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TMEM70 deficiency: Novel mutation and hypercitrullinemia during metabolic decompensation

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Abstract

Respiratory chain disorders comprise a heterogeneous group of diseases that are the result of mutations in nuclear or mitochondrial genes. TMEM70 encodes a nuclear protein involved in the assembly of respiratory chain complex V. Although mutations in various genes can result in isolated complex V deficiency; *TMEM70* mutations represent the most common reported etiology.

TMEM70 deficiency is known to cause a syndrome of neonatal mitochondrial encephalocardiomyopathy, accompanied by elevated lactate and hyperammonemia.

Elevated citrulline has been reported previously in different inborn errors of metabolism, although uncommonly associated with TMEMT70 deficiency.

We present a series of two siblings diagnosed with TMEM70 deficiency, and describe hypercitrullinemia during decompensation as a new finding in this condition. The cause of hyperammonemia in TMEM70 deficiency was previously assumed to be related to carbamoyl phosphate synthase 1 deficiency, but our finding of hypercitrullinemia rules out this possibility. We thus propose a different etiology for the hyperammonemia seen in these patients.

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URLs

gnomAD, http://gnomad.broadinstitute.org

Conflict of Interest

The authors declare no conflict of interest.

SUPPLEMENTARY ONLINE MATERIAL

Supplementary material may be found in the online version of this article at the publisher's website (wileyonlinelibrary.com/journal/ajmg).

Keywords

Hypercitrullinemia; TMEM70 deficiency

INTRODUCTION

Mitochondrial disorders affecting the respiratory chain lead to disturbances of energy production. These disorders comprise a heterogeneous group of diseases, which result from mutations in nuclear or mitochondrial genes, given the combined genomic origin of the mitochondrial respiratory chain. *TMEM70* encodes a nuclear protein involved in the assembly of mitochondrial ATP synthase, also called respiratory chain complex V. Mutations in various genes can result in isolated complex V deficiency; *TMEM70* mutations represent the most common reported etiology of isolated complex V deficiency [Čížková et al., 2008].

Mutations in *TMEM70* are known to cause a syndrome of neonatal mitochondrial encephalocardiomyopathy, presenting antenatally as intrauterine growth restriction (IUGR), and at birth with poor feeding, hypotonia, lethargy and heart failure, accompanied by lactic acidosis and hyperammonemia [Honzík et al., 2010].

Elevated concentrations of plasma citrulline have been reported in patients with argininosuccinate synthetase deficiency, argininosuccinate lyase deficiency, arginase deficiency, citrin deficiency, pyruvate carboxylase deficiency, lysinuric protein intolerance [Rabier and Kamoun, 1995], and dihydrolipoamide dehydrogenase deficiency (*DLD*) [Haviv et al., 2014]. The association with TMEM70 deficiency is not well documented.

We present a series of two siblings diagnosed with a novel *TMEM70* frameshift mutation, presenting with hypercitrullinemia as part of decompensation episodes in addition to the previously reported lactic acidosis and hyperammonemia.

METHODS

Patients and informed consent

The study was approved by the Soroka Medical Center Institutional Review Board (IRB) (approval #5071G). Two family members of consanguineous Bedouin kindred were studied (Figure 1A). DNA samples were obtained following legal guardians' informed consent. Clinical phenotyping was determined by an experienced team of pediatricians and geneticists for all affected individuals. Muscle biopsy was performed in the first affected sibling.

Sequencing analysis

Chromosomal microarray (CMA) and homozygosity mapping: Genome-wide copy-number variation (CNV) and homozygosity mapping of the proband (IV-4) was performed using CytoScan™ 750K Array (Affymetrix, Inc.), which includes 750,000 markers for copy number analysis, including 200,000 gene-centric SNP and 550,000 non-polymorphic probes.

Data were analyzed using Chromosome Analysis Suite (ChAS) (Affymetrix, Inc.), enabling detection of CNV and loss off heterozygosity, marking homozygous segments.

Whole exome sequencing (WES) was performed by Centogene (Rostock, Germany), with 70–100x average coverage (~95% targeted bases covered >10x). Library preparation was done using Nextera® Rapid Capture Exome Kit V2.1 and sequencing was performed with Hiseq 2000 sequencing system (Illumina, Inc.). Data were analyzed using QIAGEN's Ingenuity® Variant Analysis™ software from QIAGEN Redwood City as previously described [Perez et al., 2017]: using their filtering cascade, we excluded variants that are observed with an allele frequency greater than or equal to 1.0% of the genomes in the 1000 genomes project, NHLBI ESP exomes (All), or the Allele Frequency Community. In addition, we excluded variants that appeared in a homozygous state in our in-house WES database of 153 Bedouin control samples. Furthermore, we kept variants which are predicted to have a deleterious effect upon protein coding sequences (e.g., frameshift, in-frame indel, stop codon change, missense or predicted to disrupt splicing by MaxEnt Scan) and variants which were experimentally observed to be associated with a phenotype: pathogenic, possibly pathogenic, or disease-associated according to the Human Gene Mutation Database (HGMD). Homozygous and biallelic variants were prioritized as recessive heredity was suspected. Genes previously correlated with elevated levels of plasma citrulline, such as *ASS1*, *SLC25A13*, *PC* and *DLD* were given special attention and directly visualized using Integrative Genomics Viewer (IGV; Broad institute). Validation and segregation analysis of the *TMEM70* frameshift mutation was done via Sanger sequencing using the following primers: forward 5′- ctgtttctggcgttgggcag −3′; reverse 5′- tcccgaaggccccgtactc-3′ (171 bp amplicon). PCR parameters included an annealing temperature of 60°C, extension time of 30 seconds, and 40 cycles.

RESULTS

The clinical phenotype of the two affected siblings is summarized in table 1.

Patient 1

Patient 1 was the first child of consanguineous parents of Bedouin origin, born at term following a pregnancy with intrauterine growth retardation (IUGR). Fetal echocardiography revealed mild cardiac hypertrophy. Birth weight was 2100 grams and head circumference 31 cm. The patient developed severe lactic acidosis on the first day of life, which did not resolve, and he died after 3 days. As part of his work-up a muscle biopsy was done, which ruled out pyruvate dehydrogenase deficiency and demonstrated normal activity of mitochondrial respiratory complexes (see table S1).

Patient 2

Patient 2, sibling of Patient 1, was the fourth child of consanguineous Bedouin parents. She was born at term following a pregnancy suspected of IUGR and mega cisterna magna. The birth weight was 2,195 grams and the head circumference 33.5 cm. Fetal echocardiography revealed mild hypertrophy. At birth, mild axial hypotonia was noted, with elevation of lactate (18.7 mM; norm – 0.50-2.20 mM). Due to her hypotonia, she

was fed by a nasogastric tube during the first days of life. The lactate normalized after a week. During hospitalization she started oral feeding, gained weight and was discharged at one month. Her work-up included: brain imaging which disclosed normal ventricles with mega cisterna magna and hypoplastic vermis; failed hearing screen; echocardiography with right ventricular hypertrophy; normal Holter; normal upper gastrointestinal series. She was diagnosed with buphthalmos of the right eye. Urine organic acid analysis at 2 months revealed a qualitative moderate elevation in 3-methylglutaconic acid.

The patient was readmitted at 4 months due to vomiting and encephalopathy. Laboratory studies showed lactic acidosis (8.4mM) with elevated ammonia (494 umol/L, norm<50), and persistent hypoglycemia. Sepsis work-up was negative. Plasma amino acids revealed markedly elevated citrulline (225 umol/L; norm- 4-44) with elevation of glutamine (1,108 umol/L, norm- 211-829), elevated alanine (1,596 umol/L; norm- 116-584) and elevated lysine (651 umol/L; norm- 48-284). The patient did not have renal failure; urinary orotic acid was not measured. Echocardiography disclosed concentric left ventricle hypertrophy. The patient was treated with glucose 10% infusion, ammonia scavengers, sodium bicarbonate and antibiotics and her lab test values normalized over the course of the next seven days. Repeat amino acid levels after the crisis subsided were normal; in particular, citrulline levels were within the reference range on two separate occasions (Figure 3). The patient had a percutaneous gastrostomy insertion at 17 months of age due to severe failure to thrive. Her weight at 2 years is 8.3 kg. She started walking at two years of age and speaks 5 words.

Genetic Analysis

Chromosomal microarray (CMA) and homozygosity mapping-: normal female karyotype was observed for individual IV-4. Genome-wide homozygosity mapping identified 44 segments with loss off heterozygosity (table S1 and figure S1). High number of small homozygous loci is expected due to a longstanding custom of consanguineous marriage. Of the 44 segments, two were larger than 20MB, in 8q12.1q21.3(59,565,072-88,769,465) and 4q12q22.2(58,760,785-94,674,019) [hg19].

Whole-exome sequencing (WES) data of individual IV-4 (Figure 1A) were filtered for normal variants as described in Methods. Based on the WES data visualized by IGV, complemented by Sanger sequencing in areas of coverage lower than 10X, no mutations were found in coding regions of genes previously associated with elevated levels of plasma citrulline. It should be noted that a heterozygous variant was found in the 5'UTR of *ASS1* (NM_000050.4): c.-4C>T. This variant (dbSNP ID- rs138350285) was previously suggested as a disease-causing mutation in a homozygous state [Engel et al., 2009], although it was later found at a homozygous state in two individuals in gnomAD (which is unlikely to include individuals with severe pediatric disease). More importantly, the allele frequency of this variant is quite high at 3.3% in our in-house database (10 heterozygous carriers out of 153 ethnically-matched control individuals). Based on all of the above, this variant is unlikely to be pathogenic at a homozygous state, and there is no doubt that its pathogenicity in the heterozygous state, as seen in individual IV-A, can be ruled out.

Within the homozygous locus of 8q12.1q21.3(59,565,072-88,769,465 [hg19]), a homozygous frameshift mutation was identified in a gene previously associated with neonatal mitochondrial encephalocardiomyopathy: *TMEM70* (NM_017866.5). The c.105dupT (p.Val36Cysfs*52) variant was further assayed through Sanger sequencing, and was found in both affected family members (Figure 1B). The mutation resides within the first exon of both validated *TMEM70* transcripts (NM_017866.5 and NM_001040613.2), and is predicted to cause loss-of-function of the encoded protein (via nonsense-mediated decay). This variant has not been previously reported in the clinical databases HGMD® and ClinVar, nor in the publicly available databases dbSNP 150 or ExAC, but was found with an allele frequency of 0.0009248% in gnomAD (two carriers out of 216,272 alleles, both non-Finnish Europeans; no homozygotes reported), last accessed August 27, 2018. Screening of the mutation in 153 ethnically matched controls (306 chromosomes) identified no carriers and no homozygotes (data not shown).

DISCUSSION

We describe two siblings with a novel homozygous truncating variant in *TMEM70* found by whole exome sequencing in the proband, and validated by Sanger sequencing in both.

Elevation of plasma citrulline was noticed in the proband during a metabolic crisis at 4 months. The association of hypercitrullinemia with TMEM70 deficiency is not well documented, although a review of the literature reveals one publication in which the values of plasma citrulline were found to be transiently elevated in two patients. Table 2 describes the laboratory findings of our Patient 2 compared to those of these two previous patients in the literature.

The finding of hypercitrullinemia is perplexing, given that the disease is known to be associated with energy deficiency from impaired complex V assembly. Mitochondrial disorders are known to be associated with hypocitrullinemia [Atkuri et al., 2009], and this is particularly true of a specific mutation (m.8993T>G) in *MT-ATP6*, encoding a subunit of complex V [Rabier et al., 1998]. The prevailing hypothesis is that the deficiency of complex V leads to a decrease in intramitochondrial ATP, which in turn leads to deficient activity of any of a few ATP-dependent enzymes located in the mitochondrial matrix that participate in citrulline synthesis in the enterocytes (Fig. 2). In particular, carbamoylphosphate synthetase 1 (CPS1) and δ-1-pyrroline-5-carboxylate synthetase (P5CS) are ATP-dependent enzymes that participate in citrulline synthesis within the enterocyte mitochondria, the primary source of circulating citrulline [Windmueller and Spaeth, 1981]. However, the activity of P5CS, and of ornithine transcarbamylase (OTC) in enterocytes has been shown to be normal in patients with complex V deficiency [Parfait et al., 1999]. Thus, it is presumed that the deficiency of CPS1 is responsible for hypocitrullinemia in these patients. It would be easy to make the assumption that the hyperammonemia seen in patients with TMEM70 deficiency is related to decreased CPS1 activity. However, this is unlikely to be the case, given the fact that the two previously reported patients with hypercitrullinemia also had concurrent elevation of orotic acid during the acute hyperammonemic crisis [Honzík et al., 2010], indicative of excess carbamoylphosphate availability. One possible explanation for the combination of hyperammonemia, hypercitrullinemia, and elevated orotic acid is

that argininosuccinate synthetase 1, another ATP-dependent enzyme [Diez-Fernandez et al., 2017], might be deficient due to the ATP depletion.

Why would, however, ATP depletion differently affect citrulline synthesis, if both the m.8993T>G mitochondrial DNA mutation and TMEM70 deficiency lead to low ATP synthase activity? The answer is not known. One hypothesis might be that TMEM70 deficiency is known to be associated with overexpression of the adenine nucleotide translocase (ANT), as fibroblasts from patients with TMEM70 deficiency show a statistically significant increase in protein content (over 3x) as compared to controls [Nůsková et al., 2015]. ANT normally exports ATP from and imports ADP to the mitochondrial matrix; thus, a compensatory increase in ANT would lead to increased intramitochondrial ADP. ADP is a strong activator of intestinal glutaminase; increased glutaminase activity in enterocytes' mitochondria would lead to increased glutamate, a substrate both for ornithine synthesis via P5CS and ornithine aminotransferase, and for N-acetylglutamate (NAG) synthesis via NAGS. An increase in NAG, a known activator of CPS1, could also explain the lack of CPS1 deficiency seen in these patients.

TMEM70 deficiency is associated with persistent pulmonary arterial hypertension in the newborn (PPHN) in a significant number of patients [Catteruccia et al., 2014], reported in 22% of cases [Magner et al., 2015]. The concentration of arginine, although still within normal limits, has been found to be significantly lower in patients with PPHN than in controls. In one study, the plasma arginine concentration was 32 ± 14 vs 52 ± 20 umol/L in infants with PPHN and controls, respectively ($p = 0.02$) [Vosatka et al., 1994]. A similar finding was found in another study, with plasma arginine concentrations of 20.2 ± 8.8 umol/L in PPHN patients vs 39.8 ± 17.0 umol/L in controls ($p<0.001$) [Pearson et al., 2001]. Consequently, we evaluated the plasma arginine concentration in patient 2 during a crisis as compared to baseline, and found decreased levels during the episode of metabolic decompensation (Figure 3). Thus, it is possible that PPHN in patients with TMEM70 deficiency could be related to decreased availability of arginine, a substrate for nitric oxide synthesis. We urge clinicians to obtain and carefully interpret plasma amino acid profiles of additional patients with TMEM70 deficiency, as this approach can help further elucidate the pathomechanism of this condition.

In summary, we present a series of two siblings diagnosed with a novel frameshift mutation in *TMEM70*, and we wish to call attention to the importance of proper interpretation of amino acid profiles in patients with mitochondrial disorders in general, and with TMEM70 deficiency in particular.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Atkuri KR, Cowan TM, Kwan T, Ng A, Herzenberg LA, Herzenberg LA, Enns GM. 2009. Inherited disorders affecting mitochondrial function are associated with glutathione deficiency and hypocitrullinemia. Proc. Natl. Acad. Sci. U.S.A 106: 3941–3945. [PubMed: 19223582]

Catteruccia M, Verrigni D, Martinell D, Torraco A Agovino T, Bonafé L, D'Amico A, Donati MA, Adorisio R, Santorelli FM, Carrozzo R, Bertini E, Dionisi-Vici C 2014. Persistent pulmonary arterial hypertension in the newborn (PPHN): a frequent manifestation of TMEM70 defective patients. Molecular Genetics and Metabolism, 111(3), 353–359. 10.1016/j.ymgme.2014.01.001 [PubMed: 24485043]

Čížková A, Stránecký V, Mayr JA, Tesařová M, Havlíčková V, Paul J, Ivánek R, Kuss AW, Hansíková H, Kaplanová V, Vrbacký M, Hartmannová H, Nosková L, Honzík T, Drahota Z, Magner M, Hejzlarová K, Sperl W, Zeman J, Houštěk J & Kmoch S. 2008. TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. Nature Genet. 40, pages 1288–1290 [PubMed: 18953340]

Diez-Fernandez C, Rüfenacht V, Häberle J. 2017. Mutations in the Human Argininosuccinate Synthetase (ASS1) Gene, Impact on Patients, Common Changes, and Structural Considerations. Hum. Mutat 38: 471–484. [PubMed: 28111830]

Engel K, Höhne W, Häberle J. 2009. Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. Hum. Mutat 30: 300–307. [PubMed: 19006241]

Haviv R, Zeharia A, Belaiche C, Haimi Cohen Y, Saada A. 2014. Elevated plasma citrulline: look for dihydrolipoamide dehydrogenase deficiency. Eur. J. Pediatr 173: 243–245. [PubMed: 23995961]

Honzík T, Tesarová M, Mayr JA, Hansíková H, Jesina P, Bodamer O, Koch J, Magner M, Freisinger P, Huemer M, Kostková O, van Coster R, Kmoch S, Houstêk J, Sperl W, Zeman J. 2010. Mitochondrial encephalocardio-myopathy with early neonatal onset due to TMEM70 mutation. Arch. Dis. Child 95: 296–301. [PubMed: 20335238]

Magner M, Dvorakova V, Tesarova M, Mazurova S, Hansikova H, Zahorec M, Brennerova K, Bzduch V, Spiegel R, Horovitz Y, Mandel H, Tuba Eminoğlu F, Mayr JA, Koch J, Martinelli D, Bertini E, Konstantopoulou V, Smet (UGent) J ,Rahman S, Broomfield A, Stojanović V, Dionisi-Vici C, Van Coster (UGent) R, Morava E, Sperl W, Zeman J,Honzik T. 2015. TMEM70 deficiency: long-term outcome of 48 patients. Journal of Inherited Metabolic Disease, 38(3), 417–426. 10.1007/s10545-014-9774-8 [PubMed: 25326274]

Nůsková H, Mráček T, Mikulová T, Vrbacký M, Kovářová N, Kovalčíková J, Pecina P, Houštěk J. 2015. Mitochondrial ATP synthasome: Expression and structural interaction of its components. Biochem. Biophys. Res. Commun 464: 787–793. [PubMed: 26168732]

Parfait B, de Lonlay P, von Kleist-Retzow JC, Cormier-Daire V, Chrétien D, Rötig A, Rabier D, Saudubray JM, Rustin P, Munnich A. 1999. The neurogenic weakness, ataxia and retinitis pigmentosa (NARP) syndrome mtDNA mutation (T8993G) triggers muscle ATPase deficiency and hypocitrullinaemia. Eur. J. Pediatr 158: 55–58. [PubMed: 9950309]

Pearson DL, Dawling S, Walsh WF, Haines JL, Christman BW, Bazyk A, Scott N Summar ML. 2001. Neonatal pulmonary hypertension--urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. The New England Journal of Medicine,344(24), 1832–1838. 10.1056/NEJM200106143442404 [PubMed: 11407344]

Perez Y, Wormser O, Sadaka Y, Birk R, Narkis G, Birk OS. 2017. A Rare Variant in PGAP2 Causes Autosomal Recessive Hyperphosphatasia with Mental Retardation Syndrome, with a Mild Phenotype in Heterozygous Carriers. Biomed Res Int 2017: 3470234. [PubMed: 29119105]

Rabier D, Diry C, Rotig A, Rustin P, Heron B, Bardet J, Parvy P, Ponsot G, Marsac C, Saudubray JM, Munnich A, Kamoun P. 1998. Persistent hypocitrullinaemia as a marker for mtDNA NARP T 8993 G mutation? J. Inherit. Metab. Dis 21: 216–219. [PubMed: 9686360]

Rabier D, Kamoun P. 1995. Metabolism of citrulline in man. Amino Acids 9: 299–316. [PubMed: 24178879]

Vosatka RJ, Kashyap S, & Trifiletti RR. 1994. Arginine deficiency accompanies persistent pulmonary hypertension of the newborn. Biology of the Neonate, 66(2–3), 65–70. 10.1159/000244091 [PubMed: 7993949]

Windmueller HG, Spaeth AE. 1981. Source and fate of circulating citrulline. Am. J. Physiol 241: E473–480. [PubMed: 7325229]

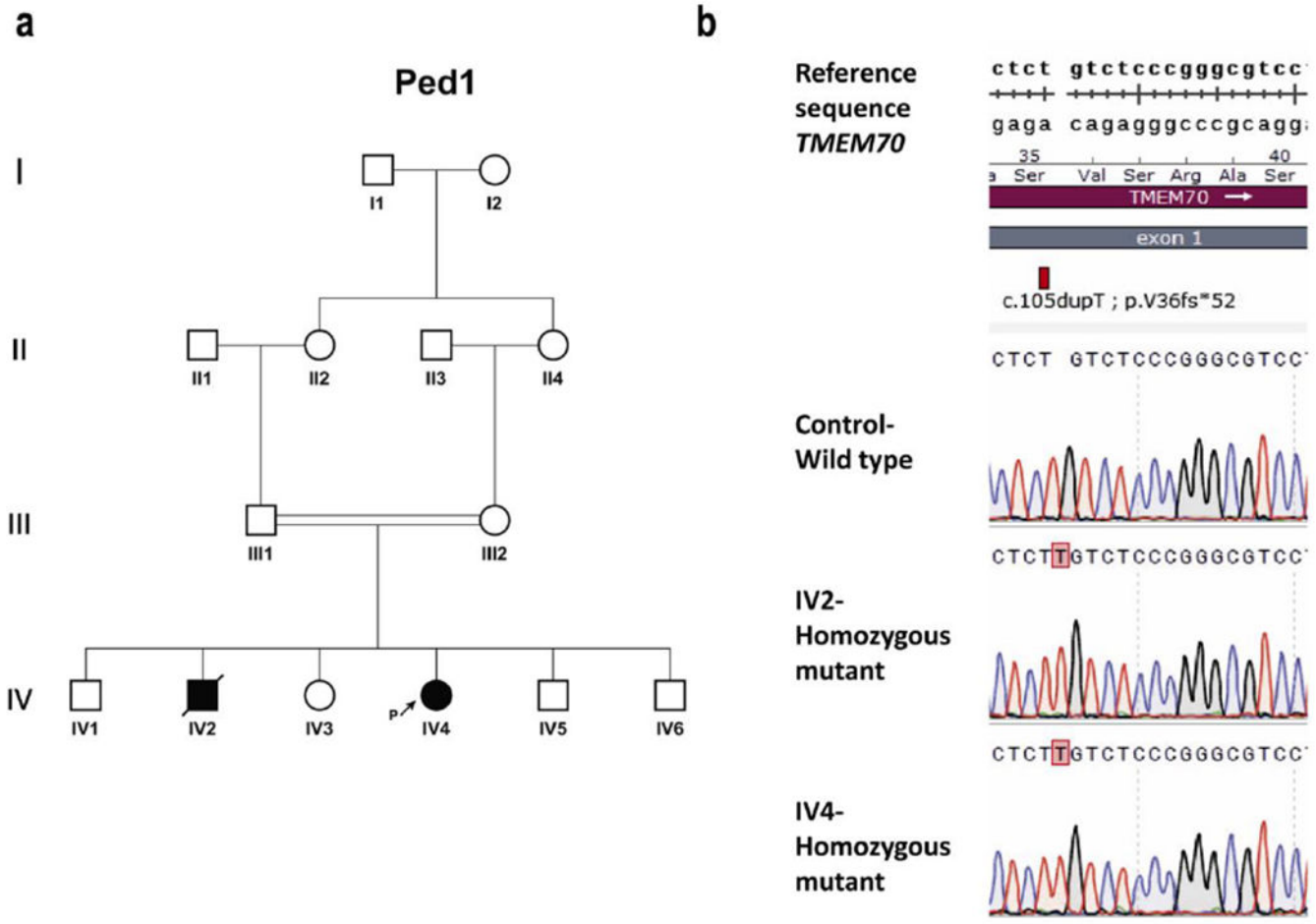

Figure 1:

Pedigree and Sanger sequencing. a. Pedigree of consanguineous Bedouin kindred studied. Genomic DNA from individual IV4 was analyzed through chromosomal microarray (CMA) and whole-exome sequencing (WES). b. Targeted Sanger sequencing of the *TMEM70* (NM_017866.5) c.105dupT; p.(V36fs*52) mutation in an unrelated control individual and in both affected family members

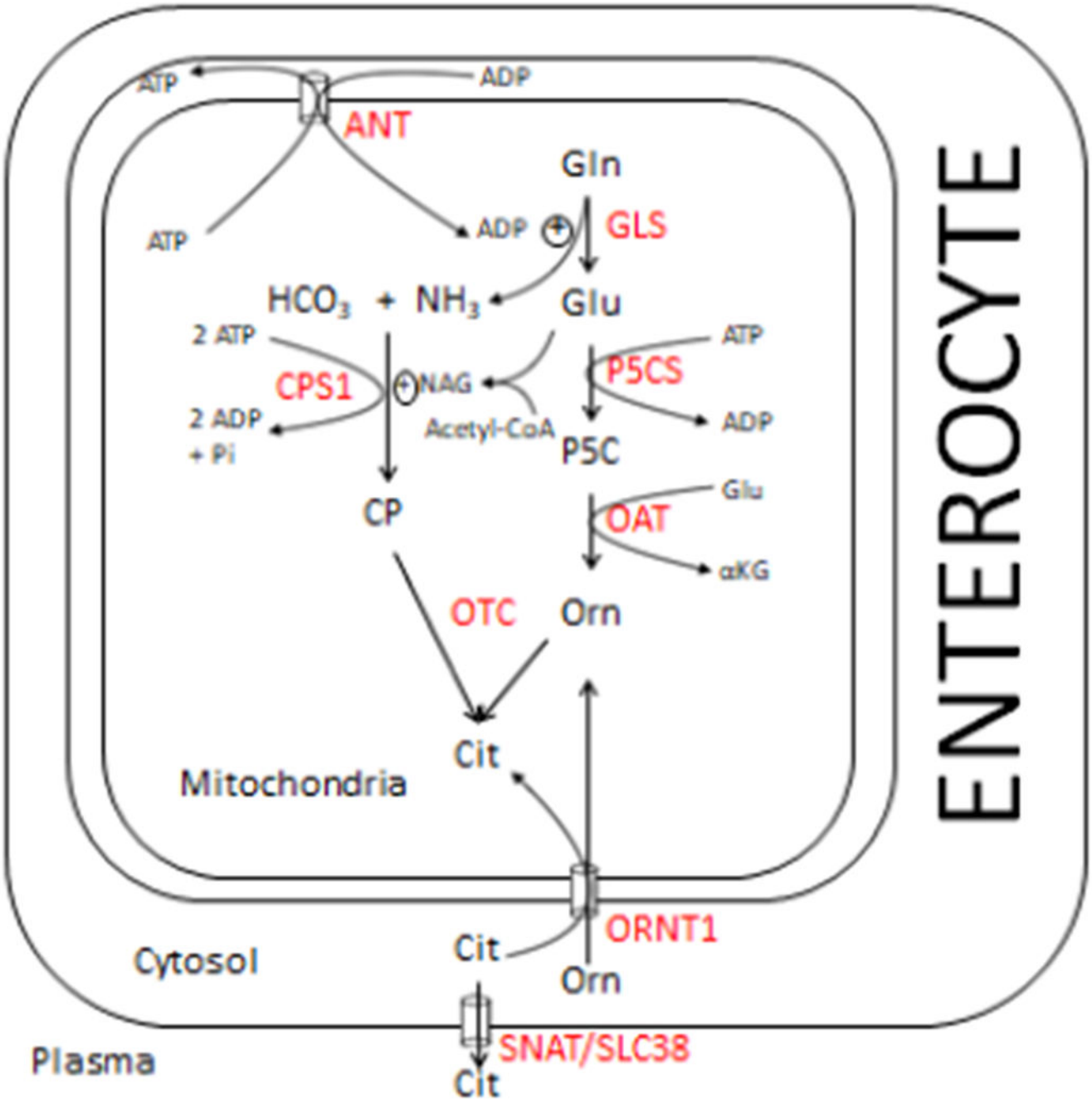

Fig 2.
Citrulline synthesis in the mitochondria of enterocytes

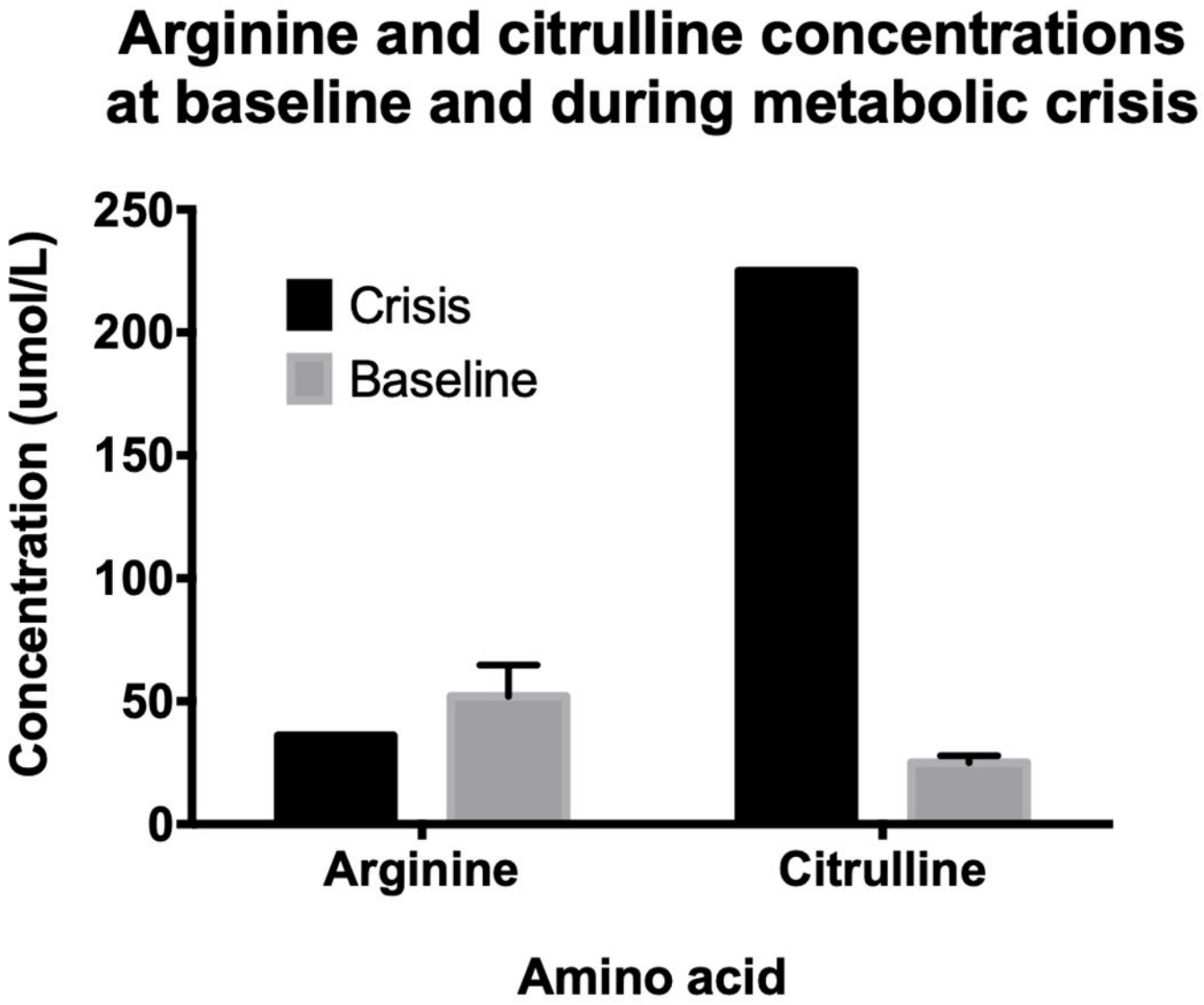

Figure 3.
Citrulline and arginine concentrations during a metabolic crisis compared to baseline.

Table 1.

Clinical features of patients

	Patient 1	Patient 2
Age	3 d	27 m
Sex	M	F
Dysmorphic Features	Micro-retrognathia, wide nasal bridge with anteverted nares and borderline low-set ears with increased posterior angulation	Micro-retrognathia, wide nasal bridge with anteverted nares and borderline low-set ears with increased posterior angulation
Hypotonia	Present	Present
Developmental delay	NA	Present
Eye manifestation	NA	Buphthalmos
Brain anomalies		Mega cysterna magna and hypoplastic vermis
Congenital heart defects	Mild cardiac hypertrophy	Concentric left ventricle hypertrophy
Antenatal	IUGR	IUGR

Table 2.

Plasma amino acid concentrations in patients with TMEM70 deficiency.

Plasma levels	Patient 2 (current report)		Tomas Honzik et al. (2010) Pt.10		Tomas Honzik et al. (2010) Pt.18		Norms
	Admission	Check-up	Admission	Check-up	Admission	Check-up	
Ammonia (umol/L)	494	79.4	870	39-49	226	ND	<60 uM
Lactate (mmol/L)	8.4	3.1	16	1.6	16	2.1-4.9	<2.3 mM
Glucose (mmol/L)	4.7	5.1	2.5	4.4	3	4.3-6.1	3.3-5.2 mM
Alanine (umol/L)	1,595	435-552	7,173	192-2,039	3,982	350-550	150-500 uM
Citrulline (umol/L)	225	23-27	151	7-29	143	5-50	5-50 uM
Arginine (umol/L)	36	43-61	22-83	22-162	90	27-124	10-150 uM
Glutamine (umol/L)	1,108	539-665	2,000	256-1,008	1,534	415-706	200-900 uM