Clinical and mutational spectrum of 4 Chinese families with glutaric aciduria type 1

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Xiaorong Shi  xswxsw2016@163.com
The First Affiliated Hospital of Fujian Medical University
Corresponding Author
ORCID: 0000-0002-0910-0660

Qiaoyan Shao
The First Affiliated Hospital of Fujian Medical University

Aidong Zheng
The First Affiliated Hospital of Fujian Medical University

Wenghuang Xie
The First Affiliated Hospital of Fujian Medical University

DOI: 10.21203/rs.2.13326/v1

SUBJECT AREAS
Medical Genetics

KEYWORDS
Polymerase chain reaction (PCR); Glutaric aciduria type I (GA-I); Mutation spectrum;
Abstract

Background To investigate clinical presentation and molecular aspects of five patients suffered from glutaric aciduria type I (GA-I), a rare neurometabolic disorder caused by glutaryl-CoA dehydrogenase deficiency due to GCDH gene mutations. Methods All five patients were diagnosed by elevated urinary glutaric acid and GCDH gene analysis. Low protein diet supplemented with special formula, GABA analog and L-carnitine were initialed following laboratory confirmation of diagnosis. The clinical and biochemical features were analyzed, and mutational analysis of GCDH was conducted using Sanger sequencing. Results Clinical manifestations of 5 patients varied from macrocephaly to severe encephalopathy, with notably different phenotype between siblings with the same mutations. Three members present complex heterozygous mutations, while two sisters present homozygous mutations. Among them, four mutations in GCDH were identified (c.1133C>T c.1244-2A>C c.339delT c.406G>T). Of these four mutations, c.1244-2A>C was found in four patients and c.339delT and c.1133C>T has not yet been reported until now. Conclusions In 5 Chinese patients with GA1, two novel mutations of GCDH gene were identified, which may expand the mutation spectrum of GCDH gene. What we found confirm that there is no correlation between clinical phenotype and genotype in GA-I patients, and c.1244-2A>C may be mutation hotspot in Southern China.

Background

Glutaric acid type 1 (GA-I , OMIM 231670) is an autosomal recessive disorder caused by glutaryl-CoA dehydrogenase (GCDH) deficiency due to GCDH gene mutations. Glutaryl-CoA dehydrogenase deficiency affects the metabolism of lysine, hydroxyl lysine and tryptophan, which triggers glutaric acid and 3-hydroxyglutaric acid accumulation[1-2]. Glutaryl-CoA is esterified with carnitine by carnitine acyltransferase, resulting in increased
glutaryl carnitine and secondary carnitine deficiency in plasma, and considered to be the significant changes biochemical in biochemical metabolism. In the early stage, the main symptoms were those of macrocephaly and mild non-specific nerve system disorders, which were easily ignored. In most cases, the initial episode of acute encephalopathy crisis induced by infection or vaccination, mainly manifested as motor dysfunction, dystonia, convulsions, coma and other neurological disorders, occurs between ages 6 to 18 months after a normal initial development. And it may cause severe neurological sequela such as non-reversible basal ganglia lesions, extra-pyramidal symptom and cerebral atrophy. Fortunately, early diagnosis and early treatment could slow the progression of encephalopathy. That is why low tryptophan diet combined with oral supplementation of L-carnitine to correct metabolism disorder timely is very important. It should be noted that urine organic acids in non-acute episode is normal in some GA-I patients, and the genetic analysis of GCDH is important for diagnosis. GCDH gene encodes glutaryl-CoA dehydrogenase, which spans approximately 7kb of the chromosome 19p13.2 and is composed of 12 exons, 11 of which are coding exons. More than 200 mutations in GCDH have been reported worldwide, however, there is no evident genotype-phenotype relationship in GA-I.

In this study, we report five Chinese cases of glutaric aciduria type I who were clinically diagnosed of an accumulation of glutaric acid and 3-hydroxyglutaric acid and confirmed by Sanger sequencing, the clinical, biochemical, and GCDH mutation profiles of them were investigated.

Subjects And Methods

2.1. Subjects

In this study, five GA-I patients (4 females, 1 male) from the First Affiliated Hospital of Fujian Medical University from 2013 to 2015 were enrolled. The onset age was from 5
months to 27 months. All five patients presented macrocephaly, of these, four cases suffered from fever and diarrhea followed by drowsiness and dystonia, and the other one suffered from recurrent seizure after trauma (Table1). All the five patients suffered from variant basal ganglia lesions, of these, three had subdural hematoma and three had subarachnoid cyst.

The parents of all five enrolled cases were healthy and non-consanguineous. For the genetic analysis, 220 DNA samples from healthy Chinese volunteers were simultaneously collected as normal controls. This study was approved by the Ethics Committee of The First Affiliated Hospital of Fujian Medical University. And written informed consents were obtained from all patients.

2.2 Routine tests, metabolic analyses and image examination

No obvious abnormality was observed in blood routine, urine routine, stool routine tests, and clinical biochemistry test. In addition, brain Magnetic Resonance Imaging (MRI) scan was also performed (Table1). Urinary organic acid levels were analyzed with gas chromatography-mass spectrometry (GC/MS) (Shimadzu GC/MS QP2010). Analysis of blood amino acids and acylcarnitines was performed using an Applied Biosystems API 3200 MS/MS analyzer, the significant elevation of glutaric acid in urinary and glutaryl carnitine and 3-hydroxyglutaric acid in blood were detected in our patients (Table 2).

2.3. Methods

2.3.1. Genomic DNA Extraction

Genomic DNA was extracted from peripheral blood leucocytes using peripheral blood DNA Extraction Kit (QIAamp, Germany).

2.3.2. GCDH gene analysis

The entire coding sequence of the GCDH gene was amplified by polymerase chain reaction (PCR) using primers designed through Primer Premier 5 software and were produced by
In 50 μl PCR reaction system: containing 0.2 mol/L primers, 100 μmol/L of each dNTP, 10 mmol/L Tris-HCl, 50 mmol/L KCl, 1.5 mmol/L Mg^{2+}, 0.1X TritonX-100, 2U of Taq polymerase, and 50~100 ng DNA. Cycling conditions were as follow: 95℃ pre-denaturation for 7 min, then run 35 cycles of 94℃ denaturation for 30 s, target-specific annealing at 65℃ for 1 min, followed by a final extension step at 72℃ for 7 min. PCR products were preserved at 25℃. The purified PCR products were sequenced by Guang Zhou Jin Yu Medical Examination Center using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), and 220 healthy controls will be further screened by Sanger Sequencing to confirm these novel mutations. DNA sequencing results were analyzed using Mutation Surveyor V4.0.5 (demo) software (American Soft genetics company), and compared with GCDH gene sequence in NCBI database.

2.4. Treatments and follow-up

Only one patient failed to take metabolic analyses timely for the poor technical conditions more than a decade ago, and the other four patients were placed on a protein-restricted diet, supplemented with vitamin B2, L-carnitine, free tryptophan, special lysine milk powder. Even though, some patients received antiepileptic therapy for recurrent seizures, who were diagnosed as secondary epilepsy. Follow-up of all patients was performed regularly.

Results

3.1. Clinical features

In our series, all cases had young onset age (5-19 months), whose initial symptom varied in severity. Among of these, three cases were onset with encephalopathy, two were misdiagnosed as "viral encephalitis (Case 1)" and "purulent encephalitis "(Case 2), and accepted a long time anti-infectious treatment. Case 2 was admitted to hospital because
of macro-cephaly, and diagnosed as GA-I after testing blood amino acids and urine organic acid by GC-MS, and accepted treatment immediately. However, Case 2 got recurrent seizure, and the glutaryl carnitine was still high after one and a half year, the data of electroencephalogram (EEG) showed epilepsy discharge, so secondary epilepsy was diagnosed and the seizure was controlled after levetiracetam taken. Case 1, the elder sister of Case 2 was admitted to our hospital with drowsiness, malaise and hyper-myotonia when 9 months old. Case 1 was diagnosed as viral encephalitis, and got better but with sequela of dyskinesia after treatment for half a year. She failed to take metabolic analyses timely and get special treatment due to financial disadvantage. Now Case 1 was 20 years old with head circumference 58.5cm, suffered from claudication in left lower extremity, while the others were normal. Case 1 was just an average student in academic performance since childhood, and now attended a vocational school with average score. Urine organic acids and blood amino acids were analyzed in Case 1 twice (16.5 years old, 18 years old), and nothing abnormal detected. In addition, brain MRI Scan revealed that the subdural hematoma was absorbed, while the remaining lesions showed no significant abnormality.

Case 3 was admitted to local hospital for coma and vomit caused by trauma, and got better after symptomatic treatments. However, two months later, Case 3 went into convulsion, and was admitted to our hospital. Electroencephalogram (EEG) showed no significant abnormality, but brain MRI scan analysis met changed characteristics of GA-I, therefore, GC/MS for urinary organic acids and MS-MS for plasma acylcarnitine were advised for clinical diagnoses of GA-I, but the parents refused to accept further treatment. Subsequently, Case 3 was admitted to emergency department several times due to convulsions, her parents finally agreed to conduct GC/MS and MS-MS analyses eight months after onset, and the results supported the clinical diagnosis of GA-I. Convulsions
were effectively controlled after routine treatment.

Case 4 was diagnosed as “purulent meningitis”, whose first symptom was fever and convulsion, in addition, subdural hematoma was detected by cranial computer tomography (CT). When given with the treatment of anti-infection and reducing cranial hypertension for twenty three days, fever of Case 4 disappeared, but still remained convulsing. Brain MRI scan analysis met changed characteristics of GA-I, and GC/MS was used to diagnosis of GA-I definitely. Case 4, admitted to hospital again two months later because of persistent crying and irritability for three days, with higher glutaryl-carnitine than before indicated that the early treatment effect is not ideal. Nine month later, Case 4 suffered from anasarca for one month was admitted to our hospital once again. Biochemical test indicated multiple organ damage such as hypo-albuminemia, coagulation dysfunction, electrolyte disturbances and liver and renal damage, however, the parents refused to accept symptomatic treatment for Case 4, who died in a week.

Case 5, suffered from fever that lasted for two days and seizures for three times, was admitted to our hospital, and definitely diagnosed as GA-I. While treated with symptomatic treatment, the seizure of Case 5 was effectively controlled, and Case 5 developed normally during seven-month follow up.

3.2. GCDH gene mutations

Four mutations (c.1244-2A>C, c.406G>T, c.339delT, and c.1133C>T) were identified in different exons of GCDH gene (Table 2), two of which were novel (c.1133C>T and c.339delT) in all the five cases. Among of these five cases, there existed 2 homozygous mutations (c.1244-2A>C, c.1244-2A>C) and 3 compound heterozygous mutations (Figure 1).

All parents were carriers of pathogenic mutations of GCDH gene. Homology analysis revealed that the two novel mutations, which could not be detected in 220 healthy
volunteers, were highly conserved.

Discussion

GA-I is a rare neuro-metabolic disease with high morbidity and mortality. The incidence rate is about 1/100000\(^3\). This disease is caused by glutaryl-CoA dehydrogenase deficiency due to GCDH gene mutation, manifested as megalencephaly, dystonia, and athetosis. Megalencephaly is the earliest and the most common symptom found in 75% of GA-I patients, but not specific \(^4\text{-}^5\). Neurologic abnormalities often appear at the age of 6 to 18 months, with low intellectual impairment \(^6\). Recently, cognitive study on GA-I patients who had got early treatment revealed that these GA-I patients suffered from dysphonia \(^7\). Acute striatal necrosis, occurred frequently in infancy, is an important early symptom of GA-I, while infections, dehydration and delayed treatment are considered to be high risk factors. Early prophylactic treatment should be taken to reduce the morbidity of nervous system in children before two years old. However, in our study, case 4, a five-month-old baby boy accepted diet control and drug therapy when diagnosed as GA-I one month later after the onset, however, the effect was poor with malnutrition and multiple organ damage during the follow-up period, which was probably correlated with the severe basal ganglia lesion (Figure 2). Macrocephaly or mild infection may result in encephalopathic crisis, which may cause metabolic diseases. It is vital to analyze brain MRI manifestations to make initial diagnosis of GA-I.

Classic imaging characteristics of GA-I reveals fissured lateral cerebral, widened longitudinal fissure, decreased cerebral white matter, undeveloped fronto-temporal brain, temporal arachnoid cyst, bilateral lateral ventricular dilated and chronic subdural hematoma of temporal partial dura. Symmetric low signal on T1-weighted images and high signal on T2-weighted images and high signal intensity on FLAIR sequences could be found
in bilateral basal ganglia, thalamus, cerebral peduncles, and mainly in striatum \cite{8}. In our study, basal ganglia lesion appeared in all the five cases, and existed in three patients at onset. Arachnoid cyst and subdural hematoma were seen in brain MRI scans of case 1 and case 2 (case 1 and case 2 are sisters) when they were infancy. During the 16-month follow-up period, cranial MRI showed that subdural hematoma was absorbed and abnormal signal in basal ganglia also disappeared of case 1 with early diagnose and special treatment. While case 2 had no special treatment till now, although the cranial MRI showed subdural hematoma was gone, lesions in bilateral basal ganglia, periventricular white matter and corpus callosum still existed. It suggested that subdural hematoma disappeared even without special treatment. Abnormal striatal signal disappeared in case 1 with early treatment, nevertheless, case 2 still presented striatal degeneration.

Therefore, we thought early treatment may reduce brain damage. Notably, intracranial arachnoid cysts (IAC) combined with GA-I is significantly different from independent IAC. The former is usually bilateral, and accompanied with symptom of striatum and cerebral dysplasia. In addition, anesthesia and neurosurgery should be avoided on patients of IAC combined with GA-I for the poor tolerance of GA-I children.

Although the head CT and MRI scan may be specific methods to diagnose GA-I, the clinical diagnosis of GA-I mainly depends on the GC/MS for urinary organic acids and MS/MS for plasma acylcarnitine. Early GA-I patients are often neglected for their no obvious symptoms before clear diagnosis, consequently, early screening and early diagnosis are important \cite{9}. However, the urine organic acid analysis may appear false negative for the intermittent metabolites discharge in urine, that is, GA-I may not be detected in non-acute phase \cite{9}. In our study, four cases showed increased urinary glutaric acid, 3-hydroxy glutaric acid and glutaryl carnitine, and one case with no special treatment showed no
significant abnormality after carrying out GC/MS or MS/MS (at the age of 16.5 years and 18 years), which would be caused by false negative, or age dependent of GA-I.

GA-I is an autosomal recessive disorder, caused by glutaryl-CoA dehydrogenase deficiency due to GCDH gene mutations. There exists several different kinds of GCDH gene mutations, and hot mutation differs in races and regions. R402W was confirmed to be the most common mutation in Europeans \([10-11]\). E365K and c.1296C>T were the main mutations in Irish and Pennsylvania Amish respectively \([12]\). Mutation of IVS10-2A>C (c.1244-2A>C) in children was reported in Taiwan, and also one IVS10-2A>C carrier was detected among 120 normal persons. In our study, four parents were carriers of c.1244-2A>C mutation, which would probably be hot mutation site in Chinese \([13-15]\). The c.1244-2A>C mutation causes splicing error in 10th intron and 11th exon, resulting in deletion of 5' terminal of 11th exon, which make GCDH enzyme activity disappeared. The children with homozygous mutation manifested as mild clinical symptom, and had a good recovery after treatment, which were probably due to partially retained GCDH enzyme activity in homozygous mutation patients. In our study, urine organic acid analysis was normal in case 1, but GCDH mutations were detected. Therefore, it is necessary to perform gene test, not just rely on GC/MS to diagnose GA-I patients, especially in older children. No obvious correlation was found between genotype or biochemical phenotype (enzyme function) with clinical manifestation in case 3 and case 4 that presented different phenotypes with same genotypes (Table 1). Five cases got worse after infection, indicating that environmental factors such as viral infection, which induced tryptophan to produce quinolinic acid, played a role in pathogenesis of GA-I. Two novel mutations [c.1133C>T (p.Ala378Val) and c.339delT ] were detected in the five cases, and not detected in 220 normal persons.
Nowadays, the main mechanism involved in the abnormal metabolites mediated neuronal damage remains unclear. Impairment of energy metabolism caused by decreased activity of Na+/K+-ATPase and phosphocreatine in brain could affect the energy metabolism of neurons, and the excessive excitation of N-methyl-D-aspartic acid (NMDA) receptor induced by glutaric acid would cause neurotoxicity [16-17]. Previous studies indicated children in 6 years old would suffer from striatal damage, or even aggravation in 6-year-old children. In age dependent GA-I patients, Lysine amino acid catabolism level would decrease with increasing age, in addition, young children are less tolerated to organic acid toxic metabolites. Fortunately, diet control and drug treatment before encephalopathy could partially reduce extrapyramidal sequela caused by degenerated striate. Strict treatment management of protein diet (especially lysine and tryptophan acid), L-carnitine supplementation and vitamin B2 before 6 years old is considerably necessary [18]. When suffered from encephalopathy, patients should accept high energy treatment (glucose infusion and low dose insulin), then reduce or suspend the natural protein intake 1-2 days later, and accept intravenous supplementation of L-carnitine, meanwhile maintain electrolyte and acid-base balance. In this study, case 4 died for malnutrition and multiple organ injury, and there existed notable phenotypic differences between siblings (Case 1 and Case 2). Case 1 suffered from lower extremity claudication in 18-year follow up with no special treatment at an early stage, however, her younger sister case 2 presented no movement disorder while received early treatment. The other two cases had no convulsions till now, but long-term follow-up is needed for their prognosis assessment.

Conclusions

Here, we report two novel mutations of GCDH gene, which may expand the mutation spectrum of GCDH gene. Our study implies that there is no correlation between genotype
and phenotype of GA-I. In addition, c.1244-2A>C mutation could be the hot spot in Southern China. It is necessary to perform metabolic analysis in the patients with neurological diseases or even newborn screening. To detect the hot spot mutation sites with GC/MS and PCR is a practical method to detect GA-I for prenatal diagnosis, genetic counseling and newborn screening effectively.

**Abbreviations**

GA-I: Glutaric aciduria type I  
PCR: Polymerase chain reaction  
GCDH: Glutaryl-CoA dehydrogenase  
GC/MS: Gas chromatography·mass spectrometry

**Declarations**

**Acknowledgements**

We thank all the patients and their families for their cooperation and contribution.

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of The First Affiliated Hospital of Fujian Medical University (ID:IEC-F0M-013-2.0). And informed consent for participation in the study was obtained from their parent or guardian.

**Consent for publication**

Written informed consent was obtained from the parents for publication of this study.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

No funding was obtained for this study.

**Availability of data and materials**
All data and materials used in this manuscript, except patient’s private information, are available to readers from the corresponding author by a reasonable request.

Authors' Contributions

XS designed study protocol and approved the final manuscript; QS: followed up patient’s information, analyzed data, prepared figures and tables; ZA: analyzed data and drafted the manuscript; WX: analyzed data and prepared figures and tables.

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Table 1: Clinical features of 5 patients with GA-1.

| Cases | Gender | Age at Onset | Age at diagnosis | Current age | Triggering Event | Clinical Symptoms | Macr ocephaly | Neuroimaging |
|-------|--------|--------------|------------------|-------------|-----------------|-------------------|--------------|--------------|
| 1     | female | 9m           | 18y              | 20y         | Diarrhea        | encephalopathy paralysis of left lower extremity onset of Macrocephalyencephalopathy; drowsiness vomiting seizure | Yes          | abnc suba   |
| 2     | female | 7m           | 7m               | 4y3m        | Diarrhea        | Macr ocephalyencephalopathy; drowsiness vomiting seizure | Yes          | The right fronto-parieto-temporal external hydrocephalus. The left fronto-parieto-temporal subdural hematoma, bilateral middle cranial brain atrophy, abnormal signals of corpus striatum.1 year and 5 months after treatment, brain MRI scan was taken again: posterior horn of ventricular and bilateral frontal lobe, bilateral middle cranial brain atrophy, abnormal signals of corpus striatum. |            |              |
| 3     | female | 1y7m         | 2y3m             | 3y2m        | trauma          | encephalopathy seizure | Yes          | The left fronto-parieto-temporal subdural hematoma, abnormal signals of brainstem and basal ganglia |            |              |
| 4     | Male   | 5m           | 6m               | die1y1m     | Infection with fever | Motor development, retardation, seizure, edema, multiple organ failure | Yes          | hydrocephalus, bilateral temporal arachnoid cysts, abnormal signals of basal ganglia, external hydrocephalus |            |              |
| 5     | female | 5m           | 5m               | 1y          | Infection With fever | Seizure subarachnoid tension | No           | bilateral cysts, external hydrocephalus |            |              |

Table 2: Biochemical features and GCDH gene mutations of the five patients with GA-1.

For the condition at that time, no hematuria genetic metabolic screening was done to the child and 18 years after the examination showed no abnormal genetic metabolic hematuria.

*: Novel mutation
| Case | Urine Glutaric acid | Plasma 3-Hydroxyglutaric acid | Glutaryl carnitine (lmol/L) | GCDH gene mutations | Mutation | Amino acid change |
|------|---------------------|-------------------------------|----------------------------|---------------------|----------|------------------|
| Normal | 0-4.0 | 0 | 0-0.2 | - | - |
| 1 | - | - | - | c.1244-2A>C | c.1244-2A>C | |
| 2 | 178 | 3.8 | 1.98 | c.1244-2A>C | c.1244-2A>C | |
| 3 | 130 | 4.0 | 0.34 | c.1133C>T | Ala378Val* |
| 4 | 126 | 4.7 | 1.50 | c.1133C>T | Ala378Val* |
| 5 | 232.2 | 7.3 | 0.77 | c.339delT | c.406G>T | Tyr113* |
|      |       |   |     |           |       | Gly136Cys      |

Table 3: Primers for amplification of the GCDH gene.

| Exton | Primer name | Primer sequence (5'–3') | Product sizeb | bp |
|-------|-------------|--------------------------|--------------|----|
| 1     | 1F          | TCGTTGCTCCGCTCGCTCTG     |              | 216 |
|       | 1R          | AGTCCCTAAACCCCGAGTTCC    |              |    |
| 2     | 2F          | AGTGTGGGGTGTCGGGAGTGTG   |              | 260 |
|       | 2R          | CGGGCAGTCTCGGATTCTG      |              |    |
| 3     | 3F          | GAAGGGGAGGGCAGTGAT       |              | 280 |
|       | 3R          | GCGGAGGAGCAGTCTCAG       |              |    |
| 4     | 4F          | ATAGCCACCCACCTCAAG       |              | 261 |
|       | 4R          | AAGGGAGGAAGGCTTTCAGA     |              |    |
| 5     | 5F          | TGCTCCTATTCAGGCCCCGTC    |              | 338 |
|       | 5R          | GACTGTCTTTCTTCCACCA      |              |    |
| 6     | 6F          | GCCAGCTTGTGACCTTCTG      |              | 272 |
|       | 6R          | AGTCGTTGAGGGGCTGAGTAC    |              |    |
| 7     | 7F          | TGCGCAGTGTGAGACAG        |              | 373 |
|       | 7R          | CCGCATCCGCGAGTAC         |              |    |
| 8     | 8F          | CTTTCCCTTGCTTTAGAT       |              | 264 |
|       | 8R          | CCACACCCCGAGAAAC         |              |    |
| 9     | 9F-a        | GACGGGGTGAGGGAGTGA       |              | 359 |
|       | 9R-a        | AGCCCATCGAGAGAAGAG       |              |    |
| 9     | 9F-b        | GCCCTTCTCTCGCTCTCAC      |              |    |
|       | 9R-b        | CTCCAGAAGACAAAGGTC       |              |    |
| 10    | 10F         | CCCACTGTCCTCTTCATTG      |              | 378 |
|       | 10R         | TACCCCCCTCCAGACACT       |              |    |
| 11    | 11F-a       | AAAACTCCAAACCAGACTCTGT   |              | 418 |
|       | 11R-a       | GAAGCTGCTATTCTCAGGTA     |              |    |
| 11    | 11F-b       | AAAACTCCAAACCAGACTCTGT   |              |    |
|       | 11R-b       | CGCCACCTCCCTTTCTAAG      |              |    |

Figures

![c.1244-2A>C/Normal](image1)

![c.1244-2A>C/Normal](image2)
Case 4

\[ c.1133C>T/c.1244-2A>C \]

\[ c.339\text{del}T/\text{Normal} \quad c.406G>T/\text{Normal} \]

Figure 1

Mutation analysis of GCDH in the families of five cases. (A) Case 1 and Case 2; (B) Case 3; (C) Case 4; (D) Case 5.
Abnormal signals in bilateral basal ganglia, arachnoid cyst of bilateral temporal pole, and subdural hematoma in Case 4.