Received: 3 February 2022 | Revised: 1 March 2022 | Accepted: 2 March 2022

DOI: 10.1111/cge.14127

CLINICAL GENETICS WILEY

SHORT REPORT

A novel homozygous variant in *COX5A* causes an attenuated phenotype with failure to thrive, lactic acidosis, hypoglycemia, and short stature

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Funding information
Ministero della Salute, Grant/Award Number: RF-2016-02361241; Regione Puglia

Abstract

Genetic defect in the nuclear encoded subunits of cytochrome *c* oxidase are very rare. To date, most deleterious variants affect the mitochondrially encoded subunits of complex IV and the nuclear genes encoded for assembly factors. A biallelic pathogenic variant in the mitochondrial complex IV subunit COX5A was previously reported in a couple of sibs with failure to thrive, lactic acidosis and pulmonary hypertension and a lethal phenotype. Here, we describe a second family with a 11-year-old girl presenting with failure to thrive, lactic acidosis, hypoglycemia and short stature. Clinical exome revealed the homozygous missense variant c.266 T > G in COX5A, which produces a drop of the corresponding protein and a reduction of the COX activity. Compared to the previous observation, this girl showed an attenuated metabolic derangement without involvement of the cardiovascular system and neurodevelopment. Our observation confirms that COX5A recessive variants may cause mitochondrial disease and expands the associated phenotype to less severe presentations.

KEYWORDS
COX5A, cytochrome *c* oxidase, mitochondrial disorders, supercomplexes

1 | INTRODUCTION

Cytochrome *c* oxidase (complex IV) is the terminal enzyme of the mitochondrial respiratory chain (MRC) and catalyzes the formation of two molecules of water using molecular oxygen. The mammalian complex IV (CIV) is composed of 14 subunits, three of which (COX1, COX2, and COX 3) are encoded by the mitochondrial DNA (mtDNA) and have catalytic function whereas the remaining are encoded by the nuclear DNA (nDNA) and have a not yet well defined function. In addition, CIV is the only MRC complex that has several subunits with multiple tissue-specific isoforms and whose function is still under debate.[1]

The biogenesis of complex IV is highly regulated and assisted by more than 40 nuclear encoded proteins. Its construction is a modular process where the maturation of the three mtDNA encoded subunits occurs independently.[2] Mitochondrial CIV does not exist only as a standing-alone complex, but it can form dimers or assemble into supercomplexes (SCs) with different stoichiometry (SC $I + III_2 + IV_{1-4}$; SC $I_2 + III_2 + IV_2$; SC $III_2 + IV_1$).[3] Because of the dual genetic origin, the complexity of CIV biogenesis and the tissue specific regulation of its subunits, deleterious variants in the various components of the CIV (MIM# 220110) lead to highly heterogeneous phenotypes, spanning from the most severe forms of early-onset, often lethal encephalopathy and myopathy, to milder presentations

with attenuated multisystemic phenotype or organ/system-restricted disease.

Genetic defects in the nuclear encoded structural subunits of CIV are very rare. To date, most deleterious variants have been reported in mitochondrially encoded subunits and in the nuclear encoded assembly factors of CIV.

In this scenario, involvement of COX5A is exceptionally rare. To date, only a couple of sibs have been reported with a lethal mitochondrial disease featuring failure to thrive, lactic acidosis and pulmonary hypertension due to a biallelic deleterious variant in *COX5A*.[4] Here, we report the second case of *COX5A*-associated mitochondrial disease in an 11-year-old girl manifesting growth delay, mild lactic acidosis and hypoglycemia.

2 | METHODS

2.1 | Family enrollment

The family provided signed informed consent to participate in this study. This study has been conducted in accordance with the 1984 Helsinki declaration and its following modifications.

2.2 | DNA extraction and molecular investigations

Genomic DNA was extracted from the peripheral blood using Bio Robot EZ1 (Qiagen, Solna, Sweden).

Proband's DNA was analyzed by Clinical Exome using SureSelect Constitutional Clinical Panel 17 Mb (Agilent Technologies, Santa Clara, CA), according to manufacturer instructions as specified in Appendix S1, supporting information.

2.3 | Cytochemistry and dipstik assay

For COX cytochemical studies, cells were grown on glass coverslips, and processed as described elsewhere.[5] The coverslips were mounted in glycerin–gelatin and examined with a Leica microscope with bright-field optics.

Dipstick analysis to measure CI and CIV activity in fibroblast homogenate was performed according to the manufacturer's instructions (ab109876 and ab109720 from Abcam).

2.4 | Electrophoresis and immunoblotting

Blue Native Gel electrophoresis (BNGE) was performed as specified elsewhere.[6] The 40–80 μg of proteins were loaded on a precast 4%–16% Bis-Tris Native Page Gels (Invitrogen). Then, the gels were either processed for WB (Supporting information) or subjected to *in-gel* activity assay.[7] For SDS analysis, 35 μg of proteins were loaded on a 3%–12% denaturating Bis-Tris gel (Invitrogen) and subjected to WB analysis.

3 | RESULTS

3.1 | Patient

The patient is a 11-year-old girl born from nonconsanguineous healthy parents coming from a small town (5000 residents) in the Northern Puglia (Italy). The patient presented failure to thrive since the 3rd month of life. Clinical exams at 5 months revealed mild elevation of lactate dehydrogenase (738 IU/L) and aspartate aminotransferase (76 IU/L). Full cardiological exam and heart Doppler ultrasound resulted normal. At 3 years and 10 months of age, fasting blood test revealed ketotic hypoglycemia unresponsive to glucagone and metabolic acidosis. Brain magnetic resonance imaging (Figure 1A, B) at age

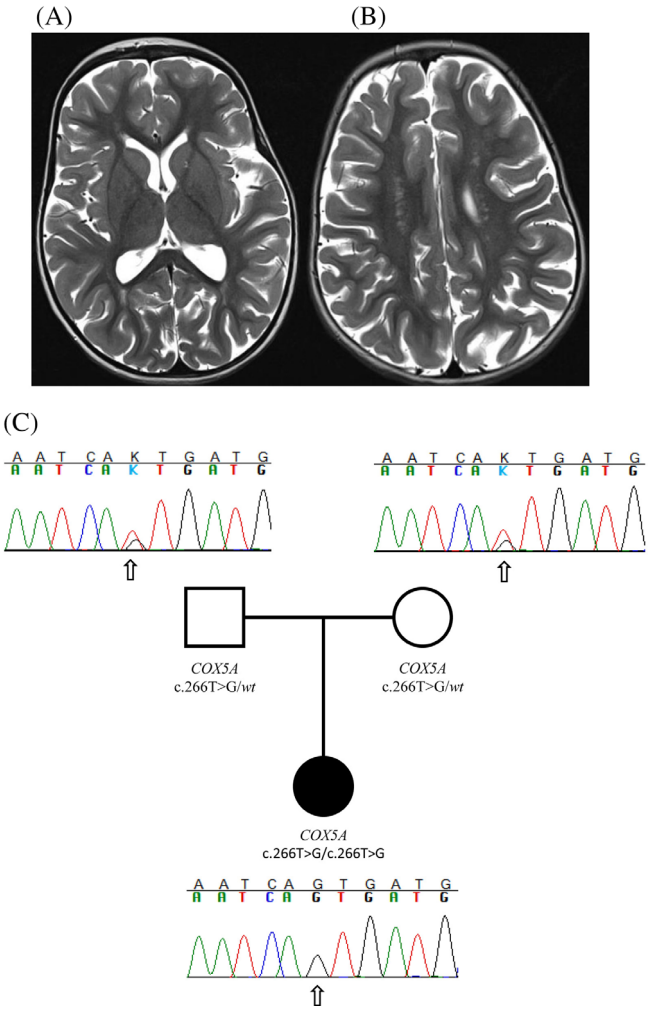

FIGURE 1 Brain MRI and genetic features. (A, B) Brain MRI of the patient at age 4 years, showing axial T2 weighted images. The images in 1A and B show enlarged subarachnoid spaces suggesting moderate cortical atrophy; basal ganglia show no abnormalities. 1B is another axial T2 weighted image showing periventricular abnormal T2 hyperintensity. (C) Family tree and electropherograms showing the segregation of the missense variant c.266 T > G in COX5A within the family members. White arrows indicate the nucleotide change [Colour figure can be viewed at wileyonlinelibrary.com]

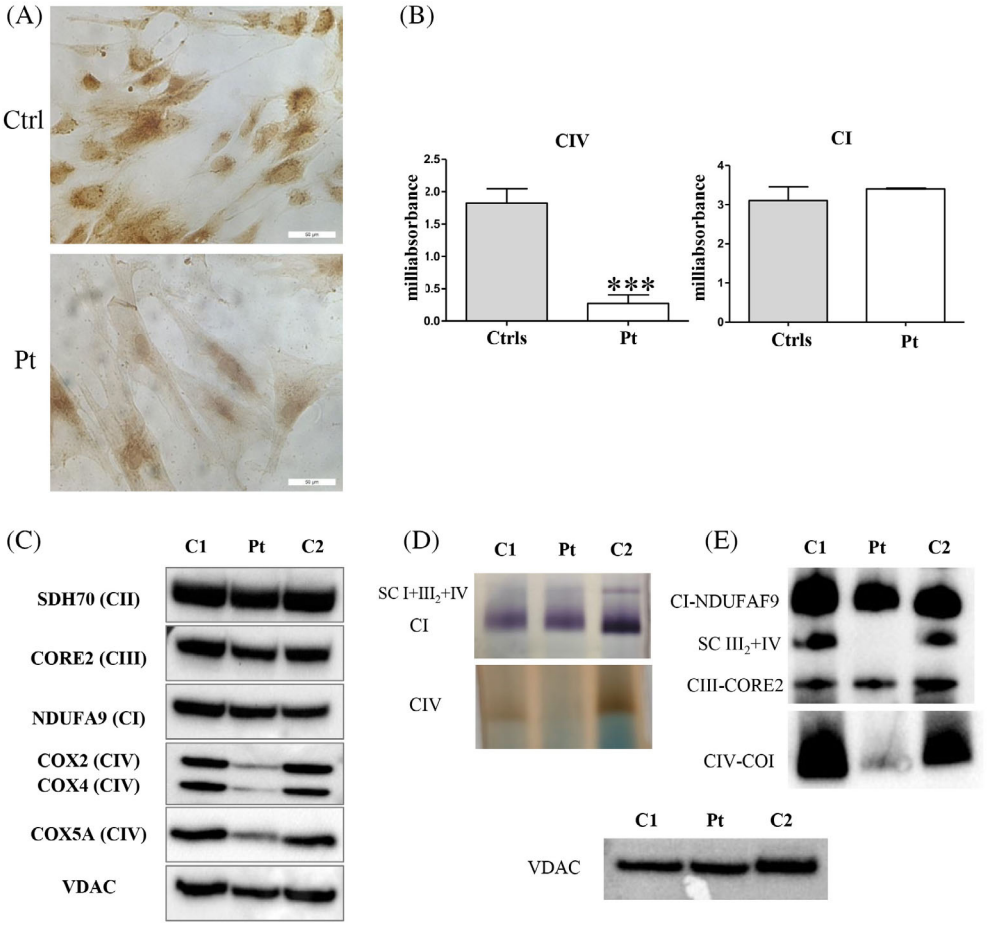

FIGURE 2 Biochemical and Protein evaluation. (A) Cytochemistry. Upper panel: control (Ctrl) fibroblast showing normal COX activity. Lower panel: patient fibroblasts displaying a diffuse reduction of COX activity. (B) Dipstick analysis. Histograms showing CI and CIV activity of controls (Ctrls) and patient (Pt) in total cells homogenate. (C) SDS-PAGE and WB analysis of fibroblasts derived mitochondria of patient (Pt) and controls (C1 and C2), using antibodies directed against the single subunits of the MRC complexes (CI, CII, CIII, CIV); BNGE followed by (D) in-gel activity assay for CI (upper panel) and CIV (lower panel) and (E) WB analysis using specific antibodies. For all the experiments, VDAC was used as loading control [Colour figure can be viewed at wileyonlinelibrary.com]

4 years showed pineal gland cyst with a maximum diameter of 10 mm, and moderate signs of cortical atrophy, with periventricular hyperintensities, while magnetic resonance spectroscopy did not reveal any significant change. Full ophthalmological and audiological exams resulted negative. Recently, the girl was investigated by a thorough endocrinologic evaluation for the considerable short stature. At 11 year and 9 months she was 115.2 cm tall (−5.01 SD), her weight was 19 kg (−4.25 SD), BMI 14.2 (−2.26 SD). A clear initial ovarian insufficiency was detected by FSH 112 mIU/ml (normal value 1.9–7.8), LH 21.5 mIU/ml (normal value >10.1), and 17β estradiol <5 pg/ml which documented hypergonadotropic hypogonadism.

3.2 | Molecular analysis

NGS analysis revealed a homozygous c.266 T > G variant (Figure 1C) which is predicted to produce a p.(Ile89Ser) aminoacidic substitution in a highly conserved region (Figure S1A) of *COX5A* (NM_004255.4). No other rare or unique variants in genes associated with the above listed HPO terms were found. Both parents resulted heterozygous for the same variant (Figure 1C). This variant was absent in gnomAD and had a REVEL score of 0.915.

3.3 | Biochemical and protein evaluation

To assess the biochemical defect of CIV we performed a cytochemical assay on primary fibroblasts. Indeed, we found a reduction of the COX activity in the patient when compared to control cells (Figure 2A). These data were supported by a dipstick assay performed on total fibroblasts homogenate that showed a selective reduction of CIV activity in the patient, whereas the activity of CI was expressed equally in the patient and control subjects (Figure 2B).

To analyze the impact of the c.266 T > G variant on COX5A steady state level and, consequently, on the CIV assembly we performed first SDS page on fibroblasts-derived mitochondria and found a 50% reduction of COX5A protein in the patient compared to controls (Figure 2C). Expression analysis of the other MRC complex subunits displayed a selective reduction of the CIV subunits (MTCOII 70% and COXIV 75% reduction, respectively) whereas the other MRC complex subunits were expressed within the normal range (Figure 2C). Analysis of the assembly state status of the MRC complexes using BNGE followed by WB confirmed the selective reduction of the holocomplex CIV (20% residual amount) as well as an almost undetectable CIV activity (Figure 2D, E). Interestingly, SC I + IV was reduced but still present in the patient, whereas SC $III_2 + IV_1$ was totally absent (Figure 2D, E) confirming that CI is essential to stabilize

TABLE 1 Demographic, genetic and clinical features in individuals with deleterious variants in the *COX5A* gene (NM_004255.4)

Individual	Patient 1	Patient 2	This patient
Reference	Baertling et al., 2017	Baertling et al., 2017	Present report
Family	Family 1	Family 1	Family 2
Age at examination	7 months	16 months	11 years
Sex	Female	Female	Female
Nucleotide change	c.319C > T	c.319C > T	c.266 T > G
Allelic state	Homozygous	Homozygous	Homozygous
Aminoacid change	p.[Arg107Cys]	p.[Arg107Cys]	p.[Ile89Ser]
Age at onset	3 months	Neonatal period	3 months
Early demise	+	+	−
Respiratory distress	+	+	−
Pulmonary arterial hypertension	+	+	−
Hypotonia	+	+	−
Failure to thrive	+	+	+
Hepatomegaly	+ (mild)	NA	−
Elevated transaminases	+ (mild)	+ (mild)	+ (mild)
Elevated lactate	+	+	+ (occasional)
Acidosis	+	+	−
Ketotic hypoglycemia	−	−	+ (occasional)
Hypergonadotropic hypogonadism	NA	NA	+
Mild brain cortical atrophy	NA	NA	+
Pineal gland cyst	NA	NA	+

Abbreviation: NA, not available.

CIV containing supercomplexes, while CIV is necessary to stabilize SC $III_2 + IV$.[3,8]

4 | DISCUSSION

The mitochondrial complex IV deficiency nuclear type 20 (MC4DN20; MIM #619064) is caused by homozygous pathogenic variant in the *COX5A* gene (603773) and to date deleterious variants have only been reported in a single family.[4] The two affected siblings of this family showed hypotonia, failure to thrive, lactic acidemia, and early-onset pulmonary arterial hypertension, which may result in cardiorespiratory failure and early death. Here, we report the second family harboring a homozygous deleterious variant in *COX5A*. Compared to the previously reported family (Table 1 summarize the clinical phenotype of the previously reported patients), this subject showed an attenuated metabolic derangement without involvement of the cardiovascular system and neurodevelopment, with the main complain of failure to thrive. In the last month (at 11 year and 9 months) a complete endocrinologic evaluation is indicative for a hypergonadotropic hypogonadism. Hypogonadism and failure to thrive are known to be frequently associated with a mitochondrial disease,[9] although the simple association of failure to thrive and hypergonadotropic hypogonadism is rather unusual for a mitochondrial disorder. Moreover, the endocrinological workup has not been able to detect any laboratory abnormality to explain the severe growth defect. The early identification of ketotic hypoglycemia in this patient is not unusual for mitochondrial disorders in general. In fact, although malfunctioning mitochondria would increase glycolysis rate and increase lactate production to regenerate NAD+, it is normal to observe reduced circulating glucose levels in individuals with dysfunctional respiratory chain.

In our patient, a homozygous missense variant in *COX5A* was detected by whole-exome sequencing. Several lines of evidence clearly indicate this variant to be the disease cause: isolated complex IV deficiency was present and the clinical presentations were suggestive of a mitochondrial disease. There were no other variants in any genes known or predicted to be complex IV associated except for the *COX5A* variant, which was predicted to be pathogenic by several independent mutation prediction softwares. The *COX5A* gene encodes a subunit of mitochondrial respiratory complex IV, which is the terminal component of the respiratory chain complex of most aerobic organisms. COX5A plays a role as an assembly factor in complex IV biogenesis[4] (Figure S1B). The c.266 T > G induced a decrease of COX5A that, however, was still present and was sufficient to sustain the formation of the holocomplex IV, although at a highly reduced amount. The low amount of COX5A together with the reduction we demonstrated of COX2 and COX4 support the initial idea[10,11] that the three subunits are part of the S2 assembly intermediate and the reduction of COX5A impairs complex IV assembly. Interestingly, the low level of the unbound CIV was insufficient to form the scarce and not well characterized SC $III_2 + IV$ whereas the SC $I + III_2 + IV$ was still present. This result can be explained considering that COX5A interacts

with different subunits of CIII both in the matrix side, the intermembrane space and within the mitochondrial inner membrane,[12] thus a strong reduction of COX5A protein can destabilize the scarce representative supercomplex SC $III_2 + IV$ that, however, do not impact deeply on the disease pathogenesis of our patient. On the other hand, the more abundant supercomplexes, such as SC I $+ III_2 + IV$, which contain low amounts of CIV are still present and functionally active. We do not have reports about the functionality of the supercomplexes in the previously reported patients but, in our opinion, differences in the supercomplexes efficiency may partially explain phenotypes differences amongst COX5A patients.

In conclusion, we have expanded the clinical and genotypic spectrum of *COX5A* defects, adding the observation of a second patient. Moreover, we have further highlighted the importance of this subunit in CIV stability and function.

ACKNOWLEDGEMENTS

We are grateful to the patients and their families for their support and cooperation. This work was supported by the Italian Ministry of Health Ricerca Corrente, the Italian Ministry of Health Ricerca Finalizzata (RF-2016-02361241) and Regione Puglia.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Silvia Morlino and Luigi Bisceglia performed NGS experiments and the related bioinformatics analyses. Alessandra Torraco, Teresa Rizza, Michela Di Nottia and Arianna Montanari performed functional analysis on cultured fibroblasts. Giorgia Bottaro and Marco Cappa performed endocrinologic evaluation. Alessandra Torraco, Silvia Morlino, Marco Castori, Enrico Bertini, and Rosalba Carrozzo are responsible for the integrity of the data, accuracy of the data analysis and for writing the manuscript. All Authors revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. All data relevant for the study are included in the article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Torraco A, Morlino S, Rizza T, et al. A novel homozygous variant in *COX5A* causes an attenuated phenotype with failure to thrive, lactic acidosis, hypoglycemia, and short stature. *Clinical Genetics*. 2022;1-5. doi:10.1111/cge.14127