Novel Insights from Clinical Practice

Molecular Syndromology

**A New, Atypical Case of Cobalamin F Disorder Diagnosed by Whole Exome Sequencing**

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**Established Facts**
- Cobalamin F disorder is caused by homozygous or compound heterozygous mutation in *LMBRD1*.
- Typical presenting features include poor growth, developmental delay and macrocytic anaemia with neutropaenia and thrombocytopaenia.

**Novel Insights**
- Cleft palate, renal agenesis, prominent metopic suture and neonatal liver dysfunction may be features of the disorder.
- Whole exome sequencing is a non-invasive route to diagnosis in rare developmental disorders where neonatal clinical and biochemical findings are inconclusive.

**Key Words**
Cobalamin F disorder · *LMBRD1* mutation

**Abstract**
Cobalamin F (cblF) disorder, caused by homozygous or compound heterozygous mutations in the *LMBRD1* gene, is a recognised cause of developmental delay, pancytopenia and failure to thrive which may present in the neonatal period. A handful of cases have been reported in the medical literature. We report a new case, diagnosed at the age of 6 years through whole exome sequencing, with atypical features including prominent metopic suture, cleft palate, unilateral renal agenesis and liver abnormalities, which broaden the phenotypic spectrum.

Disorders of intracellular cobalamin metabolism include methylmalonic acidemia and homocystinuria. These inborn errors of metabolism can manifest with dysmorphism, developmental delay, encephalopathy and megaloblastic anaemia. Cobalamin F (cblF) is a co-factor in the vitamin B\(_12\) metabolism pathway, encoded by the *LMBRD1* gene on chromosome 6q13 [Rutsch et al., 2009]. Deficiency is inherited in an autosomal recessive fashion, characteristically associated with intratertiary growth restriction, failure to thrive, congenital heart disease, developmental delay, macrocytic anaemia, neutropaenia, thrombocytopaenia, hyperhomocysteinaemia and methylmalonic aciduria [Alfadhel et al., 2011]. It is usually diagnosed through urine organic acid and plasma amino acid analysis. Molecular analysis of *LMBRD1* can be used to confirm the genetic basis of the disease.
We present a 6-year-old child with an atypical presentation of cblF disorder, diagnosed by whole exome sequencing (WES).

**Case Report**

The proband was referred to our genetic service at the age of 1 month due to cleft palate, thrombocytopenia and renal agenesis. The pregnancy history was notable for pregnancy-associated hypertension, for which the mother was managed with methyldopa. Prenatal ultrasonography demonstrated limb shortening and decreased liquor volumes. Delivery was by emergency lower segment Caesarian section at 32 weeks’ gestation for fetal distress. APGAR scores were 8 at 1 min and 9 at 5 min after delivery. Growth parameters at birth were: weight 1,100 g (1.4th centile for gestational age), length 34 cm (<0.4th centile), and head circumference 28 cm (8th centile). Physical examination revealed a prominent metopic ridge, midline cleft soft palate, low-set ears, broad nasal tip, micrognathia and undescended testes with a right-sided inguinal hernia (fig. 1). Ophthalmological assessment revealed abnormal retinal streaking pigmentation. Imaging demonstrated thoraco-lumbar scoliosis and right renal agenesis. A small patent ductus arteriosus with mild peripheral pulmonary artery stenosis was identified, which resolved spontaneously by the age of 3 years. He had feeding difficulties and required parenteral nutrition during the first 6–8 weeks of life. Thrombocytopenia and neutropenia were evident, initially attributed to sepsis and treated with antibiotics. A bone marrow examination was normal apart from neutrophil matura-

![Figure 1. Frontal view of the facial characteristics. a Shortly after birth. b At age 3 years. c At age 6 years.](image-url)
biochemical findings (table 1). There has been no change in his developmental status in the 2 years since starting therapy although growth parameters have improved slightly (fig. 2).

**Discussion**

We present a new case of cblF disorder, presenting in an atypical manner with a prominent metopic suture, cleft palate, unilateral renal agenesis, feeding difficulties, and early liver abnormalities, which was diagnosed late by the use of WES. To date, a total of 15 cases have been reported in the literature [Rosenblatt et al., 1986; Shih et al., 1989; MacDonald et al., 1992; Wong et al., 1992; Waggoner et al., 1998; Rutsch et al., 2009; Gailus et al., 2010; Miousses et al., 2011; Oladipo et al., 2011] in addition to a recent case of combined cblF/cblG disorder suggestive of digenic inheritance [Farwell Gonzalez et al., 2015]. The typical clinical phenotype of cblF disorder is failure to
thrive and feeding difficulties, developmental delay and haematological features including megaloblastic anaemia, neutropaenia and thrombocytopaenia (table 2). All but one of the previously described cases carry the c.1056delG; p.Leu352fs*18. Of the 7 cases that are homozygous for this mutation, the majority displayed developmental delay, haematological findings and failure to thrive, though their phenotypes were otherwise variable. Hepatic involvement and prominent metopic suture or dysmorphism as found in our patient have been infrequently described [Oladipo et al., 2011, Rutsch et al., 2009]. Cleft palate has not been described previously in cblF deficiency, but has been reported with other disorders of vitamin B12 and folic acid metabolism [Natsume et al., 1998; Weingärtner et al., 2007; Boyles et al., 2008]. Renal tubulointerstitial dysfunction has been described in disorders of cobalamin metabolism [Morath et al., 2013] as has reduced renal growth [Kruszka et al., 2013]. Renal agenesis has, however, not been described.

Early institution of intramuscular hydroxocobalamin corrects the biochemical abnormalities of hyperhomocysteinaemia and methylmalonic acidaemia and appears to limit the severity of cognitive and neurological impairment [Alfadhel et al., 2011]. Although late institution of intramuscular hydroxocobalamin corrected the biochemical profile in our patient, there has unfortunately been no effect on the severity of the patient’s developmental delay, though there appears to be a trend of improvement in growth parameters. The initial biochemical abnormalities might have guided towards the diagnosis, but were instead attributed to the effects of parenteral nutrition as they were partly corrected with modification of the regimen. In retrospect, the resolution of the haematological abnormalities also coincided with the institution of enteral nutrition and vitamin supplementation, but did not recur when vitamin supplementation was subsequently reduced. By virtue of his continued gastrostomy feeding, some vitamin supplementation has continued throughout his childhood, and this may explain why the usual haematological and biochemical features have not reappeared. It should be noted that both prematurity and parenteral nutrition are associated with hepatic biochemical abnormalities [Schutzman et al., 2008]. We cannot be certain how much the cblF disorder contributed to the hepatic dysfunction in this case. This study supports the utility of a genomic testing approach to make an early diagnosis for neonates with a complex clinical presentation, as has been previously suggested elsewhere [Saunders et al., 2012].

The first 1,133 family trios from the Deciphering Developmental Disorders Study have now been reported, with an overall diagnostic yield of 27% [Deciphering Developmental Disorders Study, 2014; Wright et al., 2015]. One of the outcomes from the initial analysis has been the broadening of the phenotypic spectrum of previously well-described monogenic developmental disorders. The importance of high-quality or deep clinical phenotyping of rare diseases in the genomic era cannot be underestimated. It will improve our understanding of the natural history of these disorders, and may reveal findings that will affect clinical management. As in this case, it may reveal new genotype-phenotype correlations, giving a better understanding of the molecular bases of developmental disorders and hopefully facilitating the development of targeted therapies.

In summary, this new case of cblF deficiency, diagnosed via WES, broadens the phenotypic spectrum of the condition. It is likely that the incorporation of WES into clinical practice will highlight wider phenotypic variability in previously defined disorders.

| Features                              | Number of affected patients | Our patient |
|---------------------------------------|-----------------------------|-------------|
| Developmental delay                   | 7                           | +           |
| Failure to thrive                     | 7                           | +           |
| Haematological features               | 6                           | +           |
| Congenital cardiac anomalies          | 6                           | +           |
| Small for gestational age             | 6                           | +           |
| Stomatitis +/- glossitis              | 5                           |             |
| Gastric upset                         | 3                           |             |
| Dental anomalies                      | 3                           |             |
| Feeding difficulties                  | 3                           | +           |
| Microcephaly                          | 2                           | +           |
| Seizures                              | 2                           |             |
| Hypotonia                             | 2                           |             |
| Torticollis                           | 1                           |             |
| Pes equinovarus                       | 1                           |             |
| Tracheoesophageal fistula              | 1                           |             |
| Intrauterine growth retardation       | 1                           | +           |
| Arthritis                             | 1                           |             |
| Hepatic involvement                   | 1                           | +           |
| Rash                                  | 1                           |             |
| Recurrent infection                   | 1                           |             |
| Facial dysmorphism                    | 1                           | +           |
| Recurrent apnoea                      | 1                           |             |
| Encephalopathy                        | 1                           |             |
| Cleft palate                          | 0                           | +           |
| Unilateral renal agenesis            | 0                           | +           |
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Statement of Ethics

The study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC).

Disclosure Statement

The authors have no conflicts of interest to declare.

References

Alfadhel M, Lillquist YP, Davis C, Junker AK, Stockler-Isroiglu S: Eighteen-year follow-up of a patient with cobalamin F disease (cblF): report and review. Am J Med Genet A 155A:2571–2577 (2011).

Boyles AL, Wilcox AJ, Taylor JA, Meyer K, Fredriksen A, et al: Folate and one-carbon metabolism gene polymorphisms and their associations with oral facial clefts. Am J Med Genet A 146A:440–449 (2008).

Deciphering Developmental Disorders Study: Large-scale discovery of novel genetic causes of developmental disorders. Nature 519:223–228 (2015).

Farwell Gonzalez KD, Li X, Lu HM, Lu H, Pellegro E, 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 15:29–37 (2015).

Gailus S, Suormala T, Malerczyk-Aktas AG, Toliat MR, Wittkampf T, et al: A novel mutation in LMBRD1 causes the cblF defect of vitamin B12 metabolism in a Turkish patient. J Inherit Metab Dis 33:17–24 (2010).

Kruszka PS, Manoli I, Sloan JL, Kopp JB, Venditti CP: Renal growth in isolated methylmalonic aciduria. Genet Med 15:990–996 (2013).

MacDonald MR, Wiltsie HE, Bever JL, Rosenblatt DS: Clinical heterogeneity in two patients with cblF disease. Am J Hum Genet 51:A353 (1992).

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 102:505–507 (2011).

Morath MA, Hörster F, Sauer SW: Renal dysfunction in methylmalonic aciduria: review for the pediatric nephrologist. Pediatr Nephrol 28:227–235 (2013).

Natsume N, Nagatsu Y, Kawai T: Direct effect of vitamins at the time of palatal fusion. Plast Reconstr Surg 102:2512–2513 (1998).

Oladiop O, Rosenblatt DS, Watkins D, Miousse IR, Spietsma L, et al: Cobalamin F disease detected by newborn screening and follow-up on a 14-year-old patient. Pediatrics 128:e1636–1640 (2011).

Rosenblatt DS, Laframboise R, Pichette J, Lanugvin P, Cooper BA, Costa T: New disorder of vitamin B12 metabolism (cobalamin F) presenting as methylmalonic aciduria. Pediatr 78:51–54 (1986).

Rutsch F, Gailus S, Miousse IR, Suormala T, Sagné C, et al: Identification of a putative lysosomal cobalamin exporter altered in the cblF defect of vitamin B12 metabolism. Nat Genet 41:234–239 (2009).

Saunders CJ, Miller NA, Soden SE, Dinwiddie DL, Noll A, et al: Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. Sci Transl Med 4:154ra135 (2012).

Schutzman DL, Porat R, Salvador A, Janecko M: Neonatal nutrition: a brief review. World J Pediatr 4:248–253 (2008).

Shih VE, Axel SM, Tewksbury JC, Watkins D, Cooper BA, Rosenblatt DS: Defective lysosomal release of vitamin B12 (cblF): a hereditary cobalamin metabolic disorder associated with sudden death. Am J Med Genet 33:555–563 (1989).

Waggoner DJ, Ueda K, Mantia C, Dowton SB: Methylmalonic aciduria (cblF): case report and response to therapy. Am J Med Genet 79:373–375 (1998).

Wingertner J, Lotz K, Fanghänel J, Gedrange T, Bienengräber V, Proff P: Induction and prevention of cleft lip, alveolus and palate and neural tube defects with special consideration of B vitamins and the methylation cycle. J Orofac Orthop 68:266–277 (2007).

Wong TK, Rosenblatt DS, Applegarth DA: Diagnosis and treatment of a child with cblF disease. Clin Invest Med 15:A111 (1992).

Wright CF, Fitzgerald TW, Jones WD, Clayton S, McRae JF, et al: Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. Lancet 385:1305–1314 (2015).