MMA 5066 times increased), and after 4 days of injection Cobamamide, MMA 526.5 times decreased.

Case 4

A 9-month and 19-days-old boy presented sitting instability, intermittent lethargy, somnolence, and vomited for 9 months. The EGG video showed that slow background activity with DQ41. The patient presented aware consciousness, weak myodynamia and muscle tone of four limbs by clinical examination. Plasma amino acid test showed higher C3, C3/C16, and homocysteine of 27.13 μmol/L. Methylmalonic aciduria increased to 15601.8 times of the normal level by urine organic acid test.

Genomic DNA analysis

Since the publication of the first graft of the human genome sequence, the field of genomics has been changed dramatically; and with the development of high-throughput methods that could be used to interrogate the wealth of data available in the human genome. In the present study, we used 48 genes related to organic acid metabolic disease (**Table 1**), including: MUT, MMAA (*cblA*), MMAB (*cblB*), MMACHC (*cblC*), MMADHC (*cblD*), LMBRD1 (*cblF*) and other genes. This technology combined the designed gene capture probe and customer DNA library. The gene fragments were hybridized to the probe, and adsorbed to the beads through biotin and streptavidin-biotin; and then the nonspecific binding DNA fragments were washed out. Finally the captured target DNA fragment was identified by new-generation sequencing platform in pediatrics, which can quickly distinguishe the targeted gene (MMACHC) and determine its mutation locus. In order to avoid the false positive errors. The pathogenic mutation was further confirmed by Sanger direct sequencing the whole target gene.

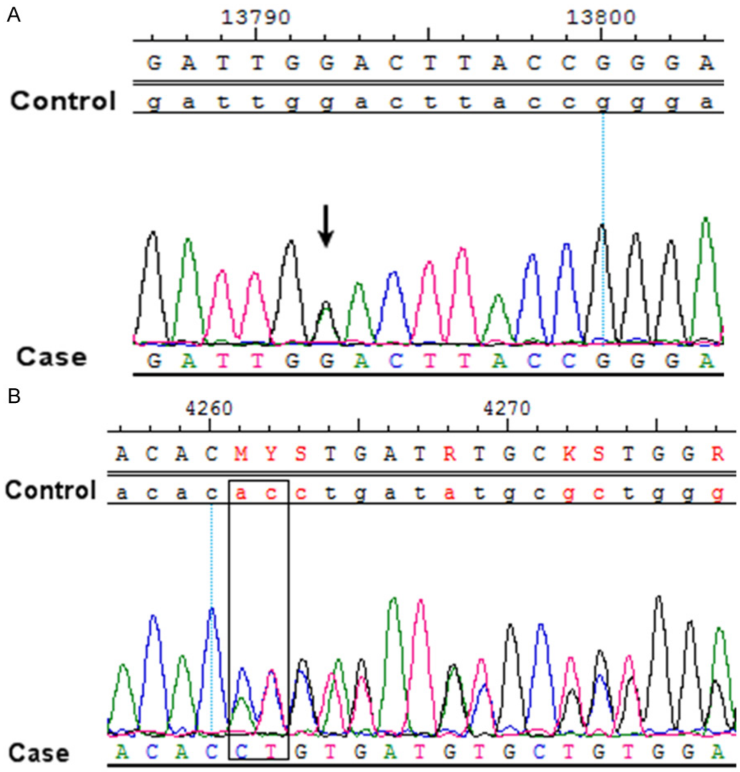

Figure 1. MMACHC gene mutations of case 1. (point mutation is indentified by arrow and deletions and insertions are marked by black frame).

Discussion

Individuals with *cblC* disease caused by mutations in MMACHC gene have aroused attentions from all over the world. While most mutations are private, clear ethnic observations have been made for more common mutations. Clinical heterogeneity in *cblC* disease has been clearly started by Morel et al [6]. The mutations of MMACHC gene can lead to onset of *cblC* disease at different age.

Herein, we have applied a facility to distinguished mutated genes, which can carefully sequence the MMACHC gene in affected individuals and has a greater than 95% chance of definitively confirming the diagnosis of a suspected case of *cblC* disease.

We reported four cases here, the first three cases were treated with betaine, folate, lysine inosite, and intramuscular cyanocobalamine (vitamin B12). On the 11th day, the patients showed no headache and lethargy, no vomiting and other main symptoms. In addition, the biochemical parameters in plasma were improved clearly. In these cases, low homocysteine level after treatment of B12 was observed which indicates that the patient is responded to B12, a characteristic of the *cblC* disease type of combined methylmalonic aciduria and homocystinuria. However, the fourth case showed no response to treatment of vitamin B12. His mum did not showed lack of Vitamin B12 during pregnancy. All gDNA samples of these four cases were checked for molecular diagnostics.

In the first case, two mutations c.609G>A p.W203Term (**Figure 1A**) and c.440_441del (**Figure 1B**) were identified in the MMACHC gene; the deletion mutation (c.440_441del) sited on the binding domain of cyanocobalamine has not been reported previously for resulting in cblC disease. In the second case, two mutations in gene MMACHC were identified, one insertion mutation c.567_568insT p.Ile190fs (**Figure 2A**) in exon 4 lead to the frame shift and early termination of its allele. The other mutation is c.482G>A p.R161Q (**Figure 2B**) which has been reported in previous cases disease [1, 7]. In third case, one pair of homozygosis mutation was indentified, c.609G>A p. W203Term (**Figure 3**). Similar to the finding was reported by Lerner-Ellis et al. It is indicated that this mutation c.609G>A p.W203Term is not ethnic. In the fourth case, we did not found any mutation of his MMACHC gene, but this patient displays clinical manifestations of cblC type. Therefore, we speculate that the mutation might occur in the regulatory regions of MMACHC gene. In order to illustrate the real reason, we should evaluate the expression level of MMACHC protein and determine the biochemical characteristic of its activity in fibroblast. It is regrettable that the patient is gone and no sample was available.

Although much experience is available [8-10], the optimal mode of treatment of cblC disease remains unclear. Finding best method to detect and choosing the optimal treatment to cblC dis-

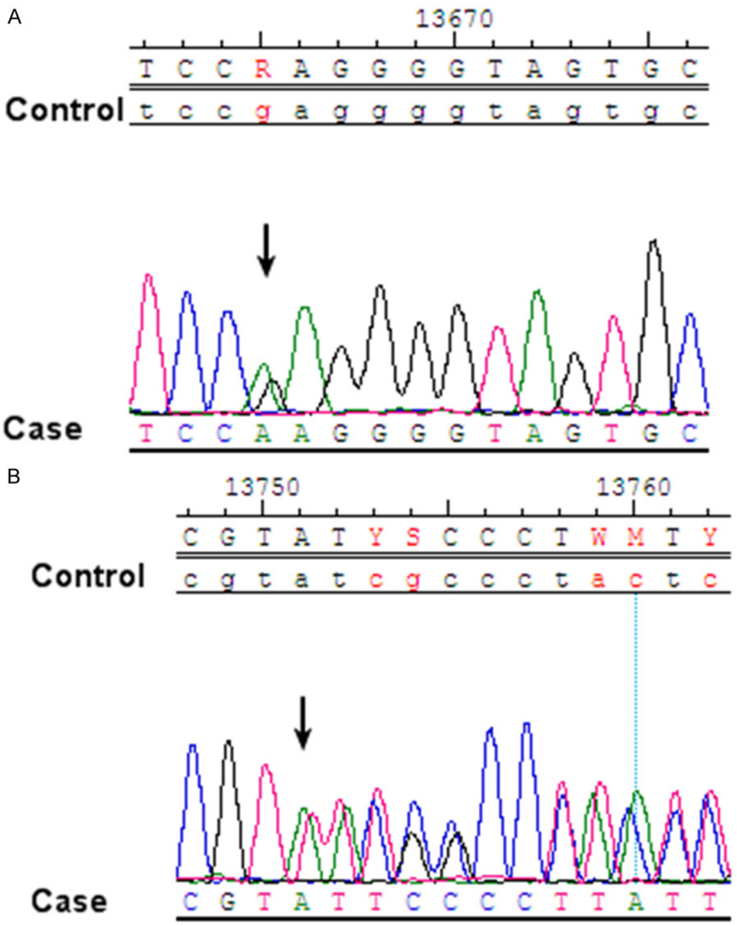

Figure 2. MMACHC gene mutations of case 2 (point mutation is indentified by arrow).

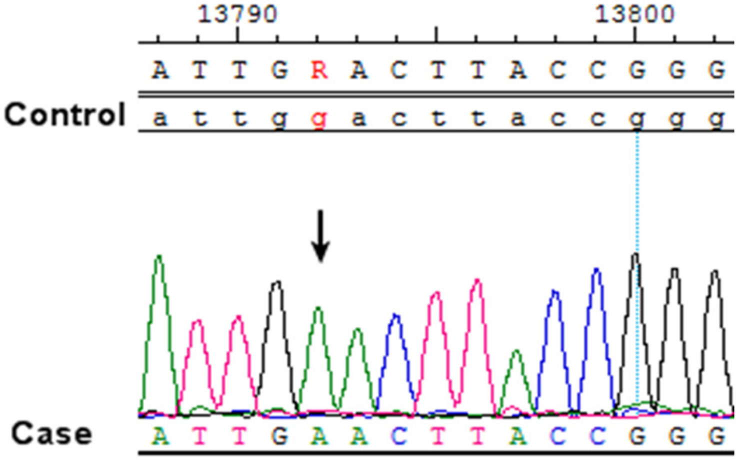

Figure 3. MMACHC gene mutations of case 3 (point mutation is indentified by arrow).

ease become especially important now. The cbIC disease can be detected symptomatically by spreading the routine newborn screening [11]. In conclusion, this information will be useful for identifying different cases related to gene MMACHC and would contribute to get insight into cbIC disease.

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Disclosure of conflict of interest

None.

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