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*Case Report*

# The First Case of Cobalamin F Disorder in China: Report and Literature Review

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**Abstract**

***Objectives:*** To investigate the phenotype, genotype and prognosis of cblF disorder (CblF). ***Methodology:*** Data including newborn screening and clinical features, *LMBRD1* mutations, treatment and prognosis of the first CblF case in China and 17 reported CblF cases were collected and analysed. ***Results:*** This case was picked up by newborn screening (NBS) due to elevated blood propionylcarnitine (C3) (5.50 μmol/L), propionylcarnitine / acetylcarnitine (C3/C2) ratio (0.47), propionylcarnitine / free carnitine C3/C0 ratio (0.25) and decreased methionine (Met) (5.27 μmol/L) identified by tandem mass spectrometry (MS-MS), then decreased serum Vitamin $B_{12}$ (cobalamin; Vit $B_{12}$) (116 pmol/L), elevated homocysteine (tHCY) (82.9 μmol/L), macrocytic anaemia and atrial septal defect, and patent ductus arteriosus were confirmed. This is similar to what has been reported in the literature. Besides, two novel truncated *LMBRD1* mutations of p.R277* and p.C29* were confirmed. The data of all the 18 cases show that the common clinical features are macrocytic anaemia, failure to thrive, developmental delay, congenital heart disease, and small for gestational age. Similar biochemical characters were identified in all 3 cases from NBS, and a total of 35 *LMBRD1* mutations in 10 different sites including frame-shift mutations (30/35), splice site mutations (2/35), nonsense mutations (2/35), and large fragment deletions (1/35) were observed, among which c.1056delG mutation was reported the most frequently (24/35). After Vit $B_{12}$ injection and supportive treatment, all of the biochemical abnormalities and most of the clinical presentations have been significantly improved within 3 months in all cases. ***Conclusion:*** The biochemical characters of CblF are similar to the combined methylmalonic acid (MMA), except for decreased serum Vit $B_{12}$, which should be distinguished from Vit $B_{12}$ deficiency. Typical phenotype includes macrocytic anaemia, growth and development problems, and congenital malformations, all of which are nonspecific. The c.1056delG mutation is the common mutation. The concentrations of C3, C3/C2, C3/C0 and Met by MS-MS and the target genetic panel of MMA should be chosen for suspected MMA cases. CblF is a treatable disease, and injection of Vit $B_{12}$ is effective, but the appropriate dosage remains unclear.

**Key words** Cobalamin; Genotype; Phenotype; Prognosis; Treatment

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## Introduction

Methylmalonic acid (MMA) is a heterogeneous disease caused by different genes of complementation groups including *cblA-G*, *cblJ*, *cblX,* and *mut*, which is divided as isolated MMA (*cblA*, *cblB*, *cblD*, *mut*) and combined MMA with hyperhomocysteinaemia (*cblC*, *cblD*, *cblF*, *cblJ*).

First reported by Rosenblatt in 1985, CblF (OMIM 277380) is an autosomal recessive disorder, and the *LMBRD1* has been identified as the cause of the disease.

The number of people suffering from CblF is scarce, to the best of our knowledge, there are only 17 reported patients worldwide and no reported case from Chinese population so far.

We summarised all information of the first CblF case in China and other 17 cases published in journals that can be obtained reviewed the phenotype, genotype, treatment and prognosis.

## Case Report

The patient was a girl born at 41 weeks of gestation with a birth weight of 3.6 kg. She is the first child of her parents, from a non-consanguinity Han Chinese family without genetic disease history. newborn screening (NBS) by MS-MS was undertaken on Day 3 after her birth. Her abnormal metabolic profile included elevated blood C3 (5.50 μmol/L), C3/C2 ratio (0.47), C3/C0 ratio (0.25) and decreased Met (5.27 μmol/L). Besides, decreased serum Vit $B_{12}$, elevated tHCY, and macrocytic anaemia were identified (Table 1). At the same time, serum folic acid, blood ammonia, blood lactate, fasting blood glucose and liver/kidney function were tested and proven to be normal. Her mother's serum levels of Vit $B_{12}$ were normal, and atrial septal defect (ASD), patent ductus arteriosus (PDA) were confirmed by cardiac ultrasonography.

Thus, the infant was highly suspected to be Vit $B_{12}$ deficiency or MMA. Daily intramuscular injection of 1 mg of cyanocobalamin (CNCbl), oral 100 mg/kg levocarnitine, and 250 mg/kg betaine was performed, then C3 and tHCY values were rapidly dropped within the normal reference interval after 5 days of medication (Table 1). Hence, the levocarnitine and betaine were terminated.

Molecular testing was performed after informed consent from the parents was gained on Day 15 after birth. Genetic panel of MMA (including *cblA*, *cblB*, *cblC*, *cblD*, *cblE*, *cblF, cblG, cblJ,* and *mut*), were tested by next generation sequencing (Beijing Gold Gene Technology Co., Ltd, Beijing, China), and compound heterozygous novel mutations of *LMBRD1* were found in the infant. Sanger sequencing confirmed these two nonsense mutations, i.e. a heterozygous c.829C> T from her mother and a heterozygous c.87C> A from her father (Figure 1). There was no report about c.87C> A and c.829C> T mutations in the ExAC database, both of which result in truncated *LMBD1* proteins. SIFT, LRT and MutationTaster softwares demonstrated that the both mutations were located in the conserved region of LMBD1 protein, and it is presumed that they have a great, destructive effect on the structure and function of LMBD1 protein. As a result, the infant was diagnosed as CblF finally.

Subsequently, the medication was adjusted to 1 mg of hydroxocobalamin (OHCbl) through intramuscular injection twice a week. Ten days later, anaemia parameters were significantly improved and recovered on the 75-day-old infant after continuing treatment with OHCbl (Table 1). The case has been followed up for 23 months until now, all

**Table 1** All measurements of the Chinese case

| Age | Measurements | Patient values | Reference interval |
|---|---|---|---|
| 3d (NBS) | C3 | 5.50 μmol/L | 0.43-3.8 μmol/L |
| | C3/C2 | 0.47 | 0.03-0.27 |
| | C3/C0 | 0.25 | 0.02-0.15 |
| | Met | 5.27 μmol/L | 7.18-41.35 μmol/L |
| 15d | C3 | 4.66 μmol/L | 0.43-3.8 μmol/L |
| | C3/C2 | 1.42 | 0.03-0.27 |
| | C3/C0 | 0.34 | 0.02-0.15 |
| | Met | 5.33 μmol/L | 7.18-41.35 μmol/L |
| | Vit $B_{12}$ | 116 pmol/L | 156-672 pmol/L |
| | tHCY | 82.9 μmol/L | 5.0-15.0 μmol/L |
| | Hb | 87 g/L | 110-155 g/L |
| | MCV | 102.5fL | 75.0-92.0 fL |
| | MCH | 34.6 pg | 26.0-31.0 pg |
| | MCHC | 337 g/L | 315-365 g/L |
| 20d | C3 | 1.35 μmol/L | 0.43-3.8 μmol/L |
| | C3/C2 | 0.04 | 0.03-0.27 |
| | C3/C0 | 0.02 | 0.02-0.15 |
| | Met | 19.25 μmol/L | 7.18-41.35 μmol/L |
| | Vit $B_{12}$ | 1867 pmol/L | 156-672 pmol/L |
| | tHCY | 8.3 μmol/L | 5.0-15.0 μmol/L |
| 45d | Hb | 94 g/L | 110-155 g/L |
| | MCV | 97 fL | 75.0-92.0 fL |
| | MCH | 30.7 pg | 26.0-31.0 pg |
| | MCHC | 317 g/L | 315-365 g/L |
| 75d | C3 | 1.12 μmol/L | 0.43-3.8 μmol/L |
| | C3/C2 | 0.02 | 0.03-0.27 |
| | C3/C0 | 0.02 | 0.02-0.15 |
| | Met | 24 μmol/L | 7.18-41.35 μmol/L |
| | Vit $B_{12}$ | 924 pmol/L | 156-672 pmol/L |
| | tHCY | 5.6 μmol/L | 5.0-15.0 μmol/L |
| | Hb | 109 g/L | 110-155 g/L |
| | MCV | 83 fL | 75.0-92.0 fL |
| | MCH | 26.7 pg | 26.0-31.0 pg |
| | MCHC | 322 g/L | 315-365 g/L |

Hb: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration

biochemical measurements and mile-stone are normal. The results of cardiac ultrasonography indicated that both the ASD and PDA recovered, and the assessment of Bayley Scales of Infant Development was normal when the patient was 8 months old.

## Literature Review

A keyword-based retrieval of "*LMBRD1*" and "cblF" was performed in PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Ovid (http://ovidsp.ovid.com/autologin.cgi), Medline (http://apps.webofknowledge.com/MEDLINE_GeneralSearch_input.do?product=MEDLINE&search_mode), Wanfang Data (http://g.Wanfangdata.com.cn), Weibu periodicals (http://lib.cqvip.com/), and China National Knowledge Infrastructure (http://epub.cnki.net/KNS/brief/result.aspx?dbprefix=CJFQ). A total of 18 cases with cblF disorder were selected in our study, among which, 17 cases came from the research previously reported.

All clinical presentations were displayed in Table 2. A total of 35 *LMBRD1* mutations in 10 different sites were found (Table 3).

All cases have received continuing injection of Vit $B_{12}$ (OHCbl or CNCbl), with or without levocarniting, betaine, and other supportive treatment. The dosage of Vit $B_{12}$ ranged from 1 mg per day to 1 mg every 2 months. All of the abnormal biochemical changes and most of the clinical features have been significantly improved after medication,

**Table 2** Summary of the clinical presentations in all cases

| Clinical presentations | Patients number |
|---|---|
| Haematological features | 9/18 |
| Developmental delay | 8/18 |
| Failure to thrive | 8/18 |
| Congenital heart disease | 8/18 |
| Small for gestational age | 7/18 |
| Stomatitis +/- glossitis | 5/18 |
| Feeding difficulties | 4/18 |
| Gastric upset | 3/18 |
| Dental anomalies | 3/18 |
| Microcephaly | 3/18 |
| Facial dysmorphism | 2/18 |
| Hepatic involvement | 2/18 |
| Rash | 2/18 |
| Seizures | 2/18 |
| Hypotonia | 2/18 |
| Torticollis | 1/18 |
| Pesequinovarus | 1/18 |
| Tracheoesophageal fistula | 1/18 |
| Arthritis | 1/18 |
| Recurrent infection | 1/18 |
| Recurrent apnoea | 1/18 |
| Encephalopathy | 1/18 |
| Cleft palate | 1/18 |
| Unilateral renal agenesis | 1/18 |

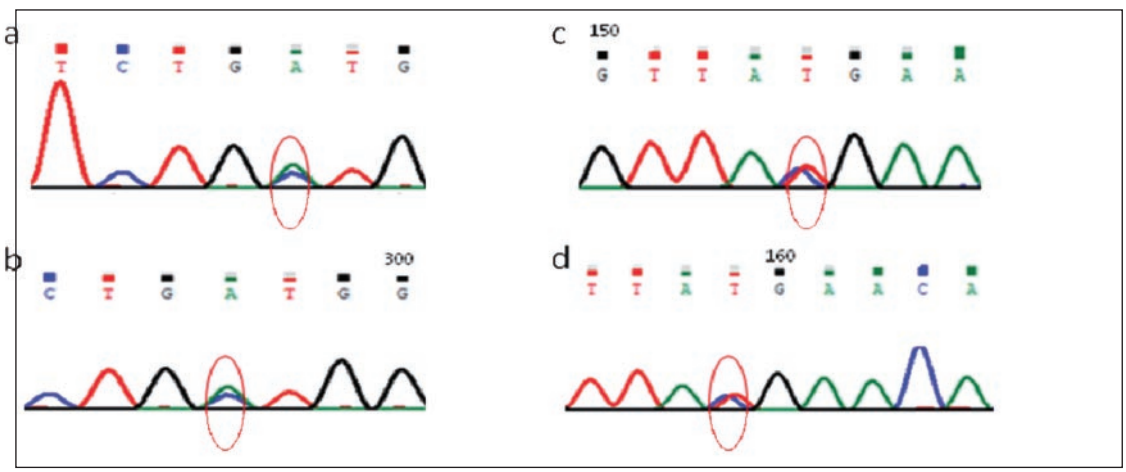


**Figure 1** Mutations of LMBRD1 gene of the infant and parents. Red circle represents the mutation loci. Figure 1a shows the mutation c.87C>A of the infant, Figure 1b shows the mutation c.87C>A of the father, Figure 1c shows the mutation c.829C>T of the infant, and Figure 1d shows the mutation c.829C>T of the mother. Sanger sequencing results demonstrated that both mutations c.87C>A and C.829C>T of the infant were inherited from the father and the mother, respectively.

except for congenital malformations.

Among all cases, three were picked from NBS and treated with early Vit $B_{12}$ injection, their C3 (5.73-15 μmol/L) and tHCY(39-82.9 μmol/L) were increased, and their serum Vit $B_{12}$ (62-148 pmol/L)was decreased before treatment. A total of 6 *LMBRD1* mutations were detected in these infants, including c.1339-G>T (2/6), c.1056delG, c.246+3476G>T, c.829C>T, and c.87C>A.

## Discussion

CblF disturbs lysosomal release of cobalamin into the cytoplasm, causing cobalamin unavailable for the synthesis of adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), and leading to combined MMA. This typical biochemistry was confirmed by our study. In addition, we discovered another interesting biochemical feature, decreased serum Vit $B_{12}$ concentration, which could be easily misdiagnosed as Vit $B_{12}$ deficiency. These two diseases should be carefully identified due to different treatment regimens: the former requires life-long treatment, while nutritional Vit $B_{12}$ deficiency could terminate Vit $B_{12}$ after serum Vit $B_{12}$ level returns to normal.

There are considerable wide clinical spectrum in CblF, whose common clinical features include macrocytic anaemia, failure to thrive and developmental delay, and congenital heart disease (CHD) and small for gestational age (SGA), all of which are nonspecific. Compared with type *cblC*, CHD and SGA are two more frequent-occurring diseases in CblF. Hence MS-MS and genetic testing are recommended for patients with unexplained macrocytic anaemia, growth and development delay, repeated infection, and feeding difficulty.

*LMBRD1,* which is located in the chromosome 6q12-13, contains 16 exons, and encoding LMBRD1 membrane protein consisting of 9 transmembrane domain sites. In our study, the frameshift mutation is the most frequent type, accounting for 85.7% of the total *LMBRD1* mutations, and c.1056delG mutation is the most common type, with a frequency of 68.6%. Up to now, there is no report related to missense mutation, indicating that the missense mutation may be neglected due to its slight effect on the structure and function of LMBD1 protein. Compound heterozygous

**Table 3** Summary of the mutations of all cases

| Patient | Mutation 1 | | Mutation 2 | |
|---|---|---|---|---|
| | Nucleotide change | Amino acid change | Nucleotide change | Amino acid change |
| 1 (This study) | c.829C>T | p.R277* | c.87C>A | p.C29* |
| 2[1] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 3[3] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 4[4] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 5[4] | c.848_851delAGAG | p.E283Gfs*3 | c.1056delG | p.N353Ifs*18 |
| 6[5] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 7[6] | c.515-516delAC | p.T172Rfs*10 | c.515-516delAC | p.T172Rfs*10 |
| 8[6] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 9[6] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 10[6] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 11[6] | c.1056delG | p.N353Ifs*18 | ? | ? |
| 12[7] | c.1405delG | p.D469Mfs*38 | c.1405delG | p.D469Mfs*38 |
| 13[8] | c.246+3476G>T | / | c.1056delG | p.N353Ifs*18 |
| 14[8] | c.916-1G>T | / | c.1056delG | p.N353Ifs*18 |
| 15[8] | c.1339-1G>T | / | c.1056delG | p.N353Ifs*18 |
| 16[6] | c.404delC | p.T135Ifs*15 | c.1056delG | p.N353Ifs*18 |
| 17[9] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |
| 18[10] | c.1056delG | p.N353Ifs*18 | c.1056delG | p.N353Ifs*18 |

https://www.ncbi.nlm.nih.gov/nuccore/NM_018368.3

novel mutations of c.87C>A and c.829C>T were detected in the Chinese case, it expands the genotype spectrum of CblF. In addition, digenic mutations have been reported for cobalamin deficiency in one study,[2] which showed that the affected patient was identified that he carried mutations of *LMBRD1* and *MTR* by exome sequencing. This study highlighted that the mutations in CblF may be more complicated, and different pathogenic genes may be involved in it. Due to the similar biochemistry among different types of combined MMA and Vit $B_{12}$ deficiency and complicated genotype of MMA, genetic panel including *cblC, cblD, cblF, and cblJ* is recommended instead of single gene sequence for timely and accurate diagnosis.

The genotype-phenotype correlations of CblF are not clear so far due to limited cases.

According to the data, all abnormal biochemical changes and most of clinical presentations in these cases have been significantly improved after Vit $B_{12}$ treatment, proving that Vit $B_{12}$ injection is an effective treatment for CblF. The dosage of Vit $B_{12}$ ranged from 1mg per day to 1 mg every 2 months, and the appropriate dosage remains to be explored.

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## Conflict of Interest

The authors declare that they have no competing interest.

## References

1. Yu HC, Sloan JL, Scharer G, et al. An X-linked cobalamin disorder caused by mutations in transcriptional coregulator HCFC1. AM J HUM GENET 2013;93:506-14.
2. Farwell GK, Li X, Lu HM, et al. Diagnostic Exome Sequencing and Tailored Bioinformatics of the Parents of a Deceased Child with Cobalamin Deficiency Suggests Digenic Inheritance of the MTR and LMBRD1 Genes. JIMD Rep 2015;15:29-37.
3. Shih VE, Axel SM, Tewksbury JC, et al. Defective lysosomal release of vitamin $B_{12}$ (cb1F): a hereditary cobalamin metabolic disorder associated with sudden death. Am J Med Genet 1989; 33:555-63.
4. MacDonald MR, Wiltse HE, Bever JL, Rosenblatt DS. Clinical heterogeneity in two patients with cblF disease (Abstract). Am J Hum Genet 1992;51(suppl):A353.
5. Waggoner DJ, Ueda K, Mantia C, et al. Methylmalonic aciduria (cblF): case report and response to therapy. Am J Med Genet 1998; 79:373-5.
6. Rutsch F, Gailus S, Miousse IR, et al. Identification of a putative lysosomal cobalamin exporter altered in the cblF defect of vitamin $B_{12}$ metabolism. Nat Genet 2009;41:234-9.
7. Gailus S, Suormala T, Malerczyk-Aktas AG, et al. A novel mutation in LMBRD1 causes the cblF defect of vitamin $B_{12}$ metabolism in a Turkish patient. J Inherit Metab Dis 2010;33: 17-24.
8. Miousse IR, Watkins D, Rosenblatt DS. Novel splice site mutations and a large deletion in three patients with the cblF inborn error of vitamin B12 metabolism. MOL GENET METAB 2011;102: 505-7.
9. Constantinou P, D'Alessandro M, Lochhead P, et al. A New, Atypical Case of Cobalamin F Disorder Diagnosed by Whole Exome Sequencing. Mol Syndromol 2016;6:254-8.
10. Wong TK, Rosenblatt DS, Applegarth DA. Diagnosis and treatment of a child with cblF disease. Clin Invest Med 1992;15: A111.