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Case report

Prenatal diagnosis of methymalonic aciduria and homocystinuria cblC type using DNA analysis

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Abstract

Methylmalonic aciduria (MMA) and homocystinuria, cblC type is the most frequent inborn error of vitamin B_{12}. CblC patients present with a heterogeneous clinical picture.

To date, the early prenatal diagnosis of MMA and homocystinuria, cblC type is performed by determination of methylmalonic acid and total homocysteine (Hcy) in amniotic fluid supernatant. In this paper we report a case of prenatal diagnosis, using genetic analysis, of MMA and homocystinuria, cblC type in an at risk couple. Direct sequencing analysis of the amplified products of chorionic villi biopsy extracted DNA showed normal sequence in the fetal DNA. Mutation analysis of the *MMACHC* gene is more cost-effective and less time-consuming than the biochemical approach. Early prenatal treatment may have an impact on the long-term complications associated with cblC disease. Future studies with the aim of determining the long-term benefits of daily parenteral OHCbl started soon after conception in at risk mothers should be considered. In this context early prenatal diagnosis could determine whether therapy needs to be continued.

Keywords

Gene, DNA, treatment, metabolism, rare, villus.

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Introduction

Methylmalonic aciduria (MMA) and homocystinuria, cblC type is the most frequent inborn error of vitamin B_{12}. Cbl-C patients present with a heterogeneous clinical picture [1]. Early-onset patients, presenting symptoms within the first year, show a multisystem disease with severe neurological, ocular, haematological, renal, gastrointestinal, cardiac, and pulmonary manifestations. Late-onset patients present a milder clinical phenotype with acute or slowly progressive neurological symptoms and behavioral disturbance. The unfavorable outcome observed in most patients confirms that actual interventions, mostly focused on improving biochemical parameters, are not sufficient to prevent organ damage and that individual differences may also influence the response to therapies.

The gene responsible for the Cbl-C defect, *MMACHC*, has been recently identified [2]. More than 40 mutations have been reported [3]. Sequencing of the *MMACHC* gene is clinically available and is more cost-effective and less time-consuming than complementation analysis for confirming the diagnosis of cblC disease. It has more than 95% chance of identifying causative mutations in affected individuals; in a recent study only six out of 109 individuals with cblC disease had one or no mutations identified through sequencing [3]. A portion of these might include exonic deletions that can be detected by deletion/duplication analysis.

Currently, the early prenatal diagnosis of MMA and homocystinuria, cblC type is performed by determination of methylmalonic acid and total homocysteine (Hcy) in amniotic fluid supernatant [4, 5]. This is a time consuming and not completely efficient approach.

In this paper we report a case of prenatal diagnosis, using genetic analysis, of MMA and homocystinuria, cblC type in an at risk couple.

Materials and methods

Case report

A family of Sardinian origin with a 5-years old child affected by MMA and homocystinuria, cblC type was referred to our Institution for genetic counseling. The patient was genetically analyzed for mutation in *MMACHC* gene and resulted homozygote, while both parents were heterozygote for c.271dupA mutation [3]. This was the second pregnancy of a nonconsanguineous couple. Following non-directive genetic counseling during which the different options available were fully discussed, the parents opted for prenatal diagnosis.

Methods

A transabdominal chorionic villi biopsy was performed at 12 weeks' gestation as previously described [6]. DNA was extracted by standard methods. Amplification of exon 2 of *MMACHC* gene was carried out in both parents the affected child using 30 ng from peripheral blood extracted DNA or purified villus DNA in the fetus using the PCR method (10 pmoles of each primer, 200 mM dNTPS, 10 mM Tris-HCL, 1.5 mM $Mgcl_2$, 50 mM KCL, and 1 U Taq I, in a 25 ml reaction). Amplification conditions were an initial denaturation in 94°C for 7 min followed by 30 cycles of 94°C for 30 s, 63°C for 20 s and 72°C for 1 min with a 7 min 72°C final extension. Sequencing was performed using dGTP technology and the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems – Perkin-Elmer, Norwalk, CT, USA) according to the manufacturer's recommendations. The sequence software package Sequencher® (version 4.2; Gene Codes, Ann Arbor, MI, USA) was used for sequence analysis.

Results

Direct sequencing analysis of the amplified products confirmed the heterozygous state for c.271dupA mutation in both parents and the homozygous state in the affected child. In addition, normal sequence was detected in the fetal DNA (**Fig. 1**). The results were confirmed postnatally on a peripheral blood sample following birth.

Discussion

The prenatal diagnosis of inherited metabolic diseases is based on the demonstration of the metabolic defect in fetal tissues or fluids. In particular, until now early prenatal diagnosis of cblC disease was possible only by metabolite measurement in the amniotic fluid and in maternal urine [4, 5]. In the last years the definition of the molecular basis of cblC disease by mutation analysis in the *MMACHC* gene has made it possible to use genetic testing for prenatal diagnosis on

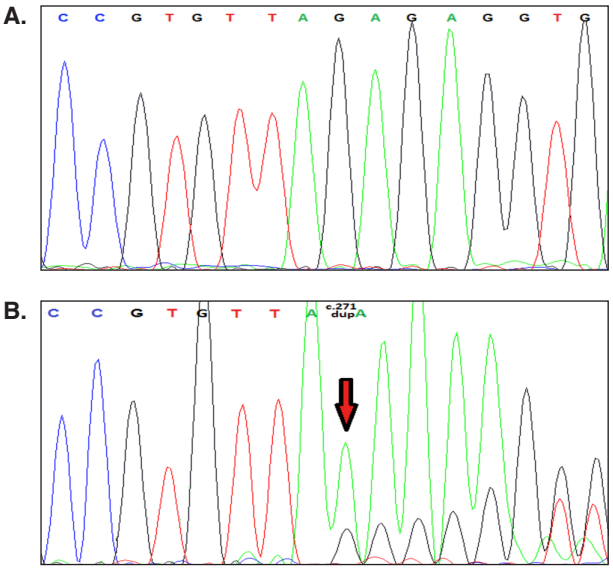

Figure 1. Electropherogram showing sequence analysis of exon 2 of *MMACHC* gene on DNA extracted from fetu's chorionic villi and peripheral blood from the mother. The upper (**A**) shows normal sequence in the fetal DNA while the lower (**B**) shows the frameshift indicated by an arrow resulting from c.271dupA mutation detected in the heterozygous mother.

chorionic villus cells extracted DNA and direct mutation detection. The c.271dupA detected in the proband in this family is the most common mutation detected in patients with cblC disease with an allelic frequency of 42% [3]. This made for simple, efficient, cost-effective and less time-consuming prenatal diagnosis. According to previous studies, c.271dupA mutation is associated with a severe phenotype in the homozygous or in the compound heterozygous state with c.331 C>T (p.R111X) mutation. Actual interventions, mostly focused on improving biochemical parameters, are not sufficient to prevent organ damage although individual differences may also influence the response to therapies. Early prenatal treatment may have an impact on the long-term complications associated with cblC disease. Prior studies have evaluated the effects of prenatal OHCbl administration and showed a decrease in maternal metabolites and, in one case, no evidence of systemic disorders or developmental delay by 9 months of age [7]. However, disease-related complications including retinopathy have been documented in patients receiving prenatal therapy [7, 8].

Conclusions

Future studies with the aim of determining the long-term benefits of daily parenteral OHCbl started soon after conception in mothers at risk should be considered. In this context, early prenatal diagnosis could determine whether therapy needs to be continued. All these informations should be fully discussed during non-directive genetic counselling. While this paper was under review, a new report describing three cases of prenatal diagnosis of MMA by mutation analysis of the *MMACHC* gene was published [9]. These data further suggest the important role of DNA analysis in prenatal diagnosis of this disease. Thus, this study, in addition to the recent published report [9], further contributes to prenatal diagnosis of MMA and homocystinuria, cblC type using DNA analysis. The results suggest the importance of molecular analysis in prenatal diagnosis of MMA and homocystinuria.

Declaration of interest

The Authors report no declarations of interest.

References

1. Carrillo-Carrasco N, Chandler RJ, Venditti CP. Combined methylmalonic acidemia and homocystinuria,cblC type. I. Clinical presentations, diagnosis and management. J Inherit Metab Dis. 2012;35:91-102.
2. Lerner-Ellis JP, Tirone JC, Pawelek PD, Doré C, Atkinson JL, Watkins D, Morel CF, Fujiwara TM, Moras E, Hosack AR, Dunbar GV, Antonicka H, Forgetta V, Dobson CM, Leclerc D, Gravel RA, Shoubridge EA, Coulton JW, Lepage P, Rommens JM, Morgan K, Rosenblatt DS. Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. Nat Genet. 2006;38:93-100.
3. Lerner-Ellis JP, Anastasio N, Liu J, Coelho D, Suormala T, Stucki M, Loewy AD, Gurd S, Grundberg E, Morel CF, Watkins D, Baumgartner MR, Pastinen T, Rosenblatt DS, Fowler B. Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations. Hum Mutat. 2009;30:1072-81.
4. Huemer M, Simma B, Fowler B, Suormala T, Bodamer OA, Sass JO. Prenatal and postnatal treatment in cobalamin C defect. J Pediatr. 2005;147:469-4.
5. Chadefaux-Vekemans B, Rolland MO, Lyonnet S, Rabier D, Divry P, Kamoun P. Prenatal diagnosis of combined methylmalonic aciduria and homocystinuria (cobalamin CblC or CblD mutant). Prenat Diagn. 1994;14:417-8.
6. Brambati B, Lanzani A, Oldrini A. Transabdominal chorionic villus sampling, clinical experience of 1159 cases. Prenat Diagn. 1988;609-13.
7. Huemer M, Simma B, Fowler B, Suormala T, Bodamer OA, Sass JO. Prenatal and postnatal treatment in cobalamin C defect. J Pediatr. 2005;147:469-72.

8. Patton N, Beatty S, Lloyd IC, Wraith JE. Optic atrophy in association with cobalamin C (cblC) disease. Ophthalmic Genet. 2001;21:151-4.

9. Zong Y, Liu N, Zhao Z, Kong X. Prenatal diagnosis using genetic sequencing and identification of a novel mutation in MMACHC. BMC Med Genet. 2015;16:48.