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JIMD Reports

Volume 19

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ISSN 2192-8304 ISSN 2192-8312 (electronic)
JIMD Reports
ISBN 978-3-662-46189-1 ISBN 978-3-662-46190-7 (eBook)
DOI 10.1007/978-3-662-46190-7

Springer Heidelberg New York Dordrecht London
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Printed on acid-free paper

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JIMD Reports
DOI 10.1007/8904_2014_349

RESEARCH REPORT

A De Novo Variant in Galactose-1-P Uridylyltransferase (*GALT*) Leading to Classic Galactosemia

Thanh-Thanh (Claire) V. Tran · Ying Liu · Michael E. Zwick · Dhanya Ramachandran · David J. Cutler · Xiaoping Huang · Gerard T. Berry · Judith L. Fridovich-Keil

Received: 14 June 2014 / Revised: 27 July 2014 / Accepted: 31 July 2014 / Published online: 15 February 2015
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Abstract Classic galactosemia (CG) is a potentially lethal genetic disease that results from profound impairment of galactose-1-P uridylyltransferase (GALT), the middle enzyme in the Leloir pathway of galactose metabolism. Patients with CG carry pathogenic loss-of-function mutations in both of their *GALT* alleles; the parents of patients are considered obligate carriers. We report here a first exception to that rule – a *de novo* *GALT* variant in a patient with classic galactosemia. The new variant, c.563A>C (p.Q188P), which introduces a missense substitution near the active site of the GALT enzyme, was found in the compound heterozygous state in a child with classic galactosemia, but not in either of her parents. Extensive genomic studies of DNA from the child and both parents confirmed the expected degrees of relationship in the trio as well as inheritance of a common c.563A>G (p.Q188R) *GALT* mutation from the mother. This result demonstrates that not all pathogenic *GALT* mutations are inherited and raises concern that *GALT* may have a higher new mutation rate than previously believed.

Communicated by: John H Walter, MD FRCPCH

Competing interests: None declared

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Introduction

Classic galactosemia (CG) is a potentially lethal genetic disorder that affects close to 1/50,000 live births in the United States (Pyhtila et al 2014). Patients with CG demonstrate profound impairment of galactose-1-phosphate uridylyltransferase (GALT), the second enzyme in the Leloir pathway of galactose metabolism (Fridovich-Keil and Walter 2008). Classic galactosemia is inherited as an autosomal recessive trait. Patients carry loss-of-function mutations in both of their *GALT* alleles, and their parents are considered obligate heterozygotes for those same mutations. The carrier risk for a loss-of-function *GALT* mutation in the general population is estimated using the Hardy–Weinberg equation at about 1 in 112 people.

Sequencing the *GALT* alleles in patients with classic galactosemia reveals striking allelic heterogeneity with >250 distinct causal or ostensibly causal variants and a small number of ostensibly neutral polymorphisms reported as of January 2013 ((Calderon et al 2007), http://arup.utah.edu/database/GALT/GALT_welcome.php). Most variants are coding missense substitutions, but some are small insertions, deletions, or indels. Finally, some variants impact only noncoding sequence, and two are large deletions that remove most or all of the *GALT* gene (e.g., (Calderon et al 2007; Coffee et al 2006; Gort et al 2006; Papachristoforou et al 2014; Tyfield et al 1999), http://arup.utah.edu/database/GALT/GALT_welcome.php).

Some *GALT* mutations are common, especially in certain populations. For example, c.563A>G (p.Q188R) accounts for the vast majority of CG patient alleles in Northern

Europe and >60% of CG patient alleles in the United States (Tyfield et al 1999). The mutation c.855G>T (p.K285N) accounts for a majority of CG patient alleles in Eastern Europe (Lukac-Bajalo et al 2007). Finally, c.404C>T (p.S135L) accounts for a majority of CG patient alleles in parts of Africa (Henderson et al 2002). Other mutations, such as c.584T>C (p.L195P), are less common, accounting for only a small percentage of reported patient alleles, and yet others are "private" – reported only in one affected patient or family. The heterogeneity of CG patient alleles identified today stands as a striking record of the many new *GALT* variants that have arisen over the course of human history, but until now there have been no reported examples of *de novo GALT* variants identified in a living patient or family. We report here the first such case.

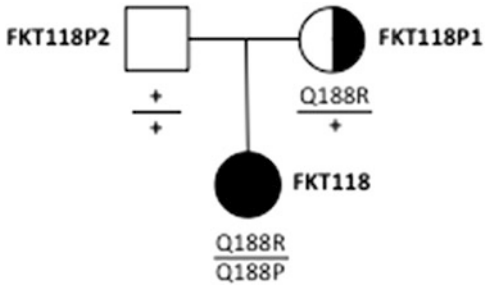

Fig. 1 The family described in this study. The child, FKT118, has biochemically confirmed classic galactosemia and a *GALT* genotype of c.563A>G (p.Q188R)/c.563A>C (p.Q188P). The mother, FKT118P1, is a heterozygous carrier of the c.563A>G (p.Q188R) mutation. The father, FKT118P2, does not show any *GALT* mutations in his DNA. The biological relationships illustrated were confirmed by extensive marker analysis. The couple also has another unaffected biological child (not illustrated) who has not been genotyped

Methods

Study Volunteers

The volunteers in this study were a child with classic galactosemia and both of her parents (Fig. 1). All three participated following appropriate informed consent/assent in Emory IRB Protocol # 00024933 (PI: JL Fridovich-Keil). The child was also a consented volunteer in a research study of galactosemia conducted out of Boston Children's Hospital (IRB Protocol # NS09-07-0369 (PI: GT Berry)).

GALT Genotyping of DNA from the Child

DNA samples isolated from independent blood draws from the child were assessed by dideoxy sequencing of the full *GALT* locus first in a clinical genetics laboratory (at Boston Children's Hospital) and subsequently in a research laboratory (at Emory University). In the research laboratory the *GALT* gene was PCR amplified as a single ~6 kb amplicon using the primers 5′-AGTACCAGGGGAG-GAATTAATTTGAATTTT-3′ and 5′-ATTCAGT-CACTGTCCAGCCTTAGTGTGATTT-3′ as described previously (Boutron et al 2012), and the relevant region was sequenced using the following custom primers: hGALT-F4(2): 5′-AAGCTTTGGTTCTGGGGAGT-3′ (3′ end anneals to position 1324), hGALT-F03(1): 5′-CCCTGGTCGG ATGTAACG-3′ (3′ end anneals to position 1164), and hGALT-R3: 5′-GTGTCTGGTAGGGC-CATGTT-3′ (3′ end anneals to position 2111). Both the clinical and research labs identified the same two mutations: the common c.563A>G (p.Q188R) missense mutation and also a novel c.563A>C (p.Q188P) missense mutation, each found in the patient in the compound heterozygous state.

GALT Genotyping of DNA from Both Parents

DNAs from parental saliva samples collected using Oragene kits were also genotyped by dideoxy sequencing of the relevant *GALT* locus using the following primers: hGALT-F4(2): 5′-AAGCTTTGGTTCTGGGGAGT-3′ and hGALT-R3: 5′-GTGTCTGGTAGGGCCATGTT-3′. Initial studies revealed that the mother, designated FKT118P1, was a carrier of the c.563A>G (p.Q188R) missense mutation and that the father, designated FKT118P2, carried neither mutation. Concerned that there may have been some mistake, we collected an independent saliva sample from FKT118P2 and repeated the test; again we found neither *GALT* mutation.

Affymetrix 6.0 Microarray Analysis of Genomic Markers in the Trio

To confirm the biological relationships among the three study volunteers, we subjected each DNA sample to genotyping using the Affymetrix Genome-Wide Human SNP Array 6.0 that tests more than 900,000 single nucleotide polymorphisms (SNPs) scattered across the genome (http://www.affymetrix.com/catalog/131533/AFFY/Genome-Wide-Human-SNP-Array-6.0#1_1). Briefly, to be considered for downstream analyses, each array was required to pass the Affymetrix recommended quality control (QC) parameters – namely >86% call rate, >0.4 contrast QC, and gender concordance. Genotype calling was performed using Birdseed algorithm (version 2), as implemented in the Affymetrix Power Tools software (APT 1.12.0). Estimations of alleles that are identical by state (IBS) from genotype calls were performed using an in-house script (D.J.C). In comparison with the parents, the proband had approximately 73% of genotypes that were IBS at both alleles (IBS2), 27% at one allele (IBS1) and 0%

Table 1 Percentage of >900,000 marker loci in the trio interrogated using the Affymetrix Genome-Wide Human SNP Array 6.0 showing 0, 1, or 2 alleles with identity by state (IBS)

Level of IBS	0 of 2 alleles with IBS	1 of 2 alleles with IBS	2 of 2 alleles with IBS
Expected for parent and offspring	0.0010	0.2692	0.7307
Observed for FKT118P1 and FKT118	0.0002	0.2665	0.7333
Observed for FKT118P2 and FKT118	0.0002	0.2686	0.7311

with IBS0 value, thus confirming the familial relationship (see Table 1).

Results

Case Report

The proband (FKT118, Fig. 1) is a 6-year-old girl who was born at gestational age 34 weeks and diagnosed with classic galactosemia as an infant in follow-up to an abnormal newborn screening result. On day 1 of life, she was supplemented with a proprietary formula containing lactose because of a poor sucking reflex. In the first week of life, she manifested poor feeding, emesis, loose stools, and jaundice. The serum total bilirubin concentration was 23 mg%. The newborn screening test for galactosemia was positive and the infant was switched to a soy-based formula on day 5 of life. Seizures occurred at 3 years of age, including grand mal seizures. There is a history of language delay and a speech defect. Magnetic resonance imaging (MRI) of the brain conducted at 5 years of age revealed areas of patchy subcortical white matter signal increases possibly related to non-suppressed perivascular spaces. On physical examination at age five, the proband had scars on her face due to a seizure-induced fall. There was diffuse hypotonia. There were no discernible peaks of galactitol on magnetic resonance spectroscopy (MRS). The erythrocyte GALT enzyme activity was undetectable, and the galactose-1-phosphate level was 3.67 mg% while on a lactose-restricted diet (reference range <1 mg%). The urinary galactitol excretion was 178 μmol/mmol creatinine (reference range 2–36). By early childhood the serum follicle stimulating hormone (FSH) level was elevated and the anti-Müllerian hormone level was undetectable. A DEXA scan of bone density did not reveal any region with a Z score that was below two standard deviations of the reference mean.

Dideoxy Sequencing Reveals a Novel *GALT* Variant in a Child with Classic Galactosemia

Initial *GALT* genotyping was performed in 2008 in a clinical genetics lab (Emory Genetics Laboratory) using allele-specific primer extension (Luminex; http://www.premierbiosoft.com/primerplex/allele-specific-primer-extension.html) to detect the presence or absence of a panel of common mutations. The result demonstrated presence of c.563A>G (p.Q188R) and absence of the wild-type allele at that locus; no other mutations included in the panel were detected. The genotype was therefore reported, to the sensitivity of the assay, as c.563A>G (p.Q188R)/c.563A>G (p.Q188R). Of note, c.563A>C (p.Q188P) was not one of the common *GALT* mutations included in the panel.

Years later a fresh blood sample was collected (at Boston Children's Hospital) and subjected to *GALT* genotyping via full gene dideoxy sequencing. Two mutations were detected, c.563A>G (p.Q188R) and c.563A>C (p.Q188P), each in the compound heterozygous state. With permission, this result was shared with our research lab at Emory University that repeated the dideoxy sequencing on an archived DNA sample from the child; the c.563A>G (p.Q188R)/c.563A>C (p.Q188P) result was confirmed (Fig. 2). A search of the galactosemia literature and *GALT* mutation database (http://arup.utah.edu/database/GALT/GALT_welcome.php) revealed no prior reports of the c.563A>C (p.Q188P) mutation.

Dideoxy Sequencing of Parental *GALT* Loci Coupled with Comprehensive Genomic SNP Analysis of the Trio Reveals that c.563A>C (p.Q188P) is a De Novo Variant in this Family

In response to a parental request for *GALT* genotyping to determine which side of the family was at risk for carrying which *GALT* mutation, parental saliva samples were

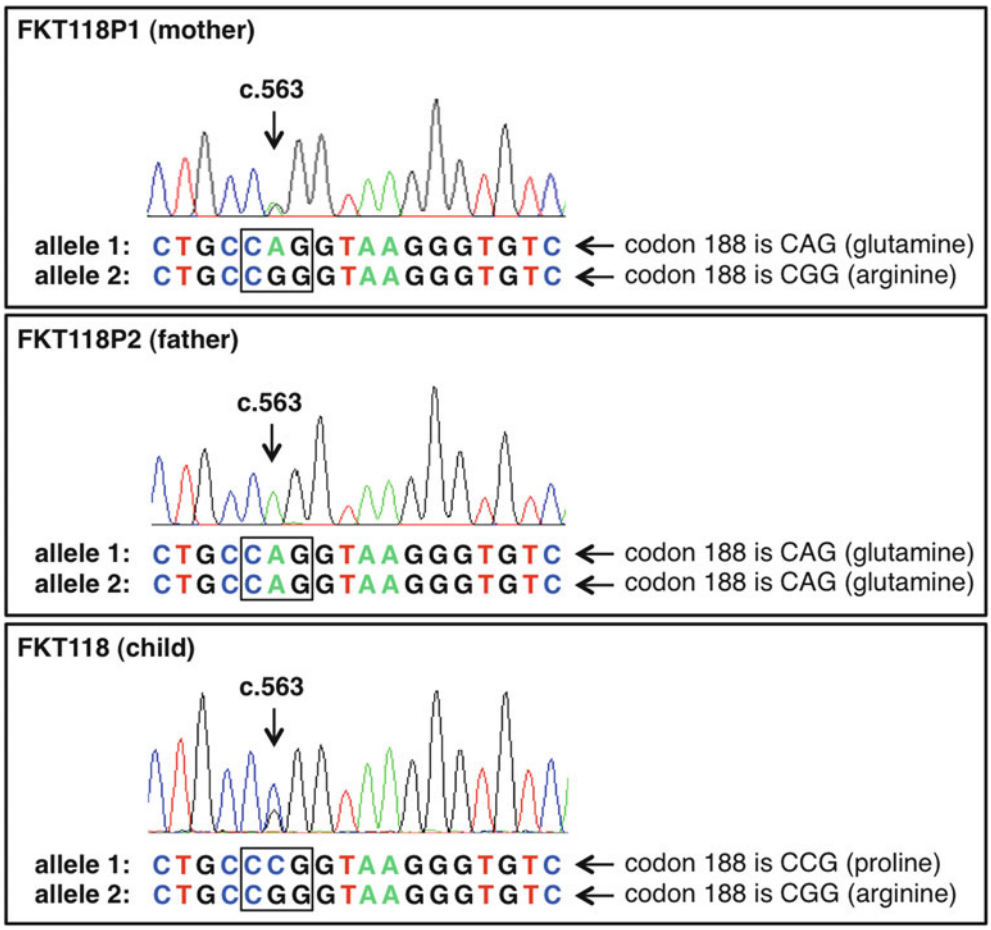

Fig. 2 *GALT* sequencing results demonstrate the presence of mutations at *GALT* position c.563 in the mother and child, but not the father. The figure presents representative dideoxy sequencing results of a relevant segment of the *GALT* locus derived from blood from the child and saliva from both parents. All sequencing results were confirmed with replicate reactions sequencing both strands. The three bases boxed in each sequence encode residue 188 in the GALT protein. The wild-type human *GALT* gene sequence can be found at NCBI accession number NG_009029 (version NG_009029.1 GI:288541293)

collected and studied. Dideoxy sequencing at the *GALT* locus in both parental samples revealed that the mother, FKT118P1, was a carrier of the common c.563A>G (p.Q188R) mutation, but no mutation was detected in the father (FKT118P2) (Fig. 2). Repeat testing of the sample confirmed the result. The family was notified and consulted regarding possible next steps, and an independent saliva sample was collected from the father and analyzed, but again no *GALT* mutation was detected.

Finally, with parental permission reaffirmed, DNA samples from the child and both parents were subjected to whole genome SNP genotyping using Affymetrix 6.0 microarrays to test the biological relationships of the samples. Comparing rates of identity by state (IBS) at each of the >900,000 SNPs genotyped for the trio confirmed, beyond question, that both parents were indeed the biological parents of the child (Table 1). This result, combined with the *GALT* genotyping result (Fig. 2), demonstrated that c.563A>C (p.Q188P) was a *de novo* variant in the child.

Discussion

We report here what is, to our knowledge, the first case of a *de novo* pathogenic variant in human *GALT* associated with classic galactosemia: a c.563A>C transversion encoding the substitution of proline (P) in place of glutamine (Q) at residue 188 in the active site of the GALT enzyme. Repeated sequencing of *GALT* loci in the patient conducted in two separate laboratories using independent samples confirmed the presence of the transversion, and repeated sequencing of independent DNA samples from both parents failed to detect the transversion. Genotyping of hundreds of thousands of polymorphic markers in the child and both parents confirmed the biological relationships among the trio.

While it is notable that the *de novo* variant in this child occurred at exactly the same location, c.563, as her inherited mutation, it remains unclear whether the presence of the inherited mutation in some way predisposed the other allele to mutate early in embryogenesis. Alternatively, it is possible that c.563 in *GALT* is intrinsically vulnerable to mutation. Finally, it is possible that the occurrence of both the inherited and *de novo* mutations at exactly the same nucleotide in *GALT* in this child was simply a striking but random coincidence.

The *de novo* variant documented here is important for two reasons: first that a *de novo* mutation event in *GALT* can lead to classic galactosemia has clear implications for genetic counseling of families, especially if the proband carries a private *GALT* mutation. In this instance, parents should not be considered obligate carriers and recurrence rates for the couple should not be predicted unless both parents have been genotyped and confirmed to be carriers. Second, this result is important because it raises the possibility that new mutations may also occur in genes associated with other autosomal recessive disorders, especially those that show strong allelic heterogeneity among patients. Consistent with this possibility, de novo mutations have been reported previously for both phenylketonuria (Eiken et al 1992) and cystic fibrosis (White et al 1991).

Finally, whether this report of a *de novo* pathogenic mutation in *GALT* leading to classic galactosemia represents the chance observation of an extremely rare event, or the first observation of what is actually a common event, remains unknown. Until family studies or population carrier screening are commonplace for galactosemia, it will be very difficult to estimate with accuracy the new mutation rate for *GALT*.

Acknowledgments We are especially grateful to the family who participated in this research study; without them none of this work would have been possible. We also thank the Emory Integrated Genomics Core who conducted the Affymetrix genotyping reported here. This work was supported in part by funds from NIH R01 DK059904 (PI: JLFK); DR was supported in part by funds from a training grant in Human Disease Genetics 1T32MH087977 (PI: ST Warren).

1-Sentence Synopsis

Genomic studies confirm a *de novo* variant in human galactose-1-P uridylyltransferase (*GALT*) leading to classic galactosemia.

Compliance with Ethical Guidelines

Conflict of Interest

Thanh-Thanh (Claire) V. Tran declares that she has no conflict of interest.

Ying Liu declares that she has no conflict of interest.

Michael Zwick declares that he has no conflict of interest.

Dhanya Ramachandran declares that she has no conflict of interest.

David Cutler declares that he has no conflict of interest.

Xiaoping Huang declares that she has no conflict of interest.

Gerard Berry declares that he has no conflict of interest.

Judith Fridovich-Keil declares that she has no conflict of interest.

Informed Consent

"All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5)."

All data from specific individuals reported here were collected following appropriate informed consent/assent and with approval by the Emory University Institutional Review Board (IRB# 00024933, PI: JL Fridovich-Keil).

Animal Rights

"This article does not contain any studies with animal subjects performed by any of the authors."

Contributions of Each Author

Thanh-Thanh (Claire) V. Tran confirmed the *GALT* genotype of the child, performed *GALT* genotyping for both parents, and also helped to edit the manuscript.

Ying Liu helped to oversee Ms. Tran's work, designed the *GALT* sequencing primers, and also helped to edit the manuscript.

Michael Zwick oversaw the Affymetrix 6.0 genotyping of the three DNA samples that confirmed both parent–child relationships and also helped to edit the manuscript.

Dhanya Ramachandran performed genotype calling and statistical analyses on Affymetrix SNP 6.0 data and also helped to edit the manuscript.

David Cutler assisted with the interpretation of the Affymetrix genotyping data and also helped to edit the manuscript.

Xiaoping Huang and Gerard Berry originally identified the Q188P variant in the child and also helped to write and edit the manuscript.

Judith Fridovich-Keil initiated the project, coordinated the efforts of the other authors, and wrote most of the manuscript.

References

Boutron A, Marabotti A, Facchiano A et al (2012) Mutation spectrum in the French cohort of galactosemic patients and structural simulation of 27 novel missense variations. Mol Genet Metab 107:438–447

Calderon F, Phansalkar A, Crockett D, Miller M, Mao R (2007) Mutation database for the galactose-1-phosphate uridyltransferase (<I>GALT</I>) gene. Hum Mutat 28:939–943

Coffee B, Hjelm L, DeLorenzo A, Courtney E, Yu C, Muralidharan K (2006) Characterization of an unusual deletion of the galactose-1-phosphate uridyl transferase (GALT) gene. Genet Med 8:635–640

Eiken H, Knappskog P, Apold J, Skjelkvåle L, Boman H (1992) A de novo phenylketonuria mutation: ATG (met) to ATA (ile) in the start codon of the phenylalanine hydroxylase gene. Hum Mutat 1:388–391

Fridovich-Keil JL, Walter JH (2008) Galactosemia. In: Valle D, Beaudet A, Vogelstein B, Kinzler K, Antonarakis S, Ballabio A (eds) The online metabolic & molecular bases of inherited disease. McGraw Hill, New York, http://www.ommbid.com/

Gort L, Boleda M, Tyfield L et al (2006) Mutational spectrum of classical galactosaemia in Spain and Portugal. JIMD 29:739–742

Henderson H, Leisegang F, Brown R, Eley B (2002) The clinical and molecular spectrum of galactosemia in patients from the Cape Town region of South Africa. BMC Pediatr 2:7

Lukac-Bajalo J, Kuzelicki NK, Zitnik IP, Mencej S, Battelino T (2007) Higher frequency of the galactose-1-phosphate uridyl transferase gene K285N mutation in the Slovenian population. Clin Biochem 40:414–415

Papachristoforou R, Petrou P, Sawyer H, Williams M, Drousiotou A (2014) A novel large deletion encompassing the whole of the galactose-1-phosphate uridyltransferase (GALT) gene and extending into the adjacent interleukin 11 receptor alpha (IL11RA) gene causes classic galactosemia associated with additional phenotypic abnormalities. JIMD Rep 12:91–98

Pyhtila B, Shaw K, Neumann S, Fridovich-Keil J (2014) Newborn screening for galactosemia in the United States: looking back, looking around, and looking ahead. JIMD Rep

Tyfield L, Reichardt J, Fridovich-Keil J et al (1999) Classical galactosemia and mutations at the galactose-1-phosphate uridyl transferase (GALT) gene. Hum Mutat 13:417–430

White M, Leppert M, Nielsen D et al (1991) A de novo cystic fibrosis mutation: CGA (Arg) to TGA (stop) at codon 851 of the CFTR gene. Genomics 11:778–779

JIMD Reports
DOI 10.1007/8904_2014_355

CASE REPORT

Refsum Disease Presenting with a Late-Onset Leukodystrophy

Flavie Bompaire · Véronique Marcaud · Emmanuelle Le Trionnaire · Frédéric Sedel · Thierry Levade

Received: 26 May 2014 / Revised: 07 August 2014 / Accepted: 18 August 2014 / Published online: 21 January 2015
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Abstract Adult Refsum disease is an autosomal recessive peroxisomal disorder characterized by phytanic acid storage. Clinical symptoms usually begin in late childhood before the age of 20. Typical clinical presentation includes nyctalopia caused by retinitis pigmentosa, and anosmia. After 10–15 years, deafness, cerebellar ataxia, polyneuropathy, ichthyosis, and cardiac arrhythmia can occur.

We report the case of a very late-onset adult Refsum disease presenting with marked cognitive decline and severe leukoencephalopathy, without peripheral nervous system involvement. Brain MRI showed a leukoencephalopathy involving the periventricular white matter, subcortical area, and the brainstem with relative sparing of juxtacortical U fibers. This was associated with severe cortical and subcortical atrophy with ventricle dilatation. MR spectroscopy showed a marked increase in the choline/NAA ratio. Elevated plasma phytanic acid level was found, whereas plasma levels of pristanic and very long chain fatty acids were normal. The patient is homozygous for a previously undescribed *PHYH* frameshift mutation. Whether the very unusual phenotype is related to this peculiar mutation remains unclear.

Communicated by: Ertan Mayatepek, MD

Competing interests: None declared

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This 72-year-old woman with a past history of left-eye congenital amaurosis, insulino-requerant diabetes, and elevated blood pressure came to medical attention because of progressive dementia and walking difficulties. She had no noticeable neurological problem until the age of 69, when she experienced balance difficulties and memory problems. A year later, after a fall, a C2 vertebra fracture was discovered. Within the next months, apragmatism (inability to wash or dress) was noticed along with a severe memory loss (she forgot the number and names of her children), nycthemeral rhythm inversion, and urinary incontinence. Neuropsychological tests showed severe frontal syndrome, dementia with spatiotemporal disorientation, and memory loss. Clinical examination disclosed gait apraxia reminiscent of frontal lobe dysfunction with no clear cerebellar ataxia.

A brain magnetic resonance (MR) imaging showed a leukoencephalopathy involving the periventricular white matter, subcortical area, and the brainstem with relative sparing of juxtacortical U fibers (Fig. 1a, b). This was associated with severe cortical and subcortical atrophy with ventricle dilatation. MR spectroscopy showed a marked increase in the choline/NAA ratio (Fig. 1c).

Electromyography showed no polyneuropathy but only bilateral carpal tunnel syndrome. Electroretinogram was normal in the right eye (not evaluable on the left). Cardiac

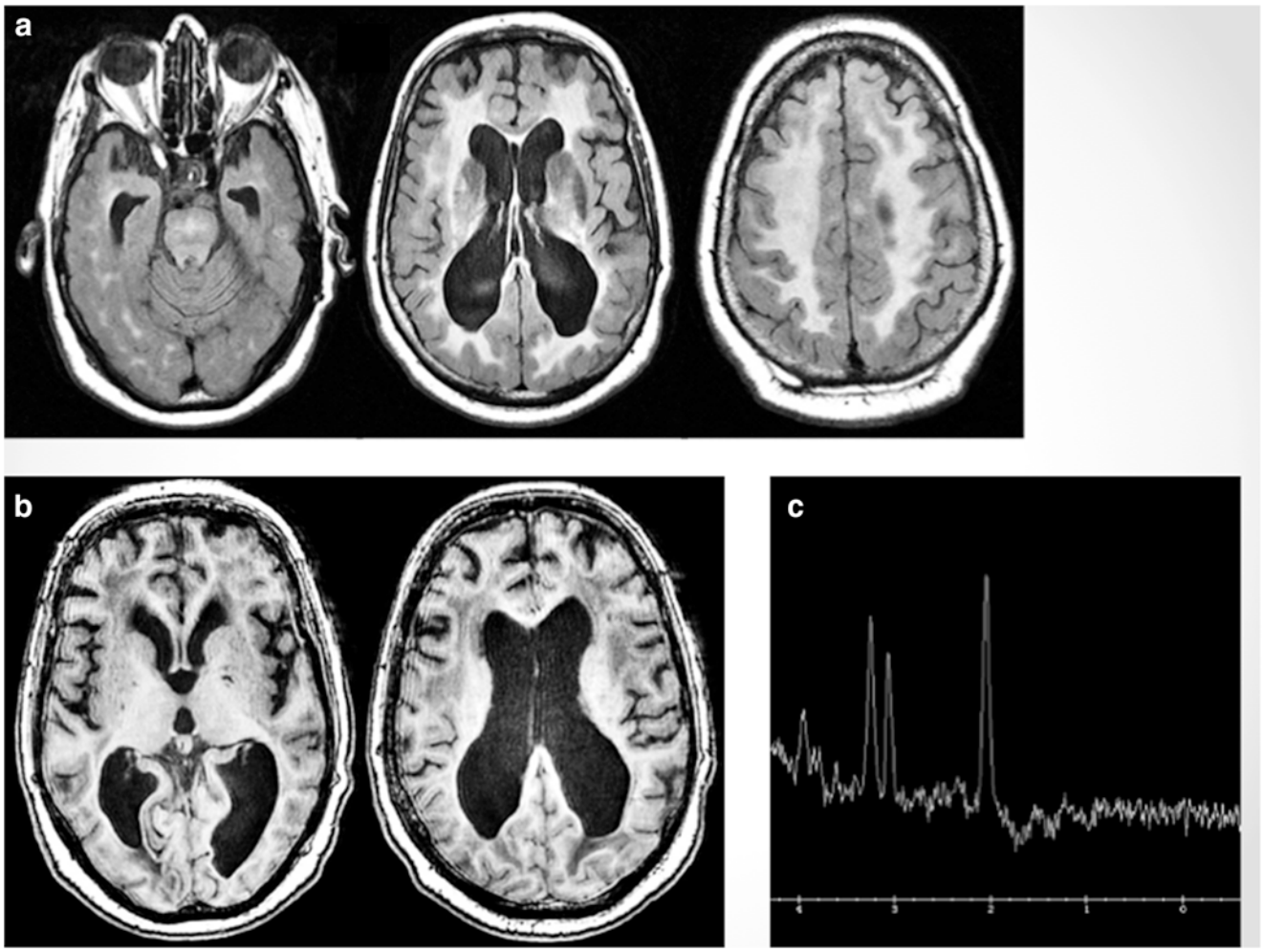

Fig. 1 Brain magnetic resonance imaging. (**a**) Axial FLAIR: extensive leukoencephalopathy, (**b**) axial T1: subcortical atrophy with ventricle enlargement, (**c**) spectroscopy: increased choline/NAA ratio

echography only showed left ventricle hypertrophy consistent with her past history of elevated blood pressure.

Cerebrospinal fluid (CSF) showed two white cells/mm^3, with slightly increased protein content (0.46 g/L).

A metabolic workup disclosed very high plasma phytanic acid level (458 μmol/L; control <10) later confirmed at 704 μmol/L. In contrast, plasma pristanic acid level was normal (0.27 μmol/L) as were very long chain fatty acids.

Genetic analysis of the *PHYH* gene revealed a novel homozygous mutation in exon 1, c.42-60dup, a frameshift mutation resulting in a premature stop codon at position 56 of the mutated sequence (which corresponds to codon 50 of the normal sequence). The patient also had 3 *PHYH* polymorphisms: the first was homozygous in exon 3 (c.153C>T, p.Asn51Asn), the second was heterozygous in intron 4 (c.415-11delT), and the third was homozygous in exon 6 (c.636A>G, p.Thr212Thr).

The severe cognitive decline was not compatible with a phytanic acid-restricted diet. LDL apheresis was not performed because of the patient's age with little expectable benefit.

Discussion

We report the very unusual case of a 72-year-old woman presenting with 2 years' history of progressive dementia, walking problems, and diffuse leukoencephalopathy. Diagnosis of Refsum disease was established by elevated plasma levels of phytanic acid and *PHYH* mutations.

Adult Refsum disease is an autosomal recessive peroxisomal disorder characterized by phytanic acid storage, due to deficient activity of phytanoyl-CoA hydroxylase that catalyzes the first step of phytanic acid alpha-oxidation (Wanders and Komen 2007). Phytanic acid accumulates in fat-containing tissues (i.e., nerve, brain, retina, and adipose tissue).

Clinical symptoms usually begin in late childhood before the age of 20, but late-onset forms (around 50 years) have

also been described. The disease often starts with nyctalopia caused by retinitis pigmentosa, and anosmia. After 10–15 years, deafness, cerebellar ataxia, polyneuropathy, ichthyosis, and cardiac arrhythmia can occur. About one third of the patients present with shortened metacarpal and 4th metatarsal bones (Wierzbicki et al. 2000, 2002; Rüether et al. 2010).

Two causative genes have been identified. The first one is *PHYH* encoding phytanoyl-CoA hydroxylase. The second one is *PEX7* encoding peroxin 7, a receptor required to import several proteins into the peroxisomal matrix. Although in most cases *PEX7* mutations lead to rhizomelic chondrodysplasia punctata, some mutations have been associated with a Refsum disease phenotype (Van den Brink et al. 2003).

Treatment of Refsum disease may consist in a special diet avoiding phytanic acid-rich food (dairy products, fish, meat, and fat of ruminant animals). Plasma exchange or LDL apheresis can be used in addition to the diet when phytanic acid plasma level is dangerously high (above 300 μmol/L) (Weinstein 1999).

Interestingly, the classical clinical features of Refsum disease were absent in our patient: she had no retinitis pigmentosa, no polyneuropathy, no cerebellar ataxia, no anosmia, no ichthyosis, no hyperproteinorachia, no cataract, no hearing loss, and no skeletal abnormalities. In addition, the association of marked cognitive decline and severe leukoencephalopathy is uncommon and not yet reported. This very unusual phenotype might be related to the particular (yet undescribed) mutation carried by our patient. The homoallelic c.42-60 duplication event in exon 1 found on the genomic DNA creates a premature stop codon. There was no notion of consanguinity in the family. The homozygous polymorphisms in exons 3 and 6, which do not change the amino acid sequence (Jansen et al. 2004), have probably no consequence on the phenotype. The c.42-60 duplication could result in nonsense-mediated mRNA decay. However, this was not the case as the duplication was also detected on cDNA prepared from the patient's lymphocytes. Such a mutation is expected to result in the synthesis of a markedly truncated (only 55 amino acids out of the normal 338 residues), very small, and therefore nonfunctional polypeptide. Thus, this mutation appears as a severe molecular defect. This translates into an increased plasma phytanic acid level, which demonstrates the causal link between the molecular defect and the metabolic abnormality. Nevertheless, at the moment, we have no clear explanation for the absence of the classical symptoms. One possibility to account for the unusual and late presentation of the disease would be that for a long period of her life the patient had a phytanic acid-poor diet, which would have delayed the onset (and possibly changed the symptoms) of the disease.

This observation expands the spectrum of Refsum disease that may also present as a very late onset leukodystrophy with no peripheral nervous system involvement.

One Sentence Take-Home Message

A 69-year-old female patient, homozygous for a novel *PHYH* mutation, presented with an unusual Refsum disease phenotype including progressive dementia, gait apraxia, and memory loss, associated with diffuse leukoencephalopathy.

Compliance with Ethics Guidelines

Conflict of Interest

Dr. Flavie Bompaire, Dr. Véronique Marcaud, Mrs. Emmanuelle Le Trionnaire, Dr. Frédéric Sedel, and Prof. Thierry Levade declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the patient for being included in the study.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

Details of the Contributions of Individual Authors

Drs. Bompaire, Marcaud, and Sedel took care of the patient and diagnosed adult Refsum disease. Mrs. Le Trionnaire and Prof. Levade performed the genetic analysis and its interpretation. Dr. Bompaire wrote the text under the supervision of Dr. Sedel and Prof. Levade, with the help of Dr. Marcaud.

References

Jansen GA, Waterham HR, Wanders RJ (2004) Molecular basis of Refsum disease: sequence variations in phytanoyl-CoA hydroxylase (PHYH) and the PTS2 receptor (PEX7). Hum Mutat 23 (3):209–218

Rüether K, Baldwin E, Casteels M et al (2010) Adult Refsum disease: a form of tapetoretinal dystrophy accessible to therapy. Surv Ophthalmol 55(6):531–538

Van den Brink DM, Brites P, Haasjes J et al (2003) Identification of PEX7 as the second gene involved in Refsum disease. Am J Hum Genet 72(2):471–477

Wanders RJ, Komen JC (2007) Peroxisomes, Refsum's disease and the alpha- and omega-oxidation of phytanic acid. Biochem Soc Trans 35(Pt 5):865–869

Weinstein R (1999) Phytanic acid storage disease (Refsum's disease): clinical characteristics, pathophysiology and the role of therapeutic apheresis in its management. J Clin Apher 14(4):181–184

Wierzbicki AS, Mitchell J, Lambert-Hammill M et al (2000) Identification of genetic heterogeneity in Refsum's disease. Eur J Hum Genet 8(8):649–651

Wierzbicki AS, Lloyd MD, Schofield CJ, Feher MD, Gibberd FB (2002) Refsum's disease: a peroxisomal disorder affecting phytanic acid alpha-oxidation. J Neurochem 80(5):727–735

JIMD Reports
DOI 10.1007/8904_2014_356

RESEARCH REPORT

Making the White Matter Matters: Progress in Understanding Canavan's Disease and Therapeutic Interventions Through Eight Decades

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Received: 26 March 2014 / Revised: 05 August 2014 / Accepted: 12 August 2014 / Published online: 21 January 2015
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Abstract Canavan's disease (CD) is a fatal autosomal recessive pediatric leukodystrophy in which patients show severe neurodegeneration and typically die by the age of 10, though life expectancy in patients can be highly variable. Currently, there is no effective treatment for CD; however, gene therapy seems to be a feasible approach to combat the disease. Being a monogenic defect, the disease provides an excellent model system to develop gene therapy approaches that can be extended to other monogenic leukodystrophies and neurodegenerative diseases. CD results from mutations in a single gene aspartoacylase which hydrolyses *N*-acetyl aspartic acid (NAA) which accumulates in its absences. Since CD is one of the few diseases that show high NAA levels, it can also be used to study the enigmatic biological role of NAA. The disease was first described in 1931, and this review traces the progress made in the past 8 decades to understand the disease by enumerating current hypotheses and ongoing palliative measures to alleviate patient symptoms in the context of the latest advances in the field.

Clinical Description

Canavan's disease (CD) is a fatal pediatric leukodystrophy in which the central nervous system (CNS) white matter shows progressive spongy degeneration (Matalon et al. 1993). It results from deficiency of aspartoacylase (*N*-acetyl-L-aspartate amidohydrolase; EC 3.5.1.15) (Matalon et al. 1988), an enzyme required for catabolism of *N*-acetylaspartate (NAA), the second most abundant amino acid derivative in the CNS. The disease was thought to be ethnically confined to Ashkenazi Jews; however, a growing number of non-Jewish patients have been identified worldwide (Elpeleg and Shaag 1999).

Patients show vacuolization in the subcortical white matter (which becomes gelatinous), edema and demyelination, and thin delicate meninges. Swollen cortical protoplasmic astrocytes containing membrane-bound cytoplasmic vacuoles, elongated mitochondria with distorted cristae, degraded axonal medullary sheaths, and a prominent increase in protoplasmic astrocytes characterize the diseased brain. There also seems to be a significant loss of proteolipid protein and total lipids in the white matter (Beaudet 2001).

Clinical manifestations include atonia of neck muscles, hypotonia, severe psychomotor and mental retardation, seizures, NAAduria, blindness, and megalencephaly. Previously, the disease was classified based on onset and severity of symptoms into congenital, infantile, and juvenile forms, congenital being the most severe with symptom onset in the first few weeks of life (Adachi et al. 1973).

Aspartoacylase: Biochemistry and Genetics

CD was described as an enzyme deficiency disease (Matalon et al. 1988) when NAA-rich patient plasma and urine samples on incubation with extracts of normal fibroblasts increased aspartate levels suggesting a block in NAA catabolism. Aspartoacylase was detected by immunohistochemistry in oligodendrocytes and nonreactive microglia but not neurons (Madhavarao et al. 2004).

Communicated by: Ivo Barić, M.D., PhD, Professor of Pediatrics

Competing interests: None declared

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However large reticular and motor neurons in the brainstem and spinal cord showed moderate staining for aspartoacylase (Klugmann et al. 2003).

Intensive genetic studies identified human aspartoacylase cDNA from kidney based on sequence information from bovine aspartoacylase. The gene was localized to 17p13-ter, comprises six exons encoding a 313 amino acid long polypeptide chain with a molecular mass of 36 kd (Kaul et al. 1993). Aspartoacylase deacetylates NAA (Birnbaum et al. 1952) with a K_m of 8.5×10^{-4} mol/L and V_{max} of 43,000 nmol/min per mg of protein (Kaul et al. 1991). Isolation of the aspartoacylase cDNA sequence and hence identification of causative mutations for CD was a landmark in the field as it made molecular diagnosis and gene therapy of CD possible (Kaul et al. 1994). Currently, >54 mutations are associated with Canavan's disease (Hershfield et al. 2007) with new mutations being identified on a regular basis. Several mutations have been identified to result in a loss of aspartoacylase activity as well as lack of expression (Sommer and Sass 2012) though all mutations have not been completely characterized; hence, DNA analysis limits prenatal diagnosis to carrier couples in populations with known mutations. The previous method of measuring AspA activity in the chorionic villi or amniocytes from fetuses was deemed unreliable (Matalon and Michals-Matalon 1999a). Currently quantification of NAA in the amniotic fluid by chromatography coupled with mass spectrometry (Jakobs et al. 1991; Bennett et al. 1993; Al-Dirbashi et al. 2009) is considered to be a reliable alternative for diagnostic testing. Patients, however, follow a similar course of disease irrespective of the residual aspartoacylase activity, and as life expectancy is highly variable in CD patients even with the same genotype, hence there are no defined correlations between the disease phenotype and mutations (Matalon and Michals-Matalon 1999b).

Characterization of the Substrate

NAA is an abundant (5–10 mM) amino acid derivative in the vertebrate CNS with a molecular mass of 175.1 Da (Birken and Oldendorf 1989). Most neuropsychiatric disorders like schizophrenia show a decrease in NAA levels (Mondino and Saoud 2013); however, CD is a rare example along with sickle cell disease (Steen 2005), tardive dyskinesia (Tsai et al. 1998), and multiple sclerosis (Tortorella et al. 2011) that shows elevated NAA levels. NAAduria is a reliable and specific biochemical index for CD (Matalon et al. 1988). Additionally CD patients show very high amounts of NAA in their blood and CSF (Matalon et al. 1988).

NAA serves as a marker of neuronal integrity as high levels indicate brain injury and disease (Birken and Oldendorf 1989); however, the biological function of NAA remains enigmatic. It is metabolically compartmentalized, being exclusively produced in neuronal mitochondria by L-aspartate *N*-acetyltransferase (AspNAT) [EC 2.3.1.17] (Ariyannur et al. 2010), and metabolized in oligodendrocytes (Madhavarao et al. 2003) by aspartoacylase.

Theories Behind the Molecular Etiology of CD

A comprehensive investigation of aspartoacylase regulation in oligodendrocytes is essential to understand CD pathogenesis because the specific connection between aspartoacylase deficiency and the failure of proper CNS development and myelination remains unclear. Listed here are existing theories that attempt to offer an explanation.

Molecular Water Pump (MWP) and Osmolyte Imbalance Theory

The adult brain is a major source of metabolic water and uses about 20 % of the daily caloric intake. Additionally, two major CD symptoms – increased CSF pressure and intramyelinic edema – are hallmarks of profound fluid imbalance suggesting the existence of an efficient MWP (Baslow 1999).

The theory suggests that NAA accumulation could result in osmolytic imbalance in the brain since it is similar in nature to taurine (Taylor et al. 1995), an important CNS osmolyte. Furthermore, NAA has two juxtaposed anabolic (neurons) and catabolic (oligodendrocytes) compartments that suggest a mechanical framework for a MWP in the brain.

A MWP involves the synthesis and facilitated diffusion of a hydrated intracellular osmolyte (NAA with its ion-dipole and dipole–dipole associated water) down its gradient. At maturity, intraneuronal NAA concentration is ~10 ± 14 mM, while the interstitial concentration is only 80–100 μm (Sager and Hansen 1997), indicating a large outward-directed transport gradient. To maintain tissue–ECF osmotic balance (in the periaxonal space), the osmolyte (NAA) is rapidly hydrolyzed (by aspartoacylase to form aspartate and acetate) to maintain the concentration gradient. The metabolic products are dehydrated as they are once again taken up by an active transport mechanism to complete the cycle (Baslow 1999). Presumably, the acetate is taken up by astroglial processes at axon internodes and synapses (Tsacopoulos 1996) recycling the hydrolyzed products to produce more NAA (Fig. 1).

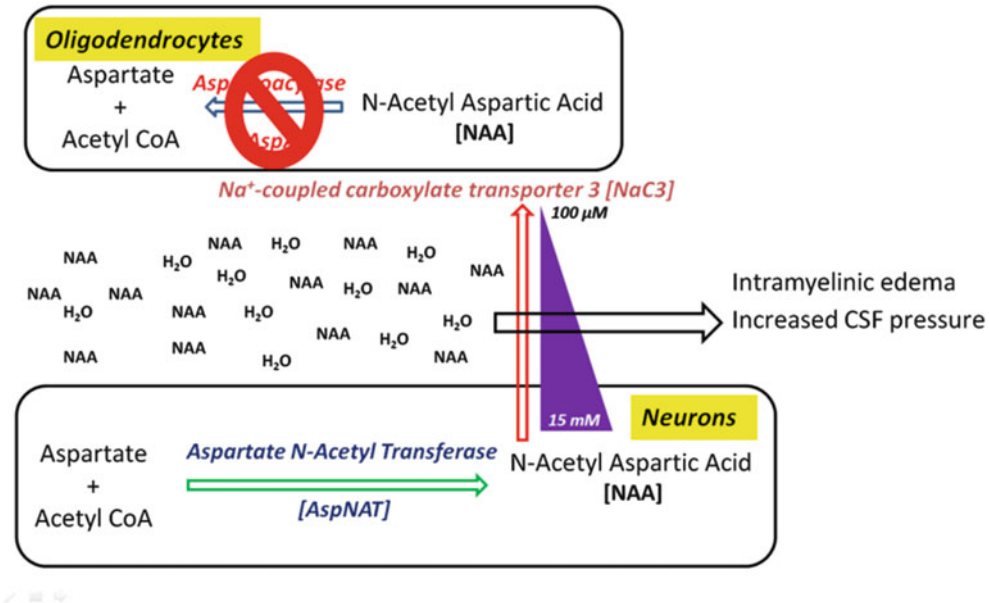

Fig. 1 Molecular water pump theory: accumulation of the L-enantiomer of NAA in interstitial space draws out water from surrounding cells causing edema in the brain as well as shrinking nearby cells to give rise to vacuoles

Paranodal seals connecting oligodendrocytes to axons could serve as the site for the NAA inter-compartmental bidirectional cycle (Baslow 1999). In CD, aspartoacylase deficiency would lead to accumulation of NAA and water and result in increased hydrostatic pressure that could loosen the tight junctional seals separating interlamellar spaces from the extracellular periaxonal and parenchymatous spaces resulting in intramyelinic edema (Hirano 1981). Subsequent demyelination could create vacant spaces within the white matter leading to the spongy brain phenotype.

Dysmyelination Theory

NAA-derived acetyl groups were shown to be involved in fatty acid synthesis as acetyl-labeled NAA injection into rats resulted in maximum fatty acid incorporation just before and during myelination (D'Adamo Jr et al. 1968). Later studies indicated that NAA was transferred from the axon to myelin, and NAA-derived acetate was incorporated into myelin lipids (Chakraborty et al. 2001).

This theory proposes that deficiency of NAA-derived acetate decreases the synthesis of myelin-associated lipids in CD leading to dysmyelination (Madhavarao et al. 2005) (Fig. 2). Additionally, temporal correlations have been shown between developmental increases in aspartoacylase activity and myelination (Bhakoo et al. 2001). Similar to human CD patients, brain acetate levels are reduced by ~80% in aspartoacylase knockout (ASPAKO) mice during peak postnatal myelination, while myelin lipids such as cerebrosides and sulfatides are reduced (Madhavarao et al. 2005; Ahmed et al. 2013). These data speculate that NAA-derived acetate is essential during postnatal myelination to supply substrate for some proportion of the lipids that make up myelin sheaths in the developing brain.

Studies on the Nur7 KO mouse (Traka et al. 2008) suggest that spongy degeneration is not dependent on disrupted myelin synthesis. Even though Nur7 mice are heterozygous for a null allele of a galactolipid-synthesizing enzyme which could further reduce brain cerebroside content, they do not show more severe myelin pathology implicating additional mechanisms in the pathophysiology of aspartoacylase deficiency (Madhavarao et al. 2009). Animal models lacking functional aspartoacylase show significant albeit structurally abnormal myelination probably because parallel pathways for myelination exist during initial stages of myelinogenesis (Wang et al. 2009).

Deficiency of AspA-Derived Acetate Compromises Oligodendrocyte Differentiation

Studies on oligodendrocyte maturation have highlighted the importance of epigenetic control in differentiation (Copray et al. 2009). Since neurons transfer NAA to oligodendrocytes (Chakraborty et al. 2001), NAA-derived acetate is probably important for histone acetylation reactions that regulate chromatin structure and gene transcription in these cells. Dramatic reduction of acetate resulting from aspartoacylase deficiency could impact histone reactions required for epigenetic gene regulation preventing normal differentiation leading to oligodendrocyte cell death and neuronal injury possibly contributing to vacuole formation.

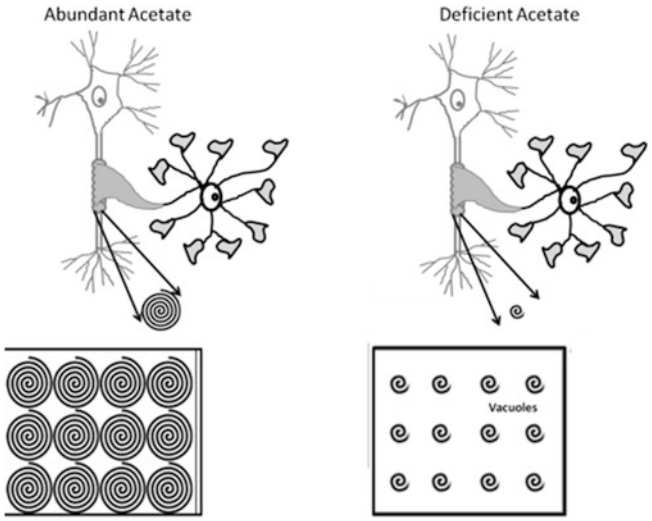

Fig. 2 Demyelination/dysmyelination theory: abundant acetate allows for proper formation of myelin bilayers, while in acetate deficiency, the layers may not be completely and tightly bound giving rise to vacuoles in interstitial space

Protein Folding and Stabilization Theory

Cells like oligodendrocytes, which have active protein secretory pathways, are sensitive to disorders of protein misfolding. Acetyl CoA is an important substrate for acetylation and deacetylation of nascent polypeptide chains in the endoplasmic reticulum (ER) required for stabilization and correct folding of proteins (Spange et al. 2009). Reduced acetyl CoA availability due to aspartoacylase deficiency could negatively impact protein folding and stabilization, targeting proteins for degradation. Oligodendrocytes are highly susceptible to ER stress associated with disruptions in protein synthesis and trafficking (Lin 2009). In the ASPAKO mouse, a severe loss of myelin basic protein and PLP/DM20 proteolipid proteins has been observed, combined with a decrease in myelinated fibers (Kumar et al. 2009).

Oxidative Stress Theory

Intracerebroventricular administration of NAA induces seizures in normal rats, probably by neuronal overexcitation (Akimitsu et al. 2000); further, tardive dyskinesia patients also have significantly higher CSF concentrations of NAA like CD patients (Tsai et al. 1998). Animal studies show that epileptic seizures result in free radical production and oxidative damage to cellular proteins, lipids, and DNA (Bruce 1995) implicating oxidative stress as one of the possible causes of neurological impairment. Recent work suggests that chronic mitochondrial oxidative stress and resultant dysfunction can render the brain more susceptible to epileptic seizures (Patel 2004). Hence it seems that there is a role for oxidative stress both as a cause and a consequence of epileptic seizures.

NAA probably promotes oxidative stress by decreasing nonenzymatic antioxidant defenses and stimulating oxidative damage to both lipids and proteins by enhancing reactive species in cerebral cortex (Fig. 3). To define the role of oxidative stress in NAA neurotoxicity, its effects on the antioxidant enzymes including catalase, superoxide dismutase (SOD) ,and glutathione peroxidase (GPX) were studied. NAA inhibited the functions of catalase and GPX enzymes indicating impaired detoxification of hydrogen peroxide, but it had no effect on SOD. Acute administration of NAA also enhanced levels of hydrogen peroxide in vitro which could possibly be involved in the progression of the characteristic neurodegeneration in CD (Pederzolli et al. 2007). Though these results could not be extrapolated to humans, they do reveal probable mechanisms since the oxidative stress parameters occurred with concentrations of NAA (~4fold higher) observed in the plasma and cerebrospinal fluid of patients affected by CD (Tsai and Coyle 1995). Based on the supposition that NAA may promote oxidative stress in vitro and in vivo both by enhancing reactive species and diminishing antioxidant defenses, administration of antioxidants, especially vitamins E and C, could be considered as a potential adjuvant therapy for patients affected by CD (Pederzolli et al. 2007).

A previous study linked NAA accumulation to nitric oxide (NO) toxicity as it upregulates inducible nitric oxide synthase (iNOS) and stimulates neuronal and endothelial nitric oxide synthase (Surendran 2009). Increased NO levels lead to disturbances in DNA structure and enhance protein interaction (Lee 2007). Discrepancies in the molecular weight of aspartoacylase when prepared using different methods (Kaul et al. 1993) suggest that it may

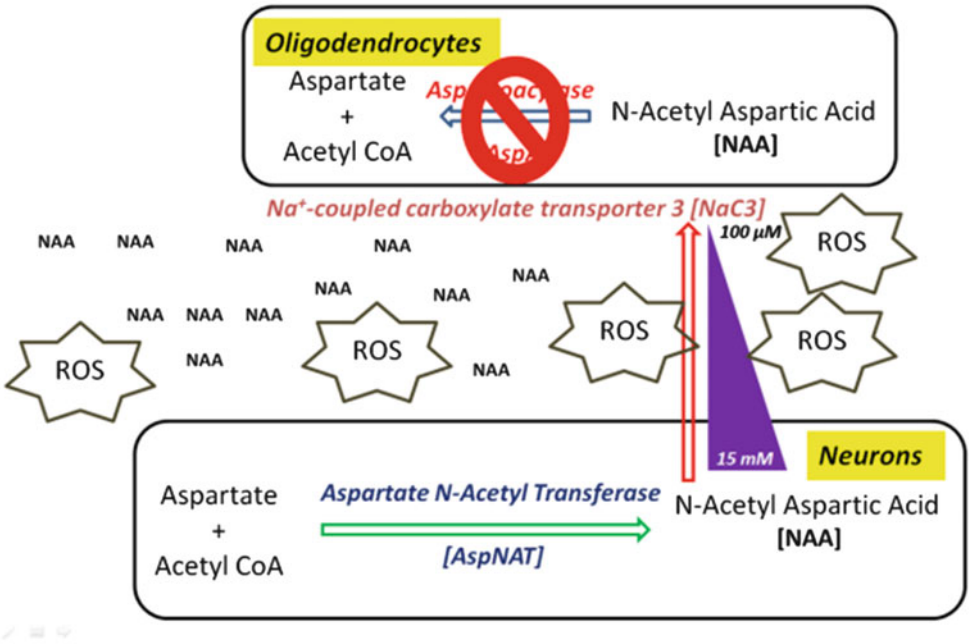

Fig. 3 Oxidative stress theory: accumulation of excess L-enantiomer of NAA could lead to generation of reactive oxygen species that could potentially generate vacuoles in brain interstitium

dimerize based on its concentration. It is possible that upregulation of NO synthase as a direct result of NAA accumulation may nitrosylate AspA causing it to dimerize. This hypothesis proposes that NAA upregulation and aspartoacylase malfunction could be cyclically linked.

Treatment Strategies

CD was described as the only neurological disease which manifests increased NAA levels in the brain; most other neurological diseases like schizophrenia show low NAA levels (Mondino and Saoud 2013). Since an increasing number of substances can affect NAA levels in the brain (Baslow and Resnik 1997), slowing down the anabolic portion of the NAA cycle using pharmaceuticals may allow the existing, albeit enzyme-deficient oligodendrocytes to produce a stable myelin sheath and restore neurological function. Surprisingly the kidney has high levels of aspartoacylase; however, since NAA levels in the kidney are very low, aspartoacylase may have additional catalytic functions. Thus, alternate approaches may be needed to deal with the pathological consequences of loss in aspartoacylase activity in such peripheral tissues.

A large number of preclinical proof-of-concept studies have been performed on various CD animal models including two rodent models – a naturally existing Tremor rat (Kitada et al. 2000) and an artificially engineered ASPAKO mouse (Matalon et al. 2000). Newer studies have created a knock-in model (Mersmann et al. 2011) and a model with a single-point mutation in Nur7 (Traka et al. 2008) making the development of therapeutic modalities much easier. Though currently the patients are mostly supported by palliative measures, existing treatment modules focus on different aspects of the disease phenotypes in CD and are described below.

Palliative Measures

Most of the palliative measures for patients of Canavan's disease follow the care provided for patients of other pediatric neurodegenerative diseases (Hunt and Burne 1995). Current palliative measures for respiratory issues in patients with Canavan's disease include suction and cough assist machines to clear secretions and mucous from the mouth, throat, nose, and upper lobes of the lungs, the Vesta system for airway clearance and oxygen concentrators to provide a continuous flow of oxygen for easy breathing. Nebulizers also help to administer medication as aerosols for these patients.

Hypotonia being a major Canavan symptom requires positioning equipment which may include foam supports, feeder seats, specialized strollers, and bath chairs to help patients with their positioning needs. Feeding pumps are also used to assist in dispensing liquid nutrients at predetermined rates for feeding.

Symptomatic Treatment of Disease

The earliest human clinical trials on CD patients used acetazolamide to reduce water concentration and NAA levels in white matter for a period of 5 months. The drug reduced the intracranial pressure, but did not reduce water content or NAA levels (Bluml et al. 1998). A ketogenic diet increased the levels of β-hydroxybutyrate in the brain but did not affect the elevated levels of NAA (Novotny Jr et al. 1999). These treatment strategies were primarily targeted to alleviate edema; however, there was little to no benefit for the patients.

In recent years, intraperitoneal injections of lipoic acid [which can cross the blood brain barrier (Samuel et al. 2005)] have also been tried in preclinical studies using tremor rats, the naturally occurring animal model for CD (Pederzolli et al. 2007) based on the fact that NAA induces oxidative stress in the CNS. The encouraging results suggest that this might be a good therapeutic approach for symptomatic treatment. With seizures being a hallmark of the disease, CD patients have canonically been targeted using anticonvulsive drugs like acetazolamide, clonazepam, and oxcarbazepine (Leone et al. 2012).

Addressing Elevated Substrate in the Context of Deficient Aspartoacylase

Dietary supplementation of acetate in newborn patients was proposed as therapeutic for CD since the primary pathogenesis is postnatal and deficiency of aspartoacylase and acetate are concurrent. This group reported phenotypic improvements in myelin galactocerebroside content and brain vacuolation and also showed a partial reversion in motor dysfunction in GTA-treated tremor rats over a course of 4 months (Madhavarao et al. 2009). In conclusion, dietary supplementation may not totally alleviate an inherent genetic metabolic disorder but may definitely offer partial symptomatic alleviation. Clinical translation of GTA to human infants showed no significant side effects or toxicity but showed no motor improvement (Segel et al. 2011).

A recent study proposed dietary triheptanoin supplementation in Nur7 mice to support fatty acid synthesis and TCA cycles and hence improve the redox status in diseased animals. It showed phenotypic improvements suggesting that the underlying pathological mechanism of CD may be a combination of several factors (Francis et al. 2013).

Neuroprotective strategies may be needed to counteract neurological damage caused by oxidative stress. Pharmacologically, lithium has been neuroprotective for dementia patients (Kessing et al. 2008) possibly by reducing expression of proapoptotic proteins (Chen 1999) and increasing the levels of antiapoptotic proteins (Chang et al. 2009). Intraperitoneal lithium administration caused a significant drop in brain NAA levels in wild-type rats (O'Donnell et al. 2000) and tremor rats (Baslow et al. 2002). After 1 year of treatment in patients, NAA levels in both urine and brain were decreased. Patients showed improved alertness and visual tracking; however axial hypotonia and spastic dysplegia were unaffected (Solsona et al. 2012).

Controlling brain NAA levels for CD (Assadi et al. 2010) may not be a highly effective therapeutic strategy since the NAA system is not universally present in the neurons (Baslow 1997). A complete inhibition or even a significant reduction in NAA production may thus be a viable option in controlling demyelination and improving the quality of life in CD patients.

Addressing the Deficiency of the Enzyme Aspartoacylase

Using enzyme replacement as a therapy for neurological disorders has been difficult because of the challenging blood–brain barrier. Surface lysyl groups of human aspartoacylase were modified through PEGylation to decrease immune response and increase circulation half-life with the intention of treating CD patients (Zano et al. 2011).

Most of these modalities as CD therapeutics mostly showed good tolerance however did not cause any significant improvement in the quality of life in the patients. However as CD is a monogenic defect with pathology being most evidently localized in the CNS, it presents an attractive target for gene therapy.

Gene Therapy Using Gene Replacement Strategy

Gene therapy is the supplementation or alteration of DNA as a therapy in organisms to alleviate disease. The most common gene therapy approach involves replacement of a mutant gene that causes a genetic disease by a fully functional gene. The therapeutic DNA is packaged into a "vector" that delivers the DNA in cells after which the cellular machinery takes over and produces the deficient protein the absence of which resulted in the disease.

Nonviral gene delivery systems are one of the conventional approaches for gene therapy; however they are limited by efficiency of gene transfer. Direct injections of plasmids (naked or in complexes) are also inefficient because of limited uptake due to aggregate formation, low rates of diffusion, endotoxin contamination and transient expression necessitating improvements in design. The first clinical trial for CD used a nonviral gene transfer technique with intraventricular injections of a non-aggregating lipid plasmid formulation LPD, composed of a recombinant

plasmid with a condensing agent [poly-L-lysine or protamine sulfate] and a liposomal formulation [DC-CHOL/DOPE] on two Canavan patients (Leone et al. 2000). The study established that the gene transfer technique worked and was safe but differences in the responses of the patients made it difficult to conclude if the gene therapy was successful.

One of the popular approaches in viral gene delivery is using adeno-associated viruses (AAVs). These are one of the smallest nonpathogenic mammalian parvoviruses that are replication incompetent, are almost completely nontoxic after CNS delivery in non-preexposed mammals (Samulski et al. 1999), and have a high level of sustained gene expression (Samulski et al. 1999) in nondividing cells. The most common serotype (AAV2) was the first to be successfully used for gene transfer (Hermonat 1984); however its use for CNS disorders was limited due to the presence of the blood–brain barrier (BBB) (which physically excludes foreign molecules and microorganisms based on size, charge, and lipid solubility from the blood to the brain) efficiently blocking rAAV diffusion into the CNS (Zlokovic 2008) rendering it incapable of marked therapeutic benefit for global neurological disorders like CD which have no cure to date.

Preclinical viral gene therapy studies involved stereotactical adenovirus-mediated delivery of aspartoacylase in tremor rats that showed reduction of seizures (Seki et al. 2002) and AAV2 mediated delivery that showed significant improvement in motor abilities and elevation of aspartoacylase expression (McPhee et al. 2005).

In another study, intraparenchymal delivery of rAAV2 to the brains of ASPAKO mice showed increased aspartoacylase activity and reduced NAA levels (Matalon et al. 2003). Vacuolation near the injection site was improved; however distant sites such as cerebellum were unaffected implying that the requirement for multisite injections to get optimal enzyme activity in the entire brain.

A second clinical trial (Janson et al. 2006) involved intracranial injections of rAAV2 vectors into the parietal, occipital, and frontal lobes in the brain of a large group of CD patients [FDA-IND#9119; NIH (RAC) #0001-381]. Clinical changes in most participants were not pronounced, relatively transient, and thought to be due to inadequacies of the vector or delivery system. Moreover, in CD patients, elevated NAA levels appear to cause white matter pathology but rAAV2 was conclusively demonstrated to transduce only neurons (McCown 2005). In a follow-up study on 13 of the 28 patients enrolled in this trial, the long-term safety, dosing parameters, and efficacy of the treatment were evaluated (Leone et al. 2012). From the study, it is evident that rAAV-mediated gene therapy is the most promising safe therapeutic modality for CD to date. The treatment stabilized the atrophy and even slowed progression of the disease in some patients, but there was a lack of a uniform response across cohorts. Standardized neurological exams and motor function tests post-gene therapy showed improvements in younger patients though the raw scores still indicated spastic quadriplegia. This result underlines the importance of defining a therapeutic window that will help improve the quality of life for the patients. This study clearly established the long-term safety of rAAVs as gene therapy vectors and indicated the need for serotypes and delivery strategies that lead to widespread and efficient transduction of the brain.

Various neurological diseases are subject of several clinical trials using rAAV (Asokan et al. 2012) and the available results indicate that the vectors mediate stable gene expression in the human brain (Muramatsu et al. 2010; Hwu et al. 2012) and are safe (Leone et al. 2012). rAAV2 vectors were the first vectors used for CNS gene transfer in animal models as well as in humans, and incidentally Canavan's disease was one of the first CNS diseases to go for clinical trials using rAAV (Leone et al. 2000; Janson et al. 2002). It was not until the discovery of a large family of novel primate-derived AAVs (Gao et al. 2002) that gene delivery to the CNS made great strides. Encouraging results with stereotactic delivery of rAAV2 to the brain led to intraparenchymal testing of other more efficient rAAV serotypes like 1,5,7,8,9, and rh.10 (Burger et al. 2004; Cearley and Wolfe 2006; Cearley et al. 2008) for CNS gene delivery. Direct infusion of the vectors in the brain parenchyma using stereotactic equipment guided needle placement is the most effective means for structure-specific gene delivery and proved to be beneficial for diseases with localized pathology.

Although intraparenchymal infusion is a great option for localized corrections of pathology, it is not suitable for widespread CNS transduction due to localized delivery and limitations in diffusion. Treatment of neurological disorders caused by single-gene defects requires global CNS transduction, intravenous injections would be extremely beneficial as the brain is rich in blood capillaries, allowing a wider distribution of the therapeutic vector concurrently being less invasive than intracranial delivery. A break-through in the field occurred when AAV9 was found to cross the BBB (Foust et al. 2009) and intravascular administration of rAAV9 in mice resulted in a widespread transduction of the CNS. Discovery of more serotypes that can cross the BBB (Zhang et al. 2011; Yang et al. 2014) paves the way for more efficient CNS gene transfer using the rAAV vectors. It is noteworthy that the rAAV vectors appear to be relatively consistent in their CNS gene transfer properties even across animal models like cats, dogs, and monkeys (Duque et al. 2009; Gray et al. 2013; Swain et al. 2013).

An exciting study recently demonstrated that single intravenous injection of rAAVs that can cross the BBB achieved sustained therapeutic benefit in ASPAKO mice even when vector was administered as late as P21 (Ahmed et al. 2013). This is extremely significant because untreated animals died by the age of 28 days. One of the most relevant issues in clinical application of gene therapy is the age of the patient. Though genetic screening allows for detection of inherited diseases in newborns, some diseases may manifest when the patient is well advanced in adulthood. In both cases gene therapy methods would need to be tailored to the specific patient type since the dosage and route of administration may not be equally therapeutic in both cases. Preclinical studies using rAAV9 also showed neuronal transduction accompanied by dramatic improvement in the diseased mice (Ahmed et al. 2013). Taken together, these observations suggest that de novo expression of aspartoacylase, regardless of the enzyme's physical location, can reduce NAA levels indicating that gene correction of every cell in the brain is not necessary (Matalon et al. 2003). The study addressed a couple of key issues, one of which was the timing of vector administration. Intravenous rAAV9 injections were performed at different ages of mouse pups to primarily document survival and motor functions. They found that although therapeutic benefits were indeed conferred upon all the treated animals, an earlier intervention was more beneficial presumably because certain developmental stages are more responsive to the therapeutics based on the stage of myelination.

It is evident that systemic delivery of rAAV9 at early neonatal stages achieves an extensive and robust transduction of neural cell types in CD that can be extended to developing therapeutics for other neurodegenerative diseases like Parkinson's disease using neurotrophic growth factors. Supraphysiological aspartoacylase levels or for that matter, growth factors, in other peripheral tissues have not been documented, may be potentially harmful and even lead to deleterious immune responses. To address this issue, the same study harnessed the endogenous miRNA machinery to limit transduction of unintended target tissues opening up newer possibilities of treatment. miRNA binding sites of specific miRNAs that are highly enriched in tissues like the liver, heart, and skeletal muscle were used to construct vectors that would detarget aspartoacylase expression from these tissues. The study showed successful alleviation of symptoms even when aspartoacylase expression was detargeted documenting the practical applications of miRNA-based therapeutics in leukodystrophies that can be extended for other diseases as well.

Although CD is defined as a leukodystrophy, it is yet to be established if the major event in this disease is demyelination or dysmyelination. Several therapeutic attempts, more notably the metabolic correction approach, seem to indicate an occurrence of demyelination. In this case, supplementation of substrates required for myelination would markedly increase the longevity as well as the quality of life of patients. However, most metabolic supplementation studies do not show any dramatic reversal of symptoms indicating that the molecular etiology might be more complicated. Thin layer chromatography (TLC) and X-ray diffraction studies conducted on ASPAKO mice indicate abnormal myelin composition in untreated animals (Ahmed et al. 2013) indicating that it would be worth exploring if the diseased oligodendrocytes are unable to synthesize normal myelin or are incapable of myelination itself. It would be appropriate to indicate here that systemic delivery of AAV9 resulted in a primarily neuronal transduction. As neurons do not divide, they would act as in situ factories that constantly produce the therapeutic protein. Additionally, since neurons are the site of NAA synthesis, expression of the metabolizing enzyme would reduce the load of NAA to a significant extent, especially when it appears that most of the CD symptoms like hydrocephaly, vacuolation, and NAAduria arise from accumulation of excess NAA. Targeting oligodendrocytes specifically would prove to be beneficial since aspartoacylase is localized in these cells, however the chances of transduced cells dividing and diluting out the therapeutic benefits seem imminent and the approach would be fraught by the danger of reduced therapeutic benefit with the progression of time.

Perspectives and Future Directions

It has been more than eight decades since CD was first described in 1931 (Canavan 1931), and there was little progress in understanding its pathogenesis until it was identified as an aspartoacylase deficiency almost five decades later (Matalon et al. 1988). Description of the human aspartoacylase cDNA sequence led to a huge leap in molecular diagnosis for CD patients (Kaul et al. 1993); however the molecular etiology has remained controversial. To resolve this, strong efforts in modeling CD in animals significantly broadened avenues to investigate pathological mechanisms. Preclinical studies began with the naturally existing tremor rats and then expanded to include the ASPAKO mouse and later the Nur7 and the LacZ knock-in mouse models. Currently the field seems poised for more revolutionary progress with the advent of newer serotypes and advances in capsid evolution for rAAVs that are specific for different neural cell types and can cross the BBB.

Currently CD falls in a category of diseases, the treatment of which could have wide implications in

developing therapeutics for a vast group of leukodystrophies and neurodegenerative diseases. Being a monogenic defect, CD is a perfect target for gene therapy attempts which could be utilized as tools to tease out the underlying pathology of leukodystrophies and develop efficient therapeutics for them. Mouse models that show a less severe phenotype resembling infantile and juvenile CD patients could be used to determine disease progression. Additionally, the unique symptom of elevated NAA levels in CD also makes it a perfect disease model to study functions of NAA. An important element for translating observations from ASPAKO mice would be to couple in vivo physiology and imaging in the mouse to functional neuroimaging in patients to help identify conserved neural circuit phenotypes and pave way to improve upon therapeutics.

In addition to existing therapeutic modalities, alternative means of treatment for CD could include partial silencing of the NAA biosynthetic enzyme AspNAT (Madhavarao et al. 2003) to control the NAA metabolism cycle. Additionally inhibition of NAA export from the neuronal mitochondria could markedly decrease NAA accumulation in interstitial spaces that probably causes osmotic dysregulation in the CNS. Pharmacological protection of oligodendrocytes against damage in demyelinating diseases could also be a promising avenue of treatment (Waksman 1999). Intravenous delivery of rAAV9 (Ahmed et al. 2013) in the ASPAKO model suggests that lowering NAA levels in the brain probably led to alleviation of NAAduria as well as a decrease in edema indicating that the MWP theory could partially explain pathogenesis in CD.

Inclusion of newer CD animal models, continuously expanding viral vector repertoire and less invasive delivery methods for pan CNS delivery, makes it likely that gene therapy for CD and other such diseases will advance rapidly in the near future. It should be noted, however, in spite of newer serotypes, that there will still remain the issue of half-life of the transduced cells. All of these factors increasingly indicate the potential for combinatorial therapy strategies that could be translated to human patients.

An important issue would be to determine how far along in disease progression can the treatment be administered to patients to achieve substantial therapeutic benefits. Neurometabolic diseases like globoid cell leukodystrophy show favorable outcomes for presymptomatic intervention but not later indicating the existence of a restricted therapeutic time window (Escolar et al. 2005). Similar outcomes were seen in preclinical studies (Ahmed et al. 2013) and follow-up study on CD clinical trials (Leone et al. 2012) indicating that future gene therapy interventions should begin before irreversible neuro-structural changes occur underlining the importance of rodent studies. However, an important issue in the translation of the intravenous rAAV therapeutic dose from mice to humans is the huge vector manufacturing burden-making treatment costs prohibitive. Tweaking the expression cassette by codon optimization would increase expression efficiency and require lower therapeutic doses. Moreover the delivery of vector to the CSF directly would entail more diffusion in the CNS and less spread in peripheral organs.

The core clinical features of CD are very distinct and provide good targets for therapeutics. Long-term studies on rAAV gene therapy-treated patients have documented that the therapy is safe and without risks and it is only a matter of time until the inefficiencies are improved. Efficient translation of the comprehensive data-driven validation of effective therapy in mouse models to humans would push forward the successful development of therapeutics like rAAVs and help to alleviate the sufferings of the hitherto helpless patients.

Acknowledgment We would like to acknowledge the grant support from Jacob's Cure, NTSAD Foundation, Canavan Foundation, and Public Health Service grants 1R01NS076991, P01 HL59407-11, P01 AI100263-01 from National Institutes of Health to GG.

One Sentence Synopsis

In this review, we follow the evolution of research on Canavan's disease and discuss current understanding of molecular pathogenesis as well as developing potential therapeutics for the disease.

Compliance with Ethics Guidelines

Conflict of Interest

Seemin Seher Ahmed declares that she has no conflict of interest.

Guangping Gao is a founder of Voyager Therapeutics and holds equity in the company. He is also an inventor on patents with potential royalties licensed to Voyager Therapeutics. Voyager Therapeutics is a newly launched gene therapy company that focuses on using the recombinant adeno-associated virus platform technology for the development of gene therapeutics to treat a wide range of CNS disorders.

Informed Consent

This article directly does not contain any studies with human subjects performed by the authors.

Animal Rights

All institutional and national guidelines for the care and use of laboratory animals were followed in the work done in the authors' laboratory.

Details of the Contributions of Individual Authors

Seemin Seher Ahmed planned and drafted the review.

Guangping Gao reviewed the drafts and provided critical inputs.

References

Adachi M, Schneck L, Cara J, Volk BW (1973) Spongy degeneration of the central nervous system (van Bogaert and Bertrand type; Canavan's disease). A review. Hum Pathol 4(3):331–347

Ahmed SS, Li H, Cao CSE, Denninger AR, Su Q, Eaton S, Liso Navarro AA, Xie J, Szucs S, Zhang H, Moore C, Kirschner DA, Seyfried TN, Flotte TR, Matalon R, Gao G (2013) A single intravenous rAAV injection as late as P20 achieves efficacious and sustained CNS gene therapy in Canavan mice. Mol Ther 12:2136–2147

Akimitsu TKK, Hanaya R, Iida K, Kiura Y, Arita K, Matsubayashi H, Ishihara K, Kitada K, Serikawa T, Sasa M (2000) Epileptic seizures induced by N-acetyl-L-aspartate in rats: in vivo and in vitro studies. Brain Res 861(1):143–150

Al-Dirbashi OY, Kurdi W, Imtiaz F et al (2009) Reliable prenatal diagnosis of Canavan disease by measuring N-acetylaspartate in amniotic fluid using liquid chromatography tandem mass spectrometry. Prenat Diagn 29(5):477–480

Ariyannur PS, Moffett JR, Manickam P et al (2010) Methamphetamine-induced neuronal protein NAT8L is the NAA biosynthetic enzyme: implications for specialized acetyl coenzyme A metabolism in the CNS. Brain Res 1335:1–13

Asokan A, Schaffer DV, Samulski RJ (2012) The AAV vector toolkit: poised at the clinical crossroads. Mol Ther 20(4):699–708

Assadi M, Janson C, Wang DJ et al (2010) Lithium citrate reduces excessive intra-cerebral N-acetyl aspartate in Canavan disease. Eur J Paediatr Neurol 14(4):354–359

Baslow M (1997) A review of phylogenetic and metabolic relationships between the acylamino acids, N-acetyl-L-aspartic acid and N-acetyl-L-histidine, in the vertebrate nervous system. J Neurochem 68(4):1335–1344

Baslow MH (1999) Molecular water pumps and the aetiology of Canavan disease: a case of the sorcerer's apprentice. J Inherit Metab Dis 22(2):99–101

Baslow MH, Resnik TR (1997) Canavan disease. Analysis of the nature of the metabolic lesions responsible for development of the observed clinical symptoms. J Mol Neurosci 9(2):109–125

Baslow MH, Kitada K, Suckow RF, Hungund BL, Serikawa T (2002) The effects of lithium chloride and other substances on levels of brain N-acetyl-L-aspartic acid in Canavan disease-like rats. Neurochem Res 27(5):403–406

Beaudet A (2001) Aspartoacylase deficiency (Canavan disease) In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, McGraw-Hill Publishers, pp 5799–5805

Bennett MJ, Gibson KM, Sherwood WG et al (1993) Reliable prenatal diagnosis of Canavan disease (aspartoacylase deficiency): comparison of enzymatic and metabolite analysis. J Inherit Metab Dis 16(5):831–836

Bhakoo KK, Craig TJ, Styles P (2001) Developmental and regional distribution of aspartoacylase in rat brain tissue. J Neurochem 79(1):211–220

Birken DL, Oldendorf WH (1989) N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. Neurosci Biobehav Rev 13(1):23–31

Birnbaum SM, Levinton L, Kingsley RB, Greenstein JP (1952) Specificity of amino acid acylases. J Biol Chem 194:455–462

Bluml S, Seymour K, Philippart M, Matalon R, Ross B (1998) Elevated brain water in Canavan disease: impact of a diuretic therapy. In: Book elevated brain water in Canavan disease: impact of a diuretic therapy. p 171

Bruce AJBM (1995) Oxygen free radicals in rat limbic structures after kainate-induced seizures. Free Radic Biol Med 18(6):993–1002

Burger C, Gorbatyuk OS, Velardo MJ et al (2004) Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. Mol Ther 10(2):302–317

Canavan MM (1931) Schilder's encephalitis periaxialis diffusa. Arch Neurol Psychiat 25:299–308

Cearley CN, Wolfe JH (2006) Transduction characteristics of adeno-associated virus vectors expressing cap serotypes 7, 8, 9, and Rh10 in the mouse brain. Mol Ther 13(3):528–537

Cearley CN, Vandenberghe LH, Parente MK, Carnish ER, Wilson JM, Wolfe JH (2008) Expanded repertoire of AAV vector serotypes mediate unique patterns of transduction in mouse brain. Mol Ther 16(10):1710–1718

Chakraborty G, Mekala P, Yahya D, Wu G, Ledeen RW (2001) Intraneuronal N-acetylaspartate supplies acetyl groups for myelin lipid synthesis: evidence for myelin-associated aspartoacylase. J Neurochem 78(4):736–745

Chang YC, Rapoport SI, Rao J (2009) Chronic administration of mood stabilizers upregulates BDNF and bcl-2 expression levels in rat frontal cortex. Neurochem Res 34(3): 536–541

Chen RWCD (1999) Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression. A prominent role in neuroprotection against excitotoxicity. J Biol Chem 274(10):6039–6042

Copray SHJ, Sher F, Casaccia-Bonnefil P, Boddeke E (2009) Epigenetic mechanisms facilitating oligodendrocyte development, maturation, and aging. Glia 57(15):1579–1587

D'Adamo AF Jr, Gidez LI, Yatsu FM (1968) Acetyl transport mechanisms. Involvement of N-acetyl aspartic acid in de novo fatty acid biosynthesis in the developing rat brain. Exp Brain Res 5(4):267–273

Duque S, Joussemet B, Riviere C et al (2009) Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. Mol Ther 17(7):1187–1196

Elpeleg ON, Shaag A (1999) The spectrum of mutations of the aspartoacylase gene in Canavan disease in non-Jewish patients. J Inherit Metab Dis 22(4):531–534

Escolar MLPM, Provenzale JM, Richards KC, Allison J, Wood S, Wenger DA, Pietryga D, Wall D, Champagne M, Morse R, Krivit W, Kurtzberg J (2005) Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. N Engl J Med 352(20):2069–2081

Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK (2009) Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol 27(1):59–65

Francis J, Markov V, Leone P (2013) Dietary triheptanoin rescues oligodendrocyte loss, dysmyelination and motor function in the

nur7 mouse model of Canavan disease. J Inherit Metab Dis 37(3):369–381
Gao GP, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM (2002) Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proc Natl Acad Sci U S A 99(18):11854–11859
Gray SJ, Nagabhushan Kalburgi S, McCown TJ, Samulski RJ (2013) Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates. Gene Ther 20(4):450–459
Hermonat PLMN (1984) Use of adeno-associated virus as a mammalian DNA cloning vector: transduction of neomycin resistance into mammalian tissue culture cells. Proc Natl Acad Sci U S A 81(20):6466–6470
Hershfield JR, Pattabiraman N, Madhavarao CN, Namboodiri MA (2007) Mutational analysis of aspartoacylase: implications for Canavan disease. Brain Res 1148:1–14
Hirano A (1981) Structure of normal central myelinated fibers. Adv Neurol 31:51–68
Hunt A, Burne R (1995) Medical and nursing problems of children with neurodegenerative disease. Palliative Med 9(1):19–26
Hwu WL, Muramatsu S, Tseng SH et al (2012) Gene therapy for aromatic L-amino acid decarboxylase deficiency. Sci Transl Med 4(134):134–161
Jakobs C, ten Brink HJ, Langelaar SA et al (1991) Stable isotope dilution analysis of N-acetylaspartic acid in CSF, blood, urine and amniotic fluid: accurate postnatal diagnosis and the potential for prenatal diagnosis of Canavan disease. J Inherit Metab Dis 14(5):653–660
Janson C, McPhee S, Bilaniuk L et al (2002) Clinical protocol. Gene therapy of Canavan disease: AAV-2 vector for neurosurgical delivery of aspartoacylase gene (ASPA) to the human brain. Hum Gene Ther 13(11):1391–1412
Janson CG, McPhee SW, Francis J et al (2006) Natural history of Canavan disease revealed by proton magnetic resonance spectroscopy (1H-MRS) and diffusion-weighted MRI. Neuropediatrics 37(4):209–221
Kaul R, Casanova J, Johnson AB, Tang P, Matalon R (1991) Purification, characterization, and localization of aspartoacylase from bovine brain. J Neurochem 56(1):129–135
Kaul R, Gao GP, Balamurugan K, Matalon R (1993) Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease. Nat Genet 5(2):118–123
Kaul R, Gao GP, Aloya M et al (1994) Canavan disease: mutations among Jewish and non-Jewish patients. Am J Hum Genet 55(1):34–41
Kessing LVSL, Forman JL, Andersen PK (2008) Lithium treatment and risk of dementia. Arch Gen Psychiatry 65(11):1331–1335
Kitada K, Akimitsu T, Shigematsu Y et al (2000) Accumulation of N-acetyl-L-aspartate in the brain of the tremor rat, a mutant exhibiting absence-like seizure and spongiform degeneration in the central nervous system. J Neurochem 74(6):2512–2519
Klugmann M, Symes CW, Klaussner BK et al (2003) Identification and distribution of aspartoacylase in the postnatal rat brain. Neuroreport 14(14):1837–1840
Kumar S, Biancotti JC, Matalon R, de Vellis J (2009) Lack of aspartoacylase activity disrupts survival and differentiation of neural progenitors and oligodendrocytes in a mouse model of Canavan disease. J Neurosci Res 87(15):3415–3427
Lee DHPG (2007) Mutagenesis induced by the nitric oxide donor sodium nitroprusside in mouse cells. Mutagenesis 22(1):63–67
Leone P, Janson CG, Bilaniuk L et al (2000) Aspartoacylase gene transfer to the mammalian central nervous system with therapeutic implications for Canavan disease. Ann Neurol 48(1):27–38
Leone P, Shera D, McPhee SW et al (2012) Long-term follow-up after gene therapy for Canavan disease. Sci Transl Med 4(165):165ra163
Lin WPB (2009) Endoplasmic reticulum stress in disorders of myelinating cells. Nat Neurosci 12(4):379–385
Madhavarao CN, Chinopoulos C, Chandrasekaran K, Namboodiri MA (2003) Characterization of the N-acetylaspartate biosynthetic enzyme from rat brain. J Neurochem 86(4):824–835
Madhavarao CN, Moffett JR, Moore RA, Viola RE, Namboodiri MA, Jacobowitz DM (2004) Immunohistochemical localization of aspartoacylase in the rat central nervous system. J Comp Neurol 472(3):318–329
Madhavarao CN, Arun P, Moffett JR et al (2005) Defective N-acetylaspartate catabolism reduces brain acetate levels and myelin lipid synthesis in Canavan's disease. Proc Natl Acad Sci U S A 102:5221–5226
Madhavarao CN, Arun P, Anikster Y et al (2009) Glyceryl triacetate for Canavan disease: a low-dose trial in infants and evaluation of a higher dose for toxicity in the tremor rat model. J Inherit Metab Dis 32(5):640–650
Matalon R, Michals-Matalon K (1999a) Biochemistry and molecular biology of Canavan disease. Neurochem Res 24(4):507–513
Matalon R, Michals-Matalon K (1999b) Prenatal diagnosis of Canavan disease. Prenat Diagn 19(7):669–670
Matalon R, Michals K, Sebesta D, Deanching M, Gashkoff P, Casanova J (1988) Aspartoacylase deficiency and N-acetylaspartic aciduria in patients with Canavan disease. Am J Med Genet 29(2):463–471
Matalon R, Kaul R, Michals K (1993) Canavan disease: biochemical and molecular studies. J Inherit Metab Dis 16(4):744–752
Matalon R, Rady PL, Platt KA et al (2000) Knock-out mouse for Canavan disease: a model for gene transfer to the central nervous system. J Gene Med 2(3):165–175
Matalon R, Surendran S, Rady PL et al (2003) Adeno-associated virus-mediated aspartoacylase gene transfer to the brain of knockout mouse for Canavan disease. Mol Ther 7(5 Pt 1): 580–587
McCown T (2005) Adeno-associated virus (AAV) vectors in the CNS. Curr Gene Ther 5(3):333–338
McPhee SW, Francis J, Janson CG et al (2005) Effects of AAV-2-mediated aspartoacylase gene transfer in the tremor rat model of Canavan disease. Brain Res Mol Brain Res 135(1–2):112–121
Mersmann N, Tkachev D, Jelinek R et al (2011) Aspartoacylase-lacZ knockin mice: an engineered model of Canavan disease. PLoS One 6(5):e2033
Mondino M BJ, Saoud M. (2013) N-acetyl-aspartate level is decreased in the prefrontal cortex in subjects at-risk for schizophrenia. Front Psychiatry 4:Article 99
Muramatsu S, Fujimoto K, Kato S et al (2010) A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. Mol Ther 18(9):1731–1735
Novotny EJ Jr, Hyder J, Rothman DI (1999) Cerebral amino acids and metabolites in aminoacylase II deficiencies. J Mol Neurosci 12(3):174–175
O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH (2000) Chronic lithium and sodium valproate both decrease the concentration of myo-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Res 880(1–2):84–91
Patel M (2004) Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. Free Radic Biol Med 37(12):1951–1962
Pederzolli CDMC, Scapin F, Rockenbach FJ, Sgaravatti AM, Sgarbi MB, Wyse AT, Wannmacher CM, Wajner M, Dutra-Filho CS (2007) N-acetylaspartic acid promotes oxidative stress in cerebral cortex of rats. Int J Dev Neurosci 25(5):317–324

Sager TNF-JA, Hansen AJ (1997) Transient elevation of interstitial N-acetylaspartate in reversible global brain ischemia. J Neurochem 68(2):675–682

Samuel SKR, Jayavelu T, Chinnakkannu P (2005) Protein oxidative damage in arsenic induced rat brain: influence of DL-a-lipoic acid. Toxicol Lett 155:27–34

Samulski RJ, Sally M, Muzyczka N, TF eds (1999) Adeno associated viral vectors: the development of human gene therapy. Cold Spring Harbor Press, New York, pp 131–172

Segel RAY, Zevin S, Steinberg A, Gahl WA, Fisher D, Staretz-Chacham O, Zimran A, Altarescu G (2011) A safety trial of high dose glyceryl triacetate for Canavan disease. Mol Genet Metab 103(3):203–206

Seki T, Matsubayashi H, Amano T et al (2002) Adenoviral gene transfer of aspartoacylase into the tremor rat, a genetic model of epilepsy, as a trial of gene therapy for inherited epileptic disorder. Neurosci Lett 328(3):249–252

Solsona MDFL, Boquet EM, Andrés JL (2012) Lithium citrate as treatment of Canavan disease. Clin Neuropharmacol 35(3):150–151

Sommer A, Sass JO (2012) Expression of aspartoacylase (ASPA) and Canavan disease. Gene 505(2):206–210

Spange SWT, Heinzel T, Krämer OH (2009) Acetylation of non-histone proteins modulates cellular signalling at multiple levels. Int J Biochem Cell Biol 41(1):185–198

Steen RGOR (2005) Abnormally high levels of brain N-acetylaspartate in children with sickle cell disease. AJNR Am J Neuroradiol 26(3):463–468

Surendran S (2009) Upregulation of N-acetylaspartic acid alters inflammation, transcription and contractile associated protein levels in the stomach and smooth muscle contractility. Mol Biol Rep 36(1):201–206

Swain GP, Prociuk M, Bagel JH et al (2013) Adeno-associated virus serotypes 9 and rh10 mediate strong neuronal transduction of the dog brain. Gene Ther 21(1):28–36

Taylor DLDS, Obrenovitch TP, Doheny MH, Patsalos PN, Clark JB, Symon L (1995) Investigation into the role of N-acetylaspartate in cerebral osmoregulation. J Neurochem 65(1):275–281

Tortorella C, Ruggieri M, Di Monte E et al (2011) Serum and CSF N-acetyl aspartate levels differ in multiple sclerosis and neuromyelitis optica. J Neurol Neurosurg Psychiatry 82:1355–1359

Traka M, Wollmann RL, Cerda SR, Dugas J, Barres BA, Popko B (2008) Nur7 is a nonsense mutation in the mouse aspartoacylase gene that causes spongy degeneration of the CNS. J Neurosci 28(45):11537–11549

Tsacopoulos MMP (1996) Metabolic coupling between glia and neurons. J Neurosci 16(3):877–885

Tsai G, Coyle J (1995) N-acetylaspartate in neuropsychiatric disorders. Prog Neurobiol 46(5):531–540

Tsai GGD, Chang RW, Flood J, Baer L, Coyle JT (1998) Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psychiatry 155(9):1207–1213

Waksman B (1999) Demyelinating disease: evolution of a paradigm. Neurochem Res 24(4):491–495

Wang J, Leone P, Wu G et al (2009) Myelin lipid abnormalities in the aspartoacylase-deficient tremor rat. Neurochem Res 34(1):138–148

Yang B, Li S, Wang H et al (2014) Global CNS transduction of adult mice by intravenously delivered rAAVrh.8 and rAAVrh.10 and nonhuman primates by rAAVrh.10. Mol Ther 22(7):1299–1309

Zano S, Malik R, Szucs S, Matalon R, Viola RE (2011) Modification of aspartoacylase for potential use in enzyme replacement therapy for the treatment of Canavan disease. Mol Genet Metab 102(2):176–180

Zhang H, Yang B, Mu X et al (2011) Several rAAV vectors efficiently cross the blood–brain barrier and transduce neurons and astrocytes in the neonatal mouse central nervous system. Mol Ther 8(19):1440–1448

Zlokovic B (2008) The blood–brain barrier in health and chronic neurodegenerative disorders. Neuron 57:178–201

JIMD Reports
DOI 10.1007/8904_2014_359

RESEARCH REPORT

Disordered Eating and Body Esteem Among Individuals with Glycogen Storage Disease

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Received: 30 April 2014 / Revised: 23 July 2014 / Accepted: 27 August 2014 / Published online: 10 February 2015
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Abstract Glycogen storage disease (GSD) is an inherited disorder that requires a complex medical regimen to maintain appropriate metabolic control. Previous research has suggested the disease is associated with decreased quality of life, and clinical experience suggests that patients are at risk for disordered eating behaviors that may significantly compromise their health. The current study assessed eating attitudes, eating disorder symptoms, and body image among 64 patients with GSD ranging from 7–52 years old ($M = 18.5$ years old). About half the participants were male ($n = 33$, 51.6%). Most participants were diagnosed with GSD Type I ($n = 52$, 81.3%). Quantitative and qualitative analyses were utilized. Results indicated that 14.8% of children and 11.1% of adolescents/adults with GSD met the clinical cutoff for dysfunctional attitudes toward eating, suggesting high likelihood for presence of an eating disorder. However, traditional eating disorder symptoms (e.g., binging, purging, fasting, etc.) were less prevalent in the GSD sample compared to population norms ($t = -6.45$, $p < 0.001$). Body esteem was generally lower for both children and adolescents/adults with GSD compared to population norms. These results were consistent with interview responses indicating that GSD patients experience negative feedback from peers regarding their bodies, especially during childhood and adolescence. However, they reported growing acceptance of their bodies with age and reported less negative attitudes and behaviors. Assessing mental health, including symptoms of disordered eating and low body esteem, among individuals with GSD should be an important component of clinical care.

Communicated by: Anita MacDonald, PhD, BSc

Competing interests: None declared

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Introduction

Glycogen storage disease (GSD) is a complicated disease with a complex medical regimen. For many patients, the recommended treatment includes restricted intake of non-glucose sugars, including lactose, fructose, and sucrose (Rake et al 2002a, b), and individuals with GSD Ia may require supplemental cornstarch every 4–5 h around the clock (Weinstein and Wolfsdorf 2002). This rigid feeding schedule can interfere with daily activities and interrupt sleep schedules of patients and their families. Patients may also require additional daily dietary supplements and medications to combat associated morbidities (Chen 2011).

Relatively little research has examined the mental health impact of living with GSD. One notable study of GSD Ia and Ib patients found that they reported decreased quality of life (QOL) related to physical health, psychosocial health, and social functioning compared to the general population (Storch et al. 2008). Yet, it remains unknown what aspect(s) of GSD might negatively impact well-being. One hypothesis is that GSD may negatively influence self-esteem, which is an important predictor of life satisfaction and mental health (Diener and Diener 1993). Body esteem, a particular domain of self-esteem, is of particular interest in the GSD population. Characterized as the self-evaluation of physical appearance, body esteem has been shown to be lower in

overweight and obese individuals (Mendelson et al. 1996; O'Dea 2006). For individuals with GSD not under optimal metabolic control, hepatomegaly can lead to abdominal distention and increased BMI (Özen 2007). Additionally, the regular consumption of uncooked cornstarch as part of treatment necessitates a large and sometimes excessive caloric intake. This is compounded in some patients by exercise intolerance due to myopathy (Kollberg et al. 2007), restrictions on allowable exercise (Mundy et al. 2005), as well as the need to compensate for increased activity with additional cornstarch therapy (Shin 2006). Certain forms of GSD also present with physical manifestations including poor growth, short stature, distended abdomen, and thin extremities (Wolfsdorf et al. 1999). Body dissatisfaction and low self-esteem are considered risk factors for the development of disordered eating (Furnham et al. 2010; Olmsted et al. 2008).

Importantly, clinical experience suggests that many patients with GSD exhibit some form of disordered eating. Given how crucial dietary compliance is for patients with GSD, the serious risks of maladaptive eating behaviors can have even more detrimental consequences. Assessing for low body esteem and addressing contributing factors may provide insight into ways to improve quality of life for GSD patients and prevent further complications. This study examined body esteem, eating attitudes, and disordered eating in patients with GSD. Specifically, we hypothesized that 1) patients with GSD would demonstrate lower levels of body esteem, more dysfunctional eating attitudes, and higher rates of disordered eating than population norms, and 2) patients with more severe forms of GSD (i.e., Types Ia and Ib) would demonstrate lower levels of body esteem, more dysfunctional eating attitudes, and higher rates of disordered eating than patients with less severe forms.

Methods

Participants

Participants were 64 individuals with glycogen storage disease who were being followed by the University of Florida GSD Program. All participants had a diagnosis of GSD based upon demonstration of abnormal enzyme activity on a liver biopsy or genetic mutation analysis. The sample included 33 male participants (51.6%) and 31 female participants. They ranged in age from 7 to 52 years old ($M = 18.5$). Half the sample ($n = 32$) were children aged 7–12; the others were 13 years or older. The majority of participants ($n = 52$, 81.3%) were GSD Types Ia or Ib. Other represented types included Type IIIa (6.3%), Type VI (1.6%), Type IX (7.8%), and unclassified (3.1%).

Procedure

All procedures were approved by the University of Florida Institutional Review Board. All GSD patients ages 7 and older were invited to participate in this study during their scheduled medical monitoring. A trained research assistant obtained informed consent/assent from patients and/or their parents. Next, participants completed a semi-structured interview and a number of age-appropriate self-report psychological assessment questionnaires. Family members and medical staff were asked to leave the room in order to allow honest responding. Participants were reminded at regular intervals that their families and clinical care team would not have access to their responses.

Measures

Eating Disorders Inventory-3 (EDI-3)

This self-report inventory is useful in assessing individuals suspected of having an eating disorder (Garner 2004). The 91 items are rated on a 6-point Likert-type scale. A sample item is "I exaggerate or magnify the importance of weight." The EDI-3 has demonstrated strong psychometric properties.

Eating Disorders Inventory-Child (EDI-C)

The EDI-C is a multidimensional self-report questionnaire that is specially designed for children and young adolescents (Garner 1991). Based on the EDI-2, it consists of 91 items, scored on a 6-point Likert-type scale. Sample items include "I think my stomach is too big" and "I am very afraid of getting fat." The EDI-C has demonstrated good reliability and validity.

Eating Attitudes Test (EAT)

The EAT is used to assess eating attitudes among adults (Garner and Garfinkel 1979). The 26 items, rated on a 6-point Likert-type scale, include "I eat diet foods" and "I display self-control around food." The EAT yields scores on 3 subscales: Dieting, Bulimia and Food Preoccupation, and Oral Control. Using the cutoff score of 20, it has been used to identify individuals with any DSM-IV-defined eating disorder with a 90% accuracy rate, though it does not predict specific diagnoses (Mintz and O'Halloran 2000).

Children's Eating Attitude Test (ChEAT)

The ChEAT is a 26-item version of the Eating Attitudes Test that is used to assess eating attitudes that are common

in youth with eating disorders (Maloney et al. 1988). Items are scored on a 6-point Likert-type scale using the guidelines developed by Anton et al. (2006). Sample items include "[I] feel guilty after eating" and "[I] exercise to burn energy." The ChEAT yields subscale scores for Body Concern, Dieting, Food Preoccupation, Weight Gain, Vomiting, and Calories.

Body Esteem Scale (BES)

The BES is used to identify dissatisfaction with one's body (Mendelson et al. 2001). Adapted from the Body Esteem Scale for Children (BES-C; see below), the 30 items are rated on a 5-point scale with three subscales: Appearance (general feelings about their own appearance including face, hair, etc.), Weight (satisfaction specifically related to body size and weight), and Attribution (beliefs about how others evaluate the respondent's appearance). Sample items include "I like what I see when I look in the mirror" and "I feel I weigh the right amount for my height."

Body Esteem Scale for Children (BES-C)

The BES-C is a reliable and valid measure of body dissatisfaction for children (Mendelson and White 1985). It consists of 20 items rated on a 4-point Likert-type scale, which measure Appearance, Weight, and Attribution. Respondents rate statements such as "There are lots of thing I'd change about my looks if I could." Due to low reliability ratings for the Attribution subscale, only the Appearance and Weight subscales are recommended for interpretation (Mendelson et al. 1996).

Open-Ended Interview

All subjects participated in a short semi-structured interview about their experiences living with GSD. One of the questions was, "Everyone feels differently about their bodies. Tell me a little about how you feel about your body." Optional prompts included, "What are the things you like about your body? What are the things you don't like about your body?".

Data Analysis

Descriptive statistics were computed for study variables. Questionnaire scores were transformed into *z*-scores to allow for comparisons across age groups. One-sample t-tests compared GSD patient scores to population norms. Independent samples t-tests compared GSD Type I patient scores to scores for other types. In order to be sensitive to factors that may be affected by normal development, data were generally analyzed separately for children (ages 7–12) versus adolescents/adults (13 and older). Semi-structured interview responses were transcribed from digital audio recordings. The grounded theory method was used to assess important themes (Corbin and Strauss 2008).

Results

On measures of traditional eating disorder symptoms (EDI-3/EDI-C), GSD patients overall scored lower than the published population norms ($t = -6.45$, $p < 0.001$). Patients with more severe forms of GSD (i.e., Type Ia or Ib) demonstrated a trend toward lower levels of eating disorder symptoms than patients with less severe forms ($t = 1.77$, $p = 0.08$).

With regard to eating attitudes, 14.8% of the children scored above the cutoff score of 20 on the ChEAT ($M = 13.29$; SD = 6.56), indicating dysfunctional attitudes toward eating and likely presence of an eating disorder. Although the ChEAT does not yield specific diagnostic information for particular disorders, these results indicate clinically significant symptoms. Average scores were highest for the Dieting subscale among children in this sample. Similarly, 11.1% of the adolescents/adults scored above the cutoff score of 20 on the EAT-26 ($M = 12.8$; SD = 6.48), suggesting clinically significant symptoms. Average scores on the 3 subscales were not significantly different. There were no significant differences between male and female patients with GSD on either the ChEAT or EAT.

On measures of body esteem, GSD patients generally scored lower than population norms, indicating less healthy beliefs. Specifically, although scores for children with GSD were not significantly lower for body esteem related to their overall Appearance ($t = -1.07$, *ns*), they reported lower body esteem related to Weight ($t = -3.01$, $p = 0.006$). In addition, adolescents/adults with GSD reported lower body esteem related to others' perceptions of their bodies (Attribution: $t = -2.86$, $p < 0.007$) and trends toward lower body esteem for overall Appearance ($t = -1.90$, $p < 0.07$) and Weight ($t = -1.82$, $p < 0.08$). When comparing standardized scores that were calculated based on gender norms, male and female patients with GSD did not differ in their self-reported body esteem scores.

The sample BMI ranged from 15.3–39.9. In total, 49.2% of the sample had a "healthy" BMI between 18.5 and 25.0, whereas 38.1% had BMI in overweight/obese category. Among the children, BMI was unrelated to scores on the ChEAT or total score on the EDI-C. Higher BMI was positively correlated with scores on the "Body Dissatisfaction" subscale of the EDI-C ($r = 0.52$, $p = 0.006$) and also correlated with lower body esteem for Weight ($r = -0.42$, $p < 0.04$) and overall Appearance ($r = -0.36$, $p < 0.07$). In the adolescent/adult group, BMI was not significantly

related to scores on the EAT. However, BMI positively correlated with eating disorder symptoms on the EDI-3 total score ($r = 0.54$, $p = 0.002$), as well as the "Drive for Thinness" subscale ($r = 0.46$, $p = 0.10$) and the "Body Dissatisfaction" subscale ($r = 0.60$, $p = 0.001$). Higher BMI also correlated with lower body esteem for Weight ($r = -0.68$, $p < 0.001$) and overall Appearance ($r = -0.44$, $p < 0.02$). Though results demonstrated that adolescent/adult height ranged from 3′11″ to 5′8″ [$M = 5'3''$ (SD = 4.3″)], height was not related to body esteem. The association between height and body esteem was not analyzed in participants under 13.

From the semi-structured interviews, six major themes associated with body esteem arose, including (1) issues with bullying/teasing, (2) weight concerns, (3) height concerns, (4) positive body image, (5) negative body image, and (6) age-related acceptance of GSD and its effects on the body.

Bullying/Teasing

Some younger patients and several adult patients reported bullying and teasing while in school. Weight and appearance were particular topics of concern. For example, one girl mentioned that other kids at school "are really skinny and it is kind of embarrassing not being able to be like that... Sometimes they will call you fat," while a young woman acknowledged that "it wasn't easy dealing with weight issues when I was younger... I used to get kind of made fun of and ... called fat." Others indicated bullying occurred because of GSD-related differences from peers. One male teenager remarked, "I got a lot of [flak] in school for actually doing the routine, and taking the medicine, and not eating the same stuff they were," while a middle-aged woman noted, "growing up I was always mocked because I had a big liver and I was clumsy and bad in sports."

Weight

Weight concern was a common theme across age and gender. For some, it appeared to be entirely self-perpetuated [e.g., "I think I'm ... a little overweight and don't know why I feel like that" (female child) and "I think that I'm fat, but everyone says I'm not" (male child)]. However, several patients were particularly concerned with how they believed others perceived them. One young woman remarked, "I wish people knew I had GSD, that I don't just eat everything in sight, and thus am overweight ... because I work out four times a week, and I try to eat well and I'm still on the much much higher side. I know if people saw me they would just come to the conclusion that obviously I must not eat right." Another exclaimed, "I hate and I will cry every time... I am asked if I am pregnant... I hate that my body looks like this and it's not my fault because it is this genetic disease" (young woman). Of concern, a few patients specifically related their weight concerns to their cornstarch treatment, saying, "I'm getting like 1,500 calories from cornstarch alone right now, and no matter how much I exercise I will never be a certain amount of shape I want (sic) ... and there is absolutely nothing I can do about it" (male teenager). One young woman even admitted to dangerously reducing her treatment, saying, "I wish I could be thinner ... like any other women. I got really thin last summer ... because I reduced my cornstarch more than I should have."

Height

Though often ameliorated by appropriately aggressive treatment and management, short stature is a common complication of the glycogen storage diseases. Surprisingly, several patients viewed their height as a positive attribute. A young woman noted, "I was always teased about being short, but it made me special and people gave me attention. I love attention." Similarly, an adolescent girl reported, "I like being short." However, other participants viewed their height negatively or expressed a desire to be taller. For example, a middle-aged woman stated, "Wish I was taller." A young man noted that his height affected participation in group sports saying, "If I played with my peers, since I was so much smaller, I would get dominated. So what they did is that they ... put me in a group setting that was a year or two years younger, so I could be more competitive."

Negative Body Image

When asked what they did not like about their bodies, patients' responses ranged from overall dissatisfaction [e.g., "I don't like my body ... a problem since I was a little kid. I really wish I didn't have this disease" (middle-aged woman), and "I have long been at odds with my body because it would never do what I wanted it to do... Even when I was older I still always thought I was fat and then now, it's like failing ... the muscles are failing" (middle-aged woman)] to more specific complaints. For example, several individuals commented on the GSD side effect of having large livers/stomachs [e.g., "I guess you always feel self-conscious because you have the tendency to have the larger liver, which makes your stomach larger" (young woman), and "When I was younger, I had a little pot belly and I resented that, but that's behind me now" (young man)]. Other GSD-related issues were also mentioned, including "I have a scar from my liver biopsy. I don't like that!" (young woman). One patient expressed frustration with her thin limbs, "Fractures are frustrating. I have spindly arms and legs. I wish they were more muscular"

(middle-aged woman). Another young man who displayed typical Type Ia physiology expressed, "I don't like my gut. I mean I've got skinny little arms. My nickname's the Tick with everyone I know." Notably, none of the patients identified common body concerns that were unrelated to GSD (e.g., amount/type of hair, acne, size/shape of nose, feet, breasts, etc.)

Positive Body Image

A few respondents did report positive attributes of their bodies, including GSD treatment-related improvements. One young girl said, "I'm really proud about my body because . . . my liver is really healthy, and I breathe really well, and I think really well, so I'm really proud of that." Another expressed happiness with her overall appearance saying, "My core body I actually feel pretty good about . . . I feel I am a pretty attractive person, cute . . . I'm pretty tall for a girl of GSD, sort of above my age . . . when I'm fit, I actually feel pretty darn good about myself" (young woman).

Age-Related Acceptance

Many of the adult participants spoke about age-related acceptance of their condition and reported becoming more comfortable with their appearance in adulthood. As one young man explained, "Now that I'm older, I don't really care. But when I was in my teenager years I didn't like my big belly, I was self-conscious to go to the pool, take my shirt off. . . Now that I'm old, it's normal to be out of shape so it doesn't bother me that much. . ." Another commented, "With GSD, you know most people aren't a double zero. . . I did have a hard time with that in high school. . . I've had a baby now. So I mean, anytime I have any sort of bodily issue, it's like, well, you know, I've had a baby. And that to me is more important than squeezing into a double zero" (young woman)]. Finally, another young adult woman remarked, "as you get older, you really accept the way you are."

Discussion

In this study, GSD patients reported fewer classic symptoms of eating disorders (e.g., binging, purging, fasting, etc.) than population norms, with Type I patients demonstrating fewer symptoms than patients with less severe forms. However, the GSD patients also reported elevated dysfunctional attitudes toward food and eating, as well as lower body esteem. These results are concerning, as both increase the risk for disordered eating (Mintz and O'Halloran 2000; Littleton and Ollendick 2003; Verplanken and Velsvik 2008).

Unhealthy habits may be adopted by patients with GSD because healthy weight management options are limited due to dietary requirements and restrictions on exercise, particularly for patients with GSD Type I (Mundy et al. 2005). The current results suggest that a significant minority (11–15%) of GSD patients have clinically significant disordered eating symptoms and may meet diagnostic criteria for Eating Disorder Not Otherwise Specified. Given how crucial food is as medicine for GSD patients, even subclinical eating disorders pose a severe threat for this population. It is possible that traditional measures of eating disorder symptoms are not appropriate for GSD patients, given that the items primarily assess symptoms that could have catastrophic or even fatal consequences for GSD patients (e.g., fasting, purging, excessive exercise, etc.). As a result, GSD-specific measures may be needed to fully examine the extent of disordered eating and dysfunctional attitudes in this population. Items should assess behaviors such as skipping cornstarch doses, decreasing cornstarch without medical supervision, eating foods that are not well absorbed to manage weight, and unhealthy exercise habits.

In this sample, negative attitudes related to eating and body image appeared to be particularly problematic for younger patients, with higher average scores on the ChEAT and increased frequency of scoring above the clinical cutoff for children with GSD compared to non-clinical samples. Scores in the adolescent/adult group of GSD patients were more similar to body esteem scores in the general population, though the GSD sample still reported lower body esteem (Maloney et al. 1988; Maloney et al. 1989). These findings are consistent with the qualitative descriptions provided by adult GSD patients in this study, who reported more negative views during childhood, with improvements to their body esteem as they entered adulthood.

Associations between BMI and body esteem in the current sample were similar to those seen in a study of healthy young adults, where correlations between BMI and Weight Esteem ranged from $r = -0.43$ to -0.58 ($p < 0.05$), based on gender, and BMI and Appearance Esteem correlated $r = -0.36$ ($p < 0.05$) for both men and women (Streeter et al. 2012). A study of healthy children (Bernier et al. 2010) also demonstrated similar correlations between BMI percentile and overall body esteem ($r = -0.36$ to -0.45, $p < 0.01$); however, those children demonstrated a negative correlation between BMI percentile and scores on the ChEAT ($r = -0.22$ to -28, $p < 0.01$). When comparing to patients with chronic metabolic disease, previous research involving patients with Type I diabetes has noted that body dissatisfaction is associated with poor glycemic control, whereas good metabolic control is linked to better quality of life (Meltzer et al. 2001; Hoey et al. 2001). Poor metabolic control in GSD leads to greater complications and comorbid factors including hypoglycemic episodes, hepatomegaly, hyperlipidemia, hyperlactatemia, nephropathy, and hepatic adeno-

mas, which may negatively impact quality of life. Future research should explore links between metabolic control, body image, and quality of life, particularly in GSD patients suffering from overtreatment obesity.

The results of the present study should be interpreted within the context of some important limitations. First, though the sample was relatively large considering GSD is a rare disease, the small sample size likely affected statistical significance, particularly with regard to exploration of gender or age discrepancies. Second, the sample was overwhelmingly comprised of GSD Type I patients, precluding meaningful analyses among the subtypes. Third, the sample was comprised of individuals who attend regular clinic appointments, suggesting higher rates of compliance. This may affect their psychological response to living with GSD, including the burdens of the intensive medical regimen and the likelihood of fewer medical complications, resulting in somewhat biased results. Fourth, there are concerns about the appropriateness of certain study measures (i.e., measures assessing classic eating disorder symptoms) for this particular population. Fifth, the self-report nature of the data may have introduced response bias. Despite these limitations, this study is the first to examine the important topic of disordered eating and body esteem among patients with GSD.

Conclusions

Focus on food as essential medicine in patients with GSD may be protective against development of classic eating disorder symptoms, even in the presence of dysfunctional eating attitudes and low body esteem. The GSD patients in this study generally reported lower rates of eating disorder symptoms such as binging, purging, and fasting, with Type I patients, who have the strictest dietary guidelines and increased reliance on cornstarch therapy, reporting the fewest symptoms. Despite this, some patients reported concerning symptoms, and food preoccupation may have a negative impact on body esteem and quality of life for patients with GSD. Screening for dysfunctional eating attitudes, disordered eating behaviors, and negative body esteem should be included in the standard of care for patients with GSD. Questionnaires assessing the particular concerns of this population, as have been developed for individuals with diabetes, are needed to better understand their level of risk. Negative body image appears to be problematic in this population, and the medical team should assess and address this with young patients, in particular. Referral to qualified mental health professionals may be indicated for patients who are struggling with these concerns. At minimum, the medical team should provide patients with age-appropriate suggestions on how to talk with others about their disease and its impact on their body in order to reduce feelings of shame and decrease the potential for bullying or discrimination.

Compliance with Ethics Guidelines

Synopsis

Patients with Glycogen Storage Disease are at risk for disordered eating behaviors and low body esteem; these areas should be evaluated as part of standard clinical care.

Conflict of Interest

All the authors of this chapter declare that there are no conflicts of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Author Contributions

Theresa B. Flanagan – Contributed to data analysis and the writing of the initial draft of the manuscript. Jill A. Sutton – Conducted statistical analysis and assisted with the write-up of results. Laurie M. Brown – Assisted with data collection and reviewed the final draft of the manuscript. David A. Weinstein – Provided the initial idea for the study and gave feedback on study design, elicited funding, and provided edits to the final manuscript. Lisa J. Merlo – Designed the study, supervised data collection, conducted statistical analysis, and contributed to the writing of the manuscript/guarantor.

References

Anton SD, Han H, Newton RL Jr et al (2006) Reformulation of the Children's Eating Attitudes Test (ChEAT): factor structure and scoring method in a non-clinical population. Eat Weight Disord Stud Anorexia Bulimia Obesity 11(4):201–210

Bernier CD, Kozyrskyj A, Benoit C, Becker AB, Marchessault G (2010) Body image and dieting attitudes among preadolescents. Can J Diet Pract Res 71(3):e34–e40

Chen M (2011) Glycogen storage diseases. In: SPS Monga (ed) Molecular pathology of liver diseases, vol 5. Springer, New York, 677–681

Corbin J, Strauss AL (2008) Basics of qualitative research: techniques and procedures for developing grounded theory, 3rd edn. Sage Publications, Inc., Thousand Oaks

Diener E, Diener M (1993) Cross-cultural correlates of life satisfaction and self-esteem. JPers Soc Psychol 68(4):653–663

Furnham A, Badmin N, Sneade I (2010) Body image dissatisfaction: gender differences in eating attitudes, self-esteem, and reasons for exercise. J Psychology 136(6):581–586

Garner DM (1991) Eating disorders inventory-C. Psychological Assessment Resources, Lutz

Garner DM (2004) EDI-3 eating disorder inventory-3 professional manual. Psychological Assessment Resources, Inc., Odessa

Garner DM, Garfinkel PE (1979) The Eating Attitudes Test: an index of the symptoms of anorexia nervosa. Psychol Med 9:273–279

Hoey H, Aanstoot HJ, Chiarelli F et al (2001) Good metabolic control is associated with better quality of life in 2,101 adolescents with type 1 diabetes. Diabetes Care 24(11):1923–1928

Kollberg G, Tulinius M, Gillijam T et al (2007) Cardiomyopathy and exercise intolerance in muscle glycogen storage disease 0. N Engl J Med 357:1507–1514

Littleton HL, Ollendick T (2003) Negative body image and disordered eating behavior in children and adolescents: what places youth at risk and how can these problems be prevented? Clin Child Fam Psychol Rev 6(1):51–66

Maloney M, McGuire J, Daniels S (1988) Reliability testing of a children's version of the Eating Attitudes Test. J Am Acad Child Psychiatry 5:541–543

Maloney M, McGuire J, Daniels S et al (1989) Dieting behavior and eating attitudes in children. Pediatrics 84:482–489

Meltzer LJ, Bennett Johnson S, Prine JM et al (2001) Disordered eating, body mass, and glycemic control in adolescents with type I diabetes. Diabetes Care 24(4):678–682

Mendelson BK, White DR (1985) Development of self-body-esteem in overweight youngsters. Dev Psychol 21:90–96

Mendelson BK, White DR, Mendelson MJ (1996) Self-esteem and body esteem: effects of gender, age, and weight. J Appl Dev Psychol 17:321–346

Mendelson BK, White DR, Balfour L (2001) The body-esteem scale for adolescents and adults. J Pers Assess 76(1):90–106

Mintz LB, O'Halloran MS (2000) The Eating Attitudes Test: validation with DSM-IV eating disorder criteria. J Pers Assess 74(3):489–503

Mundy HR, Georgiadou P, Davies LC, Leonard JV, Lee PJ (2005) Exercise capacity and biochemical profile during exercise in patients with glycogen storage disease type I. J Clin Endocrinol Metab 9(5):2675–2680

O'Dea JA (2006) Self-concept, self-esteem and body weight in adolescent females: a three-year longitudinal study. J Health Psychol 11(4):599–611

Olmsted MP, Colton PA, Daneman D et al (2008) Prediction of the onset of disturbed eating behavior in adolescent girls with type 1 diabetes. Diabetes Care 31(10):1978–1982

Özen H (2007) Glycogen storage diseases: new perspectives. World J Gastroenterol 13(18):2541–2553

Rake JP, Visser G, Labrun P et al (2002a) Glycogen storage disease type I: Diagnosis, management, clinical course and outcome. Results of the European study on glycogen storage disease I. Eur J Pediatr 161:S20–S34

Rake JP, Visser G, Labrun P et al (2002b) Guidelines for management of glycogen storage disease type I – European study on glycogen storage disease I. Eur J Pediatr 161:S112–S119

Shin YS (2006) Glycogen storage disease: clinical, biochemical, and molecular heterogeneity. Semin Pediatr Neurol 13(2):115–120

Storch E, Keeley M, Merlo L et al (2008) Psychosocial functioning in youth with glycogen storage disease type I. J Pediatr Psychol 33 (7):728–738

Streeter V, Milhausen RR, Buchholz AC (2012) Body image, body mass index, and body composition: In young adults. Can J Diet Pract Res 73(2):78–83

Verplanken B, Velsvik R (2008) Habitual negative body mage thinking as psychological risk factor in adolescents. Body Image 5:133–140

Weinstein DA, Wolfsdorf JI (2002) Effect of continuous glucose therapy with uncooked cornstarch on the long-term clinical course of type 1a glycogen storage disease. Eur J Pediatr 161: S35–S39

Wolfsdorf JI, Holm IA, Weinstein WA (1999) Glycogen storage diseases—phenotypic, genetic and biochemical characteristics, and therapy. Endocrinol Metab Clin 28(4):801–823

JIMD Reports
DOI 10.1007/8904_2014_361

CASE REPORT

One Year Experience of Pheburane® (Sodium Phenylbutyrate) Treatment in a Patient with Argininosuccinate Lyase Deficiency

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Received: 29 May 2014 / Revised: 28 August 2014 / Accepted: 01 September 2014 / Published online: 10 February 2015
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Abstract Argininosuccinate lyase deficiency (ASLD) is a urea cycle disorder (UCD) treated with dietary adjustment and nitrogen scavenging agents. "Pheburane®" is a new tasteless and odour-free formulation of sodium phenylbutyrate, indicated in the treatment of UCD.

A male patient diagnosed with ASLD was put on treatment with the new formulation of sodium phenylbutyrate (granules) for a period of one year, at 500 mg/kg orally in 3 intakes/day. Plasma glutamine, arginine, citrulline, argininosuccinate, serum sodium, potassium, liver function tests and urine orotate all remained unchanged over this period. There was no difference in mean ammonia levels before and after treatment, and no hyperammonemia episode occurred during treatment with Pheburane®. An improvement in a measurement of quality of life (QOL) was noted after treatment with Pheburane®.

Conclusion: Good metabolic control and improved QOL were achieved throughout the treatment period.

Abbreviations

ASLD	Argininosuccinate lyase deficiency
NaPB	Sodium phenylbutyrate
QOL	Quality of life
UCD	Urea cycle disorder

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Competing interests: None declared

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Argininosuccinate lyase deficiency (ASLD), caused by the deficiency of the enzyme argininosuccinate lyase, is the second most common urea cycle disorder. Dietary restriction and arginine supplementation combined with an ammonia scavenger such as sodium phenylbutyrate (NaPB) are the main therapeutic modalities used in the chronic treatment of ASLD (Erez 2013). The unpleasant taste and odour of the medicine is a significant burden adding to the stress of the families faced with the daily challenge of getting the children to take a medicine for a life-threatening disease (Brusilow and Maestri 1996). Pheburane® is a new, taste -masked and odour-free formulation of NaPB (developed by Lucane Pharma, France). Results following the use of Pheburane in patients with UCD under a French cohort temporary utilisation protocol have been recently published (Kibleur et al. 2014). NaPB is an established treatment for UCD, available for over three decades (Brusilow et al. 1980). NaPB, as a precursor of sodium phenylacetate, provides an alternative pathway of nitrogen excretion by conjugation with glutamine. The decrease in urea cycle flux and hence decrease in the production of argininosuccinate may also mitigate the hepatic injury described in ASLD (Smith et al. 2013). Pheburane® (granules) consists of small spherical sugar cores which are then coated with NaPB and ethylcellulose sequentially in two separate layers. Ethylcellulose is a well-known taste-masking agent for active substances (Guffon et al. 2012).

Here we report 1 year usage of Pheburane® in a case of late onset ASLD (asymptomatic until the first three months of life) prescribed because it is a tasteless and odour-free formulation. A male patient first presented at three months of age with symptoms of retarded development, strabismus and vomiting. He was the first offspring of parents both working as high school teachers. There was no

Table 1 Levels of plasma ammonia, glutamine, arginine, citrulline, argininosuccinate, serum sodium, potassium, liver function tests and urine orotate at diagnosis and during periods of different therapies

Parameters	Normal values	At diagnosis (mean ± SD)	Na-benzoate (mean ± SD)	Na-phenylbutyrate (mean ± SD)	Pheburane® (mean ± SD)
Ammonia (p) (μg/dL)	21–50	150	48 ± 10	49 ± 9	33 ± 5
Glutamine (p) (μmol/L)	333–809	760	570.8 ± 52	549.3 ± 51	540.6 ± 49
Arginine (p) (μmol/L)	12–112	10	102.6 ± 28	49.6 ± 18	46 ± 19
Citrulline(p) (μmol/L)	3–35	55	35.6 ± 10	33.2 ± 8	30.1 ± 5
Argininosuccinate(p) (μmol/L)	0.0	0.1	0.0	0.0	0.0
Potassium (s) (mEq/L)	3.5–5.0	4.1	3.8 ± 1.1	3.9 ± 0.9	3.8 ± 0.8
Sodium (s) (mEq/L)	136–145	140	138 ± 2.1	139 ± 1.8	138 ± 1.9
Aspartate aminotransferase (AST,SGOT) (s) (U/L)	15–55	22	25 ± 3	28 ± 5	26 ± 4
Alanine aminotransferase (ALT, SGPT) (s) (U/L)	5–45	51	28 ± 5	25 ± 7	26 ± 5
Orotic acid (u) (mmol/mol creatinine)	0.02–3.6	4.2	0.01	0.01	0.01

p plasma, *s* serum, *u* urine. Mean levels of parameters ±SD were presented

consanguinity between the parents. Born as a healthy baby (weight, 3,000 g (50th percentile); length, 50 cm (50th percentile); head circumference, 38 cm (50th percentile); Apgar, 10/10) after an uneventful pregnancy, the patient was found to have hyperammonemia during an attack of bronchiolitis. ASLD was diagnosed based on moderately elevated plasma citrulline (55 μmol/L, *N*: 3–35) and argininosuccinate (0.1 μmol/L, *N*: 0.0) levels and decreased argininosuccinate lyase activity measured in red blood cells (9.0 nmol/min/gHb, *N*: 50–140). Treatment was initiated with a diet containing 1.25–2 g/kg/day protein and supplementation of L-arginine (250 mg/kg/day) and Na-benzoate (500 mg/kg/day). Based on the recommendation to reduce the L-arginine dose at the age of four, this was decreased from 250 to 100 mg/kg/day (Nagamani et al. 2012). This was followed by an increase in ammonia levels, and Na-benzoate (500 mg/kg/day) was replaced with NaPB (500 mg/kg/day) to improve scavenging. At the age of six, weight was 18 kg (3–10th percentile range), height 103 cm (<3th percentile) and head circumference 48 cm (3th percentile). Clinical examination revealed mild mental retardation (IQ 69) (References: IQ 50–70 according to ICD10) and strabismus. The family reported refusal to eat or drink because of the taste and smell of the accompanying drugs, particularly NaPB. Attempts by the parents to mask the taste in food (particularly in yogurt) were unsuccessful. The consequences of the child not taking the medication added a significant burden to the stress already experienced by the parents caring for a child with a history of hyperammonemia (three episodes of hyperammonemia and hospitalisation (one in intensive care unit) during febrile infections in infancy). The quality of life (QOL) score was 23.9 (Uneri et al. 2008).

The new formulation of NaPB was started and continued at the same dose range (500 mg/kg/day orally) in 3 intakes/day as before. No hyperammonemia episode occurred over one year of treatment with Pheburane®. There was no significant difference in the mean ammonia level before and after treatment with Pheburane® (Table 1). Mean levels of plasma glutamine, arginine, citrulline, argininosuccinate, serum sodium, potassium, liver function tests and urine orotate remained unchanged (Table 1). During the first week of administration, the parents reported vomiting during administration. However, successful posology adjustment to ensure rapid swallowing, minimising the time with the medicine in the mouth, was achieved by the end of the first week. The patient became fully compliant with the prescribed doses of Pheburane® at the end of the first month. A decrease in family stress was observed during regular outpatient visits. A repeat of the QOL measurement indicated an improved quality of life; the QOL score increased from 23.9 to 57.6. Additionally, a detailed interview with the parents performed by a psychologist revealed decreased anxiety and improved compliance.

Considering that new aspects of NaPB usage are emerging which may indicate further therapeutic activity (enhancement of pyruvate dehydrogenase complex enzymatic activity in vitro and in vivo by increasing the proportion of unphosphorylated enzyme through inhibition of pyruvate dehydrogenase kinase), the development of a new NaPB formulation which is far more palatable is extremely important (Ferriero et al. 2013).

In conclusion, despite the limited observational time of one year in this single patient, we would like to share our experience of use of Pheburane® with the aim of informing

clinicians about this new option for treatment in UCD which may improve QOL for these patients.

Acknowledgments We thank Pamela Lewis and Yves Kibleur for editorial assistance.

Compliance with Ethics Guidelines

Conflict of Interest

Sema Kalkan Uçar, Burcu Özbaran,Yasemin Atik Altinok, Melis Köse, Ebru Canda, Mehtap Kağnc, Mahmut Coker declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from the patient for being included in the study.

Authors' Contributions

SKU had primary responsibility for protocol development, patient enrollment, outcome assessment, data analysis and writing the manuscript. BO contributed to the psychological evaluation and report of the work. YAA participated in patient's nutritional management. MK, EC, MK participated in patient follow-up. MÇ supervised the design, performed the final data analyses and contributed to the writing of the manuscript. All authors read and approved the final manuscript.

References

Brusilow SW, Maestri NE (1996) Urea cycle disorders: diagnosis, pathophysiology, and therapy. Adv Pediatr 43:127–170

Brusilow SW, Tinker J, Batshaw M (1980) Amino acid acylation: a mechanism of nitrogen excretion in inborn errors of urea synthesis. Science 207:659–661

Erez A (2013) Argininosuccinic aciduria: from a monogenic to a complex disorder. Genet Med 15:251–257

Ferriero R, Manco G, Lamantea E et al (2013) Phenylbutyrate therapy for pyruvate dehydrogenase complex deficiency and lactic acidosis. Sci Transl Med 175:1–26

Guffon N, Kibleur Y, Copalu W et al (2012) Developing a new formulation of sodium phenylbutyrate. Arch Dis Child 97:1081–1085

Kibleur Y, Dobbelaere D, Barth M et al (2014) Results from a nationwide cohort temporary utilization authorization (ATU) survey of patients in France treated with Pheburane® (sodium phenylbutyrate) taste-masked granules. Paediatr Drugs. doi:10.1007/s40272-014-0081-5

Nagamani SC, Lee B, Erez A (2012) Optimizing therapy for argininosuccinic aciduria. Mol Genet Metab 107:10–14

Smith W, Diaz GA, Lichter-Konecki U et al (2013) Ammonia control in children ages 2 months through 5 years with urea cycle disorders: comparison of sodium phenylbutyrate and glycerol phenylbutyrate. J Pediatr 162:1228–1234

Uneri OS, Agaoglu B, Coskun A et al (2008) Validity and reliability of pediatric quality of life nventory for 2- to 4-year-old and 5- to 7-year-old Turkish children. Qual Life Res 17:307–315

JIMD Reports
DOI 10.1007/8904_2014_362

RESEARCH REPORT

Growth Hormone Deficiency and Lysinuric Protein Intolerance: Case Report and Review of the Literature

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Received: 06 March 2014 / Revised: 19 August 2014 / Accepted: 03 September 2014 / Published online: 23 January 2015
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Abstract *Background*: Lysinuric protein intolerance (LPI; MIM# 222700) is a rare metabolic disorder caused by a defective cationic amino acids (CAA) membrane transport leading to decreased circulating plasma CAA levels and resulting in dysfunction of the urea cycle. Short stature is commonly observed in children with LPI and has been associated with protein malnutrition. A correlation between LPI and growth hormone deficiency (GHD) has also been postulated because of the known interaction between the AA arginine, ornithine, and lysine and growth hormone (GH) secretion. Our report describes a case of GHD in an LPI patient, who has not presented a significant increase in growth velocity with recombinant-human GH (rhGH) therapy, suggesting some possible pathogenic mechanisms of growth failure.

Case Presentation: The proband was a 6-year-old boy, diagnosed as suffering from LPI, erythrophagocytosis (HP) in bone marrow, and short stature. Two GH provocative tests revealed GHD. The patient started rhGH therapy and a controlled-protein diet initially with supplementation of oral arginine and then of citrulline. At 3-year follow-up, no significant increase in growth velocity and in insulin-like growth factor-1 (IGF-1) levels was observed. Inadequate nutrition and low plasmatic levels of arginine, ornithine, lysine, and HP may have contributed to his poor growth.

Conclusion: Our case suggests that growth failure in patients with GHD and LPI treated with rhGH could have a complex and multifactorial pathogenesis. Persistently low plasmatic levels of lysine, arginine, and ornithine, associated with dietary protein and caloric restriction and systemic inflammation, could determine a defect in coupling GH to IGF-1 production explaining why GH replacement therapy is not able to significantly improve growth impairment. We hypothesize that a better understanding of growth failure pathophysiology in these patients could lead to the development of more rational strategies to treat short stature in patients with LPI.

Abbreviations

AA	Amino acids
CAA	Cationic amino acids
GH	Growth hormone
GHD	Growth hormone deficiency
HP	Erythrophagocytosis
IGF-1	Insulin-like growth factor-1
IUGR	Intrauterine growth restriction
LDH	Lactic dehydrogenase
LPI	Lysinuric protein intolerance
MPH	Midparental height
NO	Nitric oxide

Communicated by: Jean-Marie Saudubray

Competing interests: None declared

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Introduction

Lysinuric protein intolerance (LPI; MIM 222700) is a rare autosomal recessive metabolic disorder caused by a defective CAA lysine, arginine, and ornithine membrane transport. Urinary excretion of CAA is increased and CAA are poorly adsorbed from the intestine leading to decreased

circulating plasma CAA levels (Borsani et al. 1999; Torrents et al. 1999). This defect results in dysfunction of the urea cycle, causing episodes of postprandial hyperammonemia and spontaneous protein aversion (Ogier de Baulny et al. 2012; Sebastio et al. 2011; Palacin et al. 2004). The *SLC7A7* gene, which encodes the y+L amino acid transporter-1 (y+LAT-1), is the only gene in which mutations are currently known to cause LPI (Torrents et al. 1999; Ogier de Baulny et al. 2012; Sebastio et al. 2011).

Typically, patients with LPI are asymptomatic while breast-feeding and develop symptoms later in life after weaning. Recurrent vomiting and episodes of diarrhea are observed as acute symptoms after protein ingestion; however, the diagnosis of LPI may be missed during infancy unless the presence of neurologic involvement triggers a diagnostic laboratory evaluation. Chronic symptoms of LPI are short stature, enlarged liver and spleen, osteoporosis, muscle hypotonia, and sometimes moderate mental retardation. Renal insufficiency, pulmonary alveolar proteinosis, and hematologic anomalies including HP are recurrent clinical features and are suggestive of multisystem involvement (Ogier de Baulny et al. 2012; Sebastio et al. 2011).

Current long-term treatment of LPI consists of controlled-protein diet and supplementation with oral L-citrulline, L-lysine, or L-arginine, in order to replenish the urea cycle and prevent hyperammonemia and orotic aciduria (Palacin et al. 2004; Awrich et al. 1975; Mizutani et al. 1984; Brosnan and Brosnan 2007).

Failure to thrive and short stature are commonly observed in children with LPI and have been associated with protein malnutrition (Sebastio and Nunes 2006). A correlation between LPI and GHD was also postulated because of the known interaction between the amino acids (AA) arginine, ornithine, and lysine and GH secretion (Alba-Roth et al. 1988; Knopf et al. 1965; van Vught et al. 2008; Isidori et al. 1981).

There are only a few cases in the literature dealing with LPI patients in whom the GH/IGF-1 axis was investigated (Awrich et al. 1975; Goto et al. 1984; Esposito et al. 2006; Niinikoski et al. 2011), and a true GHD was observed in only one case (Esposito et al. 2006). In that case the patient was a 12-year-old severely stunted girl with renal tubular disease as well; she responded well to GH replacement therapy, but she had an age close to puberty and the follow-up period was only 1 year.

Our report describes a case of GHD in an LPI patient, who has not presented a significant increase in growth velocity with recombinant-human GH (rhGH) therapy, suggesting some possible pathogenic mechanisms of growth failure.

Case Presentation

Case Report

Our patient was a 6-year-9-month-old boy born at 38 weeks after an uneventful pregnancy from two unrelated healthy parents. His birth weight was 2,750 g (between 10th and 25th percentile on the Italian Neonatal Study growth charts (INeS) www.inescharts.com) and length was 47 cm (between 10th and 25th percentile on the INeS growth charts). He underwent normal weaning but subsequently showed recurrent vomiting, diarrhea, and an aversion to protein-rich foods that his parents gradually eliminated from his diet. In the first years of life, the boy suffered from failure to thrive and many upper respiratory infections. While assessing his problems, laboratory tests revealed high serum ferritin (244 ng/mL) and lactic dehydrogenase (LDH) values (1017 U/L).

He was brought to our attention for short stature. He was in good general conditions and his clinical examination was normal. His weight was 16.8 kg (<3rd percentile on the growth charts of the Center for Disease Control, CDC, and Prevention www.cdc.gov/growthcharts) and his height was 107 cm (<3rd percentile on CDC growth charts) (Fig. 1). The height velocity during the year before our evaluation was of 5 cm/year. Tanner stages were of G1, PH1. Midparental height (MPH) was used as an indicator of genetic growth potential, and the genetic target height of the patient was 172.5 cm (between 25th and 50th percentile on CDC growth charts) (Fig. 1). His bone age was delayed, being 4 years and 6 months according to Greulich and Pyle Method.

Two GH provocative tests were performed to measure GH peak serum levels, revealing in both cases GH values well below 10 μg/L; in particular, the arginine stimulation test induced a GH peak level of 1.1 μg/L and the insulin stimulation test of 6.8 μg/L. IGF-1 value was very low (3 nmol/L, reference range 9–30 nmol/L). Abdominal ultrasonography revealed only a mild hepatosplenomegaly. Blood cell and platelet count, renal and liver function tests, and urine analysis were all normal.

After the first laboratory tests, we found again high ferritin (770 ng/mL) and LDH levels (1,007 U/L). Therefore, a bone marrow aspiration was performed, evidencing HP. Given the history of poor growth associated with aversion to protein-rich food, HP in bone marrow, and hepatosplenomegaly, we assessed plasma and urinary AA as well. We found lower than normal *plasmatic* levels of lysine (62 μmol/L; normal values 115–278 μmol/L), ornithine (13.9 μmol/L; n.v. 25–159 μmol/L), and arginine (22.1 μmol/L; n.v. 38–135 μmol/L) and higher than normal

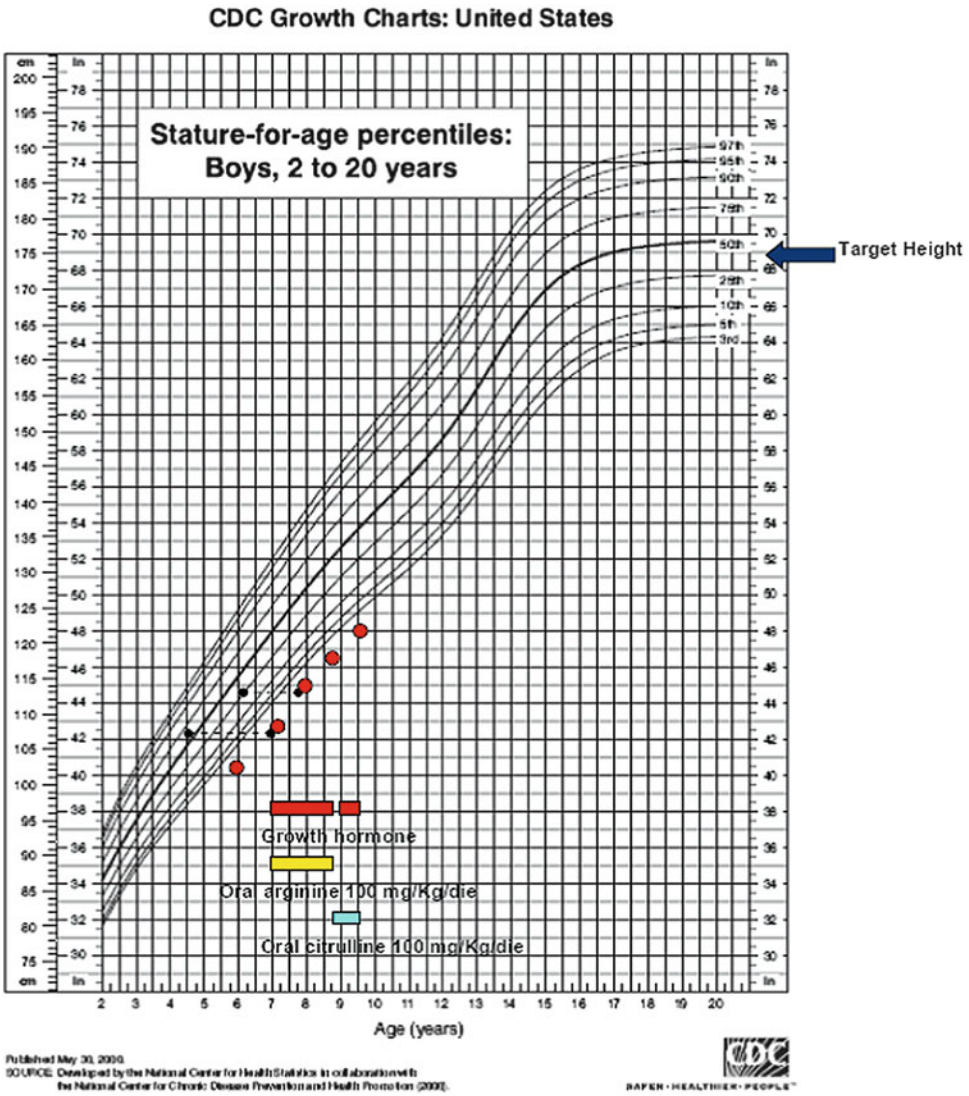

Fig. 1 Growth chart of the patient, plotted by CDC charts percentiles. The time when GH treatment and oral supplementation with arginine and citrulline were started are indicated

urinary levels of lysine (247 mmol/mol of creatinine; n.v. 10–56 mmol/mol of creatinine), arginine (89.5 mmol/mol of creatinine; n.v. 0–6 mmol/mol of creatinine), and ornithine (20.5 mmol/mol of creatinine; n.v. 0–6 mmol/mol of creatinine) (Tables 1 and 2). Based on these results, we diagnosed LPI with HP in bone marrow and GHD.

After PCR-amplified fragments sequencing, the patient was reported as a homozygote for a known missense mutation 1001T>G in the *SLC7A7* gene, leading to a premature stop codon in exon 8. His parents were heterozygous carriers of the same mutation.

At 7 years (height 108 cm), the patient started a controlled-protein diet (1 g/kg/day) and an oral supplementation with L-arginine (100 mg/kg/die) and vitamin D (800 IU/day). Following magnetic resonance imaging to rule out abnormalities of the hypothalamo-pituitary axis, the patient began daily subcutaneous rhGH therapy at a dose of 30 μg/kg/day for 6 days a week.

After 12 months from the institution of the rhGH therapy, despite good compliance with therapy and diet, the patient showed only a mild acceleration of growth velocity (6.5 cm/year). GH therapy promoted appetite, but his protein, in gram per kilogram per day, and his caloric intake, in Kcal per kilogram per day, showed no clear differences. IGF-1 level increased only from 3 to 10 nmol/L and bone age was of 6 years.

After 12 months, GHD was retested; L-dopa stimulation test revealed a GH peak level of 5.6 μg/L, confirming GHD. Because of the gastrointestinal symptoms experienced by the patient, in particular intestinal bloating, we stopped oral L-arginine and started oral supplementation with L-citrulline (100 mg/kg/day). Lysine supplement was

Table 1 Results of *plasma* cationic amino acids analyses at LPI diagnosis and after 24 and 36 months of therapy

Amino acids	Proband: at diagnosis (μmol/L)	Proband: after 24 months of therapy (μmol/L)	Proband: after 36 months of therapy (μmol/L)	Reference range (μmol/L)
Lysine	62	73.81	36.96	115–278
Arginine	22.1	53.70	18.96	38–135
Ornithine	13.9	16.21	17.45	25–159
Glutamine	1,025	1,041	1,061	399–823

Reference range is also indicated

Table 2 Results of *urinary* amino acids analyses at LPI diagnosis

Amino acids	Proband: at diagnosis (mmol/mol of creatinine)	Reference range (mmol/mol creatinine)
Lysine	247	10–56
Arginine	89.5	0–6
Ornithine	20.5	0–6

Reference range is also indicated

not given because of his experienced gastrointestinal symptoms.

At 10 years, after 36 months of good compliance with rhGH therapy and diet, his height was 125.4 cm, still under 3rd percentile on CDC growth charts, and his growth velocity was of 5.4 cm/year.

Therapy was monitored on the basis of plasma ammonia, plasma AA, and urinary orotic acid excretion. Postprandial plasma ammonia levels and orotic aciduria were always in the normal range. Plasmatic levels of arginine, ornithine, and lysine remained low. Glutamine levels remained around 1,000 μmol/L (Table 1).

Although a gradual GH dosage adjustment, IGF-1 levels persisted low (11.7 nmol/L).

During the whole follow-up period of 3 years, the patient maintained good general conditions and no acute episode of metabolic derangement was reported. His renal and pulmonary function was normal.

Discussion

Among the several metabolic-endocrine disorders causing short stature, especially in cases of aversion to protein-rich foods, LPI must always be considered. The clinical onset of the disease is usually delayed by breast-feeding or by the use of milk-based infant formula due to their relatively low protein content (Ogier de Baulny et al. 2012; Sebastio et al. 2011; Palacin et al. 2004), but in the case of our patient, the diagnosis was further delayed because his parents gradually eliminated protein-rich foods from his diet. Moreover, during the first 6 years of his life, failure to thrive and the gastrointestinal symptoms misled the diagnosis toward gastrointestinal disorders.

The growth failure frequently observed in children with LPI appears to be a postnatal phenomenon, because intrauterine development is usually normal (Bröer 2007), as observed in our patient, and poor growth is generally attributed to protein malnutrition (Sebastio and Nunes 2006). Nevertheless, Sperandeo et al. (Sperandeo et al. 2007) reported on the generation of a slc7a7$^{-/-}$ mice that experienced intrauterine growth restriction (IUGR) by downregulating IGF-1 expression in fetal liver.

In humans with LPI, there are only a few cases in whom the GH/IGF-1 axis was investigated (Awrich et al. 1975; Goto et al. 1984; Esposito et al. 2006; Niinikoski et al. 2011), and a true GHD was documented in only one of these patients (Esposito et al. 2006). Esposito et al. in 2006 described the first case of a 12-year-old LPI patient with documented GHD that was tested with arginine (GH peak 6.4 μg/L) and clonidine stimulation test (GH peak 2.7 μg/L). After 1 year of GH replacement therapy (35 μg/kg/day) with oral citrulline supplementation (150 mg/kg/day) and protein-restricted diet (1.2 g/kg/day), the authors observed a significant acceleration of growth velocity (from 2 to 8 cm/year) associated with a significant increase in IGF-1 levels (from 95 to 379 μg/L). Nevertheless, the follow-up period of this patient was only 1 year and the patient had an age close to puberty and a renal tubular disease as well.

Some observations suggest a possible link between pathophysiology of LPI and alterations in GH/IGF-1 axis. Niinikoski et al. (2011) observed in four LPI children very low IGF-1 concentrations (5–13 nmol/L), despite arginine-insulin GH provocative test revealed an abnormal GH peak level (< 10 ug/L) in only one of the patients. Nevertheless, a normal GH response to provocative tests in patients with auxology suggestive of GHD does not definitively rule out GHD because they may have a low spontaneous GH secretion over 24 h, likely reflecting GH neurosecretory dysfunction (Gasco et al. 2008). Moreover, today, there is no gold standard for estimating GH secretion in LPI

patients. In our case a very low GH peak was obtained with the arginine intravenous (IV) stimulation test; it is likely that this test may not be an appropriated test for GHD evaluation in patients with LPI because of their increased renal clearance of arginine.

Some studies suggest that GH secretion in LPI patients could be altered due to specific lysine, ornithine, and arginine deficiencies (Awrich et al. 1975; Goto et al. 1984). Goto et al. (1984) observed in a 14-year-old LPI boy a better response to the same GH stimulation test after treatment with arginine. Awrich et al. (1975) observed in a 9-year-old girl with LPI – but without GHD – an improvement of growth after oral supplement of arginine, lysine, and citrulline.

Although studies on slc7a7$^{-/-}$ mice exclude CAA depletion as a primary cause of IUGR (Sperandeo et al. 2007), it is not possible to exclude its role in postnatal growth delay. In fact, it is well known that GH secretion can be promoted by intravenous administration of various AA, and the stimulatory effect of some AA on GH is clinically used as a method to assess the responsiveness of the GH secretory system. In particular, it is known that IV arginine increases GH release by suppressing endogenous somatostatin secretion (Alba-Roth et al. 1988), and infusion of lysine also promotes relatively large increases (~8- to 22-fold) in circulating GH levels (Knopf et al. 1965). IV administration of ornithine stimulates GH release as well (Evain-Brion et al. 1982). The stimulatory effect of these AA on GH secretion is also evident upon oral administration (Chromiak and Antonio 2002), suggesting a role of dietary AA in the regulation of plasma GH concentrations (van Vught et al. 2008; Isidori et al. 1981).

CAA depletion could also compromise IGF-1 production. In fact, it has been observed that in humans, lysine sufficiency increased IGF-1 concentrations (Jansen 1962), and in animals low lysine intakes decreased muscle protein accretion and significantly downregulated hepatic IGFBP-1, as well as muscle GH-R and IGF-II (Ishida et al. 2011; Hevry et al. 2007). Furthermore, arginine has been demonstrated to stimulate IGF-1 production (Chevalley et al. 1998).

It is interesting to note that, although a gradual rhGH dosage adjustment, in our patient, IGF-1 levels remained very low, suggesting a defect in coupling GH to IGF-1 production.

Inadequate nutrition, in particular protein malnutrition and low caloric intake, may well contribute to the poor growth, because the functioning of GH/IGF-1 axis appears optimal only with adequate dietary intake. In fact, infants and young children having higher energy demands are especially vulnerable to nutritional imbalances. In particular, nutritional status is a critical element in regulating the GH/IGF-1 system, modulating the responsiveness of the liver to GH (Zamboni et al. 1996; Trobec et al. 2011; Hintz et al. 1978) and influencing IGF-1 gene expression also in extra-hepatic tissues (Naranjo et al. 2002). Moreover, malnutrition and protein deprivation are associated to high levels of GH accompanied by marked decrease in circulating IGF-1 levels (Zamboni et al. 1996; Trobec et al. 2011), and in specifically protein-malnourished children, it has been observed that serum IGF-1 bioactivity is low (Hintz et al. 1978). Furthermore, caloric intake is positively correlated with the increment in growth velocity during GH therapy (Zadik et al. 2005).

These points suggest that it is very important to optimize nutrition to improve growth in LPI patients, even more in GHD patients treated with GH. In fact, because GH therapy can potentially induce a rapid acceleration in growth, it should be suspected that a child who does not increase accordingly could experience compromised nutritional intake or a deficiency in some essential nutrients.

Systemic disorder-related manifestations of LPI (e.g., hematological abnormalities, immune dysfunction, pulmonary and renal involvement) could also exacerbate growth failure in these patients.

In fact, chronic inflammatory diseases have been associated with growth retardation in children (Pozzo and Kemp 2012). Moreover, the increased energy expenditure observed in chronic inflammatory disorders could impair linear growth, and inflammatory cytokines can adversely affect a number of components of growth plate chondrogenesis (De Benedetti 2009; Ahmed and Sävendahl 2009) and can also disrupt the GH/IGF-1 axis (Savage 2013).

A role of an unbalanced metabolism of intracellular arginine, which causes intracellular arginine accumulation and increased nitric oxide (NO) production, has been hypothesized to explain especially the inflammatory complications affecting lung and immune and hematologic systems in LPI patients (Sebastio et al. 2011), but currently, it is not clear if this mechanism could play a role in the pathophysiology of alterations in GH/IGF-1 axis and growth failure.

A good and precocious treatment of both GHD and LPI is also very important. Several studies have demonstrated that growth response on GH treatment is positively influenced by a young age at the start of treatment (Ranke et al. 2007). Moreover, optimal long-term outcome, such as stature, in inherited disorders depends on early diagnosis and good metabolic control (MaCdonald et al. 2012). Our patient was treated by 7 years of age, and chronic dysmetabolism and delayed rhGH therapy could be responsible for growth impairment.

Nevertheless, delayed puberty and prolonged growth due to delayed bone age, frequently observed in LPI patients, may explain why the final height in treated LPI patients is anyhow usually subnormal or low normal (Palacin et al. 2004).

In conclusion, our case suggests that growth failure in patients with GHD and LPI treated with rhGH has a

complex and multifactorial pathogenesis. Persistently low IGF-1 levels, observed in our patient although a gradual rhGH dosage adjustment, suggest that CAA deficiency, associated with dietary protein and caloric restriction and systemic inflammation, could determine a defect in coupling GH to IGF-1 production explaining why GH replacement therapy is not able to significantly improve growth impairment. CAA deficiency could be responsible also for reduced spontaneous GH secretion.

Pathogenesis of growth failure in LPI is still partially understood. We hypothesize that patients with LPI and GHD could better elucidate the biochemical causes of growth failure pathophysiology leading to the development of more rational strategies to treat short stature in LPI.

Acknowledgements The Authors are grateful to CEINGE Laboratories of University of Naples Federico II (Italy) for testing SLC7A7 gene, and to Alice Dianin for dietetic assessment of the patient to Ms. Judyth Dillon for editing the English version of the manuscript.

Conflict of Interest

Maines Evelina, Morandi Grazia, Olivieri Francesca, Camilot Marta, Cavarzere Paolo, Gaudino Rossella, Antoniazzi Franco, and Andrea Bordugo declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Written informed consent was obtained from the parents of the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Animal Rights

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Author's Contributions

Dr. Gaudino and Dr. Cavarzere conceived and designed the study and ensured the accuracy of the data and analysis. Dr. Olivieri collected the data and revised the manuscript critically for important intellectual content. Dr. Maines and Dr. Morandi ensured the accuracy of the data and analysis, wrote the initial draft, and critically revised the manuscript for important intellectual content. Dr. Camilot ensured the accuracy of the draft and edited the English version of the manuscript. Dr. Antoniazzi conceived and designed the study, ensured the accuracy of the data analysis, and critically revised the manuscript for important intellectual content. Dr. Bordugo revised the manuscript critically for important intellectual content.

References

Ahmed SF, Sävendahl L (2009) Promoting growth in chronic inflammatory disease: lessons from studies of the growth plate. Horm Res 72(Suppl 1):42–47

Alba-Roth J, Muller OA, Schopohl J et al (1988) Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. J Clin Endocrinol Metab 67:1186–1189

Awrich AE, Stackhouse WJ, Cantrell JE et al (1975) Hyperdibasicaminoaciduria, hyperammonemia, and growth retardation: treatment with arginine, lysine and citrulline. J Pediatr 87:731–738

Borsani G, Bassi MT, Sperandeo MP et al (1999) SLC7A7, encoding a putative permease-related protein, is mutated in patients with lysinuric protein intolerance. Nat Genet 21:297–301

Bröer S (2007) Lysinuric protein intolerance: one gene, many problems. Am J Physiol Cell Physiol 293:C540–C541

Brosnan ME, Brosnan JT (2007) Orotic acid excretion and arginine metabolism. J Nutr 137:1656–1661

Chevalley T, Rizzoli R, Manen D et al (1998) Arginine increases insulin-like growth factor-1 production and collagen synthesis in osteoblast-like cells. Bone 23:103–109

Chromiak JA, Antonio J (2002) Use of amino acids as growth-hormone-releasing agents by athletes. Nutrition 18:657–661

De Benedetti F (2009) The impact of chronic inflammation on the growing skeleton: lessons from interleukin-6 transenic mice. Horm Res 72(Suppl 1):26–29

Esposito V, Lettiero T, Fecarotta S et al (2006) Growth hormone deficiency in a patient with lysinuric protein intolerance. Eur J Pediatr 165:763–766

Evain-Brion D, Donnadie M, Roger M, Job JC (1982) Simultaneous study of somatotropic and corticotropic pituitary secretions during ornithine infusion test. Clin Endocrinol 17:119

Gasco V, Corneli G, Beccuti G et al (2008) Retesting the childhood-onset GH-deficient patient. Eur J Endocrinol 159(Suppl 1): S45–S52

Goto I, Yoshimura T, Kuroiwa Y (1984) Growth hormone studies in lysinuric protein intolerance. Eur J Pediatr 141:240–242

Hevrøy EM, El-Mowafi A, Taylor RG et al (2007) Lysine intake affects gene expression of anabolic hormones in Atlantic salmon, *Salmo salar*. Gen Comp Endocrinol 152:39–46

Hintz RL, Suskind R, Amatayakul K et al (1978) Plasma somatomedin and growth hormone values in children with protein-calorie malnutrition. J Pediatr 92:153–156

Ishida A, Kyoya T, Nakashima K et al (2011) Muscle protein metabolism during compensatory growth with changing dietary lysine levels from deficient to sufficient in growing rats. J Nutr Sci Vitaminol 57:401–408

Isidori A, Lo Monaco A, Cappa M (1981) A study of growth hormone release in man after oral administration of amino acids. Curr Med Res Opinion 7:475

Jansen GR (1962) Lysine in human nutrition. J Nutr 76:1–35

Knopf RF, Conn JW, Fajanss SS, Floyd JC et al (1965) Plasma growth hormone response to intravenous administration of amino acids. J Clin Endocrinol Metab 25:1140–1144

MaCdonald A, van Rijn M, Feillet F et al (2012) Adherence issues in inherited metabolic disorders treated by low natural protein diets. Ann Nutr Metab 61:289–295

Mizutani N, Kato T, Maehara M et al (1984) Oral administration of arginine and citrulline in the treatment of Lysinuric protein intolerance. Tohoku J Exp Med 142:15–24

Naranjo WM, Yakar S, Sanchez-Gomez M et al (2002) Protein calorie restriction affects nonhepatic IGF-a production and the lymphoid system: studies using the liver-specific IGF-1 gene-deleted mouse model. Endocrinology 143:2233–2241

Niinikoski H, Lapatto R, Nuutinen M et al (2011) Growth hormone therapy is safe and effective in patients with lysinuric protein intolerance. JIMD Reports 1:43–47

Ogier de Baulny H, Schiff M et al (2012) Lysinuric protein intolerance (LPI): a multi organ disease by far more complex than a classic urea cycle disorder. Mol Genet Metab 106:12–17

Palacin M, Bertran J, Chillaron J et al (2004) Lysinuric protein intolerance: mechanisms of pathophysiology. Mol Gen Metab 81: S27–S37

Pozzo AM, Kemp SF (2012) Growth and growth hormone treatment in children with chronic diseases. Endocrinol Metab Clin North Am 41:747–759

Ranke MB, Lindberg A, Price DA et al (2007) Age at growth hormone therapy start and first-year responsiveness to growth hormone are major determinants of height outcome in idiopathic short stature. Horm Res 68:53–62

Savage MO (2013) Insulin-like growth factors, nutrition and growth. World Rev Nutr Diet 106:52–59

Sebastio G, Nunes V (2006) Lysinuric protein intolerance. In: Pagon RA, Adam MP, Ardinger HH et al (eds) Gene reviews™ [Internet]. University of Washington, Seattle, 1993–2014

Sebastio G, Sperandeo M, Andria G (2011) Lysinuric Protein Intolerance: reviewing concepts on a multisystem disease. Am J Med Genet Part C 157:54–62

Sperandeo MP, Anunziata P, Bozzato A et al (2007) Slc7a7 disruption causes fetal growth retardation by downregulating Igf1 in the mouse model of lysinuric protein intolerance. Am J Physiol Cell Physiol 293:C191–C198

Torrents D, Mykkanen J, Pineda M et al (1999) Identification of SLC7A7, encoding y + LAT-1, as the lysinuric protein intolerance gene. Nat Genet 21:293–296

Trobec K, von Hachling S, Anker SD et al (2011) Growth hormone, insulin-like growth factor 1, and insulin signalling-a pharmacological target in body wasting and cachexia. J Cachexia Sarcopenia Muscle 2:191–200

van Vught AJ, Nieuwenhuizen AG, Brummer RJM et al (2008) Effects of oral ingestion of amino acids and proteins on the somatotropic axis. J Clin Endocrinol Metab 93:584–590

Zadik Z, Sinai T, Zung A, Reifen R (2005) Effect of nutrition on growth in short stature before and during growth-hormone therapy. Pediatrics 116:68–72

Zamboni G, Dufillot D, Antoniazzi F et al (1996) Growth hormone-binding proteins and insulin-like growth factor-binding proteins in protein-energy malnutrition, before and after nutritional rehabilitation. Pediatr Res 39:410–414

JIMD Reports
DOI 10.1007/8904_2014_363

RESEARCH REPORT

Geographical and Ethnic Distribution of Mutations of the Fumarylacetoacetate Hydrolase Gene in Hereditary Tyrosinemia Type 1

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Received: 26 June 2014 / Revised: 04 September 2014 / Accepted: 09 September 2014 / Published online: 15 February 2015
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Abstract Hereditary tyrosinemia type 1 (HT1) (OMIM 276700) is a severe inherited metabolic disease affecting mainly hepatic and renal functions that leads to a fatal outcome if untreated. HT1 results from a deficiency of the last enzyme of tyrosine catabolism, fumarylacetoacetate hydrolase (FAH). Biochemical findings include elevated succinylacetone in blood and urine; elevated plasma concentrations of tyrosine, methionine and phenylalanine; and elevated tyrosine metabolites in urine. The HT1 frequency worldwide is about 1 in 100,000 individuals. In some areas, where the incidence of HT1 is noticeably higher, prevalence of characteristic mutations has been reported, and the estimated incidence of carriers of a specific mutation can be as high as 1 out of 14 adults. Because the global occurrence of HT1 is relatively low, a considerable number of cases may go unrecognized, underlining the importance to establish efficient prenatal and carrier testing to facilitate an early detection of the disease. Here we describe the 95 mutations reported so far in HT1 with special emphasis on their geographical and ethnic distributions. Such information should enable the establishment of a preferential screening process for mutations most predominant in a given region or ethnic group.

Communicated by: Bridget Wilcken

Competing interests: None declared

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Abbreviations

BCH	Birmingham Children's Hospital
FAH	Fumarylacetoacetate hydrolase
GTR	Genetic Testing Registry
HCC	Hepatocellular carcinoma
HT1	Hereditary tyrosinemia type 1
LGCD	Laboratory of Cell and Developmental Genetics
NTBC	2-(2-Nitro-trifluoromethylbenzoyl) 1,3-cyclohexanedione
SLSJ	Saguenay-Lac-St-Jean region

Introduction

Hereditary tyrosinemia type 1 (HT1) (OMIM 276700) is an inherited metabolic disease, mainly of childhood. This pathological condition was referred to as hereditary tyrosinemia type 1 in the mid-1960s (reviewed in Mitchell et al. 2001; Russo et al. 2001), and it was later shown to result from a deficiency in fumarylacetoacetate hydrolase (FAH), the last enzyme of the tyrosine catabolic pathway (Lindblad et al. 1977; Fällström et al. 1979; Berger et al. 1981; Kvittingen et al. 1981; Tanguay et al. 1990).

HT1 is an autosomal recessive disease characterized by severe liver dysfunction, impaired coagulation, neurological crises, renal tubular dysfunctions and a high risk of hepatocellular carcinoma (HCC). Three main clinical forms of HT1 have been described: the *acute* form, which

presents itself in the first months of life and is associated with acute liver failure; the *subacute* form (second half of the first year) that manifests a similar but less severe clinical picture presenting usually with hepatomegaly or hypophosphatemic rickets (due to tubular dysfunction); and the *chronic* form which appears after the first year of age and shows a slower progression (Tanguay et al. 1990; van Spronsen et al. 1994; Bergman et al. 1998; Russo et al. 2001). Patients affected with HT1 generally show failure to thrive and hepatic damage including hepatomegaly, cirrhosis, hepatic failure and HCC. Complications associated with liver damage include jaundice, ascites and bleeding. HT1 also disrupts kidney function causing multiple tubular dysfunctions, Fanconi-like syndrome and glomerulosclerosis. In 1992, the introduction of NTBC (2-(2-nitro-trifluoromethylbenzoyl) 1,3-cyclohexanedione, also known as nitisinone) (Lindstedt et al. 1992) has proven to be highly effective in preventing the progression of liver damage, neurological crises and kidney damages (Larochelle et al. 2012; Bartlett et al. 2014). NTBC in combination with a low-tyrosine diet represents the only treatment available for this disease. However, one of the most severe complications occurring in HT1 patients remains the development of HCC (Mitchell et al. 2001). Indeed, although regular administration of NTBC in HT1 patients, combined with a protein-restricted diet, prevents liver and kidney dysfunction, recent reports have documented the presence of HCC even under therapy (de Laet et al. 2013). Effectiveness of this treatment depends on how early the disease is recognized and treated; thus, recent retrospective studies highly recommend the implementation of newborn screening in more areas (Zytkovicz et al. 2013; Dehghani et al. 2013; De Laet et al. 2013; Mayorandan et al. 2014). For example, Mayorandan and collaborators in their retrospective study point out the necessity of neonatal programmes borne by the government or health insurance companies to allow early diagnosis and access to adequate treatment. Indeed they report that patients, who were diagnosed after the neonatal period and consequently received NTBC treatment later, had a 2–12-fold higher risk (depending on age at start of therapy) of developing hepatocellular carcinoma compared to patients treated as neonates.

Detection of succinylacetone (SA) in urine, blood and amniotic fluid is the most reliable biochemical diagnostic for HT1. Assay of FAH enzyme activity in skin fibroblasts is possible but not readily available. Advent of molecular genetic testing has greatly improved the diagnostic power for this disease. Mutation analysis is not essential for clinical management but is useful for prenatal diagnosis and reproductive counselling. In fact targeted mutation analysis for diseased alleles and sequence analysis of the entire *fah* coding region can detect mutations in more than 95% of affected individuals (Sniderman King et al. 2011). The database of the GTR (Genetic Testing Registry: https://www.ncbi.nlm.nih.gov/gtr/conditions/C0268490) reports 56 clinical tests for diagnosis and monitoring of this condition. Carrier testing for at-risk relatives and prenatal diagnosis for pregnancies at increased risk are possible if both disease-causing alleles in a family are known.

Patients and Methods

The present review is based on a current compilation of all HT1 alleles reported worldwide including those from patients identified in the Laboratory of Cellular and Developmental Genetics (LGCD), Université Laval, Quebec, Canada (Dr RM Tanguay), and the Department of Clinical Chemistry at Birmingham Children's Hospital (BCH), Birmingham, UK (Dr G Gray), mostly between 2001 and 2013 (unpublished data). Screening of genetic databases (e.g. HGMD, NCBI, ENSEMBL) and HT1 literature has been made to classify the reported mutations and to identify the ethnic group of patients. The mutations reported so far and the patients' origins are listed in Table 1.

Since there are inconsistencies in the literature of names of the mutations in this gene, we have used the Human Genome Variation Society's nomenclature for the description of sequence variations (http://www.hgvs.org/mutnomen/recs.html) as the basis of nomenclature (den Dunnen and Antonarakis 2000) and used the *fah* cDNA sequence given as GenBank accession number BT007160.1 as our reference sequence. For splice defects we have also added the historical mutation nomenclature, since this is the most common way in which they are named worldwide.

Results and Discussion

Fah Gene Characteristics and Mutations

The first mutation reported in the *fah* gene was the c.47A>T (p.Asn16Ile) in a French Canadian patient and was shown to be causative of FAH deficiency (Phaneuf et al. 1992).

The human *fah* gene is located on chromosome 15q23-q25, spans 30–35 kb and consists of 14 exons. The cDNA has an open reading frame of 1,257 bp encoding 419 amino acids (Phaneuf et al. 1991; Labelle et al. 1993). Identification of this gene (Phaneuf et al. 1991) led to mutation screening of patients and characterization of a number of disease-causing alleles, some of which were present at relatively high frequencies in specific populations (St-Louis and Tanguay 1997).

Eighty-three disease-causing mutations are presently reported on Human Gene Mutation Database (HGMD® Professional 2014.2, accessed in August 2014). Recently,

Table 1 Compilation of hereditary tyrosinemia type 1 alleles worldwide

HGVS mutation nomenclature		Effect of mutation	Origin	No. patients	Reported alleles	References
cDNA (Alias[a])	Protein					
c.1A>G	p.1Met>Val	Missense	Emirates	1	2	Al-Shamsi et al. (2014)
			Greece	1	2	Georgouli et al. (2010)
			Saudi Arabia	7	14	Imtiaz et al. (2011), Mohamed et al. (2013)
c.47A>T	p.Asn16Ile	Missense	French Canada	1	1	Phaneuf et al. (1992)
c.67T>C	p.Ser23Pro	Missense	Asia (un)	1	2	Heath et al. (2002)
c.82-1G>A	–	Splicing	Spain	1	?	Perez-Carro et al. (2013)
c.103G>A	p. Ala35Thr	Missense	Belgium	1	2	Cassiman et al. (2009)
c.185T>G	p.Phe62Cys	Missense	Japan	1	2	Awata et al. (1994)
c.191delA	–	Deletion	Turkey	1	2	Dursun et al. (2011)
c.192G>T (IVS2-1G>T)	p.Gln64His	Splicing	Asia (un)	5	10	BCH
			India	2	4	Rootwelt et al. (1994a), Rootwelt et al. (1996)
			Middle East	3	6	Rootwelt et al. (1994a), Rootwelt et al. (1996)
			Pakistan	6	11	Rootwelt et al. (1994a), Rootwelt et al. (1996)
			Pakistan	9	8	BCH
c.192+1G>T (IVS2+1G>T)	–	Splicing	Portugal	1	1	Bergman et al. (1998)
c.233G>A	p.Trp78X	Nonsense	Spain	3	4	Arranz, et al. (2002)
c.234G>A	p.Trp78X	Nonsense	Spain	1	1	Couce et al. (2011)
c.315-3C>G (IVS3-3C>G)	–	Splicing	Turkey	4	8	Dursun et al. (2011)
c.374 C>G	p.Thr125Arg	Missense	Egypt	1	2	Imtiaz et al. (2011)
c.398A>G	p.His133Arg	Missense	Asia (un)	1	2	Heath et al. (2002)
c.398A>T	p.His133Leu	Missense	Spain	1	2	Couce et al. (2011)
c.401C>A	p.Ala134Asp	Missense	Norway	2	2	Labelle et al. (1993), Rootwelt et al. (1994c), Rootwelt et al. (1996)
			Turkey	1	1	Rootwelt et al. (1994c)
c.441_448del8	–	Deletion	Turkey	1	?	Dursun et al. (2011)
c.442-1G>A (IVS4+1G>A)	–	Splicing	Egypt/Saudi Arabia	4	8	Imtiaz et al. (2011)
c.455G>A	p.Trp152X	Nonsense	China	4	3	Yang et al. (2012), Dou et al. (2013)
c.467C>A	p.Pro156Gln	Missense	Asia (un)	1	1	Heath et al. (2002)
c.473G>A	p.Gly158Asp	Missense	Germany	1	1	Bergman et al. (1998)
c.497T>G	p.Val166Gly	Missense	North America (un)	2	2	Grompe and al-Dhalimy (1993)
			Germany	1	1	Rootwelt et al. (1996)

(continued)

Table 1 (continued)

HGVS mutation nomenclature		Effect of mutation	Origin	No. patients	Reported alleles	References
cDNA (Alias[a])	Protein					
			Iran	1	1	Grompe and al-Dhalimy (1993)
			Italy	1	2	Bergman et al. (1998)
			Turkey	2	4	Dursun et al. (2011)
c.509 G > T	p.Gly170Val	Missense	Saudi Arabia	2	4	Imtiaz et al. (2011)
c.520C>T	p.Arg174X	Nonsense	North America (un)	1	1	Timmers and Grompe (1996)
			Asia (un)	1	1	Heath et al. (2002)
			Turkey	1	2	Dursun et al. (2011)
c.548_553+20del26	–	Deletion	Brno-Czech Rep	1	1	Arranz et al. (2002)
c.536A>G	p.Gln179Arg	Missense	Korea	1	?	Choi et al. (2014)
c.553+5G>A (IVS6+5G>A)	–	Splicing	America	1	1	Timmers and Grompe (1996)
c.554-1G>C (IVS6-1G>C)	–	Splicing	Yugoslavia	1	1	Bergman et al. (1998)
c.554-1G>T (IVS6-1G>T)	–	Splicing	Africa	1	2	Bergman et al. (1998)
			Brazil	1	2	LGCD
			Czech Rep	8	14	Arranz et al. (2002), Vondrackova et al. (2010)
			Europe (un)	2	2	Poudrier et al. (1999), Kim et al. (2000)
			France	3	4	Bergman et al. (1998)
			Hungary	1	2	Laszlo et al. (2013)
			Italy	2	3	Bergman et al. (1998), Arranz et al. (2002)
			Morocco	6	11	Ploos van Amstel et al. (1996), Bergman et al. (1998), Arranz et al. (2002), la Marca et al. (2011)
			North America (un)	10	13	Timmers and Grompe (1996), Poudrier et al. (1999)
			UK	1	1	BCH
			Pakistan	1	2	BCH
			Spain	36	63	Rootwelt et al. (1996), Bergman et al. (1998), Arranz et al. (2002), Couce et al. (2011)
			Turkey	11	20	Bergman et al. (1998), Dursun et al. (2011)
			Yugoslavia	1	1	Bergman et al. (1998)
			UK	4	5	BCH
			Unknown	1	2	Rootwelt et al. (1996)

c.577T>C	p.Cys193Arg	Missense	Netherlands	1	1	Ploos van Amstel et al. (1996)
c.579C>A	p.Cys193X	Nonsense	Czech Rep	1	2	Vondrackova et al. (2010)
c.607-1G>A (IVS7-1G>A)	–	Splicing	Turkey	1	2	Ploos van Amstel et al. (1996)
c.607-6T>G (IVS7-6 T>G)	–	Splicing	USA	?	?	Sniderman King et al. (2011)
c.615delTs	p.Phe205leufsX2f	Frameshift	Norway	1	1	Bliksrud, et al. (2012)
c.620G>A	p.Gly207Asp	Missense	North America (un)	1	1	Timmers and Grompe (1996)
c.648C>G (IVS8-59C>G)	p.Ile216Met	Splicing	India	3	4	Sheth et al. (2012)
c.680G>C	p.Gly227>Ala	Missense	Egypt	1	2	Imtiaz et al. (2011)
c.680G>T	p.Gly227>Val	Missense	Czech Rep	2	4	Vondrackova et al. (2010)
c.696C>A	p.Asn232Lys	Missense	Turkey	1	2	Dursun et al. (2011)
c.696C>T (IVS8-11C>T)	p.Asn232Asn	Splicing	Netherlands	1	1	Ploos van Amstel et al. (1996)
c.698A>T	p.Asp233Val	Missense	Turkey	8	15	Rootwelt et al. (1994a), Rootwelt et al. (1996), Dursun et al. (2011)
c.700T>G	p.Trp234Gly	Missense	USA	1	1	Hahn et al. (1995), Rootwelt et al. (1996)
c.707-1G>A (IVS8-1G>A)	–	Splicing	Egypt	1	2	Imtiaz et al. (2011)
			Saudi Arabia	3	6	Couce et al. (2011), Imtiaz et al. (2011)
			Spain	3	5	Arranz et al. (2002), Couce et al. (2011)
c.707-1G>C (IVS8-1G>C)	–	Splicing	Israel	8	16	Bergman et al. (1998), Elpeleg et al. (2002)
c.709C>T	p.Arg237X	Nonsense	Asia (un)	1	2	Heath et al. (2002)
			China	2	2	Cao et al. (2012)
			Morocco	1	2	la Marca et al. (2011)
			Pakistan	1	2	BCH
			Saudi Arabia	10	20	Imtiaz et al. (2011)
			Thailand	1	2	Jitraruch et al. (2011)
			Turkey	4	8	Ploos van Amstel et al. (1996), Dursun et al. (2011)
c.718 C>T	p.Gln240>X	Nonsense	Iran	1	2	Imtiaz et al. (2011)
c.726G>A	p.Trp242X	Nonsense	UK	1	1	BCH
c.744delG	p.Pro249HisfsX55	Frameshift	Norway	5	7	Bliksrud et al. (2012)
c.745C>A	p.Pro249Thr	Missense	North America (un)	1	1	Timmers and Grompe (1996)
c.775G>C	p.Val259Leu	Splicing	USA	1	1	LGCD
c.776T>A	p.Val259Asp	Missense	Turkey	1	2	Dursun et al. (2011)
c.782C>T	p.Pro261Leu	Missense	Israel	3	6	Bergman et al. (1998), Elpeleg et al. (2002)
			Israel	1	2	BCH
			Saudi Arabia	1	2	BCH

(continued)

Table 1 (continued)

HGVS mutation nomenclature		Effect of mutation	Origin	No. patients	Reported alleles	References
cDNA (Alias[a])	Protein					
			Egypt/Saudi Arabia	3	6	Imtiaz et al. (2011)
c.786G>A	p.Trp262X	Nonsense	Denmark	1	1	Rootwelt et al. (1996)
			Europe (un)	1	1	BCH
			Finland	22	40	Rootwelt et al. (1994a), St-Louis et al. (1994), Rootwelt et al. (1996), Mustonen et al. (1997)
			French Canada	1	1	LGCD
			Norway	1	1	Rootwelt et al. (1994a)
			Poland	1	1	Rootwelt et al. (1996)
			UK	2	2	BCH
			USA	3	6	LGCD
c.787G>A	p.Val263>Me	Missense	Saudi Arabia	2	4	Imtiaz et al. (2011)
c.835delC	pGln279ArgfsX25	Frameshift	Norway	2	2	Bliksrud et al. (2012)
c.836A>G (IVS9-2A>G)	p.Gln279Arg	Splicing	Spain	1	?	Perez-Carro et al. (2013)
			USA	1	1	Kim et al. (2000), Dreumont et al. (2001)
c.837+2T>C (IVS9+2T>C)	–	Splicing	Turkey	2	4	Dursun et al. (2011)
c.838-2A>G (IVS9-2A>G)	–	Splicing	Caucasus	1	1	Heath et al. (2002)
			UK	1	1	BCH
c.843 C > A	p.281Thr > Pro	Missense	Saudi Arabia	1	2	Imtiaz et al. (2011)
c.880A>C	p.Thr294Pro	Missense	North America (un)	1	1	Timmers and Grompe (1996)
			France	1	1	Bergman et al. (1998)
c.913G>C	p.Gly305Arg	Splicing	Spain	1	?	Perez-Carro et al. (2013)
c.913+5G>A	–	Splicing	Korea	1	?	Choi et al. (2014)
c.914-2A>T (IVS10-2A>T)	–	Splicing	Spain	1	1	Arranz et al. (2002)
c.938delC	p.Thr313ThrfsX60	Frameshift	Spain	1	1	Arranz et al. (2002)
c.960q1130_*1260q10539del18036	–	Deletion	Korea	3	4	Park et al. (2009)
c.961-1010del50	–	Deletion	Iran	1	2	Haghighi-Kakhki et al. (2014)
			North Europe (un)	9	8/?	Rootwelt et al. (1994d), Prieto-Alamo and Laval (1998)

c.974C>T	p.Thr325Met	Missense	Caucasus	1	1	Heath et al. (2002)
			Spain	1	1	Couce et al. (2011)
			UK	1	1	BCH
c.974_976delC AinsGC	–	Deletion	China	3	6	Yang et al. (2012)
c.982C>T	p.Gln328X	Nonsense	Spain	2	2	Arranz et al. (2002)
c.1001 C>T	p.Ser334>Phe	Missense	Saudi Arabia	1	2	Imtiaz et al. (2011)
c.1009G>A	p.Gly337Ser	Missense	Austria	1	2	Bergman et al. (1998)
			France	1	1	Rootwelt et al. (1994b)
			Iran	1	2	Haghighi-Kakhki et al. (2014)
			Norway	6	7	St-Louis et al. (1995), Bliksrud et al. (2005), Bliksrud et al. (2012)
			North Europe (un)	18	15/?	Rootwelt et al. (1994d), Rootwelt et al. (1996), Prieto-Alamo and Laval (1998)
			Portugal	1	1	Bergman et al. (1998)
			Spain	1	2	Bergman et al. (1998)
c.1022 G>C	p.Arg341Pro	Missense	Egypt	1	2	Imtiaz et al. (2011)
c.1025C>T	p.Pro342Leu	Missense	Greece	1	2	Bergman et al. (1998)
			Norway	1	1	Rootwelt et al. (1994c)
			USA	1	1	Rootwelt et al. (1996)
c.1027G>T	p.Gly343Trp	Missense	Spain	2	2	Arranz et al. (2002)
c.1027G>C	p.Gly343Arg	Missense	China	2	3	Dou et al. (2013)
			Egypt	3	6	Imtiaz et al. (2011)
c.[1035_1037del]		Deletion	China	2	3	Mak et al. (2013)
c.1043C>G	p.Ser348Gly	Missense	North Europe (un)	5	?	Prieto-Alamo and Laval (1998)
c.1056C>A	p.Ser352Arg	Missense	Asia	1	2	Heath et al. (2002)
c.1061C>A	p.Pro354Gln	Missense	Norway	1	1	Bliksrud et al. (2005)
c.1062+5G>A (IVS12+5G>A)	–	Splicing	Asia (un)	3	5	Heath et al. (2002)
			Canada	3	5	Grompe et al. (1994)
			Czech Rep	1	2	Vondrackova et al. (2010)
			Denmark	1	1	Rootwelt et al. (1994d)
			Europe (un)	23	28	Rootwelt et al. (1996)
			Finland	1	1	Grompe et al. (1994)
			French Canada	98	181	Grompe and al-Dhalimy (1993), Grompe et al. (1994), Poudrier et al. (1996), LGCD
			France	1	2	Grompe et al. (1994)
			Germany	2	3	Ploos van Amstel et al. (1996), Bergman et al. (1998)

(continued)

Table 1 (continued)

HGVS mutation nomenclature		Effect of mutation	Origin	No. patients	Reported alleles	References
cDNA (Alias[a])	Protein					
			India	2	4	BCH
			Iran	2	3	Grompe and al-Dhalimy (1993), Imtiaz et al. (2011)
			Israel	1	2	Bergman et al. (1998)
			Mexico	1	1	Grompe et al. (1994)
			Netherlands	7	10	Ploos van Amstel et al. (1996), Bergman et al. (1998)
			Norway	3	3	Grompe et al. (1994), Rootwelt et al. (1994d)
			Pakistan	2	4	Rootwelt et al. (1996), BCH
			Portugal	4	6	Bergman et al. (1998)
			Spain	6	8	Arranz et al. (2002), Couce et al. (2011), Perez-Carro et al. (2013)
			Turkey	4	10	Rootwelt et al. (1996), Bergman et al. (1998), Dursun et al. (2011)
			UK	4	8	BCH
			UK	2	2	Grompe and al-Dhalimy (1993), Rootwelt et al. (1994d)
			USA	11	13	Grompe et al. (1994), Hahn et al. (1995), Timmers and Grompe (1996), LGCD
c.1063-1G>A (IVS12 -1G>A)	–	Splicing	China	2	3	Mak et al. (2013)
c.1069G>T	p.Glu357X	Nonsense	Caucasus	1	1	Heath et al. (2002)
			French Canada	5	6	Grompe and al-Dhalimy (1993), St-Louis et al. (1995)
			Netherlands	1	1	Ploos van Amstel et al. (1996)
			Norway	1	1	Rootwelt et al. (1996)
			Poland	1	1	Rootwelt et al. (1996)
			UK	1	1	Rootwelt et al. (1994d), Rootwelt et al. (1996)
			UK	1	1	BCH
c.1090G>T	p.Glu364X	Nonsense	French Canada	7	7	Grompe and al-Dhalimy (1993), Timmers and Grompe (1996), Poudrier et al. (1999)
			UK	2	2	Rootwelt et al. (1994d), Rootwelt et al. (1996)
			USA	4	4	Timmers and Grompe (1996)
			Netherlands	2	2	Ploos van Amstel et al. (1996), Bergman et al. (1998)
			Belgium	1	1	Grompe et al. (1994)
c.1097_1099delCGT	p.Ser366del	In-frame deletion	Italy	1	1	Bergman et al. (1998)
			Netherlands	1	1	Bergman et al. (1998)
c.1100 G>A	pTrp367X	Nonsense	China	3	2	Yang et al. (2012)
c.1106G>T	p.Gly369Val	Missense	Morocco	1	1	Ploos van Amstel et al. (1996)

c.1107delG	p.Asn344Tfsx	Deletion	Turkey	1	1	Dursun et al. (2011)
c.1124T>C	p.Leu375Pro	Missense	China	2	2	Cao et al. (2012)
c.1141A>G	p.Arg381Gly	Missense	French Canada	5	5	St-Louis et al. (1995)
			Portugal	1	1	St-Louis et al. (1995)
c.1156G>C	p.Asp386His	Missense	Emirates	1	2	Al-Shamsi et al. (2014)
c.1159G>A	p.Gly387Arg	Missense	India	3	4	Sheth et al. (2012)
c.1190delA	–	Deletion	Egypt	2	4	Imtiaz et al. (2011)
c.1195G>C	p.Asn400His	Missense	Saudi Arabia	1	2	Imtiaz et al. (2011)
c.1210G>A	p.Gly404Ser	Missense	Czech Rep	1	2	Vondrackova et al. (2010)
c.1213_1214delTTinsCA	p.Phe405His	Missense	Portugal	1	1	Bergman et al. (1998)

[a] Variant designation that does not conform to current naming conventions

BCH Birmingham Children's Hospital (unpublished data), *LGCD* Laboratory of Cell and Developmental Genetics (unpublished data), *Un* Undetermined region

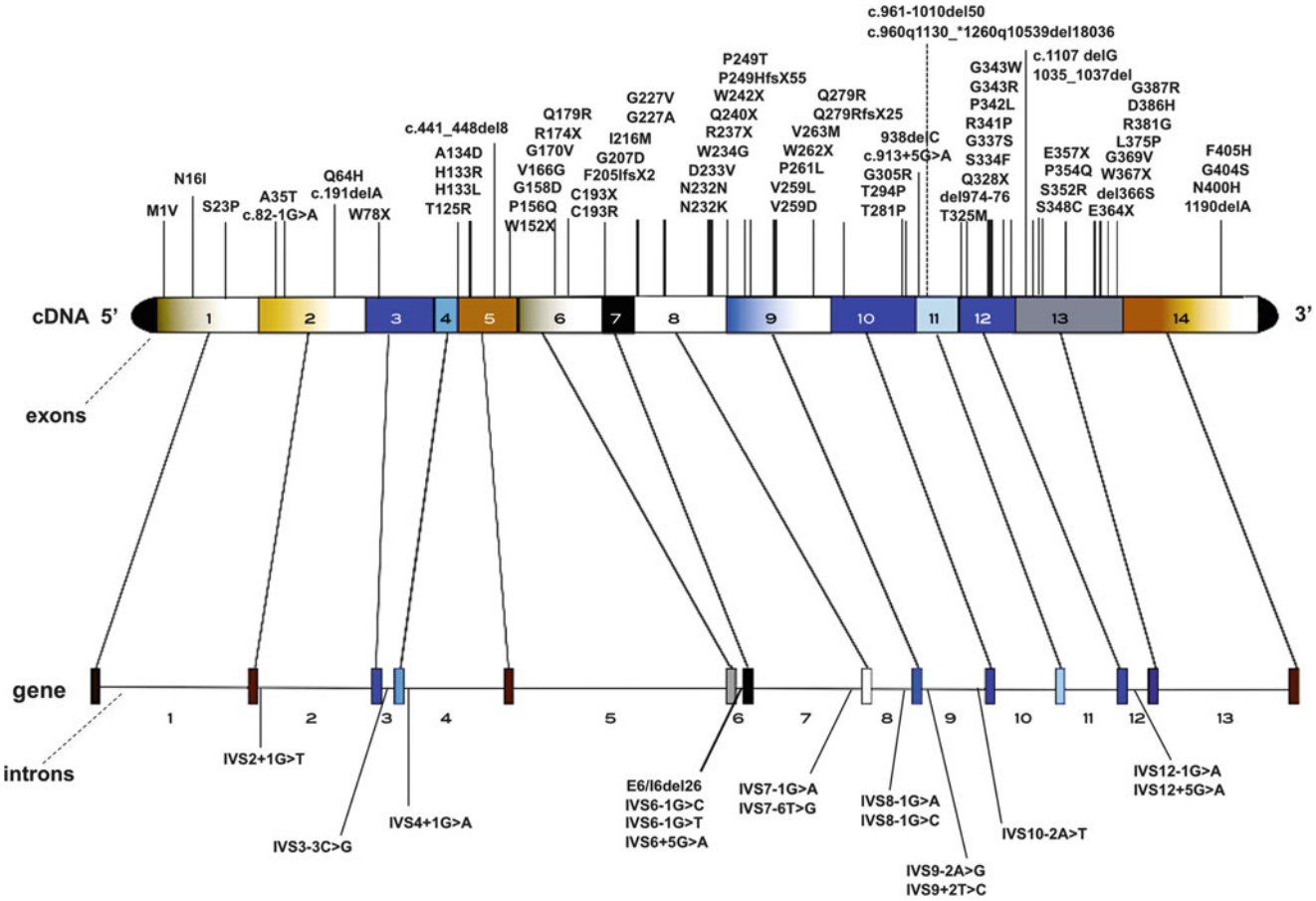

Fig. 1 Location of the 95 mutations identified on the *fah* gene. Among the known HT1 alleles causing mutations, 45 are missense mutations, 23 are splicing mutations, 13 are nonsense mutations, 10 are deletions and 4 are frameshift. Intronic mutations are illustrated at the *bottom* of the figure

two new mutations were uncovered at LGCD, Quebec, and in BCH, Birmingham (unpublished data). The first was the c.726G>A (p.Trp242X) nonsense mutation, obtained by screening one English adult patient at BCH. The second, the c.775G>C (p.Val259Leu) a potential missense mutation, was observed in an American patient at the Quebec laboratory. This patient was heterozygous for the new c.775G>C (p.Val259Leu) allele and the already reported c.554-1G>T (IVS6-1G>T) (Grompe et al. 1994). Western blot analysis of his liver obtained after transplantation revealed the absence of FAH protein and no activity was detected by enzymatic assay (data no shown). RNA analysis suggested a defect in splicing affecting exon 9, and this was confirmed using minigene constructs transfected in HeLa cells (Dreumont and Tanguay, unpublished).

Reclassification of HT1 Mutations

After cross-checking of genetic databases and the literature on HT1 from the oldest publications to the present day, we updated the number of allelic variants with the two found by our group and others recently reported (Fig. 1 and Table 1). Next we decided to reclassify them in a unique list containing number of known alleles from patients and geographical distribution of the mutations most predominant for each country (Fig. 2, and Table 1). Indeed, frequency of reported alleles and origin of patients could be useful in helping clinicians to focus on mutations specific of certain regions, facilitating the targeted detection of diseased alleles.

Overall 95 mutations are now reported within the *fah* gene in this review (Fig. 1 and Table 1). All 95 HT1 alleles are divided in 45 missense mutations, 23 splice defects, 13 nonsense mutations, 10 deletions and 4 frameshift (Table 1). In addition the missense c.1021C>T (p.Arg341Trp) sequence variant is described as a pseudodeficiency variant since individuals homozygous for this mutation are healthy (Rootwelt et al. 1994b; Bergeron et al. 2001).

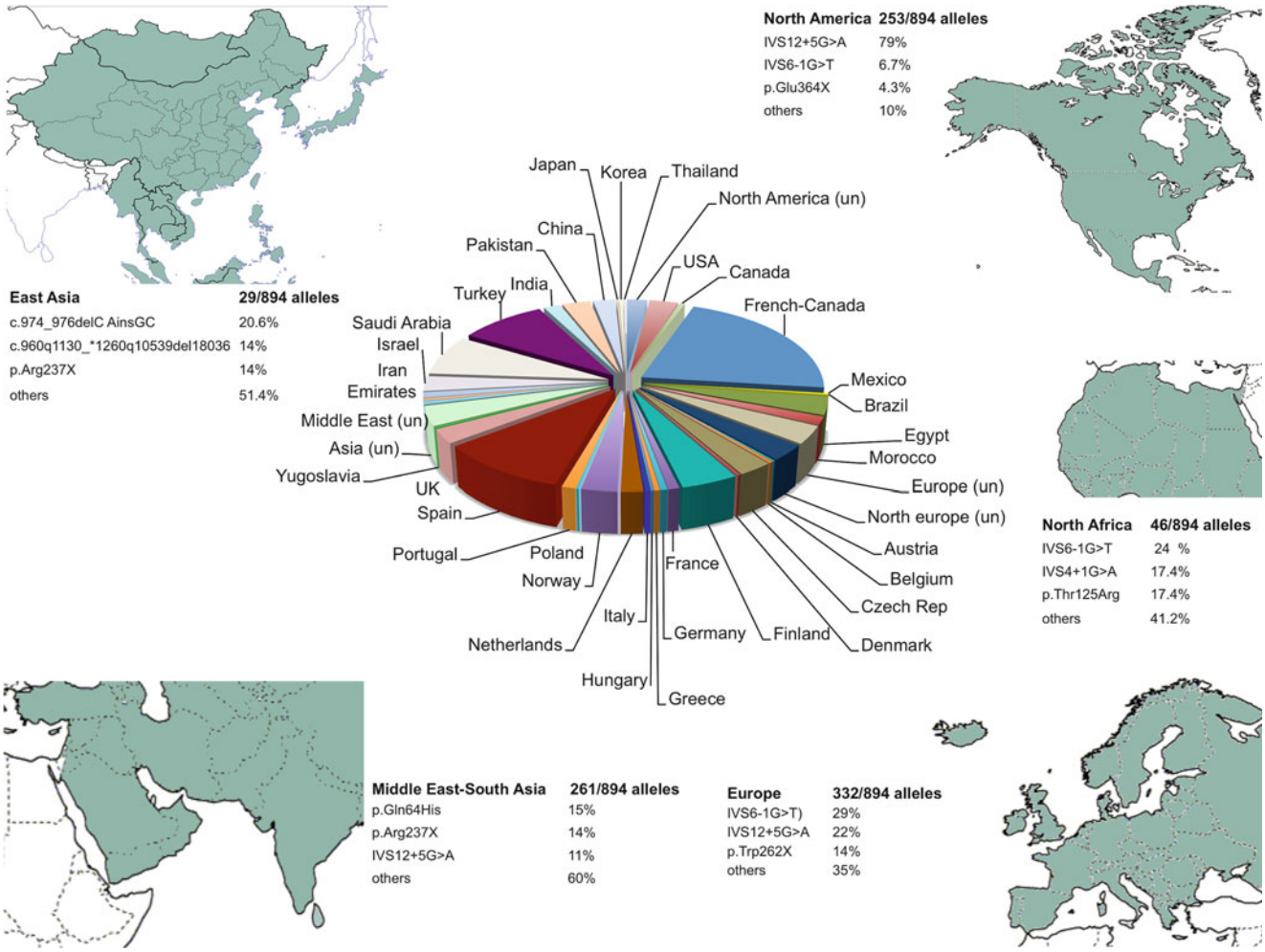

Fig. 2 Geographical distribution of the most common HT1 alleles causing mutations worldwide. *Pie chart* representing distribution of ethnic groups in HT1 alleles. Where the patient provenance was not clear, the mutation is included in the continent of origin, i.e. undefined (un) on graphic. The top three mutations and the total number of alleles for each continent are reported. There are more than 894 HT1 alleles reported worldwide. The most frequent HT1 mutation encountered is the IVS12+5G>A splice mutation, which accounts for 33.7% of all HT1 alleles, followed by the IVS6-1G>T mutation (16.4%). The French Canadian population alone accounts for as much as a third of all HT1 alleles reported. Both mutations are the most reported globally

Predominance of Ethnic Groups in HT1 Distribution

Despite the fact that the worldwide incidence of HT1 is relatively low with one affected individual in approximately 100,000 healthy individuals (Hutchesson et al. 1996), specific populations stand out as they represent small clusters of diseased alleles (Fig. 2). The population that possesses the highest incidence of HT1 is the French Canadian population of the SLSJ region, in the province of Quebec (Canada) (De Braekeleer and Larochelle 1990; Poudrier et al. 1996). The prevalence of HT1 in the SLSJ region was as high as 1/1,042 births in 1971 but dropped to 1/1,846 births in 1986, most likely due to the implementation of a screening programme for HT1 in 1970 conducted by the Quebec Network of Genetic Medicine. The most predominant mutation in this region is the c.1062 + 5G>A (IVS12 + 5G>A) accounting ~90% of all the disease-causing alleles. Furthermore, even though the Quebec population accounts for only approximately 0.12% of the world population (estimated today to number of 7 billion), it represents ~33% of all HT1 alleles worldwide. Although these data may be biased by the fact that all newborns in Quebec are screened for HT1, it is clear that this region represents the highest incidence of HT1 and that the c.1062 + 5G>A mutation is predominant in this region.

A second cluster of HT1 is found in Scandinavia (Kvittingen et al. 1981). In the Finnish population of Pohjanmaa, 1 individual out of 5,000 is affected with HT1

(St-Louis et al. 1994), whereas the overall incidence of HT1 in Finland is 1:60,000 (Mustonen et al. 1997). In this region, one single mutation (c.786G > A, p.Trp262X) represents ~88% of all reported HT1 alleles (St-Louis et al. 1994). Indeed 40 of the 46 European c.786G>A alleles have been reported in this country.

New findings show a peculiar pattern of HT1 mutations also in Norway. In a recent report, 19 Norwegian HT1 patients were investigated in the Hospital of Oslo University and three new small deletions were found: c.615delT, (p.Phe205LeufsX2), c.744delG (p.Pro249HisfsX55) and c.835delC (p.Gln279ArgfsX25). The novel mutations lead to frameshift and premature termination codons. FAH protein structure is affected, and normal folding, function and stability of the protein cannot be expected (Bliksrud et al. 2012). The c.615delT, c.744delG and the c.835delC are found in 13.5%, 3.8% and 1.9% of the alleles, respectively. Around 65% of the Norwegian HT1 patients are heterozygous for different mutations. The relatively high incidence of HT1 in Norway (1 in 74,800 live births) has not been connected with a single founder effects or high incidence of parental consanguinity as in the previous areas (Bliksrud et al. 2012).

Another cluster occurs in an immigrant population from Pakistan living in the UK, predominantly in Birmingham (Hutchesson et al. 1998). Birmingham is a city in the West Midlands Region, which has a total population of approximately 5.3 million of which nearly 3% are of Pakistani origin. We have diagnosed 44 patients from the West Midlands with this disorder of which 30 (68%) were of Pakistani origin. This is over 22-fold higher than the frequency of people of Pakistani origin in this region. Mutation analysis revealed that five out of 12 index patients (42%) in this ethno-geographic group had the c.192G>T (p.Gln64His) mutation. This mutation was not detected in patients from any other close-by region suggesting a founder effect from the region of origin of this population. Indeed the frequency of this mutation in Pakistani from the UK was comparable to that of the common pan-ethnic c.1062 + 5G>A mutation.

Most Frequent HT1 Alleles Around the World

Although Quebec, Finland, Norway and Pakistani in the UK stand out as populations with the higher frequency of HT1, reports highlight a specific tendency in mutational distribution among ethnic groups. The c.1062 + 5G>A (IVS12 + 5G>A) mutation is found frequently in patients from a wide range of ethnic groups over a large geographical distribution. Given the high frequency and wide spread of this mutation, it is likely to be a very old mutation and it was originally reported in a French Canadian patient and in two patients of Iranian origin (Grompe et al. 1994). Although this is the most frequent HT1 mutation encountered worldwide (302/894 HT1 alleles), the c.554-1G>T (IVS6-1G>T) splice mutation is also frequently observed (147/894 HT1 alleles), showing a high prevalence in the Mediterranean region and in southern Europe. In a recent cross-sectional retrospective study on 168 HT1 patients originating from Europe, Turkey and Israel, mutational analysis performed in 58/168 patients revealed the predominance of the IVS12 + 5G>A (11 patients) and IVS6-1G>T (13 patients) mutations in these ethnic groups (Mayorandan et al. 2014).

The mutation that ranks third in prevalence in Europe is the c.786G>A (p.Trp262X) nonsense mutation. This ranking is due to its predominance in the Finnish population. A number of others mutations have also been associated with specific ethnic or geographic groups, as described below (Table 1, Fig. 2). The c.1062 + 5G>A (IVS12 + 5G>A), the c.607-6T>G (IVS7-6T/G) and the c.554-1G>T (IVS6-1G>T) splicing mutations and the c.786G>A (p.Trp262X) nonsense mutation all together represent 60% of mutant alleles in the general US population (Sniderman King et al. 2011). Surprisingly, only one HT1 allele was reported until now in Mexico and this allele carried the c.1062 + 5G>A mutation most prevalent in Quebec (Table 1). 16 new cases have recently been described in Brazil (Neto et al. 2014), with only two alleles reported at this time, and these harboured the c.554-1G>T (IVS6-1G>T) mutation, most prevalent in the Mediterranean area (Table 1).

Arranz et al. in their work based on a panel of 29 patients mostly from southern Europe demonstrated a high homogeneity of the mutational spectrum in this region (Arranz et al. 2002). In a retrospective study on European HT1-affected individuals (Couce et al. 2011), mutational analysis on 34 Spanish patients reported nine different mutations in this population, documenting c.554-1G>T (IVS6-1G>T) as the most prevalent, in accordance with the previous literature (Arranz et al. 2002). Molecular genetics analysis of the *fah* gene in 11 Czech patients with HT1, diagnosed in the Medical Faculty of Charles University in Prague between 1982 and 2006, revealed three mutations not previously described: the c.579C>A nonsense mutation (p.Cys193X) and the c.680G>T (p.Gly227Val) and c.1210G>A (p.Gly404Ser) missense mutations (Vondrackova et al. 2010).

The Middle East is interesting in the sense that even though patients harbour the common c.1062 + 5G>A (IVS12 + 5G>A) and c.554-1G > T (IVS6-1G>T) mutations, many of the other mutations reported are typical to this region. One such example is the already described c.192G>T (p.Gln64His) mutation, which is thus far found only in people originating from Pakistan, the Middle East and North West India. This mutation accounts for over one

third of all HT1 alleles in these populations (Rootwelt et al. 1994a; Rootwelt et al. 1996). Another mutation that is often detected in patients from the Middle East is the c.709C>T (p.Arg237X) mutation (Imtiaz et al. 2011). In Turkey, the c.698A>T (p.Asp233Val) mutation, which has not been reported elsewhere, accounts for 20% of the reported alleles (Rootwelt et al. 1994a; Rootwelt et al. 1996; Dursun et al. 2011). Moreover, other different mutations, although not at high frequency, are peculiar for this population (Table 1).

The c.782C>T (p.Pro261Leu) missense mutation was found in 100% of Ashkenazi-Jewish examined in Israel (Elpeleg et al. 2002). Direct sequencing in 43 HT1-affected patients originating from Saudi Arabia, Egypt and Iran identified a total of 17 different homozygous mutations. Eleven of these (8 missense, 1 nonsense, 1 splice site and 1 deletion) had not been reported previously (Imtiaz et al. 2011).

Little information about the epidemiology and molecular defects in HT1 patients from East Asia is available at this time. Sakai and Kitagawa (1957) reported the first case of HT1 in a two-month-old Japanese patient, but genetic analysis was not possible at that time. The c.185T>G (p.Phe62Cys) represents the first and the only allele reported in Japan to date (Awata et al. 1994). Recent findings start to describe HT1 mutations in China. The missense mutation c.1124T>C (p.Leu375Pro) represents the first case of HT1 analysed by molecular genetics in this area (Cao et al. 2012). This mutation, affecting the secondary protein structure, decreases the stability of FAH enzyme and compromises the protein's functions. Another report represents the first case of HT1 in a two-month-old Hong Kong Chinese patient (Mak et al. 2013). Genetic analysis of this patient showed two novel mutations, the c.1063-1G>A splicing mutation and the c.1035_1037del. Recently, clinical data on 3 HT1 Chinese patients showed five mutations in the FAH gene: c.455G>A (p.Trp152X), c.520C>T (p.Arg174X), c.974_976delCGAinsGC, c.1027G>A (p.Gly343Arg) and c.1100G>A (p.Trp367X) (Yang et al. 2012; Dou et al. 2013). The c.455G>A, c.974_976delCGAinsGC and c.1100G>A mutations have not been described elsewhere. Currently, few cases of HT1 have been reported in Korea. Mutational analysis of two female neonates admitted to hospital for further work-up of an abnormal newborn screening test revealed three novel mutations (one deletion, one missense and one splice defect) that have not been reported elsewhere (Park et al. 2009; Choi et al. 2014).

To our knowledge no mutations in HT1 have yet been documented in Central America or in the Oceania continent.

Conclusions

The advent of neonatal screening, prenatal diagnosis and carrier tests for genetic disorders has shown the importance of establishing the population frequencies and ethno-geographic spread of mutations for the evaluation of future screening strategies. To highlight the prevalence of HT1 mutations in a geographical context, we compiled all reported HT1 alleles worldwide, including those not yet reported in the common databases, and another two, discovered in the screening of HT1 patients in our laboratories over a period between 2001 and 2013 (summarized in Table 1 and Fig. 2). Obvious conclusions can be drawn when we examine the incidence of HT1 worldwide (Fig. 2 and Table 1).

According to the data gathered so far, a preferential screening for those mutations in regions in which they show a higher prevalence could provide some improvement in carrier diagnostic efficiency and may enable the establishment of family pedigrees for adequate counselling in some cases. Currently, screening is carried out in Quebec, the USA and Europe (Morrissey et al. 2011; Barnby 2014). In this case it is obviously important to know the pattern of mutations in the respective populations.

However, it is necessary to bear in mind that this compilation may be partly biased by the fact that: (1) very few cases of HT1 are overlooked in some countries as in the province of Quebec due to a screening programme for HT1 established early in 1970 and (2) not all cases of HT1 are described in the literature. Many of these probably occur in countries with no or limited access to service for diagnosis of genetic disease and as a result remain undiagnosed (De Laet et al. 2013). This could lead to some geographical bias reflected in the fact that the majority of the patients whose mutations have been described are residents of Europe, the Middle East or North America. (3) Whilst we have carefully attempted to ensure that patients are not counted twice because they appear in more than one publication, this may occur in a few cases.

In summary, this report allows a detailed identification of the mutations causing HT1 worldwide, with diagnostic and methodological consequences implementing the ground-work for future carrier and prenatal testing, premarital screening and pre-implantation genetic diagnosis.

Acknowledgements Work on HT1 was supported by the Canadian Institutes for Health Research (grant to RMT, studentship to AB and postdoctoral fellowship to FA).

Synopsis

Geographical and ethnic distribution of mutations in hereditary tyrosinemia type 1

Compliance with Ethics Guidelines

Conflict of Interest

Francesca Angileri, Anne Bergeron, Geneviève Morrow, Francine Lettre, George Gray, Tim Hutchin, Sarah Ball and Robert M. Tanguay declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration as revised in 2013.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

Author's Contributions

AB and FA contributed equally to this review. AB, FA and SB did the literature review and contributed to the draft of the manuscript. FL, TH and SB performed mutational analysis in some patients. RMT, GM and GG designed the review and worked on the draft of the manuscript. All authors read and approved the final manuscript.

References

Al-Shamsi A, Hertecant JL, Al-Hamad S, Souid AK, Al-Jasmi F (2014) Mutation spectrum and birth prevalence of inborn errors of metabolism among Emiratis: a study from Tawam Hospital Metabolic Center, United Arab Emirates. Sultan Qaboos Univ Med J 14(1):e42–e49

Arranz JA, Pinol F, Kozak L et al (2002) Splicing mutations, mainly IVS6-1(G>T), account for 70% of fumarylacetoacetate hydrolase (FAH) gene alterations, including 7 novel mutations, in a survey of 29 tyrosinemia type I patients. Hum Mutat 20(3):180–188

Awata H, Endo F, Tanoue A, Kitano A, Nakano Y, Matsuda I (1994) Structural organization and analysis of the human fumarylacetoacetate hydrolase gene in tyrosinemia type I. Biochim Biophys Acta 1226(2):168–172

Barnby E (2014) Tyrosinemia type 1: an overview of nursing care. Pediatr Nursing 40(2):61–68

Bartlett DC, Lloyd C, McKiernan PJ, Newsome PN (2014) Early nitisinone treatment reduces the need for liver transplantation in children with tyrosinaemia type 1 and improves post-transplant renal function. J Inherit Metab Dis 37(5):745–752.

Berger R, Smit GP, Stoker-de Vries SA, Duran M, Ketting D, Wadman SK (1981) Deficiency of fumarylacetoacetase in a patient with hereditary tyrosinemia. Clin Chim Acta 114(1):37–44

Bergeron A, D'Astous M, Timm DE, Tanguay RM (2001) Structural and functional analysis of missense mutations in fumarylacetoacetate hydrolase, the gene deficient in hereditary tyrosinemia type 1. J Biol Chem 276(18):15225–15231

Bergman AJ, van den Berg IE, Brink W, Poll-The BT, Ploos van Amstel JK, Berger R (1998) Spectrum of mutations in the fumarylacetoacetate hydrolase gene of tyrosinemia type 1 patients in northwestern Europe and Mediterranean countries. Hum Mutat 12(1):19–26

Bliksrud YT, Brodtkorb E, Andresen PA, van den Berg IE, Kvittingen EA (2005) Tyrosinaemia type I–de novo mutation in liver tissue suppressing an inborn splicing defect. J Mol Med (Berl) 83 (5):406–410

Bliksrud YT, Brodtkorb E, Backe PH, Woldseth B, Rootwelt H (2012) Hereditary tyrosinaemia type I in Norway: incidence and three novel small deletions in the fumarylacetoacetase gene. Scand J Clin Lab Invest 72(5):369–373

Cao YY, Zhang YL, Du J et al (2012) Compound mutations (R237X and L375P) in the fumarylacetoacetate hydrolase gene causing tyrosinemia type I in a Chinese patient. Chin Med J (Engl) 125 (12):2132–2136

Cassiman D, Zeevaert R, Holme E, Kvittingen EA, Jaeken J (2009) A novel mutation causing mild, atypical fumarylacetoacetase deficiency (Tyrosinemia type I): a case report. Orphanet J Rare Dis 4:28

Choi HJ, Bang HI, Ki CS et al (2014) Two novel FAH gene mutations in a patient with hereditary tyrosinemia type I. Ann Clin Lab Sci 44(3):317–323

Couce ML, Dalmau J, del Toro M, Pintos-Morell G, Aldamiz-Echevarria L (2011) Tyrosinemia type 1 in Spain: mutational analysis, treatment and long-term outcome. Pediatr Int 53(6):985–989

De Braekeleer M, Larochelle J (1990) Genetic epidemiology of hereditary tyrosinemia in Quebec and in Saguenay-Lac-St-Jean. Am J Hum Genet 47(2):302–307

de Laet C, Dionisi-Vici C, Leonard JV et al (2013) Recommendations for the management of tyrosinaemia type 1. Orphanet J Rare Dis 8:8

Dehghani SM, Haghighat M, Imanieh MH, Karamnejad H, Malekpour A (2013) Clinical and para clinical findings in the children with tyrosinemia referring for liver transplantation. Int J Prev Med 4 (12):1380–1385

den Dunnen JT, Antonarakis SE (2000) Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat 15(1):7–12

Dou LM, Fang LJ, Wang XH et al (2013) Mutation analysis of FAH gene in patients with tyrosinemia type 1. Zhonghua Er Ke Za Zhi 51(4):302–307

Dreumont N, Poudrier JA, Bergeron A, Levy HL, Baklouti F, Tanguay RM (2001) A missense mutation (Q279R) in the fumarylacetoacetate hydrolase gene, responsible for hereditary tyrosinemia, acts as a splicing mutation. BMC Genet 2:9

Dursun A, Ozgul RK, Sivri S et al (2011) Mutation spectrum of fumarylacetoacetase gene and clinical aspects of tyrosinemia type I disease. JIMD Rep 1:17–21

Elpeleg ON, Shaag A, Holme E et al (2002) Mutation analysis of the FAH gene in Israeli patients with tyrosinemia type I. Hum Mutat 19(1):80–81

Fällström S-P, Lindblad B, Lindstedt S, Steen G (1979) Hereditary tyrosinemia-fumarylacetoacetase deficiency. Pediatr Res 13:78

Georgouli H, Schulpis KH, Michelakaki H, Kaltsa M, Sdogou T, Kossiva L (2010) Persistent coagulopathy during Escherichia coli sepsis in a previously healthy infant revealed undiagnosed tyrosinaemia type 1. BMJ Case Rep 2010. pii: bcr0720103150. doi:10.1136/bcr.07.2010.3150

Grompe M, al-Dhalimy M (1993) Mutations of the fumarylacetoacetate hydrolase gene in four patients with tyrosinemia, type I. Hum Mutat 2(2):85–93

Grompe M, St-Louis M, Demers SI, al-Dhalimy M, Leclerc B, Tanguay RM (1994) A single mutation of the fumarylacetoacetate hydrolase gene in French Canadians with hereditary tyrosinemia type I. N Engl J Med 331(6):353–357

Haghighi-Kakhki H, Rezazadeh J, Ahmadi-Shadmehri A (2014) Identification of a combined missense/splice-site mutation in FAH causing tyrosinemia type 1. J Pediatr Endocrinol Metab. doi:10.1515/jpem-2013-0489

Hahn SH, Krasnewich D, Brantly M, Kvittingen EA, Gahl WA (1995) Heterozygosity for an exon 12 splicing mutation and a W234G missense mutation in an American child with chronic tyrosinemia type 1. Hum Mutat 6(1):66–73

Heath SK, Gray RG, McKiernan P, Au KM, Walker E, Green A (2002) Mutation screening for tyrosinaemia type I. J Inherit Metab Dis 25(6):523–524

Hutchesson AC, Hall SK, Preece MA, Green A (1996) Screening for tyrosinaemia type I. Arch Dis Child Fetal Neonatal Ed 74(3): F191–F194

Hutchesson AC, Bundey S, Preece MA, Hall SK, Green A (1998) A comparison of disease and gene frequencies of inborn errors of metabolism among different ethnic groups in the West Midlands, UK. J Med Genet 35(5):366–370

Imtiaz F, Rashed MS, Al-Mubarak B et al (2011) Identification of mutations causing hereditary tyrosinemia type I in patients of Middle Eastern origin. Mol Genet Metab 104(4):688–690

Jitraruch S, Treepongkaruna S, Teeraratkul S et al (2011) Long-term outcome of living donor liver transplantation in a Thai boy with hereditary tyrosinemia type I: a case report. J Med Assoc Thai 94 (10):1276–1280

Kim SZ, Kupke KG, Ierardi-Curto L et al (2000) Hepatocellular carcinoma despite long-term survival in chronic tyrosinaemia I. J Inherit Metab Dis 23(8):791–804

Kvittingen EA, Jellum E, Stokke O (1981) Assay of fumarylacetoacetate fumarylhydrolase in human liver-deficient activity in a case of hereditary tyrosinemia. Clin Chim Acta 115(3):311–319.

la Marca G, Malvagia S, Pasquini E et al (2011) Newborn screening for tyrosinemia type I: further evidence that succinylacetone determination on blood spot is essential. JIMD Rep 1:107–109

Labelle Y, Phaneuf D, Leclerc B, Tanguay RM (1993) Characterization of the human fumarylacetoacetate hydrolase gene and identification of a missense mutation abolishing enzymatic activity. Hum Mol Genet 2(7):941–946

Larochelle J, Alvarez F, Bussieres JF et al (2012) Effect of nitisinone (NTBC) treatment on the clinical course of hepatorenal tyrosinemia in Quebec. Mol Genet Metab 107(1–2):49–54

Laszlo A, Rozsa M, Sallay E et al (2013) The fate of tyrosinaemic Hungarian patients before the NTBC aera. Ideggyogy Sz 66 (11–12):415–419

Lindblad B, Lindstedt S, Steen G (1977) On the enzymic defects in hereditary tyrosinemia. Proc Natl Acad Sci U S A 74(10):4641–4645

Lindstedt S, Holme E, Lock EA, Hjalmarrson T, Strandvik B (1992) Treatment of hereditary tyrosinemia type I by inhibition of 4-hydroxyphenylpyruvate dioxygenase. Lancet 340:813–817

Mak CM, Lam CW, Chim S, Siu TS, Ng KF, Tam S (2013) Biochemical and molecular diagnosis of tyrosinemia type I with two novel FAH mutations in a Hong Kong Chinese patient: recommendation for expanded newborn screening in Hong Kong. Clin Biochem 46(1–2):155–159

Mayorandan S, Meyer U, Gokcay G et al (2014) Cross-sectional study of 168 patients with hepatorenal tyrosinaemia and implications for clinical practice. Orphanet J Rare Dis 9(1):107

Mitchell GA, Grompe M, Lambert H, Tanguay RM (2001) Hypertyrosinemia.In: The metabolic and molecular bases of inherited diseases. McGrawHill, New York, pp 1777–1805

Mohamed S, Kambal MA, Al Jurayyan NA et al (2013) Tyrosinemia type 1: a rare and forgotten cause of reversible hypertrophic cardiomyopathy in infancy. BMC Res Notes 6(1):362

Morrissey MA, Sunny S, Fahim A, Lubowski C, Caggana M (2011) Newborn screening for Tyr-I: two years' experience of the New York State program. Mol Genet Metab 103(2):191–192

Mustonen A, Ploos van Amstel HK, Berger R, Salo MK, Viinikka L, Simola KO (1997) Mutation analysis for prenatal diagnosis of hereditary tyrosinaemia type 1. Prenat Diagn 17(10):964–966

Park HD, Lee DH, Choi TY et al (2009) Clinical, biochemical, and genetic analysis of a Korean neonate with hereditary tyrosinemia type 1. Clin Chem Lab Med 47(8):930–933

Perez-Carro R, Sanchez-Alcudia R, Perez B et al (2013) Functional analysis and in vitro correction of splicing FAH mutations causing tyrosinemia type I. Clin Genet. doi:10.1111/cge.12243

Phaneuf D, Labelle Y, Berube D et al (1991) Cloning and expression of the cDNA encoding human fumarylacetoacetate hydrolase, the enzyme deficient in hereditary tyrosinemia: assignment of the gene to chromosome 15. Am J Hum Genet 48(3):525–535

Phaneuf D, Lambert M, Laframboise R, Mitchell G, Lettre F, Tanguay RM (1992) Type 1 hereditary tyrosinemia. Evidence for molecular heterogeneity and identification of a causal mutation in a French Canadian patient. J Clin Invest 90(4):1185–1192

Ploos van Amstel JK, Bergman AJ, van Beurden EA et al (1996) Hereditary tyrosinemia type 1: novel missense, nonsense and splice consensus mutations in the human fumarylacetoacetate hydrolase gene; variability of the genotype–phenotype relationship. Hum Genet 97(1):51–59

Poudrier J, St-Louis M, Lettre F, et al (1996) Frequency of the IVS12 + 5G>A splice mutation of the fumarylacetoacetate hydrolase gene in carriers of hereditary tyrosinaemia in the French Canadian population of Saguenay-Lac-St-Jean. Prenat Diagn 16(1): 59–64

Poudrier J, Lettre F, St-Louis M, Tanguay RM (1999) Genotyping of a case of tyrosinaemia type I with normal level of succinylacetone in amniotic fluid. Prenat Diagn 19(1):61–63

Prieto-Alamo MJ, Laval F (1998) Deficient DNA-ligase activity in the metabolic disease tyrosinemia type I. Proc Natl Acad Sci U S A 95(21):12614–12618

Rootwelt H, Berger R, Gray G, Kelly DA, Coskun T, Kvittingen EA (1994a) Novel splice, missense, and nonsense mutations in the fumarylacetoacetase gene causing tyrosinemia type 1. Am J Hum Genet 55(4):653–658

Rootwelt H, Brodtkorb E, Kvittingen EA (1994b) Identification of a frequent pseudodeficiency mutation in the fumarylacetoacetase gene, with implications for diagnosis of tyrosinemia type I. Am J Hum Genet 55(6):1122–1127

Rootwelt H, Chou J, Gahl WA et al (1994c) Two missense mutations causing tyrosinemia type 1 with presence and absence of immunoreactive fumarylacetoacetase. Hum Genet 93(6):615–619

Rootwelt H, Kristensen T, Berger R, Hoie K, Kvittingen EA (1994d) Tyrosinemia type 1–complex splicing defects and a missense mutation in the fumarylacetoacetase gene. Hum Genet 94(3):235–239

Rootwelt H, Hoie K, Berger R, Kvittingen EA (1996) Fumarylacetoacetase mutations in tyrosinaemia type I. Hum Mutat 7 (3):239–243

Russo PA, Mitchell GA, Tanguay RM (2001) Tyrosinemia: a review. Pediatr Dev Pathol 4(3):212–221

Sakai K, Kitagawa T (1957) An atypical case of tyrosinosis (1-Parahydroxyphenyl-lactic aciduria) Part 1. Clinical and laboratory findings. Jikei Med J 2:1–5

Seda Neto J, Leite KM, Porta A et al (2014) HCC prevalence and histopathological findings in liver explants of patients with hereditary tyrosinemia type 1. Pediatr Blood Cancer. doi:10.1002/pbc.25094

Sheth JJ, Ankleshwaria CM, Pawar R, Sheth FJ (2012) Identification of novel mutations in FAH Gene and Prenatal Diagnosis of Tyrosinemia in Indian Family. Case Rep Genet 2012:428075

Sniderman King L, Trahms C, Scott CR (2011) Tyrosinemia type 1. Retrieved from GeneReviews® June 2014 http://www.ncbi.nlm.nih.gov/books/NBK1515/)

St-Louis M, Leclerc B, Laine J, Salo MK, Holmberg C, Tanguay RM (1994) Identification of a stop mutation in five Finnish patients suffering from hereditary tyrosinemia type I. Hum Mol Genet 3 (1):69–72

St-Louis M, Poudrier J, Phaneuf D, Leclerc B, Laframboise R, Tanguay RM (1995) Two novel mutations involved in hereditary tyrosinemia type I. Hum Mol Genet 4(2):319–320

St-Louis M, Tanguay RM (1997) Mutations in the fumarylacetoacetate hydrolase gene causing hereditary tyrosinemia type I:overview. Hum Mutat 9(4):291–299.

Tanguay RM, Valet JP, Lescault A et al (1990) Different molecular basis for fumarylacetoacetate hydrolase deficiency in the two clinical forms of hereditary tyrosinemia (type I). Am J Hum Genet 47(2):308–316

Timmers C, Grompe M (1996) Six novel mutations in the fumarylacetoacetate hydrolase gene of patients with hereditary tyrosinemia type I. Hum Mutat 7(4):367–369

van Spronsen FJ, Thomasse Y, Smit GP et al (1994) Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on dietary treatment. Hepatology 20(5):1187–1191

Vondrackova A, Tesarova M, Magner M et al (2010) Clinical, biochemical and molecular characteristics in 11 Czech children with tyrosinemia type I. Cas Lek Cesk 149(9):411–416

Yang N, Han LS, Ye J et al (2012) Analysis of clinical data and genetic mutations in three Chinese patients with tyrosinemia type I. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 29(6):648–652

Zytkovicz TH, Sahai I, Rush A et al (2013) Newborn screening for hepatorenal tyrosinemia-I by tandem mass spectrometry using pooled samples: a four-year summary by the New England newborn screening program. Clin Biochem 46(7–8):681–684

JIMD Reports
DOI 10.1007/8904_2014_364

CASE REPORT

Pathologic Variants of the Mitochondrial Phosphate Carrier *SLC25A3*: Two New Patients and Expansion of the Cardiomyopathy/Skeletal Myopathy Phenotype With and Without Lactic Acidosis

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Received: 26 April 2014 / Revised: 02 September 2014 / Accepted: 11 September 2014 / Published online: 15 February 2015
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Abstract Variants in the *SLC25A3* gene, which codes for the mitochondrial phosphate transporter (PiC), lead to a failure of inorganic phosphate (Pi) transport across the mitochondrial membrane, which is required in the final step of oxidative phosphorylation. The literature described two affected sibships with variants in *SLC25A3*; all cases had skeletal myopathy and cardiomyopathy (OMIM 610773). We report here two new patients who had neonatal cardiomyopathy; one of whom did not have skeletal myopathy nor elevated lactate. Patient 1 had a homozygous splice site variant, c.158-9A>G, which has been previously reported in a Turkish family. Patient 2 was found to be a compound heterozygote for two novel variants, c.599T>G (p.Leu200Trp) and c.886_898delGGTAGCAGTGCTTinsCAGATAC (p.Gly296_Ser300delinsGlnIlePro). Protein structure analysis indicated that both variants are likely to be pathogenic. Sequencing of *SLC25A3* should be considered in patients with isolated cardiomyopathy, even those without generalized skeletal myopathy or lactic acidosis.

Communicated by: John Christodoulou, MB BS PhD FRACP FRCPA

Competing interests: None declared

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Introduction

SLC25A3 encodes the mitochondrial phosphate transporter (PiC), which transports inorganic phosphate (Pi) across the mitochondrial membranes, thereby allowing oxidative phosphorylation by the addition of Pi to ADP to yield ATP. The driving force for this reaction is the proton gradient across the inner mitochondrial membrane generated by complexes I, III, and IV of the electron transport chain. The terminal steps of ATP production require three enzymes. First, the *SLC25A3*-encoded PiC imports Pi into the mitochondrial matrix. Next, the F_1F_0-ATP synthase catalyzes the addition of the Pi to ADP to form ATP. Finally, the adenine nucleotide translocase transports the new ATP molecule out of the matrix in exchange for an ADP molecule.

There are multiple publications describing pathogenic variants in several nuclear-encoded F_1F_0-ATP synthase components, including *TMEM70* and *ATP5E*; these lead to a wide variety of phenotypes, including cardiomyopathy, neuropathy, ataxia, and lactic acidosis (Cizkova et al. 2008; Mayr et al. 2010; Tort et al. 2011). Variants in *MT-ATP6*, a mitochondrial-encoded component of the F_1F_0-ATP synthase, can also cause a variety of phenotypes in the Leigh syndrome spectrum (OMIM 256000) (Pitceathly et al. 2012). Recently, reports have emerged describing the first examples of pathogenic variants in the mitochondrial PiC (Mayr et al. 2007; Mayr et al. 2011).

SLC25A3, located on chromosome 12q23, encodes a protein 361 amino acids in length. There are two isoforms of the protein differing by alternative splicing of exons 3A and 3B. PiC-A includes exon 3A and is expressed in heart and skeletal muscle. PiC-B contains exon 3B and is expressed in a wide variety of other tissues, including the liver, kidney, and thyroid (Dolce et al. 1996). Exons 3A and 3B are more than 70% identical and encode 42 and 41 amino acids, respectively. In the two previously reported Turkish families, one had a homozygous c.158-9A>G transition creating a novel splice acceptor site in intron 2 (family 1), and the other has a homozygous c.215G>A (p.G72E) transition within exon 3A (family 2) (Mayr et al. 2007; Mayr et al. 2011). Of the five affected children in these families, three died within the first year of life from hypertrophic cardiomyopathy and lactic acidosis (Patients 1-1, 2-1, and 2-2), and two brothers were alive at nine and seventeen years with stable hypertrophic cardiomyopathy, proximal muscle weakness, and exercise intolerance (Patients 1-2 and 1-3) (Table 1). Of note, these patients demonstrated phenotypes that were consistent with decreased PiC-A in the tissues where the isoform is known to be expressed. In addition, in these cases, the retained PiC-B isoform was unable to compensate for the loss of exon 3A despite being expressed in those tissues, which may suggest alternate functions for the two isoforms.

Materials and Methods

Patients

Patient 1

This patient was a 12-month-old male born after a 41-week gestation to a 31-year-old Guatemalan mother and father. Pregnancy, including prenatal ultrasounds, was uncomplicated. Family history was negative for neonatal death, recurrent miscarriage, significant cardiac disease, or genetic abnormalities. There were two healthy siblings. There was no known parental consanguinity, but the parents were from the same small Guatemalan town. The baby was born by routine repeat Cesarean section at a community hospital. His Apgar scores were 7 at one minute of life and 8 at five minutes of life; his birth weight was 3,990 g (75th%). He was initially transferred to the well-baby nursery but within two hours of birth was found to have decreased tone, a weak cry, and hypoxia. He then quickly decompensated with profound hypotonia, respiratory failure requiring intubation, severe lactic acidosis, a coagulopathy, and poor cardiac contractility. His initial pH was 6.8 with a base deficit of −30, which was largely unresponsive to treatment with intravenous saline and sodium bicarbonate. Urine ketones were negative, lactate was 19.2 mmol/L (reference range 0.5–2.2 mmol/L), ammonia was 24 mg/L (reference range 15–45 mg/L), and plasma betahydroxybutyrate was 0.1 mcg/mL (reference range <0.5 mcg/mL). An echocardiogram showed a structurally normal heart with a large patent ductus arteriosus and a right-to-left shunt coupled with pressures greater on the right side than on the left.

His respiratory status declined further, requiring treatment with nitrous oxide for 2 days. While intubated, he appeared strikingly and atypically alert out of proportion to his hypotonia, respiratory failure, and lactic acidosis. A repeat echocardiogram at 48 h of life showed right ventricular dilation and hypertrophy with a transitional patent ductus arteriosus. A head ultrasound showed increased echogenicity in the globus pallidus and thalami bilaterally, with normal video electroencephalogram. Brain magnetic resonance imaging (MRI) was grossly normal with MRI spectroscopy showing significantly elevated lactate levels. Normal results were obtained from newborn screening, an SNP microarray, a newborn hearing screen, and an ophthalmology exam. He was started on coenzyme Q, levocarnitine, riboflavin, thiamine, creatine, and biotin while his testing was pending.

He was extubated at 7 days of age but then had progressive severe lactic acidosis and was reintubated. Sequencing of *PHOX2B* was negative for the central hypoventilation syndrome (OMIM 209880), which was tested due to his prolonged ventilator dependence. Muscle biopsy at 2 weeks of age showed normal histology and normal mitochondrial DNA content; electron transport chain assays were not possible because of insufficient sample. His third attempt at extubation was successful and he was able to tolerate room air by ten weeks of age. Lactate levels throughout his admission ranged from 3 to 11 mmol/L (reference range 0.5–2.2 mmol/L). He did not have any significant renal, hepatic, or hematologic issues. On discharge, he was only prescribed carvedilol, sodium bicarbonate, and vitamin D supplementation. He was transferred to a long-term care facility where he has done well, although occasional readmissions were necessary for intubation when he suffered from viral infections.

Patient 2

The patient was a 10-month-old male born after a full-term pregnancy to a 32-year-old Haitian mother and a Dominican father. Pregnancy was unremarkable except for hypertrophic cardiomyopathy noted on the second trimester ultrasound examination and confirmed by fetal echocardiogram. Family history was negative for significant cardiac disease or genetic abnormalities, and there was no known parental consanguinity. He was born via scheduled Cesarean section due to the concerns of cardiac disease at a

Table 1 All reported cases of patients with variants in the mitochondrial phosphate carrier, *SLC25A3*, including the two new patients reported here (Patients 3-1 and 4-1.) Notice that patient 4-1, who carries two novel variants, does not demonstrate clinical myopathy nor lactic acidosis seen in all the previously reported patients

	Family 1			Family 2		Family 3	Family 4
	Patient 1-1	Patient 1-2	Patient 1-3	Patient 2-1	Patient 2-3	Patient 3-1	Patient 4-1
Report	Previous	Previous	Previous	Previous	Previous	New	New
Ethnicity	Turkish	Turkish	Turkish	Turkish	Turkish	Guatemalan	Haitian/Dominican
Age	Died at 6 months	9 years	17 years	Died at 4 months	Died at 9 months	12 months	10 months
Gender	Female	Male	Male	Female	Female	Male	Male
Variant	Homozygous c.158-9A>G affecting exon 3A	Homozygous c.158-9A>G affecting exon 3A	Homozygous c.158-9A>G affecting exon 3A	Homozygous c.215G-> A within exon 3A	Homozygous c.215G-> A within exon 3A	Homozygous c.158-9A>G affecting exon 3A	Compound heterozygous c.599T>G (exon 4) c. 886-898delins7 (exon 6)
Clinical course	Hypertrophic cardiomyopathy and lactic acidosis, death at 6 months. Limited information	Prenatal hypertrophic cardiomyopathy, elevated lactate. At 9 years skeletal myopathy, compensated hypertrophic cardiomyopathy	Neonatal hypertrophic cardiomyopathy, elevated lactate. At 17 years skeletal myopathy, nonprogressive hypertrophic cardiomyopathy	Neonatal hypertrophic cardiomyopathy, severe skeletal myopathy, elevated lactate, progressive cardiomyopathy resulting in death at 4 months	Neonatal hypertrophic cardiomyopathy, severe skeletal myopathy, elevated lactate, progressive cardiomyopathy resulting in death at 9 months	Persistent lactic acidosis, neonatal cardiorespiratory failure, moderate skeletal myopathy, hypertrophic cardiomyopathy, stable at 12 months of age	Prenatal hypertrophic cardiomyopathy; cardiac transplant at age 7 months, no lactic acidosis, no clinical myopathy

community hospital and then transferred to a high-acuity neonatal intensive care unit for the first month of life. His birth weight was 2,849 g (50%), and Apgar score was 8 at 1 min of life and 9 at 5 min of life.

He was initially asymptomatic and had normal tone, but echocardiogram at birth confirmed the presence of biventricular hypertrophy, left greater than right, with increased trabeculation and decreased left ventricular function, mild to moderate right ventricular dilation, and a moderate-sized atrial septal defect with left-to-right shunting. Cardiac catheterization and endomyocardial muscle biopsy at three weeks of life revealed nonspecific findings of cardiomyopathy with muscle disarray; there was no evidence of glycogen accumulation. Additional metabolic workup showed the following to be normal: plasma acylcarnitine profile, urine amino acids, plasma amino acids, plasma ammonia, serum cholesterol, plasma and urine carnitine, and serum creatinine kinase. Notable, screening lactate drawn while in cardiac failure was normal at 1.2 mmol/L (normal 0.5–1.6 mmol/L). Repeat lactate was also well within the normal range at 1.05 mM (normal range 0.80–2.0 mM), pyruvate level was slightly low at 0.02 mM (normal range 0.05–0.14 mM), and the lactate/pyruvate ratio was elevated at 53 (reference range 10–20). Newborn screening test and lysosomal screening for acid beta glucosidase, sphingomyelinase, alpha glucosidase, galactocerebrosidase, and alpha galactosidase were also normal. Imaging included normal head and renal ultrasounds. Medical treatment during the initial evaluation included courses of diuretics, beta blockers, and inotropes. He was eventually transferred at four weeks of life to a second tertiary care facility for cardiac transplant evaluation due to worsening heart failure and respiratory status. At 3 months of life, he underwent tracheostomy placement. MRI of the brain at 4 months of life showed prominence of the sulci, extra-axial spaces, and ventricles as well as thinning of the corpus callosum. Genetic and metabolic evaluation included a Noonan Spectrum panel for his cardiomyopathy, which did not reveal detectable point variants in the coding regions or intron/exon junctions of *PTPN11*, *SOS1*, *KRAS*, *NRAS*, *HRAS*, *RAF1*, *BRAF*, *SHOC2*, *MAP2K2*, *MAP2K1*, or *CBL* genes and no evidence of Pompe disease with normal acid alpha-glucosidase activity levels.

A muscle biopsy performed at 4 months of life showed no morphologic features of mitochondrial myopathy, but electron microscopy demonstrated minimal enlargement in size of mitochondria with slightly abnormal cristae. Electron transport chain enzyme function studies showed only increased citrate synthase levels suggestive of mitochondrial proliferation, thought to represent an adaptive response to mitochondrial dysfunction. Even when corrected for the increased citrate synthase levels, there were no deficiencies of the respiratory chain activities found.

At 7 months of life, the patient underwent an orthotopic cardiac transplant. The postoperative period was complicated by circulatory compromise and ventilator-dependent respiratory failure, significant gastroesophageal reflux, and seizures. He never experienced lactic acidosis during the course of his hospitalization. He was able to be discharged home at 11 months of age and was doing well with close follow-up.

Sequencing for Patient 1

Sequencing for patient 1 was performed by GeneDx (Gaithersburg, MD). Using genomic DNA from a blood specimen, all coding exons and the flanking splice junctions of a commercial panel of 101 genes involved in mitochondrial function and structure were PCR-amplified and sequenced simultaneously by next-generation sequencing. The DNA sequence was assembled, aligned against reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed. The entire mitochondrial genome from the blood sample was also amplified and sequenced using next-generation sequencing, with droplet-based multiplex PCR and library preparation with Illumina HiSeq2000. Exon level deletion/duplication analysis using Array CGH was performed concurrently. Mitochondrial DNA sequence was assembled and analyzed against the revised Cambridge Reference Sequence (rCRS) and the reported mutations and polymorphisms listed in the MITOMAP database (http://www.mitomap.org). The presence of the disease-associated sequence variant was confirmed by Sanger sequence analysis. A reference library of more than 6,000 samples from different ethnic groups and online databases for mtDNA variations was used to evaluate variants of unknown clinical significance.

Sequencing for Patient 2

The target sequences of a commercial panel of 162 genes involved in mitochondrial structure and function were enriched by using custom-designed NimbleGen SeqCap probe hybridization (Roche NimbleGen Inc., Madison, WI, USA). The captured target sequences included all coding exons and 20 bp of flanking intronic regions. The sample preparation followed the manufacturer's recommendation. Equal molar ratios of 10 indexed samples were pooled to be loaded to each lane of the flow cells for sequencing on a HiSeq2000 (Illumina Inc., San Diego, CA, USA) with 75 cycle single-end reads. The reads were aligned against human genome build hg19, and the variant calls were analyzed with the filter of dbSNP, HGMD, and NHLBI GO Exome Sequencing Project (ESP). The average sequence depth was 700× per base. All coding exons with <20× coverage were completed by PCR/Sanger sequencing to ensure 100% coverage. Sanger sequencing of the *SLC25A3* gene was also performed on the parental blood samples.

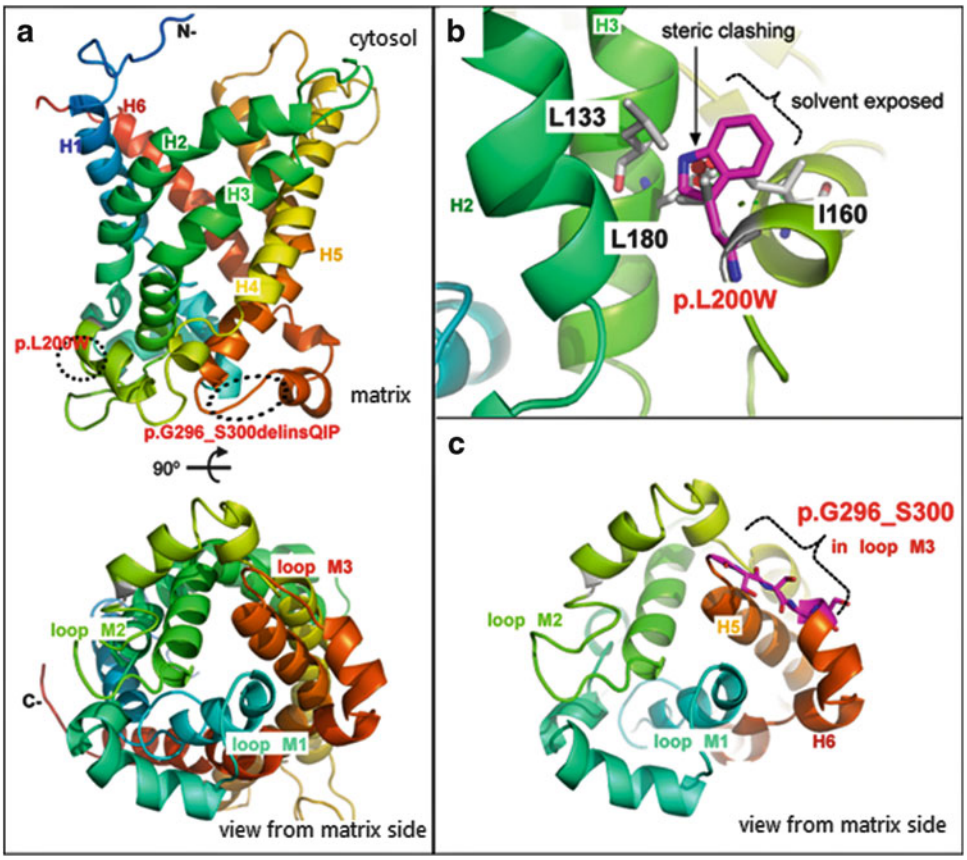

Fig. 1 Ribbon representation of human mitochondrial phosphate solute carrier (SLC25A3, NP_005879). (**a**) The overall structure of human mitochondrial phosphate solute carrier. Residues from 60 to 338 of the phosphate solute carrier are modeled using crystal structure of bovine mitochondrial ADP/ATP carrier as a template. The *top panel* is the side view, and the *lower panel* indicates the bottom view of the protein from the intermembrane space of mitochondria to the matrix. The *bottom view* illustrates the threefold symmetric organization of this protein, which is maintained through the human mitochondrial carrier superfamily. The helices and matrix loops were labeled according to the nomenclature of the original human mitochondrial ADP/ATP carrier publication. The *two dashed circles* indicate the locations of the two variants reported here. (**b**) The close-up atomic configuration of the p.L200W substitution. These hydrophobic residues L133, I160, L180, and L200 are represented as a stick model and their carbon atoms are colored in *grey*. Substitution of W (carbon atoms in purple) for L at position 200 indicates a number of small but significant steric clashes (*red*). Moreover, the large hydrophobic side chain atoms of W are exposed to hydrophilic solvent. These two unfavorable factors contribute to intolerance of W substitution for L at this position. (**c**) The GSSAS sequence (from 296 to 300) is located at matrix loop M3 which connects H5 and H6. This segment connects transmembrane helices 5 and 6 to reinforce the closed conformation of the carrier for channel formation. As indicated by the bovine mitochondrial ADP/ATP carrier protein, there are many important residues in this loop M3 dictating nucleotide-binding phosphate stoichiometry and determining the threefold symmetric organization at the matrix side

Model Building for PiC

The initial search using full-length SLC25A3 protein sequence (NP_005879) indentified the bovine mitochondrial ADP/ATP carrier as the top candidate from X-ray diffraction studies (PDB code: 1OKC at resolution of 2.20 Å). The sequence search also indicated human SLC25A3 protein shares a similar fold to other mitochondrial carrier protein from the CATH sequence domain search (www.cathdb.info). After excluding the disordered regions as predicted by the DISOPRED server (http://bioinf.cs.ucl.ac.uk/disopred), the sequence identity was improved to 19.1% and sequence similarity improved to 55.7% between human and bovine SLC25A3 proteins for residues 60–338. A model of human mitochondrial phosphate solute carrier was constructed based on the crystal structure of 1OKC with pGenTHREADER (http://bioinf.cs.ucl.ac.uk). The structure model was also subjected to energy minimization before performing structure analysis (Fig. 1a). The quality and accuracy of the stereochemical model were demonstrated with ProCheck (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) to have more than 90% favorable conformations of residues on Ramachandran plots. The key PX(D/E)XX(K/R) motif was also accurately modeled to overlap in the bovine mitochondrial ADP/ATP carrier.

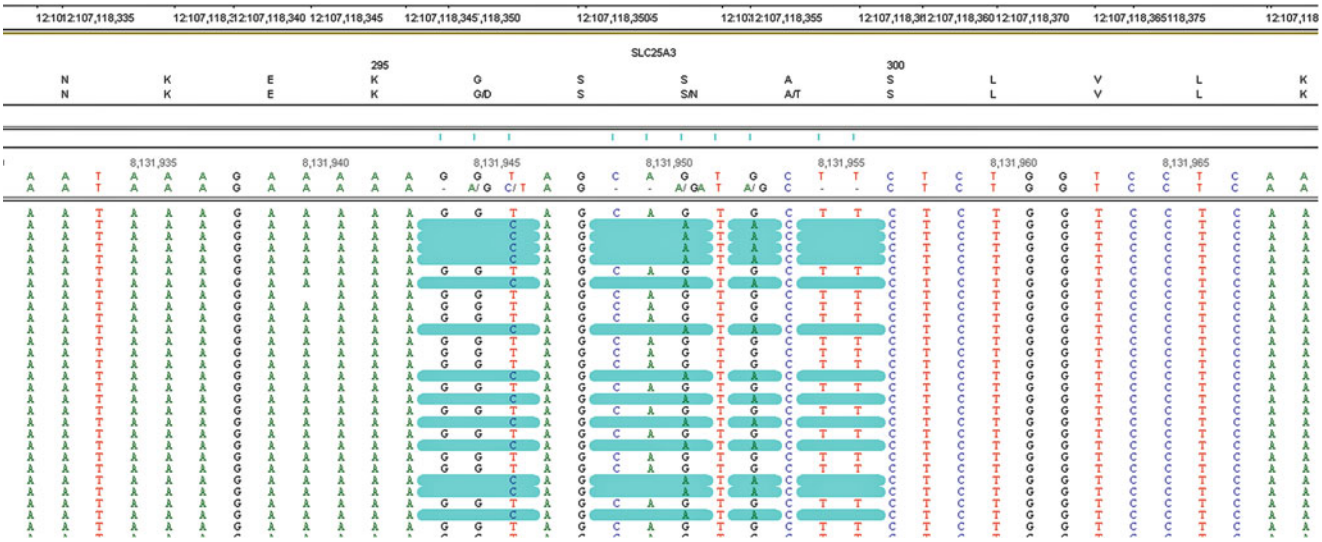

Fig. 2 Next-generation sequencing result for the c.886_898delins7 (p.G296_S300delinsQIP) mutation. Thirteen nucleotides, GGTAG-CAGTGCTT, at position c.886_898 were deleted and replaced by 7 new nucleotides, CAGATAC. This deletion/insertion change results in the replacement of 5 amino acid residues, Gly-Ser-Ser-Ala-Ser with 3 amino acids, Gln-Ile-Pro, at position p.296_300 (p.G296_S300delGS-SASinsQIP)

Results

Patient 1

Next-generation sequencing revealed a previously reported homozygous c.158-9A>G variant in *SLC25A3*. The c.158-9A>G is located in intron 2 which is next to exon 3A. This variant creates a novel splice site in intron 2 that leads to the inclusion of eight nucleotides on the 5′ side of exon 3A, predicted to result in a frame shift and early termination in the first quarter of the protein (Mayr et al. 2011). Parental testing was not available for this patient.

Patient 2

A comprehensive next-generation sequencing analysis of 162 nuclear genes and the whole mitochondrial genome revealed two novel heterozygous variants in *SLC25A3*: c.599T>G (p.L200W) and c. 886_898delGGTAGCAGTGCTTinsCAGA-TAC (p.G296_S300delGSSASinsQIP) (Fig. 2). L200 is highly conserved, and the computer-based algorithms, SIFT and PolyPhen-2, predict the p.L200W substitution to be deleterious. This in frame amino acid change is categorized as an unclassified variant but is likely pathogenic. Subsequent sequence analyses of the parents indicate that the c.599T>G (p.L200W) was maternally inherited and c.886_898delin-sCAGATAC (p.G296_S300delGSSASinsQIP) was paternally inherited; therefore, this patient's *SLC25A3* variants are in a transconfiguration.

Discussion

Here, we present two new unrelated patients with patho-logic variants in *SLC25A3* predicted to cause dysfunction of the mitochondrial PiC. These patients are informative as only two families with variants in *SLC25A3* have been previously reported, and one of our patients has two novel variants and an expanded phenotype of cardiomyopathy without skeletal myopathy nor elevated lactate (Mayr et al. 2007; Mayr et al. 2011). In addition, these are the first patients reported who are not of Turkish descent, which indicates a larger widespread prevalence.

These patients had very distinct phenotypic presentations despite variants in the same gene. Patient 1 had a normal echocardiogram at birth but had profound respiratory failure with severe lactic acidosis. Patient 2 had prenatally diagnosed hypertropic cardiomyopathy, which progressed to cardiac transplant, but never had any clinical signs of skeletal myopathy or lactic acidosis. There has not yet been any reported patients with dysfunction of the PiC without lactic acidosis, which is typically a finding that would lead clinicians to consider mitochondrial disorder. Our patient 2 had extensive testing, but mitochondrial disease was not initially considered as a differential diagnosis as he had isolated cardiomyopathy without lactic acidosis or skeletal myopathy.

We would also like to note the striking disconnect between the alert wakeful state of patient 1, which was felt to be the most unusual aspect of his presentation and is not

seen with more common diagnoses that cause neonatal lactic acidosis. We feel that this may be a distinguishing feature in the neonatal period between this disorder and other mitochondrial disorders that include more severe neurocognitive outcomes.

Patient 2, presented with a novel variant: c.886_898delinsCAGATAC (p.G296_S300delGSSASinsQIP), which resulted in a deletion of five amino acids GSSAS, at position p.296_300, and insertion of three amino acids, QIP. The five amino acid (GSSAS) sequences were located at matrix loop M3 which, according to the helix nomenclature of the ADP/ATP carrier, connects transmembrane helices 5 and 6. The relative threefold symmetric organization of this protein is maintained within the human mitochondrial carrier superfamily. It has been shown in the bovine ATP/ADP carrier structure that these connecting helices are positioned to be parallel to the membrane surface and their configuration reinforces the closed conformation of the carrier for channel formation on the matrix side (Fig. 1a, lower panel). There are many important residues in loop M3 that dictate nucleotide binding phosphate stoichiometry and interface with the threefold symmetric organization at the matrix side. These residues interact with the protein moiety as well as the hydrophobic membrane lipid bilayer. Thus, the replacement of flexible and small amino acids of GSSAS with a bulky glutamine and hydrophobic isoleucine is likely not tolerated (Fig. 1c).

The model of the human mitochondrial phosphate solute carrier suggests that L at position 200 is located at the small matrix helix which connects the transmembrane helices 3 and 4 according to the helix nomenclature of the ADP/ATP carrier. L200 interacts with adjacent hydrophobic residues L133, I160, and L180, which are in the close vicinity of the consensus sequence of all mitochondrial carrier proteins, PX(D/E)XX(K/R) motif. When L200 is changed to tryptophan, the bulky side chain is likely to introduce stereochemical clashes to these critical regions. Substitution of W (carbon atoms in purple) for L at position 200 indicates a number of small but significant steric clashes (red) (Fig. 1b). Moreover, the large hydrophobic side chain atoms of W are exposed to hydrophilic solvent. These two unfavorable factors contribute to intolerance of W substitution for L at this position. The motif, PMEAAK from amino acid position 182 to 197 in the phosphate carrier protein, also helps to maintain the protein structure as a channel for solute transport (PMID: 8132484). Replacement of L by a more bulky, aromatic W would be expected to interfere with the interaction among the segment containing W200, helix 3, and helix 4, thereby disrupting the structure of the channel and impeding solute transport (Figure 1b). Therefore, we feel that this variant would be pathogenic and responsible for patient 2's presentation.

As next-generation sequencing becomes increasingly utilized for clinical diagnosis, it will allow for improved clinical management for sick patients presenting in infancy. Many life-altering choices for these patients were predicated on having an accurate diagnosis. For example, before the genetic diagnosis was ascertained, patient 1 had a do-not-resuscitate (DNR) order. Once the genetic diagnosis was discovered, we found examples in the literature of surviving children with only mild impairment; the family then removed the DNR order. For patient 2, the molecular diagnosis allowed him to be considered for cardiac transplant, as it was previously thought he may have a global mitochondrial defect that would make him ineligible.

Both of these patients were diagnosed through next-generation sequencing with commercial panels targeting a variety of mitochondrial diseases. Neither of these patients had enough distinguishing features of PiC dysfunction to make the diagnosis clinically, especially patient 2 who presented with isolated cardiomyopathy. It is interesting that this phenotype of just hypertrophic cardiomyopathy without lactic acidosis is found in the patient with the variants that affects exons 4 and 6, which would be expected to be included in all the splice forms. All of the previously reported patients and our patient 1 had mutations affecting only exon 3A found in PiC-A, which is expressed in just skeletal and cardiac muscle. Perhaps there is more global mitochondrial dysfunction leading to lactic acidosis from a disruption of the normal ratio of the PiC-A and PiC-B isoforms than from a decrease in both isoforms as would be expected by mutations in obligate exons. It has been shown that the bovine forms of PiC-A and PiC-B have different transport affinities, with PiC-A three times as high as PiC-B, but the maximal transport rate in PiC-A is three times less than PiC-B, suggesting that appropriate tissue-specific ratios of the isoforms may play a role in total-body phosphate allocation (Fiermonte et al. 1998). It is also possible that there is a higher level of retained activity with these new variants, which is leading to the milder skeletal muscle phenotype and lack of lactic acidosis.

In this report, we describe the clinical characteristics of two new patients with variants in *SLC25A3*, which codes for the mitochondrial phosphate carrier. We also report two novel mutations in exons four and six, consider the genotype-phenotype correlation of the variants, and describe the protein modeling of the novel mutation. In conclusion, we recommend that variants in the phosphate

transporter gene should be considered in a variety of conditions, including patients with isolated cardiomyopathy like our patient 2, with skeletal myopathy and lactic acidosis like our patient 1.

Synopsis

We present two new cases of apparent disruption of the mitochondrial phosphate transporter, encoded by *SLC25A3*, which has been reported to present with cardiomyopathy, skeletal myopathy, and lactic acidosis; we describe an expanded phenotype of isolated hypertrophic cardiomyopathy.

Compliance with Ethical Guidelines

Conflict of Interest

Elizabeth Bhoj, Mindy Li, Louisa Pyle, Rebecca Ahrens-Nicklas, Colleen Clarke, Lee-Jun Wong, Jing Wang, Victor Zhang, Neal Sondheimer, Can Ficicioglu, and Marc Yudkoff declare that they have no conflict of interest.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Contributions of Each Author

Bhoj EJ, Li M, Clarke C, Sondheimer N, Ficicioglu C, and Yudkoff M were involved in the clinical care and diagnosis of these patients.

Bhoj EJ, Li M, Ahrens-Nicklas R, and Pyle LC were involved in writing the manuscript.

Wang J, Zhang VW and Wong LJ were involved in the sequencing and protein modeling.

References

Cizkova A, Stranecky V et al (2008) TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. Nat Genet 40(11):1288–1290

Dolce V, Fiermonte G et al (1996) Tissue-specific expression of the two isoforms of the mitochondrial phosphate carrier in bovine tissues. FEBS Lett 399(1–2):95–98

Fiermonte G, Dolce V, Palmieri F et al (1998) Expression in *Escherichia coli*, functional characterization, and tissue distribution of isoforms A and B of the phosphate carrier from bovine mitochondria. J Biol Chem 273:2782–2787

Mayr JA, Merkel O et al (2007) Mitochondrial phosphate-carrier deficiency: a novel disorder of oxidative phosphorylation. Am J Hum Genet 80(3):478–484

Mayr JA, Havlickova V et al (2010) Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit. Hum Mol Genet 19(17):3430–3439

Mayr JA, Zimmermann FA et al (2011) Deficiency of the mitochondrial phosphate carrier presenting as myopathy and cardiomyopathy in a family with three affected children. Neuromuscul Disord 21(11):803–808

Pitceathly RD, Murphy SM et al (2012) Genetic dysfunction of MT-ATP6 causes axonal Charcot-Marie-Tooth disease. Neurology 79 (11):1145–1154

Stappen R, Kramer R (1994) Kinetic mechanism of phosphate/phosphate and phosphate/OH- antiports catalyzed by reconstituted phosphate carrier from beef heart mitochondria. J Biol Chem 269(15):11240–11246

Tort F, Del Toro M et al (2011) Screening for nuclear genetic defects in the ATP synthase-associated genes TMEM70, ATP12 and ATP5E in patients with 3-methylglutaconic aciduria. Clin Genet 80(3):297–300

JIMD Reports
DOI 10.1007/8904_2014_366

RESEARCH REPORT

Baseline Urinary Glucose Tetrasaccharide Concentrations in Patients with Infantile- and Late-Onset Pompe Disease Identified by Newborn Screening

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Received: 26 June 2014 / Revised: 28 August 2014 / Accepted: 17 September 2014 / Published online: 15 February 2015
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Abstract *Purpose*: The urinary glucose tetrasaccharide, Glcα1-6Glcα1-4Glcα1-4Glc (Glc_4), is a biomarker of glycogen accumulation and tissue damage and is elevated in patients with Pompe disease. We report baseline urinary Glc_4 concentrations for patients with classic infantile-onset or late-onset Pompe disease, and those with a pseudodeficiency of acid alpha-glucosidase (GAA), identified through newborn screening (NBS) in Taiwan.

Methods: Infants identified through NBS with (1) classic infantile-onset Pompe disease (NBS-IOPD) ($n = 7$) defined as patients with evidence for hypertrophic cardiomyopathy by EKG, X-ray, and echocardiogram, (2) a late-onset phenotype (NBS-LOPD) ($n = 13$) defined as patients without evidence for cardiomyopathy, (3) a GAA pseudodeficiency ($n = 58$), and (4) one patient with LOPD diagnosed in infancy due to family history were consented to the study. Four infants diagnosed after the onset of clinical symptoms (CLIN-IOPD) were included for comparison. Glc_4 concentrations in dried urine samples on filter paper were determined using tandem mass spectrometry.

Results: Baseline Glc_4 concentrations were at or above the 90th centile of the age-matched reference range for the NBS-IOPD cohort. The median Glc_4 level for this group was lower than that of the CLIN-IOPD group, although not at the level of significance ($p = 0.07$), but was significantly higher than that of the NBS-LOPD group ($p < 0.05$). Baseline Glc_4 was not elevated for the NBS-LOPD and GAA pseudodeficiency cohorts and remained low for late-onset patients that did not require treatment before the age of three years.

Conclusion: Baseline urinary Glc_4 is elevated in neonates with infantile-onset Pompe disease identified through NBS.

Communicated by: Verena Peters

Competing interests: None declared

Electronic supplementary material: The online version of this chapter (doi:10.1007/8904_2014_366) contains supplementary material, which is available to authorized users.

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Introduction

Pompe disease (OMIM #232300) is caused by a deficiency of lysosomal acid α-glucosidase (GAA; EC

3.2.1.20), resulting in glycogen accumulation that has a destructive effect on muscle (Hirschhorn and Reuser 2001). Patients with a severe GAA deficiency present in infancy (infantile-onset Pompe disease; IOPD) with cardio- and skeletal myopathy and succumb to cardiorespiratory disease within 2 years (Kishnani et al. 2006). Patients with an attenuated phenotype (termed late-onset or later-onset Pompe disease; LOPD) usually have measurable residual enzyme activity and no cardiac involvement. They present anywhere from early childhood to adulthood with a myopathy that progresses to respiratory insufficiency if untreated (Hagemans et al. 2005).

Enzyme replacement therapy (ERT) with recombinant human alglucosidase alfa (rhGAA) (Myozyme®, Lumizyme®, Genzyme, Cambridge, MA) is available for IOPD and LOPD. Evidence suggests ERT has the most favorable clinical outcome when started early in the disease process (Kishnani et al. 2009; Chien et al. 2009). Recognition of the importance of early diagnosis and treatment of Pompe disease has led to the development of newborn screening (NBS) assays for this condition and a recommendation by the US Discretionary Advisory Committee for Heritable Disorders in Newborns and Children for its inclusion in the recommended uniform newborn screening panel. A NBS pilot program in Taiwan identified 7 infants with IOPD and 13 with LOPD out of 344,056 infants screened between 2005 and 2009 (Chien et al. 2008, 2009, 2011). Survival, particularly ventilator-free survival, was improved for patients with IOPD diagnosed by NBS compared with those diagnosed after onset of symptoms. A high false-positive rate was the result of a pseudodeficiency allele of the *GAA* gene, c.[1726A;2065A] which is common in the Chinese population, but has no known clinical effects (Kroos et al. 2008; Kumamoto et al. 2009; Labrousse et al. 2010).

The glucose tetrasaccharide, Glcα1-6Glcα1-4Glcα1-4Glc (Glc_4), is a limit dextrin of glycogen (Kumlien et al. 1988) and was shown to correlate with glycogen content in quadriceps biopsies in patients with IOPD (Young et al. 2009). Glc_4 can be useful in the diagnosis of Pompe disease and for monitoring the response to ERT (Young et al. 2009, 2012). We assessed the usefulness of urinary Glc_4, measured as the total hexose tetrasaccharide (Hex_4) fraction in urine, in the follow-up of infants with low GAA activity identified by the Taiwanese pilot NBS program.

Materials and Methods

Materials

Whatman grade 903 filter paper (VWR, Batavia, IL); Sep-Pak® Vac 100 mg C18 cartridges (Waters Corporation, Milford, MA); d_3-creatinine (Cambridge Isotopes, Andover, MA); creatinine standards, sodium cyanoborohydride, butyl 4-aminobenzoate, and glacial acetic acid (Sigma-Aldrich, St. Louis, MO); Glc_4 standard (Glycorex AB, Lund, Sweden); HPLC grade solvents (VWR, West Chester, PA); and [$^{13}C_6$]-labeled glucose tetrasaccharide internal standard which was synthesized as previously described (Young et al. 2003).

Patients

This study included infants identified by the Taiwan NBS program between 2005 and 2009 with IOPD (NBS-IOPD, $n = 7$) and LOPD (NBS-LOPD, $n = 13$). The diagnostic confirmation and clinical status have been reported for all patients except NBS9 (Chien et al. 2008, 2009, 2011), and subject designations (see Table Supplemental Digital Content 1) are consistent with these previous publications. Four of the thirteen patients in the NBS-LOPD cohort were started on treatment at or before the age of 3 years because of the severity of their clinical condition (NBS-LOPD early treated), whereas the remaining nine patients did not require treatment within the first 3 years of life (NBS-LOPD-Group 2) (Chien et al. 2011). An additional patient with LOPD (L14), identified at birth because of a positive family history, was also included.

Four patients with IOPD, who were not part of the newborn screening pilot study, were diagnosed in early infancy (<5 months age) after the onset of clinical symptoms (NBS-CLIN) and were included as a prospective comparison group for the NBS-IOPD patients (Chien et al. 2009).

Additionally, 58 infants identified by NBS who had a pseudodeficiency of GAA in DBS (Labrousse et al. 2010) were evaluated. These infants had the following combinations of the pseudodeficiency allele c.[1726A; 2065A] and known or putative disease-causing *GAA* mutations:

1. One mutation plus one pseudodeficiency allele (pseudodeficiency group 1, $n = 23$)
2. One mutation plus two pseudodeficiency alleles (pseudodeficiency group 2, $n = 19$)
3. Two pseudodeficiency alleles only (pseudodeficiency group 3, $n = 16$)

Baseline urine samples were collected within the first 6 weeks of life, except for NBS-L4 and NBS-L11 in the NBS-LOPD cohort on whom samples were collected at 9 and 6 months, respectively (Table Supplemental Digital Content 1). Longitudinal urine samples were collected at regular clinic visits and stored at −20°C.

This study was approved by the Institutional Review Boards of National Taiwan University Hospital and Duke University Health System. Informed consent was obtained from parents of all patients.

Control Samples

Age-specific reference ranges were determined using anonymized clinical samples ($n = 472$, median age: 1.5 years, min–max: 0.0–68 years) from the Duke Biochemical Genetics laboratory and urine specimens collected from anonymous volunteers ($n = 143$, median age: 33 years (min–max: 3–78 years)) under a Duke University Health System IRB-approved protocol.

Glc_4 Determination in Dried Urine Samples Using Stable Isotope Dilution-ESI-MS/MS

Urine specimens were soaked onto filter paper strips, dried, and mailed to the Duke Biochemical Genetics Laboratory at ambient temperature. None of the patients were on ERT at the time of the baseline sample collections. 2 × 2 cm diameter disks cut from the dried urine spot on filter paper were extracted with 1 mL DI-H_2O by shaking at room temperature for 1 h. 500 μL of the filter paper extract was dried under nitrogen at 40°C, reconstituted with 50 μL DI-H_2O and mixed with 25 μL 0.1 mmol/L [$^{13}C_6$]-labeled Glc_4 internal standard. The mixture was derivatized with butyl-*p*-aminobenzoic acid, and Glc_4 was determined as the total hexose tetrasaccharide fraction (Hex_4) in urine by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) on an Acquity UPLC-Quattro Micro tandem mass spectrometer system (Waters Corp, Milford, MA), as previously described by Young et al. (2003, 2009). Glc4 comprises ≥90 % of the total hexose tetrasaccharide fraction in most patient and control samples (unpublished observation). In keeping with previous publications on this biomarker, Hex_4 measurements will be referred to as Glc_4. Glc_4 concentrations were normalized to creatinine determined in an aliquot of the same filter paper extract using stable isotope dilution-tandem mass spectrometry (Young et al. 2009). Comparison studies have shown the equivalency of this assay when applied to liquid urine specimens and extracts of urine specimens dried on filter paper (Young et al. 2003).

Statistical Analyses

Descriptive statistics and Mann–Whitney comparison of median Glc_4 values for the study cohorts were calculated using GraphPad Prism 5.04 software (La Jolla, CA). The relationship of age to Glc_4 in the control cohort was examined using univariable linear regression with STATA 11.0 (College Station, TX) software. *p*-values ≤0.05 were considered to be significant.

Results

Urinary Glc_4 in Control Samples

A significant negative correlation with age was observed for urinary Glc_4 values in controls under aged 3 years (−2.956 [95 % confidence interval; −3.680, −2.232], $p < 0.001$, $n = 287$). No correlation with age was observed for controls over 3 years of age (−0.006 [−0.012, 0.001], $p = 0.09$, $n = 328$). Controls younger than 3 years were divided into different age bins based on a visual inspection of the data and statistical analysis. Upper limits of the reference ranges were defined as the 95th centile and were stratified according to the following age groups: 0–6 months age (95th centile: 20 mmol/mol CN, $n = 132$), 6–12 months (95th centile: 14 mmol/mol CN, $n = 78$), 1–3 years (95th centile: 8.3 mmol/mol CN, $n = 77$), and > 3 years (95th centile: 3.0 mmol/mol CN, $n = 328$). No significant correlation with age was observed for controls younger than 6 months of age (−3.217 [−9.770, 3.335], $p = 0.34$, $n = 132$), and there were no significant differences between median Glc_4 values for the following subgroups: 0–1 month, 1–3 months, and 3–6 months (Table 1).

Baseline Urinary Glc_4 Concentrations

A comparison of the median values and ranges of urinary Glc_4 concentrations and ages at baseline in the patient, pseudodeficiency, and control cohorts is shown in Table 1, and individual patient values are compared in Fig. 1 and Table Supplemental Digital Content 1. Urinary Glc_4 concentrations were at or above the 90th centile (18 mmol/mol CN) of the age-matched reference range (Table Supplemental Digital Content 1) for the six full-term infants in the NBS-IOPD group. The remaining patient in this group, born at 29 weeks gestation and started on ERT at 40 days of age because of cardiomegaly, had pretreatment Glc_4 concentrations of 32 and 16 mmol/mol CN at 0 and 1.2 months of age (not adjusted for prematurity), respectively. An appropriate

Table 1 Median and ranges for baseline urinary Glc_4 values and age at the time of sample collection for the patient cohorts (NBS-IOPD, NBS-LOPD-A, NBS-LOPD-B) and pseudodeficiency groups as described in the methods section

		Baseline Glc_4 (mmol/mol creatinine)		Age (months)	
	n	Median	Range	Median	Range
Patients with infantile- and late-onset Pompe disease identified by newborn screening					
Infantile onset (NBS-IOPD)	7	25	18–32	0.4	0.1–1.0
Late onset treated before age 3 years (NBS-LOPD: early treated)	4	13	5.2–18	0.5	0.3–1.3
Late onset not requiring treatment before age 3 years (NBS-LOPD: Group 2)	10	7.1	2.8–14	0.6	0.2–8.8
Unaffected infants with pseudodeficiency of GAA identified by newborn screening					
Group 1: 1 mutation/1 pseudodeficiency allele	23	6.7	3.4–11	1.5	0.8–3.2
Group 2: 1 mutation/2 pseudodeficiency alleles	19	7.3	3.0–12	1.4	0.7–3.4
Group 3: 2 pseudodeficiency alleles	16	6.6	1.1–12	1.5	1.0–2.6
Patients with infantile-onset Pompe disease diagnosed clinically (CLIN-IOPD)	4	38	24–42	3.0	2.0–3.9
Reference ranges					
Overall range (0–6 months age)	132	6.8	0.5–22	1.0	0.0–6.0
<1 month old controls	66	7.0	0.5–22	0.4	0.0–1.0
1 to 3 month old controls	35	6.7	1.6–22	1.7	1.0–2.8
3 to 6 month old controls	31	6.2	1.8–19	4.6	3.0–6.0

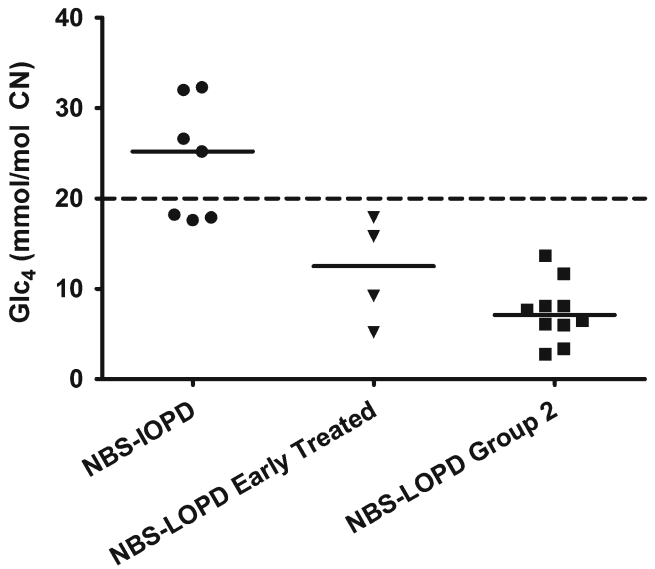

Fig. 1 Urinary Glc_4 concentrations in infants with Pompe disease identified by newborn screening. Urinary Glc_4 values at first evaluation (baseline) for individual patients with Pompe disease identified through newborn screening. *Circles*: Patients with infantile-onset Pompe disease (NBS-IOPD); *Triangles*: Patients with late-onset Pompe disease who were treated before the age of 3 years (NBS-LOPD early treated); *Squares*: Patients with late-onset Pompe disease who did not require treatment before 3 years of age (NBS-LOPD-Group 2). *Dashed line* represents the upper limit of the reference range for 0 to 6 months age ($Glc_4 < 20$ mmol/mol CN)

age-matched control range has not been evaluated for preterm infants. The NBS-IOPD group was significantly younger than the CLIN-IOPD group at the time of pretreatment assessment ($p < 0.05$) and had a lower median Glc_4 concentration, although the difference was not statistically significant ($p = 0.07$) (Table 1).

Baseline Glc_4 concentrations were within reference limits for all patients in the NBS-LOPD group, and median values were significantly lower than those of the NBS-IOPD cohort ($p < 0.05$). There was no significant difference in the median baseline Glc_4 values for the NBS-LOPD early treated and the NBS-LOPD-Group

2 cohorts. Patients NBS-L3 and NBS-L9 in the early treated group had the highest values close to the upper limit of the reference range. Urinary Glc_4 levels were within reference limits for infants with a GAA pseudodeficiency (Table 1).

Comparison of Urinary Glc_4 and Serum Creatine Kinase at Baseline

Urinary Glc_4 was significantly correlated with serum creatine kinase (CK) at the initial follow-up evaluation for patients with a confirmed diagnosis of Pompe disease (Pearson correlation coefficient $= 0.624$, $p < 0.05$; see Figure Supplemental Digital Content 2). As expected, the NBS-IOPD group had the highest CK values (excluding the premature infant, NBS9). Two of four patients in the NBS-LOPD early treated group (NBS-L3 and NBS-L9) had Glc_4 and CK values that were comparable with those for three patients in the NBS-IOPD group. Infants in the pseudodeficiency groups had CK values within the reference intervals (data not shown).

Pretreatment Monitoring of Glc_4 in Patients with a Late-Onset Phenotype

Longitudinal Glc_4 measurements for 11 of the 13 NBS-LOPD patients on whom data were available are shown in Figure Supplemental Digital Content 3. Within the NBS-LOPD early treated cohort, Glc_4 was elevated in two of the four patients prior to treatment. NBS-L3 had a persistent elevation of Glc_4 prior to treatment at age 36 months and NBS-L9 had elevated Glc_4 immediately prior to treatment at 1.5 months. In contrast, NBS-L1 and NBS-L6 did not have elevated Glc_4 prior to initiation of ERT. These trends in Glc_4 are consistent with the CK trends observed for these patients as previously reported (Chien et al. 2011), in that NBS-L3 and NBS-L9 had elevated CK prior to treatment and NBS-L1 and L6 did not. Glc_4 elevations were not observed for the NBS-LOPD group 2 during the observation period of up to 4 years of age.

Discussion

The newborn screening program for Pompe disease in Taiwan has presented a unique opportunity to evaluate Glc_4 in infants with infantile- and late-onset Pompe disease, prior to the appearance of clinical symptoms. Our results indicate that Glc_4 concentrations correlated with phenotype early in the disease process; patients with IOPD had higher Glc_4 concentrations than those with LOPD. Furthermore, median baseline Glc_4 in the NBS-IOPD group was clearly lower than that of the slightly older (by approximately 4–8 weeks) clinical comparator CLIN-IOPD group. These observations are consistent with (1) baseline clinical manifestations in the NBS-IOPD group including cardiomyopathy and elevated CK, despite a normal physical exam and tone (Chien et al. 2009), (2) the notable increase in baseline Glc_4 values with age in untreated patients with IOPD ascertained clinically before 12 months of age (Young et al. 2012), and (3) the rapidly progressive nature of the infantile form of the disease.

The variability of late-onset Pompe disease is demonstrated by differences in the age of onset of clinical signs and symptoms of the disease within the NBS-LOPD group, of which one third of patients required treatment with ERT before 3 years of age (Chien et al. 2011). Although these early treated patients with LOPD had baseline urinary Glc_4 concentrations within the reference range, two had the highest values (86th and 90th centile of the reference range) observed within the entire NBS-LOPD cohort. This observation was concomitant with an elevation of the serum CK values at baseline in these two patients, interpreted as a sign of cell damage as previously reported (Chien et al. 2011).

In conclusion, our findings suggest that urinary Glc_4 determination may be a useful component in the follow-up of a positive newborn screening result for Pompe disease, especially when the results of confirmatory enzyme and molecular testing are equivocal. An elevated Glc_4 suggests an infantile-onset phenotype, whereas a value within the reference range is consistent with a late-onset phenotype or a pseudodeficiency of GAA. However, more studies are needed to determine whether inclusion of Glc_4 in the follow-up algorithm, either as a first or later tier test, would provide additional benefit to other testing such as CK or echocardiogram. Glc_4 is probably most useful for evaluating the disease status in newly diagnosed patients, especially when combined with clinical and other laboratory assessments and for periodic monitoring of asymptomatic patients. With the possible expansion of newborn screening for Pompe disease in the United States and elsewhere, more data from larger cohorts should be accessible and will allow further assessment of the sensitivity of Glc_4 in the newborn period and its prognostic value for monitoring asymptomatic patients.

Acknowledgments The authors would like to thank the patients and their families for their participation in this study. The authors would also like to acknowledge Genzyme, A Sanofi Company for previous grant support for the development and evaluation of Glc_4 as a biomarker in Pompe disease at DUMC and for support of the Taiwan pilot newborn screening program for Pompe disease.

Synopsis

The glucose tetrasaccharide biomarker, Glc_4, determined in dried urine spots, was elevated in neonates with infantile-onset Pompe disease identified through newborn screening, compared with infants with a late-onset phenotype or a pseudodeficiency of acid alpha-glucosidase.

Details of Funding

This study was funded in part by Genzyme, a Sanofi Company

Compliance with Ethics Guidelines

Conflict of Interest

Yin-Hsui Chien reports receiving research grant support, honoraria, and travel support from Genzyme, a Sanofi Company and BioMarin Pharmaceutical Inc.

Wuh-Liang Hwu reports receiving research grant support, honoraria, and travel support from Genzyme, a Sanofi Company and BioMarin Pharmaceutical Inc.

Ni-Chung Lee reports receiving travel support from Genzyme, a Sanofi Company.

Shu-Chuan Chiang reports receiving travel support from Genzyme, a Sanofi Company.

Sarah P. Young reports receiving research grant support from Genzyme, a Sanofi Company and Amicus Therapeutics.

David S. Millington reports receiving research grant support from Genzyme, a Sanofi Company and BioMarin Pharmaceutical Inc. David S. Millington was a member of the science advisory board of and received stock options from Advanced Liquid Logics, LLC.

Priya S. Kishnani reports receiving research grant support and honoraria from Genzyme, a Sanofi Company and Amicus Therapeutics, and consulting fees from Genzyme, a Sanofi Company. P.S. Kishnani is a member of the Pompe Disease and the Gaucher Disease Registry Advisory Boards for Genzyme, a Sanofi Company.

Haoyue Zhang reports receiving research grant support from Genzyme, a Sanofi Company and BioMarin Pharmaceutical Inc.

Jennifer L. Goldstein reports receiving research grant support from Genzyme, a Sanofi Company.

P. Brian Smith, Adviye A. Tolun, and Amie E. Vaisnins declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Institutional Review Boards of National Taiwan University Hospital and Duke University Health System) and with the Helsinki Declaration of 1075, as revised in 2000 (5). Informed consent was obtained from parents of all patients.

Author Contributions

Yin-Hsiu Chien and Wuh-Liang Hwu planned the study, coordinated the collection of dried urine samples and the compliance with National Taiwan University Hospital Institutional Review Board, and participated in the data analysis and preparation and final approval of the manuscript.

Sarah P. Young supervised the analysis of Glc_4, performed data analysis and interpretation, and participated in the preparation of the manuscript and final approval of the manuscript.

Jennifer Goldstein ensured compliance with Duke Health System Institutional Review Board and participated in the preparation and final approval of the manuscript.

P. Brian Smith performed statistical analysis of the data and participated in the preparation and final approval of the manuscript

Ni-Chung Lee contributed to patient care and data collection, revision, and final approval of the manuscript.

Shu-Chuan Chiang contributed to sample management, revision, and final approval of the manuscript.

Adviye A. Tolun, Haoyue Zhang, and Amie E. Vaisnins conducted Glc_4 analysis and data processing and assisted with the data analysis and final approval of the manuscript.

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References

Chien YH, Chiang SC, Zhang XK et al (2008) Early detection of Pompe disease by newborn screening is feasible: results from the Taiwan screening program. Pediatrics 122(1):e39–e45

Chien YH, Lee NC, Thurberg BL et al (2009) Pompe disease in infants: improving the prognosis by newborn screening and early treatment. Pediatrics 124(6):e1116–e1125

Chien YH, Lee NC, Huang HJ, Thurberg BL, Tsai FJ, Hwu WL (2011) Later-onset Pompe disease: early detection and early treatment initiation enabled by newborn screening. J Pediatr 158 (6):1023–1027

Hagemans M, Winkel L, Van Doorn P et al (2005) Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. Brain 128(Pt 3):671–677

Hirschhorn R, Reuser A (2001) Glycogen storage disease type II: acid alpha-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly WS and Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 3389–3420

Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D (2006) A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. J Pediatr 148 (5):671–676

Kishnani PS, Corzo D, Leslie ND et al (2009) Early treatment with alglucosidase alpha prolongs long-term survival of infants with Pompe disease. Pediatr Res 66(3):329–335

Kroos MA, Mullaart RA, Van Vliet L et al (2008) p.[G576S; E689K]: pathogenic combination or polymorphism in Pompe disease? Eur J Hum Genet 16(8):875–879

Kumamoto S, Katafuchi T, Nakamura K et al (2009) High frequency of acid alpha-glucosidase pseudodeficiency complicates newborn screening for glycogen storage disease type II in the Japanese population. Mol Genet Metab 97(3):190–195

Kumlien J, Chester MA, Lindberg BS, Pizzo P, Zopf D, Lundblad A (1988) Urinary excretion of a glucose-containing tetrasaccharide. A parameter for increased degradation of glycogen. Clin Chim Acta 176(1):39–48

Labrousse P, Chien YH, Pomponio RJ et al (2010) Genetic heterozygosity and pseudodeficiency in the Pompe disease newborn screening pilot program. Mol Genet Metab 99 (4):379–383

Young SP, Stevens RD, An Y, Chen Y-T, Millington DS (2003) Analysis of a glucose tetrasaccharide elevated in Pompe disease by stable isotope dilution–electrospray ionization tandem mass spectrometry. Anal Biochem 316(2):175–180

Young SP, Zhang H, Corzo D et al (2009) Long-term monitoring of patients with infantile-onset Pompe disease on enzyme replacement therapy using a urinary glucose tetrasaccharide biomarker. Genet Med 11(7):536–541

Young SP, Piraud M, Goldstein JL et al (2012) Assessing disease severity in Pompe disease: the roles of a urinary glucose tetrasaccharide biomarker and imaging techniques. Am J Med Genet C Semin Med Genet 160C(1):50–58

JIMD Reports
DOI 10.1007/8904_2014_370

RESEARCH REPORT

Developmental Outcomes of School-Age Children with Duarte Galactosemia: A Pilot Study

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Received: 23 April 2014 / Revised: 26 August 2014 / Accepted: 24 September 2014 / Published online: 15 February 2015
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Abstract Duarte galactosemia (DG) is a mild allelic variant of classic galactosemia that results from partial impairment of galactose-1P uridylyltransferase (GALT). Although infants with DG are detected by newborn screening in some US states at close to 1/4,000 live births, most are discharged from follow-up very early in life and there is no consensus on whether these children are at increased risk for any of the long-term developmental delays seen in classic galactosemia. There is also no consensus on whether infants with DG benefit from dietary restriction of galactose. Reflecting the current uncertainty, some states choose to identify infants with DG by newborn screening and others do not. As a first step toward characterizing the developmental outcomes of school-age children with DG, we conducted a pilot study, testing 10 children with DG and 5 unaffected siblings from the same group of families. All children tested were between 6 and 11 years old. We used standardized direct assessments and parent-response surveys to collect information regarding cognition, communication, socio-emotional, adaptive behavior, and physical development for each child. Despite the small sample size, our data demonstrated some notable differences between cases and controls in socio-emotional development, in delayed recall, and in auditory processing speed. These results confirm that direct assessment of school-age children with DG can detect subtle but potentially problematic developmental deficits, and underscore the need for a larger study which has sufficient power to evaluate these outcomes while controlling for potentially confounding factors.

Communicated by: Gerard T. Berry, MD

Competing interests: None declared

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Introduction

Duarte galactosemia (DG) affects an estimated 1/4,000 live births every year in the United States (USA) (Fernhoff 2010; Pyhtila et al. 2014); this is close to 10 times the number who are affected by classic galactosemia (CG). Unlike the potentially lethal CG, which results from profound loss of galactose-1-P uridylyltransferase (GALT), DG occurs in patients who are compound heterozygotes for one mild (D or D2) and one severe (G) allele of *GALT*. Infants with DG demonstrate about 25% normal GALT activity (reviewed in (Fridovich-Keil and Walter 2008)) and as a result have difficulty metabolizing galactose – a sugar abundant in milk. Like patients with CG, infants with DG accumulate abnormally high levels of galactose metabolites following exposure to breast milk or milk formula (Ficicioglu et al. 2010).

Most infants diagnosed with DG in the USA come to clinical attention because of an abnormal newborn screening (NBS) result for galactosemia (Pyhtila et al. 2014). Median detection rates for DG vary widely among US states, from

essentially zero to more than 1/3,500 births; this range is believed to reflect differences in screening protocol rather than actual differences in prevalence (Pyhtila et al. 2014). Whether NBS *should* be used to identify DG infants, and whether DG infants benefit from early detection and dietary restriction of galactose in infancy, which is the practice in some states, remains unclear; doctors and public health professionals remain divided on the issue (Fernhoff 2010; Pyhtila et al. 2014).

The controversy surrounding DG stems largely from the reality that while the majority of children with classic galactosemia experience significant developmental deficits by the time they enter school (reviewed in (Fridovich-Keil and Walter 2008)), children with DG are generally discharged from follow-up as infants or toddlers so no one knows whether they are also at risk. Only two prior studies have addressed this question. One, by Ficicioglu and colleagues (Ficicioglu et al. 2008), involved direct testing of 28 toddlers and young children with DG, of whom 17 (mean age 3.5 years) consumed a lactose-restricted diet for their first year of life and 11 (mean age 2.2 years) consumed milk. Mean test scores for adaptive behavior, language, and cognitive development for both groups of children were within one standard deviation (1SD) of the reference mean, suggesting no significant deficits. However, these children were very young, and the study did not include a control group, making general conclusions difficult. The second study, by Powell and colleagues (Powell et al. 2009), involved a review of public health records to determine if children with DG were significantly overrepresented among students, 3–10 years old, receiving special educational services in the greater Atlanta metropolitan area. They were, with overrepresentation most pronounced among the older children. While provocative, this result suggesting that children with DG might be at increased risk of developmental deficits was indirect and potentially insensitive to milder developmental and educational effects.

As a first step toward assessing the specific developmental characteristics of school-age children with DG, we conducted a pilot study of 15 children ages 6–11 years – 10 with DG and 5 unaffected siblings recruited from the same group of families. Of the 10 children with DG, parents of seven had expressed concern regarding one or more developmental areas; parents of the other three said that they did not have concerns. Our goal was to assess how children with DG would perform relative to their unaffected peers in those areas of development known to be affected in children with CG (e.g., (Antshel et al. 2004; Bosch et al. 2004; Doyle et al. 2010; Potter et al. 2008; Potter et al. 2013)), using standardized tests of cognition, communication, socio-emotional, adaptive behavior, and physical development. In addition, we assessed how well a parent-response survey correlated with the results of direct testing.

Table 1 Demographic characteristics of study volunteers

Variable	Control group ($n = 5$)	DG: no parental concerns ($n = 3$)	DG: parental concerns ($n = 7$)
Child age in years, mean (SD)	7.56 (1.61)	7.26 (1.56)	9.79 (1.62)
Child gender (M:F)	1:4	1:2	3:4
Race[a,b]			
% Caucasian	80%	100%	85.7%
% Native American	20%	0	14.3%
Socioeconomic status rating[b] (Hollingshead score, mean (SD))	56.6 (5.4)	55.33 (9.24)	49.21 (9.37)
Sibs or parents with unexplained developmental, speech, or behavioral problems?	None	None	1 (yes, ADHD) 5 (no)[c]
Gross annual income[b]			
% <$100,000	20%	0%	71.4%
% ≥$100,000	80%	100%	28.6%

[a] Race was based on report of maternal background. All fathers were Caucasian

[b] For families in which two children participated (one DG and one unaffected sibling), the same parent/family information (race, income, SES) was included twice, once for each child

[c] Family history was unavailable for one child

Methods

Study Participants

Volunteers in the study included 10 children with DG and 5 unaffected siblings of children with DG, all 6–11 years old, who participated following informed consent and assent in Emory IRB protocol 00062977 (PI: ME Lynch). Demographic characteristics are presented in Table 1, and birth, diet, and diagnostic characteristics are presented in Table 2. Each child was accompanied by at least one parent. These families were recruited from among volunteers consented into a prior study of children identified by NBS as having DG (Emory IRB protocol 00024933 and GA PDH IRB protocol 130306, PI: JL Fridovich-Keil) who agreed to be recontacted and who lived within a two-hour drive of Atlanta. Of the 22 families in the recruitment pool, 14 (63.6%) responded with interest in participating, 12 were scheduled, and 11 actually participated. From parent survey responses collected in the earlier study, the 10 DG children in this study were categorized as having either no known parental concerns (3 children) or at least one developmental area of parental concern (7 children). Each child received a small prize for participating, but families were not reimbursed for travel and were not otherwise financially compensated.

Table 2 Birth and diet history, biochemical and *GALT* genotype data

Variable	DG: no parental concerns ($n = 3$)	DG: parental concerns ($n = 7$)
Birth weight and gestation	≥7 lbs, full term 6 lbs 15 oz, full term 5 lbs 10 oz (33 weeks twin)	≥7 lbs, full term (4) 6 lbs 13 oz, full term 6 lbs 10 oz, full term 4 lbs 15 oz (35 weeks, twin)
Traumatic birth or neonatal event?	None	None
Dietary galactose exposure in first year of life (soy: milk: other[a])	2:0:1	5:1:1
Available RBC GALT activity level[b] (μmol/h/g Hb)	8.9; 7.3; 7.1	8.2; 6.7; 6.1; 5.2; 4.8; 4.2; 3.3
Available *GALT* genotypes	Q188R/ N314D (2) 5 kb del/ N314D (1)	Q188R/ N314D (6) N314D/ unknown (1)
Available RBC Gal-1P (mg%) measured within 5 weeks of birth	25.1; 4.9; 0.5	15.9; 5.1; 2.1; 0.6; 0.2
Available urinary galactitol (mmol/mol creatinine) measured within 5 weeks of birth	154.6; 26.1	26.1; 27.4; <2.0

[a] Dietary galactose "other": In one case the infant drank soy until 6 months of age and then transitioned to milk; in the other case the child alternated feedings of breast milk and soy for the first year

[b] Reference range: The GALT enzyme activity reference range for unaffected controls is 22.2–45.8 μmol/h/g Hb, and for Duarte galactosemia, it is 2.5–9.5 μmol/h/g Hb

Procedures

All testing was conducted in a child development research laboratory of the Emory University Department of Psychiatry and Behavioral Sciences by psychologists and a speech-language pathologist/kinesiologist who were blinded to each child's case versus control status. Evaluations took about 2½ h per child, including breaks, and involved direct assessments of child cognitive ability, communication, auditory processing, and physical/motor development. Every child completed all of the assessments without apparent fatigue or concern. Parents were interviewed about family demographics and history as well as the child's general development, social skills, any problem behaviors, and participation, if any, in educational intervention or special education programs. Relevant biochemical and genetic lab results for study volunteers were obtained via a HIPAA waiver from the Emory Genetics Lab.

Outcome Measures

For this pilot study, we focused on developmental areas known to be affected in children with classic galactosemia (e.g., (Antshel et al. 2004; Bosch et al. 2004; Doyle et al. 2010; Potter et al. 2008; Potter et al. 2013)). Whenever a standardized measure was available, we used such an instrument to allow comparison with population norms in which standard scores (SS and *T*-scores) adjust for age and gender. The exceptions were the demographic and informational questionnaires developed for this pilot as well as the measurement of Auditory Brainstem Evoked Response (ABER), described below, and some of the measures of motor functioning. The measures used, with references, are listed in Tables 3 and 4.

To assess child socio-emotional development and behavior, we asked parents to complete interviews and rating scales concerning their child's behavior, general development, and social skills as well as to provide information about family background variables and any special education or educational intervention experiences their child may have had. Parent-response surveys included the Developmental Profile-3 (DP-3) (Alpern 2007), a developmental screening instrument; the Child Behavior Checklist (CBCL) (Achenbach and Rescorla 2001), which measures eight behavioral areas that can be problematic for school-age children; and the Social Skills Improvement System (SSIS) Rating Scales, Parent Form (Gresham and Elliott 2008), a measure of the child's social skills and problems, if any, with social interaction. Both the CBCL and the SSIS have scales that compare responses to those of children diagnosed with behavioral disorders, including autism and attention deficit hyperactivity disorder (ADHD). A number of aspects of cognitive development were assessed including global intelligence and visual-motor function, memory, working memory, processing speed, and sustained attention.

Auditory processing was measured with the ABER methodology using the Biopac STM100C stimulator (http://www.biopac.com/Manuals/app_pdf/app105b.pdf-ABER) as described previously (Kable et al. 2009; Salamy et al. 1975). This test is used in experimental contexts to measure aspects of auditory brainstem response such as latency to respond. Each ABER test was conducted in a quiet room with dim lighting by a trained tester who was blind to the case/control status of the child. Latency of the

Table 3 Measures used in direct child assessment

Variable	Measure
Cognitive skills	
Visual-motor skill	Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI) (Beery et al. 2010)
Memory	Differential Abilities Scale, 2nd Edition (DAS-II) (Elliott 2007)
	Recall of Objects, Immediate and Delayed
Working memory	DAS II – Recall of Digits Forward, Recall of Digits Backward
Processing speed	DAS-II – Speed of Information Processing Test
Sustained attention	NEPSY – Visual Attention Task (Korkman et al. 1998)
Intelligence	Wechsler Abbreviated Scales of Intelligence-II (WASI-II) (Vocabulary and Matrix Reasoning subtests) (Wechsler 2011)
Language/communication	
Articulation	Goldman-Fristoe Test of Articulation-2 (Goldman and Fristoe 2000)
Receptive and Expressive language	OWLS-II Oral and Written Language Scales, Second Edition (Carrow-Woolfolk 2011) Listening Comprehension (LC) (receptive) and Oral Expression (OE) (expressive) subtests only
Auditory processing	Auditory Brainstem Evoked Response (see Kable et al 2009)
Movement/physical	
Balance, coordination, manual dexterity	Movement Assessment Battery for Children (MABC) (Henderson and Sugden 1992)
Tongue strength	Iowa Oral Performance Test (www.IOPImedical.com)

Table 4 Child behavior and social skills measures based on parent response

Variable	Measure
Social skills	Social Skills Improvement System (SSIS) Rating Scales (Gresham and Elliott 2008) Social Skills and Problem Behavior. Subscales (social skills): communication, responsibility, cooperation, assertion, empathy, self-control, engagement. Subscales (Problem Behavior): externalizing, bullying, hyperactivity/inattention, internalizing, autism spectrum
Behavior problems	Child Behavior Checklist (CBCL) 6–18 (Achenbach and Rescorla 2001). Provides scores on three broad dimensions of problem behavior (internalizing, externalizing, and total problems) and subscale scores (anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, aggressive behavior)
Developmental problems	Developmental Profile-3 (DP-3) (Alpern 2007). Provides screening information on possible developmental delay in five areas: physical, adaptive behavior, socio-emotional, cognitive, and communication
Participation in special education or intervention	Questionnaire developed by project staff to obtain information on parent concerns; school placement of child, services and interventions child experienced; medications for behavioral problems
Potential confounding variables	Demographic Questionnaire (includes socioeconomic status)

auditory brainstem response peak from stimulus onset was determined using AcqKnowledge software from Biopac (see Biopac #AS105).

Speech articulation was assessed with the Goldman-Fristoe Test of Articulation 2 (GFTA-2; (Goldman and Fristoe 2000)). Receptive and expressive language were assessed using the Listening Comprehension and Oral Expression subtests of the Oral and Written Language Scales, Second Edition (OWLS-II). Tongue strength was assessed using the Iowa Oral Performance Instrument (IOPI) with the standard tongue bulb (IOPI Northwest 2005; (Potter et al. 2013)).

Physical/motor development measures included assessment of movement (balance, dexterity, and coordination/ball skills) and occurrence of pronounced visible tremors. Balance, manual dexterity, and ball skills were assessed using the Movement Assessment Battery for Children (MABC) (Henderson and Sugden 1992). The MABC included a shape-drawing task, which required the child to restrict his/her drawing to between the inner and outer lines. Hand tremors were scored as present or absent by the examiner (a kinesiologist) who noted if the participant exhibited an obvious kinetic tremor and was unable to draw a smooth line while completing the MABC fine motor task.

A formal assessment of kinetic tremor was not included in the pilot study protocol.

Analyses of Data

Due to the small sample size of this pilot study, we present each outcome category in descriptive terms, providing means and standard deviations (SD) as well as the proportion of children affected in each of the defined groups (controls; DG, total; DG, no parental concerns; DG, parental concerns) (Table 5). The cutoff limits for each range (e.g., normal range, borderline range, clinical range) were defined by the scoring instructions for the test – generally as a function of standard deviations from the control mean. The ABER is not a clinical test so there are no established norms; we therefore used analysis of variance (ANOVA) to assess differences between groups. Sib-sib comparisons, where available, are described in the text. To examine correlations between the parent report variables and corresponding direct testing outcomes, we used Pearson product moment correlations.

Results

Results are reported for analyses comparing variables among volunteers classified as DG, no parental concerns; DG, parental concerns; DG, total; or unaffected sibling controls. Results showing notable differences between the groups that may warrant further investigation are presented in Table 5.

Demographics and Family Information

Demographic characteristics of the study volunteers are presented in Table 1. There were no major differences in distribution of child gender, racial background, or socioeconomic status among groups; most families were European-American and of middle to upper-middle socioeconomic status. All mothers were currently married. All parents reported their children to be in good-to-excellent health at the time of the study. Child age was the only characteristic that differed notably among the groups, with children in the DG, parental concern group, being slightly older than the other groups. No child in the control group had a parent or sibling with unexplained developmental, speech, or behavioral problems. Among the DG volunteers, only one child, in the "parental concern" group, had a parent or sibling with unexplained developmental, speech, or behavioral difficulties (Table 1); family history information was unavailable for one child.

Birth and Diet History: Biochemical and *GALT* Genotype Data

In order to assess neonatal history and to better characterize the GALT deficiency and early galactose exposure for each DG child, we gathered birth and diet history information from the families and biochemical and *GALT* genotype data from the lab that performed the testing. As presented in Table 2, one child in each of the DG, no parental concern, and DG, parental concern groups, was a twin born early and at relatively low birth weight; all other children were born at term with normal birth weight. One child in the control group was also a twin born early and at low birth weight. None of the children in the study was known to have experienced a traumatic birth or neonatal event.

The majority of DG children in both the "parental concerns" and "no parental concerns" groups had experienced dietary restriction of galactose in infancy (Table 2); this was not surprising as this has been the intervention recommended for DG infants in Georgia. Biochemical lab results demonstrated a range of RBC GALT activities from 3.3 to 8.9 μmol/h/gHb and RBC galactose-1P and urinary galactitol levels in both groups ranging from normal to clearly elevated. The most elevated metabolite values were detected in one of the children in the DG, no parental concerns group.

Cognitive Outcomes

The cognitive cluster of direct assessments included measures of intelligence, visual-motor skill, memory, speed of information processing, and sustained attention. While intelligence was not affected, the pattern of results for the memory tests (Recall-Delayed and Recall of Digits Forward, a measure of auditory memory) suggested that DG children may have more difficulty in this area. For both these aspects of memory, children in the control group had higher scores than those in either the DG,no parental concerns, or the DG, parental concerns groups (Table 5). Other cognitive measures did not show notable differences in this small sample.

Auditory Processing (ABER)

Auditory processing was assessed as it is frequently associated with problems in language development and attention. When the pattern of means was examined for the auditory processing measure (Salamy et al. 1975), it showed longer latency on the initial wave for children in both the DG, no parental concerns, and the DG, parental concerns groups, than in the control group ($p < 0.04$, Table 5). Though our sample size was small, this finding suggests that children with DG may process auditory

Table 5 Notable preliminary findings from DG pilot study

Measure (construct measured)	Control group (mean ± SD) [$n = 5$]	Duarte galactosemia ($n = 10$)		
		DG combined (mean ± SD) [n = 10]	DG no parental concerns (mean ± SD) [n = 3]	DG parental concerns (mean ± SD) [$n = 7$]
Direct child assessments				
Digits forward[a] (*T*-scores) (memory) (normal range: borderline: clinical range)	58.4 ± 8.6 (5:0:0)	47.0 ± 7.8 (7:3:0)	50.0 ± 9.2 (2:1:0)	45.7 ± 7.5 (5:2:0)
Recall-delayed[a] (*T*-scores) (memory) (normal range: borderline: clinical range)	53.2 ± 4.0 (5:0:0)	49.0 ± 4. 8 (9:1:0)	43.6 ± 5.0 (2:1:0)	51.3 ± 2.4 (7:0:0)
ABER[b]: Latency Wave 1 (ms) (auditory processing)	1.45 ± 0.11	1.65 ± 0.14*	1.58 ± 0.04*	1.67 ± 0.15*
OWLS-II[c]: Listening comprehension (SS) (receptive language) (normal range: borderline: clinical range)	118.6 ± 9.8 (5:0:0)	111.7 ± 12.4 (10:0:0)	122.0 ± 4.6 (3:0:0)	107.3 ± 12.2 (7:0:0)
OWLS-II[c]: Oral expression (SS) (expressive language) (normal range: borderline: clinical range)	111.4 ± 10.5 (5:0:0)	108.2 ± 10.6 (9:1:0)	110.3 ± 4.2 (3:0:0)	107.3 ± 12.7 (6:1:0)
MABC[d]: Total (percentile scores) (motor) (normal range: borderline: clinical range)	48.0 ± 43.2 (3:0:2)	25.2 ± 36.7 (3:1:6)	50.0 ± 38.7 (2:1:0)	14.6 ± 32.9 (1:0:6)
Tremor[e]: (motor) # without:# with pronounced hand tremor	4:1	6:4	3:0	3:4
Parent-response measures				
DP-3[f]: Socio-emotional (SS) (socio-emotional) (normal range: borderline: clinical range)	101.6 ± 9.0 (5:0:0)	84.7 ± 14.9 (4:5:1)	93.7 ± 14.2 (2:1:0)	80.9 ± 14.4 (2:4:1)
CBCL[f]: Withdrawn/depressed (*T*-scores) (socio-emotional) (normal range: borderline: clinical range)	50.8 ± 1.1 (5:0:0)	57.4 ± 7.2 (9:1:0)	50.7 ± 1.2 (3:0:0)	60.3 ± 6.7 (6:1:0)
CBCL[g]: Social problems (*T*-scores) (socio-emotional) (normal range: borderline: clinical range)	52.8 ± 5.2 (5:0:0)	57.9 ± 7.5 (7:3:0)	50.7 ± 0.6 (3:0:0)	61.0 ± 6.8 (4:3:0)
CBCL[g]: Thought problems (*T*-scores) (socio-emotional) (normal range: borderline: clinical range)	51.8 ± 2.1 (5:0:0)	56.7 ± 5.0 (9:1:0)	52.0 ± 1.7 (3:0:0)	58.7 ± 4.6 (6:1:0)
CBCL[g]: Attention problems (*T*-scores) (socio-emotional) (normal range: borderline: clinical range)	54.0 ± 6.0 (5:0:0)	60.6 ± 10.1 (6:4:0)	50.7 ± 0.6 (3:0:0)	64.9 ± 9.0 (3: 4:0)
CBCL[g]: Internalizing (*T*-scores) (socio-emotional) (normal range: borderline: clinical range)	43.8 ± 9.7 (5:0:0)	56.3 ± 8.7 (7:0:3)	49.7 ± 8.5 (3: 0:0)	59.1 ± 7.5 (4:0:3)
CBCL[g] Total problems (*T*-scores) (normal range: borderline: clinical range)	45.2 ± 13.4 (4:1:0)	55.4 ± 10.4 (7:1:2)	44.3 ± 6.5 (3:0:0)	60.1 ± 7.8 (4:1:2)
SSIS[h]: Problem (SS) (social/behavior) (normal range: borderline: clinical range)	97.8 ± 17.9 (4:1:0)	105.8 ± 17.3 (6:4:0)	86.3 ± 3.5 (3:0:0)	114.1 ± 13.2 (3:4:0)
SSIS[h]: Hyperactivity/inattention (raw scores) (social/behavior) (normal range: concern)	4.2 ± 3.8 (4:1)	7.7 ± 5.2 (6:4)	2.3 ± 1.5 (3:0)	10.0 ± 4.4 (3:4)
SSIS[h]: Autism spectrum (raw scores) (social/behavior) (normal range: concern)	7.2 ± 6.3 (4:1)	11.6 ± 5.3 (7:3)	5.7 ± 2.5 (3:0)	14.1 ± 3.9 (4:3)
Educational intervention (# not receiving: # receiving educational intervention in one or more areas)	5:0	5:5	3:0	2:5
Prescription medication for behavioral issues (# not taking:# taking prescription medication for behavioral issues)	5:0	7:3	3:0	4:3

[a] Differential Ability Scales, 2nd Ed
[b] Auditory Brainstem Evoked Response
[c] OWLS-II Oral and Written Language Scales, Second Edition (standard scores, SS)
[d] Movement Assessment Battery for Children
[e] Child had observable hand tremor and was unable to draw a smooth line
[f] Developmental Profile, 3rd Ed
[g] Child Behavior Checklist
[h] Social Skills Improvement System Rating Scale; SS = standard scores
*ABER scores are not used clinically and do not have normative ranges established. Though the sample size was small, analysis of variance (ANOVA) for these ABER scores indicated that the DG and control groups were distinct ($p < 0.04$)

information more slowly and may have relative difficulties in perception of speech sounds.

Communication

All children were within normal limits on measures of expressive and receptive language applied in this pilot (standard scores (SS) of 85–134 on the OWLS-II). The pattern of means, however, suggested that children in the DG, parental concerns group, may have lower listening or receptive language skills than those in the other two groups (Table 5). Two children in the DG, parental concerns group, also demonstrated speech sound disorders in the pilot (both with SS of 81 on the GFTA-2); one of these children was later diagnosed clinically with speech delay requiring therapy. One DG child who did not demonstrate a speech sound disorder in the pilot (at age 11) had been diagnosed with speech issues early in life and received speech therapy starting at age 3. Another DG child who did not demonstrate a speech sound disorder in the pilot was later diagnosed clinically with an expressive language deficit. All other children in the pilot study had normal speech production as measured in the pilot. All the children had normal tongue strength (max pressures of 34–81 kPa).

Physical/Motor Development

We used the Movement Assessment Battery for Children (MABC) to assess physical/motor development. Six of the seven children in the DG, parental concerns group, and two of the five children in the control group had total scores at or below the 5th percentile of the reference range on the MABC, which is the most frequently used cutoff score for diagnosing a coordination disorder (Potter et al. 2013) (Table 5). All of the children in the DG, no parental concerns group, had scores above the 5th percentile on motor skills, though one scored just above the cutoff (at the 6th percentile). Of note, four of the seven children in the DG, parental concerns group, had a pronounced kinetic hand tremor observed while attempting to draw a smooth line for the MABC fine motor tasks. One child in the control group, a sister of a child with observable tremor in the DG, parental concerns group, also had pronounced kinetic hand tremor when drawing. All other children tested in the pilot study were able to complete the drawing task without difficulty and with no pronounced tremors observed.

Socio-emotional and Behavioral/Social Skills Outcomes

Results from three ratings of socio-emotional outcomes suggested that functioning in this area was strongly affected in the children with DG. On the Developmental Profile-3 (DP-3), a screening measure, parents reported lower scores on the overall socio-emotional measure for children in the DG: parental concerns group when compared to the other two groups (Table 5). This finding from the DP-3 screening measure was confirmed using the more comprehensive Child Behavior Checklist (Table 5). Children in the DG, parental concerns group, were reported to have more problems on internalizing and total problems as well as higher problem scores on some of the subscales (withdrawn/depressed, social problems, thought problems, and attention problems). Parent ratings of their children's social skills, quantified using the SSIS instrument, again showed a similar pattern, with children in the DG, parental concerns group, showing higher scores than the other two groups on the problem behavior scale as well as on the hyperactivity/inattention and autism spectrum subscales.

Educational and Intervention Status

Parent report of the child's educational history was one of the factors used to classify DG volunteers into either the "parental concerns" or "no parental concerns" groups, so it was not surprising that parents in the DG "parental concerns" group were far more likely to report that they had concerns about their child's school performance, that the child had received intervention in at least one of seven areas, or that the child was taking medications for behavioral issues (Table 5). Only one child (in the DG, parental concerns group) had repeated a grade and the same child was the only one with an Individualized Educational Plan (IEP). In total there were three children in the sample taking medication for behavioral issues; they were all from the DG, parental concerns group (Table 5).

Relationship Between Parent-Response Results (DP-3) and Direct Testing for Outcome Parameters

Correlational analyses were completed for the pilot data to examine relations between the DP-3 parent rating scales and corresponding child outcome variables from direct testing. In general, we found that direct test results assessing a given outcome, for example, cognition or language, correlated significantly with the DP-3 scale screening for a similar construct, in this case cognitive and communication skills (Table 6). As expected, cognitive and language measures also correlated with some of the other outcome domains screened by the DP-3, for example, adaptive behavior, as these outcomes are related. As expected, we did not see correlations between cognition or language test results and parent-reported measures of physical development, as these outcomes are unrelated.

Table 6 Comparison of results from the DP-3 parent-response survey and direct tests of child cognitive and language development

	Pearson correlations between DP-3 parent ratings and direct testing summary measures of cognitive and language outcomes ($n = 15$)	
DP-3 scales	WASI-II full-scale IQ (cognitive measure)	OWLS-II oral expression (language measure)
Communication	0.705** ($p = 0.003$)	0.648** ($p = 0.009$)
Adaptive	0.757** ($p = 0.001$)	0.551* ($p = 0.033$)
Socio-emotional	0.654** ($p = 0.008$)	0.461 ($p = 0.084$)
Cognitive	0.785** ($p = 0.001$)	0.673** ($p = 0.006$)
Physical	0.287 ($p = 0.299$)	0.439 ($p = 0.102$)
General development	0.736** ($p = 0.002$)	0.646** ($p = 0.009$)

To examine the relationship between DP-3 parent-response survey results and some of the direct measures used to test developmental outcomes of children in this pilot study, we completed Pearson product–moment correlations using SPSS. Correlations calculated between each pair of variables are presented together with the two-tailed p-value for that computation
*$p < 0.05$
**$p < 0.01$

Comparisons Among Siblings

Our study volunteers included four sets of siblings, with one member of each set an unaffected control and the other a child with DG (one with no parental concerns, three with parental concerns). Looking at outcome measures that showed the greatest differences between cases and controls (e.g., ABER, digits forward, DP-3 social/emotional), we examined the data to query whether DG, control sib-pairs, had scores that were more alike than unrelated pairs. To the accuracy afforded by our small data set, the answer was no.

Discussion

We undertook this pilot study to assess how school-age children with DG would perform relative to their unaffected peers in areas of development either previously implicated as problematic in this population (Powell et al. 2009) or known to be affected in children with classic galactosemia (e.g., (Antshel et al. 2004; Bosch et al. 2004; Doyle et al. 2010; Potter et al. 2008; Potter et al. 2013)). We used a combination of standardized direct tests and parent-report surveys to assess cognitive, communication, socio-emotional, adaptive behavior, and physical development of 10 children with DG and 5 unaffected siblings, all ages 6–11 years old.

While our sample size was small, limiting the ability to control for other factors that might affect outcomes, we noted several key findings. Most important, we identified a number of specific areas of development where there was evidence of a difference in performance related to DG status. Of note, sometimes these deficits were recognized by parents as evidenced by skewed distribution between the DG, parental concerns, and DG, no parental concerns groups; other times these deficits showed up in both DG groups.

The most pronounced areas of difference between cases and controls involved aspects of auditory processing, memory, and socio-emotional development. For example, children in both the DG, parental concerns, and DG, no parental concerns groups, demonstrated slower processing of auditory information (ABER) than children in the control group. This finding is of concern because slower auditory processing is frequently associated with problems in language development and attention. Consistent with this concern, two children in the DG, parental concerns group, demonstrated speech sound disorders in the pilot and three DG children from the study demonstrated speech or expressive language difficulties in clinical testing. Interestingly, a younger brother from one of the families whose DG child in the study was categorized as “no parental concerns” was later diagnosed clinically with apraxia of speech and learning delay. This child, who was too young to be included in the pilot, also has DG.

Children in both the DG, parental concerns, and the DG, no parental concerns groups, also showed evidence of less efficient auditory memory when assessed using Recall of Digits Forward, one of the memory tasks in the cognitive protocol. Children in the DG, parental concerns group, also showed evidence of lower listening or receptive language skills than children in the other two groups. These problems may be related only to auditory processing, but there may also be a contribution from memory. Further exploration of these functions in a larger sample will allow us to answer this question.

With regard to socio-emotional development, results of CBCL and SSIS ratings suggested that children in the DG, parental concerns group had greater internalizing problems (e.g., anxiety, depression) as well as difficulties with social behavior compared with controls. These problems were similar to those reported previously in individuals with classic galactosemia (Antshel et al. 2004; Bosch et al. 2004; Ryan et al. 2013; Waisbren et al. 2012). Parent reports on the SSIS Autism Spectrum subscale indicated that one of five control children and three of seven DG children in the study scored in the “concern” range. Specific symptoms of concern assessed in this section of the SSIS survey included heightened anxiety, social problems, and social withdrawal. As noted above, these same sorts of internalizing behaviors have also been reported for patients with

classic galactosemia (Antshel et al. 2004; Bosch et al. 2004; Ryan et al. 2013; Waisbren et al. 2012). Children in the DG, no parental concerns group did not exhibit these issues, which is not surprising considering that both the CBCL and SSIS are parent-response surveys, and earlier parent reports were used to stratify the DG children into "parental concern" and "no parental concern" groups.

While it is clearly possible that some of the developmental deficits we observed among our study volunteers with DG reflected ascertainment bias of the sample, the overlap between our results and those reported by Powell and colleagues (Powell et al. 2009), that were not subject to the same ascertainment bias, is concerning. That some of these deficits were found among children in both the DG, parental concerns, and DG, no parental concerns groups, also suggests that ascertainment bias cannot fully account for our observations.

Laboratory studies demonstrated that all of the children with DG had GALT activity, RBC Gal-1P, and urinary galactitol levels in the expected ranges for infants with Duarte galactosemia; in fact, the highest Gal-1P and urinary galactitol levels were reported for a child in the DG, no parental concerns group, suggesting that abnormal neonatal metabolites were not a deciding factor in determining long-term outcome. Finally, that almost all of the DG children included in our pilot study experienced dietary restriction of galactose in the first year of life raises the question of whether a population of DG children who consumed milk in infancy might have demonstrated similar or perhaps different outcomes.

Conclusion

The results of our pilot study support the hypothesis that DG patients, as a group, may experience subtle developmental deficits by mid-childhood that could impact child health and well-being, including deficits in auditory processing, memory, and socio-emotional development, among other areas. As this was a very small pilot study and confined in terms of socioeconomic status and other variables, it is not clear if these results will be generalizable to the DG population as a whole. In addition, since the sample was recruited from a state (Georgia) that generally recommends dietary restriction of galactose in the first year of life for newborns diagnosed with DG, interpretation of results cannot answer questions regarding the impact of such restrictions. The concerns raised by this pilot study underscore the need for a larger study to assess developmental outcomes of school-age children with DG who reflect the full spectrum of galactose exposure in infancy with a sample size sufficient to control for other familial and social factors that may affect outcomes. If our pilot results are confirmed in such a study, they may offer the possibility of early identification and either proactive or reactive intervention to improve the long-term outcomes of at-risk children.

Acknowledgments First and foremost, we want to thank the 11 wonderful families who participated in this pilot research study; without them none of this work would have been possible. We also thank the many researchers from the Department of Psychiatry and Behavioral Sciences at Emory University who volunteered their personal time and professional expertise, including Julie Kable PhD, Karen Howell, PhD, Molly Millians EdD, Shital Gaitonde PhD, Elles Taddeo EdD, and Jonathan Cook. We are also grateful to Kelly Shaw for her assistance gathering lab results data. Finally, we are thankful to Dr. Brad Therrell of the National Newborn Screening Information System (http://nnsis.uthscsa.edu) for his encouragement and support of this project and to the University of Texas Health Sciences Center for providing initial funding for this work (Award# 0000017221, PI: Fridovich-Keil).

1-Sentence Synopsis

Direct assessment revealed a number of subtle but concerning developmental deficits among a cohort of 10 school-age children with Duarte galactosemia.

Compliance with Ethical Guidelines

Conflict of Interest

Mary Ellen Lynch declares that she has no conflict of interest.

Nancy Potter declares that she has no conflict of interest.

Claire Coles declares that she has no conflict of interest.

Judith Fridovich-Keil declares that she has no conflict of interest.

Informed Consent

"All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5)."

All data from specific individuals reported here were collected following appropriate informed consent and with approval by the Emory University Institutional Review Board (IRB# 00024933, PI: Fridovich-Keil and IRB#00062977, PI: Lynch).

Animal Rights

”This article does not contain any studies with animal subjects performed by any of the authors.”

Contributions of Each Author

Mary Ellen Lynch coordinated the pilot study described in this manuscript including IRB requirements, recruitment, and scheduling; served a lead role in assembling and analyzing the data presented; and helped write and edit the manuscript.

Nancy Potter served a lead role in data gathering during the pilot study and also helped to write and edit the manuscript.

Claire Coles helped coordinate the pilot study, served a lead role in data analysis and interpretation, and helped write and edit the manuscript.

Judith Fridovich-Keil initiated the project, coordinated the efforts of the other authors, conducted parent interviews during the pilot, and wrote most of the manuscript.

References

Achenbach T, Rescorla L (2001) Manual for ASEBA school-age forms & profiles. University of Vermont, Research Center for Children, Youth, & Families, Burlington

Alpern G (2007) Developmental profile, 3rd edn. Western Psychological Services, Los Angeles

Antshel K, Epstein I, Waisbren S (2004) Cognitive strengths and weaknesses in children and adolescents homozygous for the galactosemia Q188R mutation: a descriptive study. Neuropsychology 18:658–664

Beery K, Buktenica N, Beery N (2010) The Beery-Buktenica developmental test of visual-motor integration, 6th edn. http://www.pearsonassessments.com

Bosch AM, Grootenhuis MA, Bakker HD, Heijmans HS, Wijburg FA, Last BF (2004) Living with classical galactosemia: health-related quality of life consequences. Pediatrics 113:e423–e428

Carrow-Woolfolk E (2011) Oral and written language scales, 2nd edn. Pearson Assessments, Minneapolis

Doyle C, Channon S, Orlowska D, Lee P (2010) The neuropsychological profile of galactosaemia. J Inherit Metab Dis 33:603–609

Elliott C (2007) Differential ability scales, 2nd edition (DAS-II). Pearson, San Antonio

Fernhoff PM (2010) Duarte galactosemia: how sweet is it? Clin Chem 56:1045–1046

Ficicioglu C, Thomas N, Yager C et al (2008) Duarte (DG) galactosemia: a pilot study of biochemical and neurodevelopmental assessment in children detected by newborn screening. Mol Genet Metab 95:206–212

Ficicioglu C, Hussa C, Gallagher PR, Thomas N, Yager C (2010) Monitoring of biochemical status in children with Duarte galactosemia: utility of galactose, galactitol, galactonate, and galactose 1-phosphate. Clin Chem 56:1177–1182

Fridovich-Keil JL, Walter JH (2008) Galactosemia. In Valle D, Beaudet A, Vogelstein B, Kinzler K, Antonarakis S, Ballabio A (eds) The online metabolic & molecular bases of inherited disease. McGraw Hill, http://www.ommbid.com/

Goldman R, Fristoe M (2000) Goldman-Fristoe test of articulation, 2nd edn. Pearson Assessments, Minneapolis

Gresham F, Elliott S (2008) SSIS (Social Skills Improvement System) Rating Scales Minneapolis, MN: NCS Pearson, Inc, http://www.pearsonassessments.com

Henderson S, Sugden D (1992) Movement assessment battery for children manual. The Psychological Corporation Ltd., London

Kable J, Coles C, Lynch M, Carroll J (2009) The impact of maternal smoking on fast auditory brain responses. Neurotoxicol Teratol 31:216–224

Korkman M, Kirk U, Kemp S (1998) NEPSY: a developmental neuropsychological assessment. Psychological Corporation, San Antonio

Potter N, Lazarus J, Johnson J, Steiner R, Shriberg L (2008) Correlates of language impairment in children with galactosaemia. J Inher Metab Dis 31:524–532

Potter N, Nievergelt Y, Shriberg L (2013) Motor and speech disorders in classic galactosemia. J Inher Metab Dis Reports 11:31–41

Powell KK, Van Naarden BK, Singh RH, Shapira SK, Olney RS, Yeargin-Allsopp M (2009) Long-term speech and language developmental issues among children with Duarte galactosemia. Genet Med 11:874–879

Pyhtila B, Shaw K, Neumann S, Fridovich-Keil J (2014) Newborn screening for galactosemia in the United States: looking back, looking around, and looking ahead. J Inher Metab Dis Reports, epub ahead of print

Ryan E, Lynch M, Taddeo E, Gleason T, Epstein M, Fridovich-Keil J (2013) Cryptic residual GALT activity is a potential modifier of scholastic outcome in school age children with classic galactosemia. J Inher Metab Dis 36:1049–1061

Salamy A, McKean C, Buda F (1975) Maturational changes in auditory transmission as reflected in human brain stem potentials. Brain Res 96:361–366

Waisbren S, Potter N, Gordon C et al (2012) The adult galactosemic phenotype. J Inherit Metab Dis 35:279–286

JIMD Reports
DOI 10.1007/8904_2014_374

CASE REPORT

Molecular Diagnosis of Hereditary Fructose Intolerance: Founder Mutation in a Community from India

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Received: 05 February 2014 / Revised: 09 October 2014 / Accepted: 13 October 2014 / Published online: 18 January 2015
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Abstract Hereditary fructose intolerance (HFI) is a difficult-to-confirm diagnosis, requiring either invasive liver biopsy-enzyme assay or potentially hazardous fructose challenge test or expensive molecular genetic analysis. Therefore, worldwide there has been a trend towards finding "common mutations" in distinct ethnic groups to simplify the process of diagnosis. The nonspecific presentation of the disease often leads to diagnostic confusion with other metabolic liver disorders such as glycogenoses, galactosemia, and tyrosinemia. This leads to much delay in diagnosis with consequent harm to the patient.

We report mutations in the *ALDOB* gene, from eleven Indian patients, seven of whom belong to the Agarwal community. Six patients from the Agarwal community and two non-Agarwal patients harbored one novel mutation, c.324+1G>A (five homozygous and one heterozygous), in the *ALDOB* gene. Haplotyping performed in families confirmed a founder effect. The community has been known to harbor founder mutations in other genes such as the *MLC1*, *PANK2*, and *CAPN3* genes, thus providing another evidence for a founder effect in the community in case of HFI. This may pave the path for a simpler and quicker test at least for this community in India. In addition to the founder mutation, we report four other novel mutations, c.112+1delG, c.380-1G>A, c.677G>A, and c.689delA, and a previously reported mutation, c.1013C>T, in the cohort from India.

Introduction

Hereditary fructose intolerance (HFI, OMIN #229600) is a potentially fatal inborn error of metabolism (IEM) resulting from deficiency of aldolase B enzyme (EC 4.1.2.13), encoded by the *ALDOB* gene (OMIM *612724) in the liver and kidneys. The disorder is extremely important to recognize as it is easy to treat and has excellent outcome on avoidance of fructose- and sucrose-containing foods and dietary products (Steinmann and Santer 2012). Therapy does not involve any expensive diets or medications. In view of its rarity and practical difficulties in diagnosis, many HFI patients remain undiagnosed and suffer permanent liver damage, increasing the morbidity and sometimes mortality. The most difficult part in HFI has been establishing a diagnosis. Traditionally, these children when suspected on clinical grounds were subjected to "fructose challenge test" which is potentially life-threatening in view of sudden hypoglycemia in patients (Steinmann and Gitzelmann 1981). It has therefore become obsolete now. Enzyme assay requires a liver biopsy, an invasive test, and

Communicated by: Gajja Salomons

Competing interests: None declared

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estimation can be performed only in a few laboratories around the world. The liver sample requires to be shipped under dry ice which adds to the cost and is not often feasible for long distances. Further, the enzyme activity in the liver may be secondarily reduced in a damaged liver (Steinmann and Santer 2012). Thus, the most accurate method to diagnose is through gene studies. Worldwide, many founder and common mutations have been identified in many communities, which has simplified the *ALDOB* molecular analysis by using population-specific custom protocols (Coffee et al. 2010; Esposito et al. 2010). India is a unique mix of many populations but marriage within communities creates the background for the presence of founder mutations in various IEMs.

We performed sequencing of the *ALDOB* gene in eleven patients from nine families, who were strongly suspected to have HFI. We report the existence of a founder mutation in the Agarwal community from North India. The single most common mutation, c.324+1G>A, in the *ALDOB* gene was observed in homozygous form in five and heterozygous state in one of seven patients from the Agarwal community. This mutation was also noted in two non-Agarwal patients in heterozygous state. Sequencing of the *ALDOB* gene also revealed presence of four new mutations in the cohort.

Materials and Methods

Consecutive patients presenting to genetics clinics at Sir Ganga Ram Hospital and All India Institute of Medical Sciences, New Delhi, with history consistent with HFI were recruited. There were 11 patients in the cohort from 9 families from North India, including a trio of mother and two children.

Clinical data was collected from each patient and family including the demographic details, the ethnic group, age of presentation, main clinical features, and investigation details that were performed prior to diagnosis of HFI in each family. Few patients had been on clinical follow-up for few years before recruitment into the study.

Molecular analysis was performed for a diagnostic purpose. Informed consent was taken for the test as well as for haplotype analysis from parents of each case. As this was a diagnostic study of a small scale, no ethical clearance was required from the hospital ethics committee.

Molecular Analysis

DNA was isolated by salting out method from 2 mL of whole blood (Miller et al. 1988). All nine exons and exon-intron boundaries of the *ALDOB* gene were amplified using intronic primers flanking the exons (primer sequences available on request). PCR was performed using 10 pmol of each primer, 1U of Taq DNA polymerase, 1.5 mM $MgCl_2$, and 0.1 mM dNTPs in the recommended buffer with the initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30s, annealing at 58°C for 30 s, and extension at 72°C for 45 s. Sequencing was carried out on ABI 3500 analyzer covering all the nine coding exons encompassing nearly 80 bp of the exon-intron boundaries (Applied biosystems, California). Sequence chromatograms obtained were analyzed with Chromas software (Technelysium, Tewantin, Australia) and blasted with UCSC ALDOB genome browser.

Haplotype analysis was performed to determine if mutation carriers shared a common ancestry. Members from five families were analyzed using eight single-nucleotide polymorphic markers (rs9299349, rs12686025, rs970385, rs10819926, rs2417251, rs10989471, rs10989412, rs1338739) spanning approximately 1 Mb interval at the chromosomal region 9q31.1, which contains the *ALDOB* gene.

Bioinformatics Analysis

Gene-prediction tools GENSCAN, AUGUSTUS, GeneID, and GeneMark were used to analyze the effect of intronic mutations c.112+1delG, c.324+1G>A, and c.380-1G>A (Burge and Karlin 1997; Stanke et al. 2004; Blanco et al. 2007; Lomsadze et al. 2005). The aldolase B protein structure was modeled based on 1XDL.pdb (a thermolabile A149P aldolase B mutant) using Modeller (Sali and Blundell 1993).

Results

In the cohort of 11 patients, 7 were from the Agarwal community. Consanguinity was noted in one family (family 8). Children presented in infancy with typical symptoms of failure to thrive with either vomiting and diarrhea or progressive liver disease. A detailed account of the cases is provided in Table 1. There was only one adult in the cohort, the mother of two children (family 3), who gave history of aversion to fruits and sugar since childhood. Few unusual features are also highlighted in the table such as the custom of offering honey to newborns resulting in much earlier clinical presentation than expected in a few children.

Molecular and Computational Analysis

Molecular genetic analysis of the *ALDOB* gene through sequencing revealed six new sequence variants in 22 mutated alleles, of which c.324+1G>A mutation was noted in 13 of 22 (5 in homozygous state and three heterozygous). Other mutations were: homozygous c.112+1 delG and

Table 1 Detailed clinical presentation and mutation analysis results of cases

Family number	1	2	3	3	3	4	5	6	7	8	9
Cases (initials)	ST	AA	PJ	BJ	BG(mother of PJ and BJ)	RS	SR	SS	MG	SYK	B
Age at onset of Symptoms	2.5 months[a]	4 months	5 months	Birth[a]	Infancy	1 year	1.6 years	10 months	4 months	7 months	9 months
Age at diagnosis	18 months	18 months	1 year	Birth	Adult	1 year	2.5 years	2.5 years	9 months	13 months	3 years
Presenting complaints	Diarrhea, failure to thrive	Abdominal distension, failure to thrive	Vomiting, failure to thrive	Vomiting	Vomiting, aversion to sugar in milk	Vomiting, failure to thrive	Vomiting, failure to thrive	Vomiting after sugar in milk, failure to thrive	Vomiting at weaning (4 months), diarrhea at 8 months	Failure to thrive, constipation, liver disease	Vomiting, diarrhea, and jaundice
Hepatomegaly	Yes	Yes	Yes	No	Not known	Yes	Yes	Yes	Yes	Yes, 4 cm BCM	Yes, 4 cm BCM
Liver dysfunction before treatment	Yes	Yes	Yes	No	Yes (childhood and pregnancy)	Yes, mildly deranged liver enzymes	Yes	Yes	Yes	Yes	Yes, mildly deranged SGPT
Renal involvement	Yes	Yes	No	No	No	No	No	No	No	Yes	No
Liver biopsy performed, findings	Yes, granulomatous hepatitis, macrosteatosis	Yes, details not available	Yes, details not available	No	No	Yes, details not available	Yes, details not available	No	Yes, details not available	Yes, disarrayed lobular architecture, porto-portal bridging fibrosis. Panlobular macro- and microvesicular steatosis	No
Fructose challenge test	Not performed	Not performed	Performed, positive	Not performed	Not performed	Performed, positive	Performed, positive	Performed, positive	Not performed	Not performed	Not performed
Hypoglycemia	No	Yes	No	Yes[a]	Never tested	Yes	No	No	No	No	Yes
Other significant finding	Developmental delay, abnormal transferrin isoforms	Occasional epistaxis, requires vit. K. LFTs normal	No	Seizure at day 17 of life. Normal development	No	No	No	Idiopathic hypokalemia at the age of 10 months	Milk protein allergy (milk allergen specific IgE 41.8 [<0.1]), hyperreactive airway disease	No	Mild gross motor delay
Diet history suggestive	Yes (in retrospect)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gene study: mutations in the *ALDOB* gene	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Compound heterozygous	Homozygous	Homozygous	Compound heterozygous	Homozygous	Compound heterozygous
	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/ intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.112+1 del G, exon 2/ intron 2 boundary	c.677G>A, exon 7	c.324+1G>A, exon 3/intron 3 boundary	c.1013C>T, exon 9	c.324+1G>A, exon 3/intron 3 boundary
						c.1013C>T, exon 9			c.689delA, exon 7		c.380-1G>A intron 4/exon 5 boundary

(continued)

Table 1 (continued)

Family number	1	2	3	3	3	4	5	6	7	8	9
Cases (initials)	ST	AA	PJ	BJ	BG(mother of PJ and BJ)	RS	SR	SS	MG	SYK	B
Current clinical status	5 years – mild developmental delay – attends normal school but learning difficulty +, hepatomegaly (12 cm span at 5 years), normal liver function and renal function	5 years – normal development, attending regular school. Mild hepatomegaly (span 9 cm at 5 years), Liver function tests normal, spleen 8 cm, mild nephrocalcinosis with normal renal functions	7.5 years – normal development, in grade 2 at regular school, mild hepatomegaly, normal liver and renal function	3 years of age – normal development, attending preschool, mild hepatomegaly, normal liver and renal function	Adult – mild hepatomegaly, normal liver and renal functions	10 years – normal development. No seizures, attends regular school. Liver 5 cm below costal margin. Liver function test normal	7 years – mild developmental delay, no seizures, doing well, normal liver and renal functions	7 year 10 months – normal development. Liver palpable 3.5 cm below costal margin. Normal liver and renal function	2 year 7 months – normal development, *poor weight gain*, liver about 3 cm below costal margin, normal liver function and renal function	3.3 years old well child. No hepatomegaly. Normal liver and renal functions	4 years – normal development, *failure to thrive*, *seizure*, liver palpable 3 cm and spleen 2 cm below costal margins, normal liver and renal function
Response to treatment	Satisfactory	Good	Very good	Very good	Good	Very good	Satisfactory	Very good	Good (also has milk protein allergy)	Excellent	Satisfactory

[a] Intake of honey

c.677G>A in one patient each, heterozygous c.689delA and c.380-1G>A in one patient each, and heterozygous and homozygous c.1013C>T mutation in one patient each.

Mutations c.324+1G>A, c.112+1delG, c.380-1G>A, c.677G>A, and c.689delA were noted to be novel, whereas c.1013C>T is a previously reported mutation. A widely used and one of the most accurate gene-prediction program, GENSCAN, predicted that all the three intronic mutations c.112+1delG, c.324+1G>A, and c.380-1G>A disrupt splicing and result in abnormal protein with 8, 4, and 6 amino acids deletion, respectively (Figs. 1 and 2). The GENSCAN predictions were in corroboration with those of three other tools (AUGUSTUS, GeneMark, and GeneID) for c.324+1G>A and c.380-1G>A mutations. However, for the c.112+1delG mutation, AUGUSTUS predicted an insertion of amino acid valine at position 36 (Fig. 1), while both GeneMark and GeneID predicted that the single aldolase B polyprotein would break into two smaller fragments (data not shown). The exonic mutation c.677G>A leads to a p.G226D mutation in the aldolase B protein (Figs. 1 and 2). Although homology modeling predicted that this alteration does not affect the protein structure (data not shown), a distinct single amino acid mutation p.A149P is known to cause losses in thermal stability, quaternary structure, and activity (Malay et al. 2005). The effects of p.A149P mutation could also not be predicted by homology modeling and underscores the difficulties in predicting perturbations due to single amino acid changes (Malay et al. 2005). On the other hand, the second exonic mutation c.689delA is a frameshift mutation that results in premature termination of the polypeptide and deletion of ~130 C-terminal amino acids of aldolase B protein (Figs. 1 and 2). All five mutations were detected to be damaging using SIFT, Polyphen II, and Mutation Taster (http://sift.jcvi.org/www/SIFT_enst_submit.html, http://genetics.bwh.harvard.edu/pph2/, http://www.mutationtaster.org/).

Homozygous mutation c.324+1G>A was noted in five of seven Agarwal patients. In addition, it also occurred in heterozygous form in one Agarwal and two non-Agarwal patients. Another child from the Agarwal community harbored a different c.112+1delG mutation in homozygous state. These findings led us to investigate the presence of a founder effect in the community, which is known to harbor other founder mutations (Gorospe et al. 2004; Bahl et al. 2005; Chabbria et al. 2007).

Haplotype Analysis

Eight single-nucleotide polymorphisms (SNPs) were analyzed flanking the mutation c.324+1G>A. These included both intragenic and flanking regions mapping up to 1 Mb. Homozygosity mapping in the families using

```
ALDOB_no_mutation          MAHRFPALTQEQKKELSEIAQSIVANGKGILAADES-VGTMGNRLQRIKVENTEENRRQF      59
ALDOB_splice_GENSCAN       MAHRFPALTQEQKKELSEIAQSIVANGKGILAADES-V--------RIKVENTEENRRQF      51
ALDOB_splice_AUGUSTUS      MAHRFPALTQEQKKELSEIAQSIVANGKGILAADESVVSTMGNRLQRIKVENTEENRRQF      60
ALDOB_c.677G>A             MAHRFPALTQEQKKELSEIAQSIVANGKGILAADES-VGTMGNRLQRIKVENTEENRRQF      59
ALDOB_c.689delA            MAHRFPALTQEQKKELSEIAQSIVANGKGILAADES-VGTMGNRLQRIKVENTEENRRQF      59
                           ********************************** *        **************

ALDOB_no_mutation          REILFSVDSSINQSIGGVILFHETLYQKDSQGKLFRNILKEKGIVVGIKLDQGGAPLAGT      119
ALDOB_splice_GENSCAN       REILFSVDSSINQSIGGVILFHETLYQKDSQGKLFRNILKEKGIV----LDQGGAPLAGT      107
ALDOB_splice_AUGUSTUS      REILFSVDSSINQSIGGVILFHETLYQKDSQGKLFRNILKEKGIV----LDQGGAPLAGT      116
ALDOB_c.677G>A             REILFSVDSSINQSIGGVILFHETLYQKDSQGKLFRNILKEKGIVVGIKLDQGGAPLAGT      119
ALDOB_c.689delA            REILFSVDSSINQSIGGVILFHETLYQKDSQGKLFRNILKEKGIVVGIKLDQGGAPLAGT      119
                           ****************************************    ************

ALDOB_no_mutation          NKETTIQGLDGLSERCAQYKKDGVDFGKWRAVLRIADQCPSSLAIQENANALARYASICQ      179
ALDOB_splice_GENSCAN       NKETTI------QERCAQYKKDGVDFGKWRAVLRIADQCPSSLAIQENANALARYASICQ      161
ALDOB_splice_AUGUSTUS      NKETTI------QERCAQYKKDGVDFGKWRAVLRIADQCPSSLAIQENANALARYASICQ      170
ALDOB_c.677G>A             NKETTIQGLDGLSERCAQYKKDGVDFGKWRAVLRIADQCPSSLAIQENANALARYASICQ      179
ALDOB_c.689delA            NKETTIQGLDGLSERCAQYKKDGVDFGKWRAVLRIADQCPSSLAIQENANALARYASICQ      179
                           ******      .*********************************************

ALDOB_no_mutation          QNGLVPIVEPEVIPDGDHDLEHCQYVTEKVLAAVYKALNDHHVYLEGTLLKPNMVTAGHA      239
ALDOB_splice_GENSCAN       QNGLVPIVEPEVIPDGDHDLEHCQYVTEKVLAAVYKALNDHHVYLEGTLLKPNMVTAGHA      221
ALDOB_splice_AUGUSTUS      QNGLVPIVEPEVIPDGDHDLEHCQYVTEKVLAAVYKALNDHHVYLEGTLLKPNMVTAGHA      230
ALDOB_c.677G>A             QNGLVPIVEPEVIPDGDHDLEHCQYVTEKVLAAVYKALNDHHVYLEDTLLKPNMVTAGHA      239
ALDOB_c.689delA            QNGLVPIVEPEVIPDGDHDLEHCQYVTEKVLAAVYKALNDHHVYLEGTLLSPTW------      233
                           ********************************************* ***.*.

ALDOB_no_mutation          CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGGMSEEDATLNLNAINLCPLPKPWKLS      299
ALDOB_splice_GENSCAN       CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGGMSEEDATLNLNAINLCPLPKPWKLS      281
ALDOB_splice_AUGUSTUS      CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGGMSEEDATLNLNAINLCPLPKPWKLS      290
ALDOB_c.677G>A             CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGGMSEEDATLNLNAINLCPLPKPWKLS      299
ALDOB_c.689delA            ------------------------------------------------------------      233

ALDOB_no_mutation          FSYGRALQASALAAWGGKAANKEATQEAFMKRAMANCQAAKGQYVHTGSSGAASTQSLFT      359
ALDOB_splice_GENSCAN       FSYGRALQASALAAWGGKAANKEATQEAFMKRAMANCQAAKGQYVHTGSSGAASTQSLFT      341
ALDOB_splice_AUGUSTUS      FSYGRALQASALAAWGGKAANKEATQEAFMKRAMANCQAAKGQYVHTGSSGAASTQSLFT      350
ALDOB_c.677G>A             FSYGRALQASALAAWGGKAANKEATQEAFMKRAMANCQAAKGQYVHTGSSGAASTQSLFT      359
ALDOB_c.689delA            ------------------------------------------------------------      233

ALDOB_no_mutation          ACYTY  364
ALDOB_splice_GENSCAN       ACYTY  346
ALDOB_splice_AUGUSTUS      ACYTY  355
ALDOB_c.677G>A             ACYTY  364
ALDOB_c.689delA            -----  233
```

Fig. 1 Alignment of aldolase B protein sequences. The intronic mutations c.112+1delG, c.324+1G>A, and c.380-1G>A result in 8, 4, and 6 amino acid deletions, respectively (shaded in yellow; GENSCAN program prediction). The residue 226 altered due to exonic mutation c.677G>A is shaded in *gray*. Alignment was performed using Clustal Omega at http://www.ebi.ac.uk/Tools/msa/clustalo/. ALDOB_no_mutation, wild-type protein sequence; ALDOB_splice_GENSCAN and ALDOB_splice_AUGUSTUS, predicted proteins of gene with intronic mutations by GENSCAN and AUGUSTUS, respectively; ALDOB_c.677G>A and ALDOB_c.689delA, protein sequence of gene with exonic mutations c.677G>A and c.689delA, respectively

trios of child and parents revealed identical haplotypes with the c.324+1G>A mutation as shown in the Fig. 3. All patients harboring homozygous mutation had identical haplotype on both chromosomes. The Agarwal patient with heterozygous mutation showed a different haplotype on one chromosome. All the markers tested seemed to be in linkage disequilibrium with the mutation c.324+1G>A in the *ALDOB* gene. This haplotype was different from haplotype in another Agarwal patient who had a different mutation, c.112+1delG. These findings confirm the presence of a founder effect in the subpopulation.

Discussion

HFI is a unique inborn error of metabolism where the major hurdle lies in making an accurate diagnosis rather than treatment which is as simple as removal of fructose and sucrose from the diet. The outcome is related to severity of initial presentation, comorbidities, and the time taken for diagnosis after onset of symptoms and commencement of therapy. In our cases, majority of children are doing well, with no developmental delay and only mild hepatomegaly. Two children have developmental delay related to the

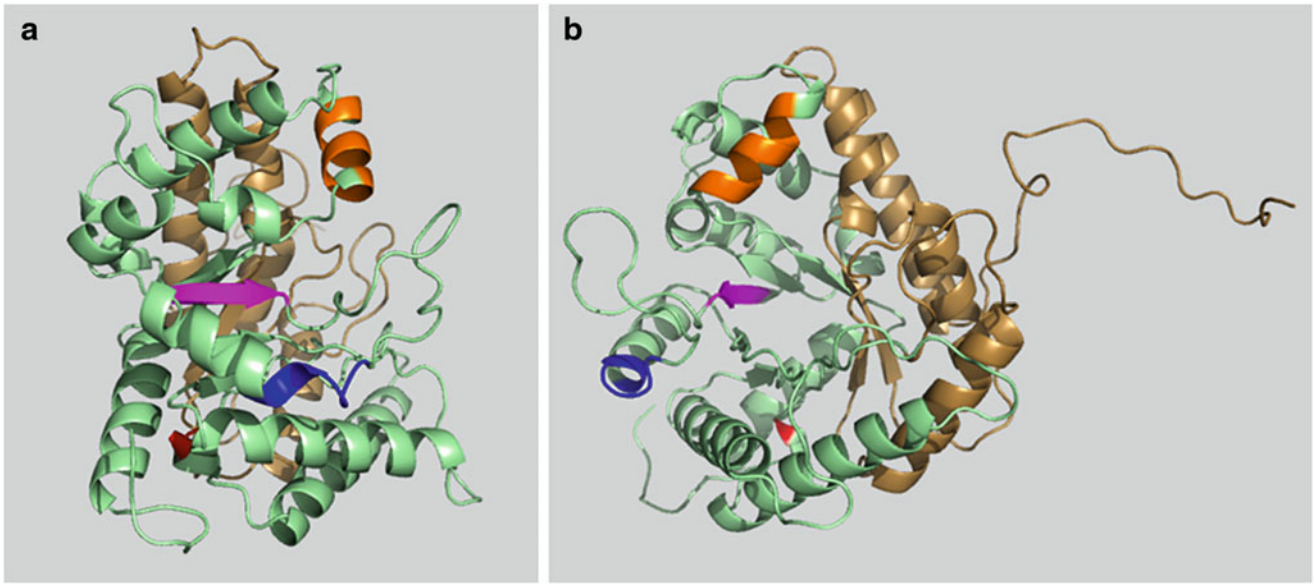

Fig. 2 Modeled structure of wild-type aldolase B. The residues that get deleted (predicted by GENSCAN program) due to intronic mutations c.112+1delG, c.324+1G>A, and c.380-1G>A have been shown in *orange*, *pink*, and *blue*, respectively. The residue 226 altered due to exonic mutation c.677G>A is highlighted in *red*. The structure marked in *brown* gets deleted in gene with c.689delA mutation. The structure in panel B is derived by rotating the panel A structure by 260° along *y*-axis

severity of the symptoms at onset and delay in diagnosis. Traditionally, the disorder has either remained unconfirmed or diagnosed only after invasive liver biopsy enzymology or a fructose challenge test which can prove hazardous in view of rapid hypoglycemia after fructose intake (Steinmann and Gitzelmann 1981). Worldwide, common mutations such as p.A149P mutation, which showed a founder effect in studies, have simplified the diagnostic strategy in certain populations (Brooks and Tolan 1993). There is a paucity of literature on HFI and *ALDOB* gene studies from India, as only few case reports, without mutations, have been reported (Bharadia and Shivpuri 2012; Ananth et al. 2003). This is the first report of mutations in the *ALDOB* gene from India.

In our cohort of patients, all patients have shown remarkable improvement in clinical condition and catch-up growth once treatment is commenced after diagnosis. This is consistent with the established literature (Steinmann and Santer 2012; Steinmann and Gitzelmann 1981) and emphasizes the need for an early and accurate diagnosis.

The Indian population is a heterogeneous mix of various communities, and the common Caucasian mutations are not expected to be present. The gene pool is expected to be heterogeneous but with pockets of homogeneity because of the general trend of marriages within closed communities. The Agarwal community is a large enterprising community consisting of affluent business-class North Indian population originating from a small town of Agroha, near Delhi and spreading all over the world (Singhal 2005). Genetic studies have suggested founder effect in Agarwals in some other genetic disorders like megalencephalic leukoencephalopathy with subcortical cysts (MLC, OMIM #604004), spinocerebellar ataxia type 12 (OMIM #183090), and pantothenate kinase-associated neurodegeneration (PKAN, OMIM#234200) (Gorospe et al. 2004; Bahl et al. 2005; Chabbria et al. 2007). Recently, two mutations have been shown to have founder effect in Agarwals in calpain-3 gene (CAPN3, OMIM *114240) in patients of LGMD type 2A (OMIM #253600) (Ankala et al. 2013).

Our molecular analysis in the Agarwal families revealed homozygous segment of DNA spanning 1 mb flanking the *ALDOB* gene, on haplotype analysis. This haplotype was in linkage disequilibrium with the mutation c.324+1G>A, as this did not match with another Agarwal patient harboring a different mutation, thus confirming a founder effect.

The information about the common mutation is likely to benefit our Indian patients who have been visiting the clinics of pediatricians, gastroenterologists, and geneticists for years without a diagnosis. It will simplify the diagnosis of this condition, at least in the Agarwal community, thus leading to an excellent outcome after early diagnosis.

We also report four other novel mutations from our cohort, including splice site mutations c.112+1 delG and c.380-1G>A as well as exonic mutations c.677G>A and c.689delA. The presence of more novel mutations than previously reported in literature is suggestive of a different

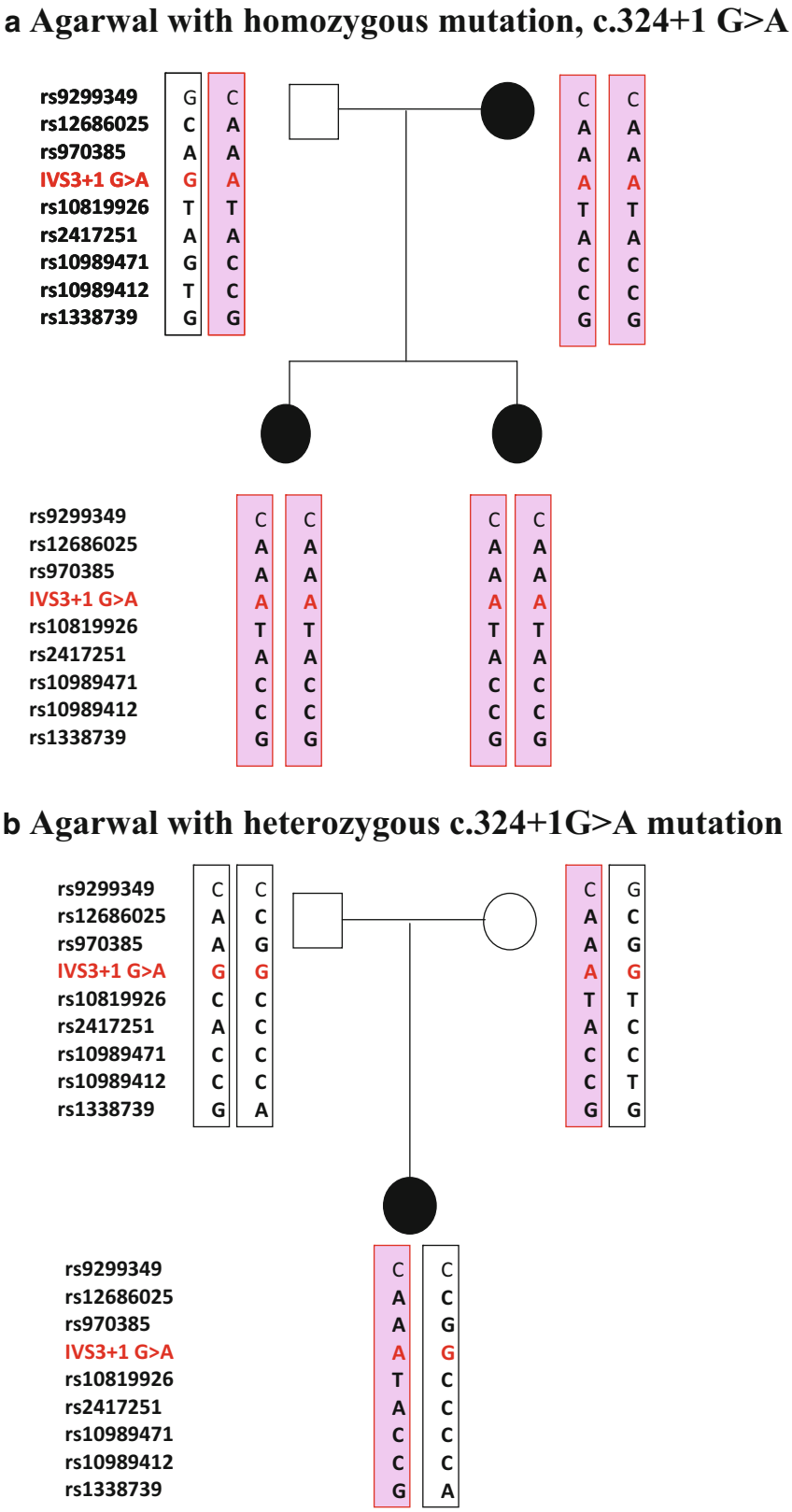

Fig. 3 Haplotype analysis in Agarwal families

gene pool among the Indian population. Only one of six mutations, c.1013C>T, is previously reported. Interestingly this mutation was noted in a child belonging to a consanguineous family from Kashmir. The Kashmir population has been noted by our group to share the gene pool with the Caucasian population based on genetic studies in other disorders, one notable example being cystic fibrosis. We propose a stepwise molecular diagnostic approach in children suspected to have HFI, based on their ethnicity within the Indian population.

Acknowledgements We would like to acknowledge the contribution of Dr Rebecca Treacy and Dr J Drummond, East Anglican Medical Genetics Service Genetics Lab, Addenbrooke's hospital, Cambridge, UK, for sequencing of the *ALDOB* gene in two patients. Deepak Sharma is thankful to his institute for the core grant for the bioinformatic study.

Take-Home Message

First report of molecular mutations in HFI from India, founder mutation in the Agarwal community. Simplifying diagnosis for easily treatable yet life-threatening metabolic condition.

Conflict of Interest

All the authors (Sunita Bijarnia-Mahay, Sireesha Movva, Neerja Gupta, Deepak Sharma, Ratna D. Puri, Udhaya Kotecha, Renu Saxena, Madhulika Kabra, Neelam Mohan and Ishwar C Verma) declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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2. Clinical management of children with HFI
3. Analysis and interpretation of data
4. Drafting the article

Sireesha Movva

1. Analysis and interpretation of data, haplotype analysis
2. Drafting the article (molecular methodology)

Neerja Gupta

1. Conception and design
2. Clinical management of children with HFI
3. Drafting the article (provided patient information)

Deepak Sharma

1. Analysis and interpretation of data (computational analysis and bioinformatics)
2. Drafting the article (methodology and results of bioinformatics)

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1. Clinical management of children with HFI
2. Revising it critically for important intellectual content)

References

Ananth N, Praveenkumar GS, Rao KA, Vasanthi, Kakkilaya S (2003) Two cases of hereditary fructose intolerance. Indian J Clin Biochem 18(2):87–92

Ankala A, Kohn JN, Dastur R, Gaitonde P, Khadilkar SV, Hegde MR (2013) Ancestral founder mutations in calpain-3 in the Indian Agarwal community: historical, clinical, and molecular perspective. Muscle Nerve 47(6):931–937

Bahl S, Virdi K, Mittal U, Sachdeva MP, Kalla AK, Holmes SE, O'Hearn E, Margolis RL, Jain S, Srivastava AK, Mukerji M (2005) Evidence of a common founder for SCA12 in the Indian population. Ann Hum Genet 69:528–534

Bharadia L, Shivpuri D (2012) Non responsive celiac disease due to coexisting hereditary fructose intolerance. Indian J Gastroenterol 31(2):83–84

Blanco E, Parra G, Guigó R (2007) Using gene id to identify genes. Curr Protoc Bioinformatics. doi:10.1002/0471250953.bi0403s18

Brooks CC, Tolan DR (1993) Association of the widespread p.A149P hereditary fructose intolerance mutation with newly identified sequence polymorphisms in the *Aldolase B* gene. Am J Hum Genet 52:835–840

Burge C, Karlin S (1997) Prediction of complete gene structures in human genomic DNA. J Mol Biol 268(1):78–94

Chabbria R, Udani V, Das S, Shah R (2007) Founder mutation in the PANK gene of Agrawal children with neurodegeneration with brain iron accumulation (NBIA). Ann Indian Acad Neurol Suppl 2 10:33

Coffee EM, Yerkes L, Ewen EP, Zee T, Tolan ER (2010) Increased prevalence of mutant null alleles that cause hereditary fructose intolerance in the American population. J Inherit Metab Dis 33(1):33–42

Esposito G, Imperato MR, Ieno L, Sorvillo R, Benigno V, Parenti G, Parini R, Vitagliano L, Zagari A, Salvatore F (2010) Hereditary fructose intolerance: functional study of two novel *ALDOB* natural variants and characterization of a partial gene deletion. Hum Mutat 31(12):1294–1303

Gorospe JR, Singhal BS, Kainu T, Wu F, Stephan D, Trent J, Hoffman EP, Naidu S (2004) Indian Agarwal megalencephalic leukodystrophy with cysts is caused by a common MLC1 mutation. Neurology 62(6):878–882

Lomsadze A, Ter-Hovhannisyan V, Chernoff YO, Borodovsky M (2005) Gene identification in novel eukaryotic genomes by self-training algorithm. Nucleic Acids Res 33(20):6494–6506

Malay AD, Allen KN, Tolan DR (2005) Structure of the thermolabile mutant aldolase B, A149P: molecular basis of hereditary fructose intolerance. J Mol Biol 347(1):135–144

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acid Res 16:1215

Sali A, Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 234(3):779–815

Singhal BS (2005) Leukodystrophies – Indian scenario. Indian J Pediatr 72(4):315–318

Stanke M, Steinkamp R, Waack S, Morgenstern B (2004) AUGUSTUS: a web server for gene finding in eukaryotes. Nucleic Acids Res 32(Web server issue):W309–W312

Steinmann B, Gitzelmann R (1981) The diagnosis of hereditary fructose intolerance. Helv Paediatr Acta 36:297–316

Steinmann B, Santer R (2012) Disorders of fructose metabolism. In: Saudubray JM, van den Berghe G, Walter JH (eds) Inborn metabolic disorders – diagnosis and treatment, 5th edn. Springer, New York, pp 159–165

JIMD Reports
DOI 10.1007/8904_2014_375

CASE REPORT

Leigh Syndrome Caused by the *MT-ND5* m.13513G>A Mutation: A Case Presenting with WPW-Like Conduction Defect, Cardiomyopathy, Hypertension and Hyponatraemia

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Received: 24 April 2014 / Revised: 19 September 2014 / Accepted: 15 October 2014 / Published online: 15 February 2015
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Abstract Mitochondrial disease can present with a wide range of clinical phenotypes, and knowledge of the clinical spectrum of mitochondrial DNA mutation is constantly expanding. Leigh syndrome (LS) has been reported to be caused by the m.13513G>A mutation in the ND5 subunit of complex I (*MT-ND5* m.13513G>A). We present a case of a 12-month-old infant initially diagnosed with tachyarrhythmia requiring defibrillation, subsequent presentation with hypertension and hyponatraemia secondary to renal salt loss and presumed inappropriate ADH secretion. Complex I activity in the muscle tissue was 54%, and mutation load in the muscle and lymphocytes was 50%. This case of Leigh syndrome caused by the m.13513G>A mutation in the ND5 gene illustrates that hyponatraemia due to renal sodium loss and inappropriate ADH secretion and hypertension can be features of this entity in addition to the previously reported cardiomyopathy and WPW-like conduction pattern and that they present additional challenges in diagnosis and management.

Communicated by: Wolfgang Sperl, MD, PhD

Competing interests: None declared

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Introduction

Leigh syndrome caused by mutations of genes affecting complex I of the respiratory chain has been increasingly recognized. We describe a case of Leigh syndrome caused by the m.13513G>A mutation in the ND5 subunit of complex I with previously unreported aspects including tachyarrhythmia requiring electrical cardioversion, hypertension and hyponatraemia. This case adds new facets to the spectrum of disease manifestation of the *MT-ND5* m.13513G>A mutation.

Case Report

A male infant was born by spontaneous vaginal birth at 39 weeks to a gravid 4 para 4 mother. He was the first child born to a non-consanguineous couple; maternal age at the time of birth was 39 years. The perinatal course was unremarkable. Of interest the infant was diagnosed on antenatal ultrasound with bilateral periventricular cysts, which were confirmed by early postnatal ultrasound and cranial MRI.

At the age of 2 and 3 months, respectively, the infant was admitted to hospital with episodes of broad-complex tachycardia with a left bundle branch block pattern and inferior axis. Both episodes required electrical cardioversion after failed attempts at pharmacological cardioversion. Echocardiography at that time suggested an apical area of possible LV non-compaction/hypertrabeculation (no images available). The infant was commenced on sotalol and subsequently propranolol. Blood pressure at that point of time was within normal limits. There was no family history of structural cardiac disease or arrhythmia and in particular no history of Wolff-Parkinson-White syndrome (WPW).

Subsequently the infant displayed failure to thrive with weight gradually falling below the third percentile for age; frequent episodes of choking were reported, which were attributed to gastro-oesophageal reflux. Mild hypotonia and inability to sit independently at 12 months suggested developmental delay. Moreover, constipation and new-onset intermittent right-sided exotropia were noted. Ophthalmological examination demonstrated that the infant followed a silent toy; media and fundus were unremarkable. Barium swallow study three days prior to the acute presentation was unremarkable as were electrolytes and blood pressure as documented 10 days prior to admission.

He then presented to our emergency department with a 2-day history of poor feeding and increasing intermittent lethargy and drowsiness. Spontaneous eye opening with response to tactile stimulation but without clearly fixing and a right-sided exotropia were noted. His skin was clammy and peripherally dusky. Capillary refill time was normal. Temperature was 34.5°C; SaO_2 in air was 99%; and respiratory rate was 22/min with irregular "sighing" respirations. Heart rate was 133/min and regular. A noninvasive blood pressure of 122/88 mmHg (mean BP 99 mmHg) was obtained (systolic, mean and diastolic blood pressure >95th percentile) (Kent et al. 2007). Cardiac examination revealed a soft ejection systolic murmur with strongly palpable pulses in both upper and lower limbs. His abdomen was soft without palpable masses.

Initial laboratory values (capillary sample, reference range in brackets) showed a serum sodium of 125 mmol/L (135–145 mmol/L), lactate of 3.4 mmol/L (0.6–2.4), glucose of 9.3 mmol/L (2.5–7.1 mmol/L), pH of 7.34 (7.32–7.42), pCO_2 of 40 mmHg (35–45), HCO_3 of 21.1 mmol/L (20–28) and base deficit of 3.8 mmol/L (–2 to +2); urea and creatinine were within normal range. Urinalysis showed glycosuria, mild proteinuria and a specific gravity of 1.020 (normal range: 1.005–1.030).

Serum osmolality was 257 mmol/kg. Serum lactate was normal on subsequent specimens (0.8–1.2 mmol/L). Blood cultures were negative after 48 h. Nasopharyngeal aspirate for respiratory pathogens, and a stool test for viral pathogens were negative.

A cranial CT performed in the emergency department during the acute presentation excluded an intracranial space-occupying lesion.

The patient developed generalized tonic-clonic seizures 8 h after admission and required intubation and ventilation for prolonged apnoeas following irregular sighing respirations. Hypertension was confirmed once arterial access had been obtained, reaching systolic levels of 120–140 mmHg and diastolic levels between 90 and 100 mmHg (both greater than 10 mmHg above 95th percentile for age).

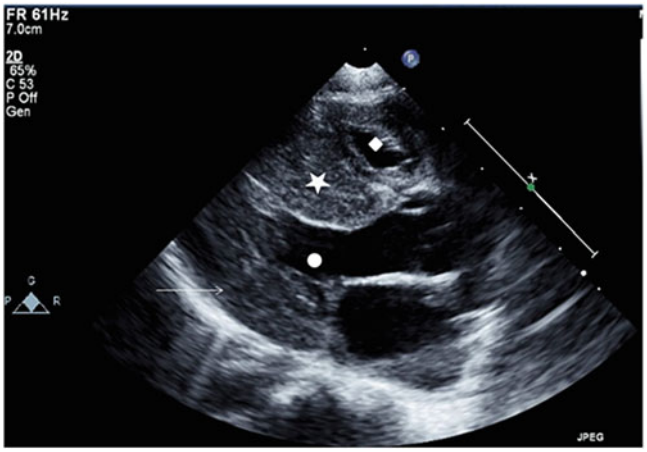

Fig. 1 Echocardiogram: parasternal long-axis view demonstrating septal (*filled star*) and left ventricular posterior wall thickening (*arrow*) with crowding of both the left (*filled circle*) and right ventricular cavity (*filled diamond*)

Antihypertensive treatment with amlodipine, captopril and intermittent nifedipine was initiated in addition to regular propranolol, which was continued. A repeat echocardiogram showed marked left ventricular hypertrophy, which had developed precipitously over 2 weeks since the previous echocardiogram (see Fig. 1).

The child was extubated on day 6 of admission but required reintubation 2 days later (day 8 of admission) for hypopnoea and respiratory failure. Neurological signs at this point included generalized hypotonia and new-onset bilateral supranuclear facial nerve palsy. Ophthalmology review confirmed the previously noted exotropia and a new-onset downbeat nystagmus with unremarkable retinae and optic nerve discs.

Clinical course

Hyponatraemia reached a minimum serum sodium of 123 mmol/L with maximum urinary sodium concentration of 286 mmol/L and maximum estimated sodium losses of 14 mmol/kg/day based on urine output. Central venous pressure was normal, and a weight gain of 730 g was recorded over 72 h. Serum sodium normalized by day 9 following admission with fludrocortisone at 0.1 mg twice daily, fluid restriction and sodium supplementation. Antihypertensive treatment with amlodipine, captopril and nifedipine was continued.

Serum catecholamines were elevated as were spot urine catecholamines (Table 1). Collection of urinary catecholamines over 24 h demonstrated a similar pattern (values not shown). The remaining endocrine investigations including

Table 1 Endocrine investigations, plasma and urine catecholamines and toxicology

Direct renin	10.2 uIU/mL	Normal range (5–100)
Aldosterone	400 pmol/L	300–1,500
Cortisol	Normal	
Thyroid function tests	Normal	
Plasma free metanephrines		
Metanephrine	700 pmol/L	<500
Normetanephrine	1,200 pmol/L	<900
Urine spot catecholamines		
MHMA	44 μmol/mmol creatinine	<13
HVA	30 μmol/mmol creatinine	<23
Adrenaline	534 μmol/mmol creatinine	<90
Noradrenaline	1,232 μmol/mmol creatinine	<371
Dopamine	3,140 μmol/mmol creatinine	<2,280
Urine toxicology screen	Negative	

renin, aldosterone and a toxicology screen were unremarkable (Table 1).

Urinalysis demonstrated marked generalized aminoaciduria, glycosuria and lactic aciduria along with elevated urinary orotic acid and Krebs cycle intermediates. A thoracic-abdominal CT angiography excluded aortic and renal artery stenosis and renal, adrenal and paraaortic masses. Cranial MRI was performed on day 5 of admission and demonstrated symmetrical hyperintensities on T2 weighted images in the tegmental area and in the peri-aqueductal grey matter (see Fig. 2). This was thought to represent changes characteristic of Leigh syndrome, which was confirmed by mtDNA mutation screening. This demonstrated the m.13513G>A mutation in the *MT-ND5* gene of complex I; mutation load from leukocytes was 50%. Coenzyme Q10, thiamine, carnitine and idebenone were commenced

Muscle biopsy demonstrated nonspecific lymphoid aggregates without evidence of small- or large-group atrophy. Histochemistry (ATP-ase pH 4.6, NADH, cytochrome oxidase, myodenylate deaminase, phosphorylase) was unremarkable as was immunohistochemistry.

Complex I activity was 54% of control mean value relative to citrate synthase in the muscle; analysis of complex II, II + III, III, and IV and citrate synthase were within normal range. Mutation load in muscle was also 50%. A CSF examination was not performed at the parents' request.

In view of the repeated unsuccessful extubation attempts, a decision was made to withdraw life-sustaining treatment in agreement with the parents' wishes, and the infant died 44 days following admission. An autopsy was not performed as per the parents' requests.

Testing of maternal tissues revealed that the *MT-ND5* m.13513G>A mutation was either not present or below the threshold of detection (~1%) in lymphocytes but was reliably detected at an estimated mutation load of 5–10% in urine sediment.

Discussion

Several case series and reports delineating features associated with the *MT-ND5* m.13513G>A mutation have been published including presentation as MELAS and Leigh syndrome and recently fatal neonatal lactic acidosis (Chol et al. 2003; Shanske et al. 2008; Ruiter et al. 2007; Van Karnebeek et al. 2011; Sudo et al. 2004; Wang et al. 2008; Monlleo-Neila et al. 2013).

Although a Wolff-Parkinson-White-like conduction pattern, paroxysmal supraventricular tachycardia, incomplete right bundle branch block and AV block have been described as a feature of this mutation, documented arrhythmia requiring electrical cardioversion has not been described as the initial manifestation with the *MT-ND5* m.13513G>A mutation (Chol et al. 2003; Ruiter et al. 2007; Sudo et al. 2004; Wang et al. 2008; Monlleo-Neila et al. 2013).

Hypertrophic cardiomyopathy has been previously associated with this mutation (Chol et al. 2003; Sudo et al. 2004; Wang et al. 2008). In one case this was transient; in a second case a change over time to a dilative type of cardiomyopathy was observed (Chol et al. 2003; Wang et al. 2008). The precipitous change in left ventricular morphology over the course of 2 weeks such as in this current case has also not been previously reported; no trigger such as a viral infection could be identified on routine testing to explain this acute presentation.

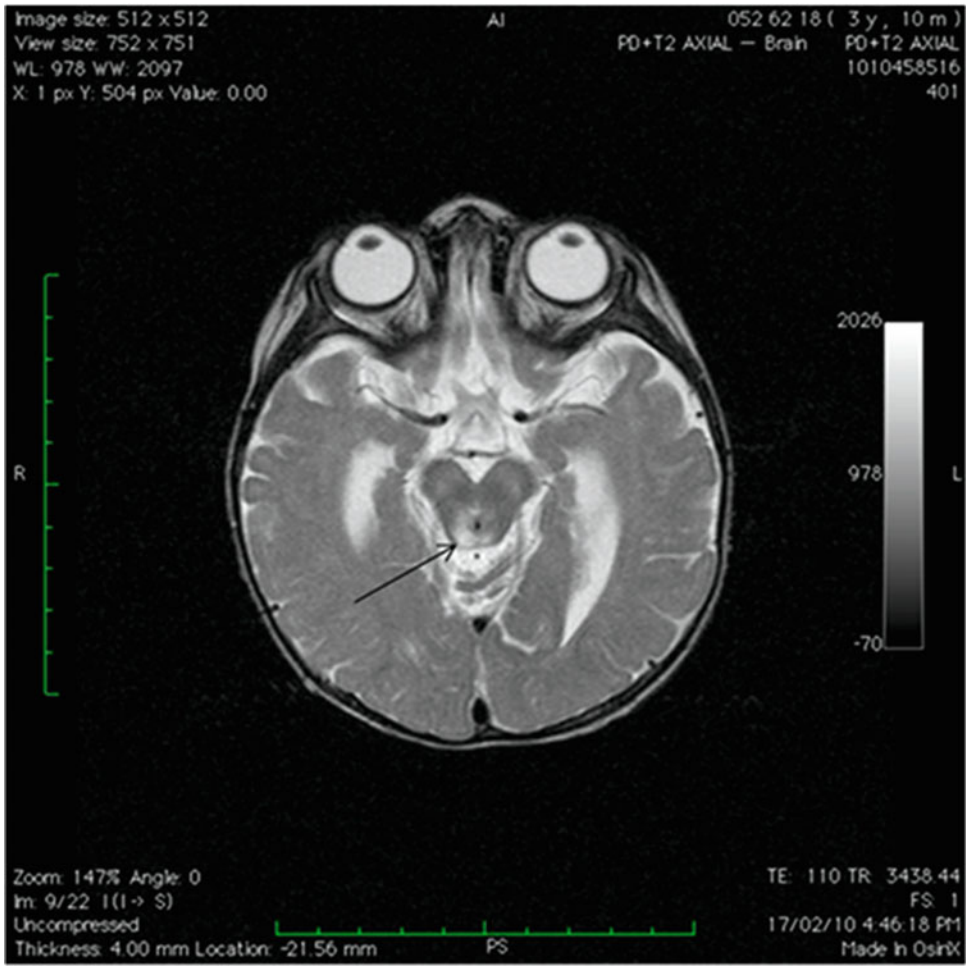

Fig. 2 Cranial MRI: Axial T2 weighted image showing hyperintensities in the peri-aqueductal grey matter (*arrow*)

A recently reported case with the *MT-ND5* m.13513G>A mutation presented with gastro-oesophageal reflux and later paralytic ileus, which is similar to our patient, who had poor weight gain due to frequent and severe episodes of regurgitation and later developed constipation (Monlleo-Neila et al. 2013). We hypothesize that the choking episodes in this current case could have been a manifestation of gastro-oesophageal dysmotility associated with the *MT-ND5* m.13513G>A mutation and that this was further complicated by involvement of the brainstem area that is central to coordination of the swallowing reflex (Monlleo-Neila et al. 2013; Saito 2009).

Hyponatraemia has been documented with other mitochondrial disorders such as MELAS and Kearns-Sayre syndrome and in these cases was thought to be secondary to transient or recurrent renal salt loss and inappropriate ADH secretion (SIADH) as evident by a positive fluid balance such as in this current case (Swiderska et al. 2010; Southgate and Penney 2000). Our case also illustrates that differentiation between SIADH and other pathologies such as cerebral salt wasting (CSW) can be difficult in the acute setting in the presence of hypertension and concurrent renal salt wasting (Rivkees 2008).

Hypertension was another presenting feature in this case and has not been described previously with the *MT-ND5* m.13513G>A mutation. Overall, hypertension has only rarely been reported as a presenting sign in mitochondrial respiratory chain disorders. Three cases reaching hypertensive crisis level with fatal outcome have been described, one of which was subsequently diagnosed with complex I deficiency (Lohmeier et al. 2007; Pamphlett and Harper 1985; Narita et al. 1998). Similarly to the patient in Lohmeier's report, our patient presented with signs of autonomic dysfunction and altered vasomotor tone such sweatiness and dusky extremities, which could support the hypothesis of centrally mediated

vasomotor dysregulation (Lohmeier et al. 2007; Zelnik et al. 1996). Interestingly, two of these reported cases displayed normal catecholamine levels, whereas in our case levels were elevated.

It seems plausible to assume a neurogenic blood pressure dysregulation as the areas of necrosis in the brainstem comprise the nucleus tractus solitarii, an area central to cardiorespiratory reflex integration (Saito 2009; Lohmeier et al. 2007). This functional area has also been implicated in the characteristic pattern of sighing respirations and post-sigh apnoeas often seen in Leigh syndrome (Saito 2009). Whether the significantly elevated catecholamine levels in our patient are indicative of this central dysregulation remains speculative. Interestingly, the onset of hypertension had coincided with onset of respiratory dysfunction and sighing respirations in the reported cases similarly to ours.

The *MT-ND5* m.13513G>A mutation has been reported to cause abnormalities at a low mutation load; in the series reported by Brautbar et al., mutation load was 20–40% and five out of their six patients positive for the mutation had normal complex I activity as had our patient (Brautbar et al. 2008).

In conclusion we report the first documented case of Leigh syndrome caused by the *MT-ND5* m.13513G>A mutation presenting with renal salt loss, proximal tubular dysfunction and SIADH in association with rapidly progressive hypertrophic cardiomyopathy, WPW-like conduction defect requiring electrical cardioversion and arterial hypertension, further extending the spectrum of presentation of this mutation.

Acknowledgement The authors thank Dr C.J. Turner, paediatric cardiologist, the Children's Hospital at Westmead, Sydney, New South Wales, Australia, for providing additional clinical information.

Compliance with Ethics Guidelines

Conflict of Interest

Marcus Brecht, Malcolm Richardson, Ajay Taranath, Scott Grist, David Thorburn and Drago Bratkovic declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Consent

Additional informed consent was obtained from the carers of the patient for whom potentially identifying information is included in this article.

Details of the Contributions of Individual Authors

Dr Brecht managed the patient during admission, conceived the article and wrote the first draft of the manuscript; Dr Richardson performed and interpreted serial echocardiograms, provided the echocardiogram and edited the manuscript; Dr Taranath interpreted the cranial MRI, selected the MRI image and edited the manuscript; Dr Grist oversaw mitochondrial DNA analysis and edited the manuscript; Prof Thorburn oversaw and interpreted the respiratory chain enzymology assay and tissue mitochondrial DNA analysis and edited the manuscript; Dr Bratkovic oversaw overall patient management, coordinated testing, discussed and revised the manuscript.

References

Brautbar A, Wang J, Abdenur JE et al (2008) The mitochondrial 13513G>A mutation is associated with Leigh disease phenotypes independent of complex I deficiency in muscle. Mol Genet Metab 94(4):485–490

Chol M, Lebon S, Bénit P et al (2003) The mitochondrial DNA G13513A MELAS mutation in the NADH dehydrogenase 5 gene is a frequent cause of Leigh-like syndrome with isolated complex I deficiency. J Med Genet 40:188–191

Kent AL, Kecskes Z, Shadbolt B, Falk MC (2007) Blood pressure in the first year of life in healthy infants born at term. Pediatr Nephrol 22(10):1743–1749

Lohmeier K, Distelmaier F, van den Heuvel LP et al (2007) Fatal hypertensive crisis as presentation of mitochondrial complex I deficiency. Neuropediatrics 38:148–150

Monlleo-Neila L, Toro MD, Bornstein B, Garcia-Arumi E, Sarrias A, Roig-Quilis M, Munell F (2013) Leigh syndrome and the mitochondrial m.13513G>A mutation: expanding the clinical spectrum. J Child Neurol 28(11):1531–1534

Narita T, Yamano T, Ohno M, Takano T, Ito R, Shimada M (1998) Hypertension in Leigh syndrome–a case report. Neuropediatrics 29(5):265–267

Pamphlett R, Harper C (1985) Leigh's disease: a cause of arterial hypertension. Med J Aust 143(7):306–308

Rivkees SA (2008) Differentiating appropriate antidiuretic hormone secretion, inappropriate antidiuretic hormone secretion and cerebral salt wasting: the common, uncommon, and misnamed. Curr Opin Pediatr 20:448–452

Ruiter EM, Siers MH, van den Elzen C et al (2007) The mitochondrial 13513G4A mutation is most frequent in Leigh syndrome combined with reduced complex I activity, optic atrophy and/or Wolff–Parkinson–White. Eur J Hum Genet 15:155–161

Saito Y (2009) Reflections on the brainstem dysfunction in neurologically disabled children. Brain Dev 31(7):529–536

Shanske S, Coku J, Lu J et al (2008) The G13513A mutation in the ND5 gene of mitochondrial DNA as a common cause of MELAS or Leigh syndrome: evidence from 12 cases. Arch Neurol 65 (3):368–372

Southgate HJ, Penney MD (2000) Severe recurrent renal salt wasting in a boy with a mitochondrial oxidative phosphorylation defect. Ann Clin Biochem 37:805–808

Sudo A, Honzawa S, Nonaka I, Goto Y (2004) Leigh syndrome caused by mitochondrial DNA G13513A mutation: frequency and clinical features in Japan. J Hum Genet 49(2):92–96

Swiderska N, Appleton R, Morris A, Isherwood D, Selby A (2010) A novel presentation of inappropriate antidiuretic hormone secretion in Leigh syndrome with the myoclonic epilepsy and ragged red fibers, mitochondrial DNA 8344A>G mutation. J Child Neurol 25(6):782–785

Van Karnebeek CD, Waters PJ, Sargent MA et al (2011) Expanding the clinical phenotype of the mitochondrial m.13513G>A mutation with the first report of a fatal neonatal presentation. Dev Med Child Neurol 53(6):565–568

Wang SB, Weng WC, Lee NC, Hwu WL, Fan PC, Lee WT (2008) Mutation of mitochondrial DNA G13513A presenting with Leigh syndrome, Wolff-Parkinson-White syndrome and cardiomyopathy. Pediatr Neonatol 49(4):145–149

Zelnik N, Axelrod FB, Leshinsky E, Griebel ML, Kolodny EH (1996) Mitochondrial encephalomyopathies presenting with features of autonomic and visceral dysfunction. Pediatr Neurol 14 (3):251–254

JIMD Reports
DOI 10.1007/8904_2014_376

RESEARCH REPORT

Deep Genotyping of the *IDS* Gene in Colombian Patients with Hunter Syndrome

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Received: 08 August 2014 / Revised: 13 October 2014 / Accepted: 15 October 2014 / Published online: 15 February 2015
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Abstract *Background*: Mucopolysaccharidosis type II (MPSII), also known as Hunter syndrome, is an X-linked disorder caused by mutations in the iduronate 2 sulfatase (*IDS*) gene. This enzyme catalyzes the initial step in the catabolism of heparan sulfate and dermatan sulfate; thus, its deficiency leads to the accumulation of these glycosaminoglycans. MPS II has significant allelic heterogeneity, making the establishment of genotype-phenotype correlations difficult. This study assessed clinical features in combination with deep genotyping of a group of Colombian patients with MPS II and attempted to establish a degree of genotype-phenotype correlation by employing bioinformatic tools.

Methods: Eighteen patients were included in this study, 11% of whom were non-neuronopathic, and the other 89% were neuronopathic. Samples were all analyzed using three molecular methodologies: MLPA, direct exon sequencing, and RFLP analysis.

Results: A total of 13 mutations were identified, 6 of which were novel (c.548_564dup16, c.477insT, c.595_607del12, c. 549_562del13, c.182delC, and a complete deletion of exon 7). The frequency of common mutations (R468Q, Q465X, K347Q, K236N, S71N, R88H, and a conversion phenomenon) was 53.85%. The S71N mutation was frequent among the attenuated phenotype, while private frameshift mutations and rearrangements were seen in patients with severe phenotypes. Molecular docking was performed on the wild-type and mutant IDS proteins, which revealed changes in the enzyme-substrate interaction for the mutant IDS.

Conclusion: The frequency of novel mutations (46.15%) is similar to what has been reported elsewhere. The use of bioinformatic tools showed differences in enzyme-substrate interactions. Studies with larger groups of patients are needed.

Communicated by: Maurizio Scarpa, M.D, Ph.D

Competing interests: None declared

Electronic supplementary material: The online version of this chapter (doi:10.1007/8904_2014_376) contains supplementary material, which is available to authorized users.

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Introduction

Mucopolysaccharidosis type II (OMIM 309900), or Hunter syndrome, is the only MPS with a recessive X-linked inheritance pattern (Yatziv et al. 1977). The primary defect in this condition is the absence or defective activity of the lysosomal iduronate 2 sulfatase enzyme (IDS; EC 3.1.6.13) due to mutations in the *IDS* gene.

The severity with which this syndrome presents ranges from a neuronopathic (Hunter A) to a non-neuronopathic phenotype (Hunter B) (Neufeld and Muenzer 2001; Young

et al. 1982; Martin et al. 2008). The neuronopathic form has an earlier onset and severe systemic and neurological involvement (Yatziv et al. 1977; Jones et al. 2009). The non-neuronopathic phenotype has minimal or no neurological deterioration, although patients manifest all the systemic complications (Young et al. 1982).

The reported incidence of MPSII is 1 in 170,000 male births (Martin et al. 2008; Nelson 1997; Poorthuis et al. 1999; Malm et al. 2008). In Japan, the incidence is estimated to be 1 in 90,000 (Ochiai et al. 2007). There are no studies regarding the incidence and prevalence of MPS II in Colombia; however, the combined frequency of all MPS cases calculated in the departments of Cundinamarca and Boyacá was 1.98 in 100,000 live births, and the estimated frequency of MPSII was 0.45 in 100,000 live births (Gómez et al. 2012).

The *IDS* gene is located at Xq27.3-q28, spans 24 Kb, and is composed of nine exons (Wilson et al. 1990). To date, over 350 mutations in this gene have been described (The Human Mutation Gene Database, http://www.hgmd.cf.ac.uk/ac/index.php), and phenotype-genotype correlations are difficult due to this abundant allelic heterogeneity (Rathmann et al. 1996; Vafiadaki et al. 1998; Lualdi et al. 2005; Lagerstedt et al. 2000; Froissart et al. 2007). A pseudogene is located approximately 25 Kb telomeric to the functional gene, with homologous regions to *IDS* exons 2 and 3 and intron 2 and with 96% homology with intron 7, explaining the susceptibility to complex recombination events (Rathmann et al. 1995; Bondeson et al. 1995; Bunge et al. 1998; Lualdi et al. 2005).

IDS is a 550-amino acid, monomeric lysosomal enzyme (Wilson et al. 1990) and is part of the sulfatase superfamily, with domains highly conserved (Diez-Roux and Ballabio 2005). It has been hypothesized that Asp45, Asp46, Cys84, Asp334, and His335 comprise the active site of the enzyme, based on homology with the active sites of arylsulfatases A and B (Dierks et al. 1999; Waldow et al. 1999). The Cys84 residue is conserved among all eukaryotic sulfatases (Dierks et al. 1999). Because complete characterization of this enzyme is ongoing and because its structure has not yet been elucidated by X-ray crystallography, most of the information on its structure and function has been inferred from homology with other better characterized sulfatases (Miech et al. 1998).

This study focuses on the clinical characterization and mutational profiling of Colombian patients with MPS II. This is achieved by deep genotyping of the *IDS* gene and assessment of the consequences in an IDS 3D model using a bioinformatic approach.

Methods

Phenotypic Analysis

This is a descriptive study without a hypothesis to prove. Diagnoses of MPS II were established clinically and confirmed by measurement of IDS activity. Eighteen patients (representing 15 families) were assessed by pediatric neurologists and clinical geneticists. The clinical assessment was performed following the criteria developed by Holt et al. for establishing the phenotype based on a severity score (Holt et al. 2011). This score takes into account 7 early clinical markers: sleep disturbance, increased activity, behavior difficulties, seizure-like behavior, perseverative chewing, and inability to achieve bowel training and bladder training. For each of these markers, a patient is assigned a 0 (zero) for "never" or a 1 for "ever." Patients with a total score $\geq$3 have a high likelihood of developing CNS disease (Holt et al. 2011). The main phenotypic variables are described; the frequencies and percentages among patients are estimated for discrete variables. For continuous variables, the same procedure was performed and expressed in terms of central tendency and dispersion measurements.

A Kolmogorov-Smirnov nonparametric test was performed on a single sample to assess whether enzymatic activity values were normally distributed (H_o). The significance level required to reject H_o was 0.05 (IC 95). Once this was completed, a chi-squared (χ^2) test with 9 degrees of freedom was performed to assess variation among the obtained data, with H_o being the existence of statistically significant differences when $p < 0.05$. These calculations were performed using the IBM SPSS free trial software v.21 (Chicago IL, USA).

Genotyping

Peripheral blood samples were obtained after written, informed consent was given. Genomic DNA extraction from 300 μL of blood was performed using the UltraClean Blood DNA Isolation Kit® (MOBIO Laboratories, USA), following the manufacturer's instructions. Obtained DNA was quantified by spectrophotometry using a Nanodrop2000®, a Thermo Scientific® instrument. For genotyping, the following three steps were performed:

Step 1. Detection of large deletions and duplications using MLPA

MLPA (multiplex ligation-dependent probe amplification) (Schouten et al. 2002) was performed on all subjects, because it has been reported from several patient series that up to 20% of patients will have

complex rearrangements (Froissart et al. 2007). SALSA® MLPA® P164 IDS (MRC-Holland) was used, and the protocol was followed according to the manufacturer's specifications. Briefly, a minimum of 5 patients and 3 control DNA samples were used in each experiment, with the same amount of DNA (10 ng) per reaction. Capillary electrophoresis was performed using an ABI Prism 310 Genetic Analyzer. *Genemapper*® software was used to process raw data. Analysis of fragments was performed using the *Coffalyser* version 8 software.

Step 2. Sequencing

Direct sequencing of the 9 *IDS* exons was performed using primers modified from the initial reports by Froissart et al. (1998) and Lin et al. (2006) (Alves et al. 2006; Lau and Lam 2008) to amplify gene segments and intron-exon boundaries (Table 1, supplementary material). We did not analyze 5′UTR-3′UTR segments or epigenetic alterations. Conventional PCR and sequencing details of this work are available in Box 1 in supplementary material. Reaction products were analyzed on an ABI Prism 3500 sequencer (Applied Biosystems), and the obtained sequences were carefully revised using BioEdit® Sequence Alignment Editor. The BLASTn tool was employed for the identification of mutations, and the amino acid sequences were obtained using the Translate Tool from Proteomics Tools (ExPASy).

Step 3. Recombinant analysis by RFLPs

For subjects whose MLPA and sequencing results were negative, a rapid PCR and RFLP method for detection of the 4 most common types of *IDS/IDSP1* rearrangements, as described by Lualdi and collaborators, was used (Lualdi et al. 2005). This method consists of two PCRs separated by digestion with restriction enzymes (*HinfI*, *SacI*, and *Eco57I-AcuI*) which recognize the breakage sites where these recombinations occur.

Bioinformatic Analysis

Bioinformatic analysis of the effects of mutations on the IDS 3D protein structure was performed as described in Galvis et al. (2014). The first step was tridimensional modeling of the IDS protein. Measurement of the root mean square deviation (RMSD) was performed by superimposing the model and a template to evaluate the model's precision; total RMSD values (the average distance between atoms in two overlapping proteins) of 1.0 or close to this value represent molecules with high structural similarity. Molecular docking of wild-type IDS was then performed, followed by modeling and docking for mutants, for which RMSD and ASA measurements were also calculated (Galvis et al. 2014).

Results

Clinical Characterization of Patients

A total of 18 Colombian patients were included in this study, and the average age of the patients was 10.8 years (SD ±7.8 years). Following the criteria of Holt et al. for determining the phenotype, a severity score was calculated for each patient (Table 1). Those patients with a score ≥3 were classified as neuronopathic, or as suggestive of a neuronopathic phenotype if they were younger than 3 years old. The severity score was 0 in only two patients, both of whom were above 5 years of age, which classified them as non-neuronopathic. All remaining patients had a score of 3 or higher.

It was found that neuronopathic Hunter syndrome is the most common, accounting for 89% of cases, while the non-neuronopathic type was seen in 11% of cases. The mean age of symptom onset for all patients was 2.5 years (SD ±1.55 years), but among those with the neuronopathic phenotype was 2.7 years (SD ±1.63 years).

The mean age at diagnosis was 6 years (SD ±4.38 years) for the whole group, 5 years (SD ±3.21) for the neuronopathic group, and 7 years (SD ± 2.13) for non-neuronopathic group. Initial symptoms commonly reported in the neuronopathic phenotype group were speech delay (81.2%) and joint rigidity (18.8%). For the non-neuronopathic group, initial manifestations included recurrent pneumonia, visceromegaly, and joint rigidity (Table 1).

Enzyme activity levels were analyzed by a nonparametric Kolmogorov-Smirnov test and Chi-squared (χ^2) test. No statistically significant differences in enzyme activity were found between individuals for the whole group.

Genotyping of MPSII Patients

Using MLPA, it was possible to detect two types of mutations in two patients. In patient MPSII001, a gain in exon 4 and in the pseudogene was identified. Direct sequencing confirmed that this gain consisted of a 1 nucleotide insertion (Table 2). For patient MPSII012, an absence of signal for exon 7 was detected, indicating a complete deletion of said exon (Fig. 1). A search of the *X chromosome gene database*® and *Human Gene Mutation Database*® from *Biobase* confirmed that deletion of exon 7 is a novel mutation.

Direct sequencing of the nine gene segments and sequence comparison to the reference genome allowed identification of point mutations (Table 2).

Table 1 Phenotypic characteristics of the study sample

Patient	Age (years)	Family history of MPSII	Age at diagnosis	IDS activity[a]	Initial symptom	Severity score (Holt et al. 2011)	PHENOTYPE
MPSII001	5	No	1	0.71	Speech delay	4	Neuronopathic
MPSII002	18	No	4	0.24	Speech delay	7	Neuronopathic
MPSII003	8	No	5	0.32	Macrocephaly/speech delay	5	Neuronopathic
MPSII004	14	Maternal uncle	8	0.19	Speech delay/coarse features	5	Neuronopathic
MPSII005	2	No	2	1.10	Speech delay	3	Suggestive of. Neuronopathic
MPSII006	17	Maternal uncle, cousins	10	0.40	Speech delay	7	Neuronopathic
MPSII007	6	No	5	0.00	Pneumonia/ visceromegaly/ umbilical hernia	0	Non Neuronopathic
MPSII008	10	Brother	6	1.6 *(dried blood spot)	Speech regression/coarse features	5	Neuronopathic
MPSII009	14	No	8	1.8[b]	Speech delay	5	Neuronopathic
MPSII010	13	No	3	0.47	Pneumonia/joint stiffness	5	Neuronopathic
MPSII011	13	Maternal Uncle	6	0.85	Speech delay/joint stiffness.	3	Neuronopathic
MPSII012	8	Maternal uncle, cousin	2	26** (dried blood spot)	Macrocephaly/speech delay	3	Neuronopathic
MPSII013	7	No	3	1.34	Hearing loss/joint stiffness	3	Neuronopathic
MPSII014	1	No	1	0.47	Pneumonia	3	Suggestive of Neuronopathic
MPSII015	31	Nephew	9	9.46*** (dried blood spot)	Joint stiffness/umbilical hernia	0	Non Neuronopathic
MPSII016	16	Uncle, cousins	9	UN	Speech delay	6	Neuronopathic
MPSII017	6	Uncle, cousins	2	UN	Speech delay	5	Neuronopathic
MPSII018	16	No	10	22.27** (dried blood spot)	Speech delay/joint stiffness	5	Neuronopathic

Reference values: [a] 7.5–55.1 nmol/mg protein/h. [b] 48–118 nmol/mg protein/h. IDS activity in dried blood spot (μmol/L h): *ref 12–23, **ref 57–149, ***ref 71–187
UN unavailable

Neither MLPA nor direct sequencing allowed for the establishment of a molecular diagnosis in patient MPSII013. For this patient, we used the PCR method described by Lualdi et al. (Lualdi et al. 2005). The product of a first PCR was digested with *HinfI* and *SacI* to identify recombination sites on intron 7, with negative results. Finally, an *AcuI* digestion of the second PCR product led to the identification of a conversion event in intron 3, where thymine 292879 is the breakage site and is replaced by a cytosine from the chimeric 3–7 introns (Fig. 2).

Employing these three molecular approaches, gross rearrangements were identified in 15.4% of patients, whereas small indels and point mutations were identified in 84.6% of patients. Novel mutations accounted for 46.15% of all the mutations found (Table 2).

Bioinformatic Results

All the results are shown in the supplementary material. The wild-type hIDS model superposed against its main template yielded an RMSD of 0.97 Å, indicating the model had high accuracy (Fig. 1, supplementary material). Wild-type hIDS docking interactions involved the O-2 sulfate of glucuronic acid (GlcA) with Cys84, Tyr165, Leu244, and Arg297 (Fig. 2, supplementary material) (Galvis et al. 2014). RMSD, ASA, and docking results for the IDS

Table 2 Genotypes of Colombian MPSII patients

Code	Mutation (cDNA)	Mutation (protein)	Phenotype	Report[a]
MPSII001	c.477 insT	P160S fsX4	N	This study
MPSII002	c.548_564dupTTGCCCTGTGGATGTG	D190P fsX13	N	This study
MPSII003	c.1403G>A	R468Q	N	Whitley (1993); Villani (2000); Sukegawa-Hayasaka et al. (2006)
MPSII004	c.263G>A	R88H	N	Rathmann et al. (1996)
MPSII005	c.708G>C	K236N	Suggest. N	Gucev (2011)
MPSII006	c.1393C>T	Q465X	N	Li (1996)
MPSII007	c. 212G>A (polymorphism c.438 C>T)	S71N	No N	Froissart et al. (1998)
MPSII008	c.1403G>A	R468Q	N	Whitley (1993), Villani (2000)
MPSII009	c.1039A>C	K347Q	N	Lissens (1997)
MPSII010	c.595_607delACAGAGCACTGA	Del.Q200_E2003 (QSTE)	N	This study
MPSII011	c. 549_562delTGCCCTGTGGATG	P185WfsX23	N	This study
MPSII012	Deletion of exon 7	R294GfsX2	N	This study
MPSII013	Conversion involving intron 3	–	N	Lualdi et al. (2005)
MPSII014	c.263G>A	R88H	Suggest. N	Rathmann et al. (1996)
MPSII015	c. 212G>A (polymorphism c.438 C>T)	S71N	Non N	Froissart et al. (1998)
MPSII016	c.1393C>T	Q465X	N	Li (1996)
MPSII017	c.1393C>T	Q465X	N	Li (1996)
MPSII018	c.182 delC	P62QfsX67	N	This study

[a] These mutations were verified against the *X Chromosome gene database*® and *Human Gene Mutation Database*® from *Biobase*, confirming whether these were novel or previously reported mutations

mutants are shown in the supplementary material (Tables 2 and 3 and Fig. 3) (Galvis et al. 2014).

Discussion

This is the first study in South America to perform a molecular analysis of MPS II with three available laboratory methodologies and in silico simulations with the identified mutations.

Clinical Characterization

Even though Holt et al. included only patients over 3 years of age in their work, the first five clinical markers are documented to have onset ages before even 2 years of age, and the first three have onset ages in the first months of life (Holt et al. 2011). We employed their methodology with all the patients of this study, but those younger than 2 years of age with scores ≥ 3 were classified as being "suggestive of neuronopathic phenotype."

According to the literature, two thirds of patients have a neuronopathic phenotype (Wraith et al. 2008b), which is similar to the neuronopathic phenotype occurrence observed in our population, which was 89%. In a Brazilian study, initial symptoms for the severe phenotype were macrocephaly, speech delay, and behavior abnormalities, with symptom onset at 1.5 years (6–24 months) (Schwartz et al. 2007). The same age at onset is reported in HOS (Wraith et al. 2008a, b). For the neuronopathic phenotype, the initial symptoms more commonly reported in our study were speech delay (81%) and joint stiffness (18.8%), but age at symptom onset was 2.7 years on average, which differs from the reported result due to an atypical age at onset (5 years old) for patient 008, as the parents recalled.

Reported first manifestations of the non-neuronopathic phenotype were skeletal abnormalities, joint stiffness, and hearing loss (Schwartz et al. 2007). We observed recurrent pneumonia, umbilical hernia, visceromegaly, and joint stiffness (Table 1).

The Hunter Outcome Survey reported a median age at diagnosis of Hunter syndrome of 3.5 years (Wraith et al. 2008a). In a group of 77 patients in Brazil, a mean age at diagnosis of 6 years was found (Schwartz et al. 2007), which is more similar to our results (6 ± 4.38 years). This is most likely a consequence of a delay in recognition of early signs, and/or difficulties in accessing health services in some geographical, cultural, or social contexts.

In Latin America, patients with an attenuated phenotype are reported to be diagnosed at age 14 on average, while those with a severe phenotype are diagnosed at 7 years old (Schwartz et al. 2007). In our study, patients with the non-

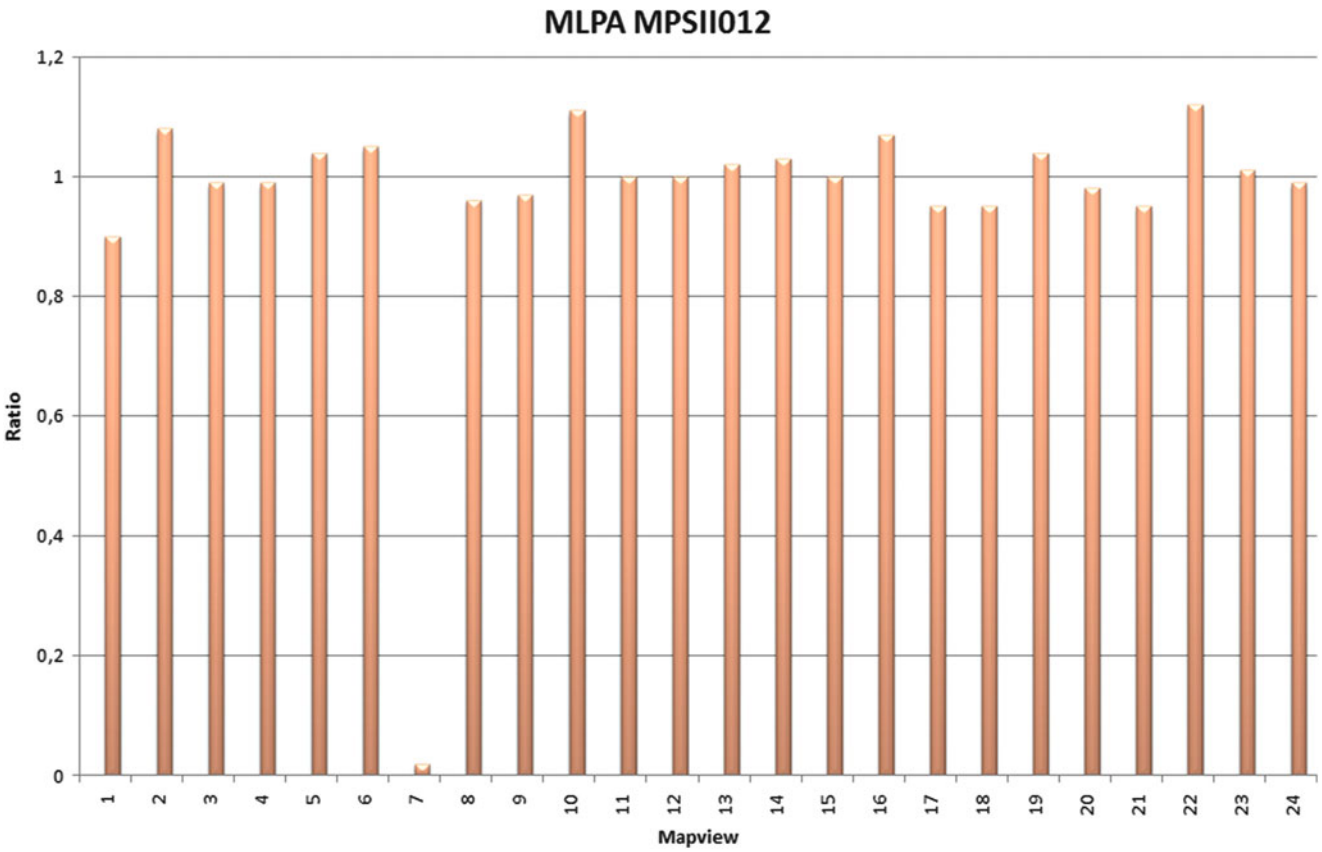

Fig. 1 Complete deletion of exon 7 in patient MPSII012. The *X*-axis of this bar diagram displays specific fragments, and the *Y*-axis shows normalized ratios. The fragment for exon 7 (7) yielded no signal (0)

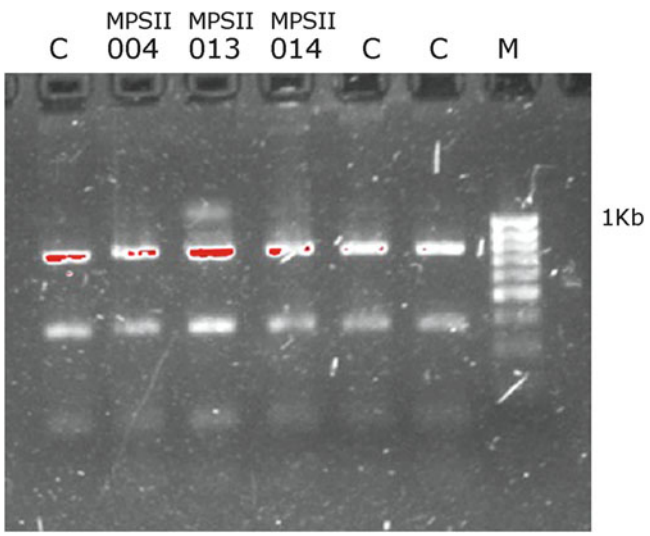

Fig. 2 PCR 2 products after digestion with *AcuI*. *C* control. Note the 1,174 bp product on well 3, corresponding to MPSII013. *M* molecular weight

neuronopathic phenotype were diagnosed at 7 years of age and those with the neuronopathic phenotype (or with symptoms suggestive of it) at 5 years of age, on average.

IDS quantity and activity do not correlate with phenotype (Parkinson et al. 2004), and the enzyme is equally deficient in both forms of the disease (Scarpa et al. 2011). Here we found no differences in IDS activity among the whole group of patients.

Mutational Profile

Using MLPA, genotyping, and RFLPs, we were able to confirm the molecular diagnosis in 100% of patients in this study. In a series of 155 European patients, it was reported that up to 64% of patients have private mutations (Froissart et al. 2007). However, novel mutations in Colombian patients were detected with a frequency of 46.15%, similar to what was observed in 38 patients from China (Zhang et al. 2011).

The most commonly reported mutation (in 3.2–11% of patients) is R468Q (Isogai et al. 1998; Vafiadaki et al. 1998; Lin et al. 2006; Froissart et al. 2007; Zhang et al. 2011), which was found in 15.4% of our study population. R88H is also pan-ethnic (2–5.4% of patients) (Rathmann et al. 1996; Froissart et al. 2007; Zhang et al. 2011; Sohn et al. 2012), with a frequency in our study of 15.4%.

Two mutations were complex rearrangements: a conversion at intron 3 detected by RFLPs (Lualdi et al. 2005) and

a deletion of exon 7 detected by MLPA, together accounting for 15.4% of all cases. This is similar to previously reported results, which have calculated that recombination phenomena account for 20% of cases (Steen-Bondeson et al. 1992; Bunge et al. 1998; Lualdi et al. 2005; Froissart et al. 2007).

A recent study of 103 unrelated South American patients reported that 29% of observed mutations were novel (Brusius-Facchin et al. 2014), which differs from the findings in our study. The same authors (Brusius-Facchin et al. 2014) found alterations such as inversions/disruptions and partial/total deletions of the IDS gene in 20/103 (19%) patients. Small (<22 bp) insertions/deletions/indels and point mutations were identified in 83/103 (88%) patients, which is very similar to our findings; in our Colombian group, we found small indels and point mutations in 84.6% of patients.

Bioinformatic Analysis and Effect of Mutations on the Tridimensional Structure of hIDS

Regarding mutant IDS, modeling revealed that even point mutations can alter the complete 3D structure, as reflected in the RMSD values (Galvis et al. 2014).

In 2005, Kato et al. performed tridimensional modeling of IDS and reported that the residues at the catalytic site are Asp45, Asn46, Cys84, Arg88, Lys135, His138, Asp334, His335, and Lys347 (Kato et al. 2005). By contrast, our docking simulation found that besides Cys84, the residues Arg297, Leu244, Tyr165, and Asn106 are part of the catalytic site as well (Galvis et al. 2014). It is likely that the cationic metal union required for the catalytic activity induces changes in the amino acids of the catalytic pocket, but these types of simulations are outside the scope of this study (Galvis et al. 2014).

In IDS mutants, docking simulations performed here showed differences with regard to interactions with substrates. Kato et al. hypothesized that a nonconservative mutation in R468Q should affect the electrostatic field for substrate entrance into the active site (Kato et al. 2005). Furthermore, Western blot analysis showed only primary precursors (Sukegawa-Hayasaka et al. 2006). In the mutation Q465X, an early stop codon causes the loss of the two potential glycosylation sites N513 and N537, with potential consequences for subcellular transport (Millat et al. 1997). For the S71N mutation, we propose that new hydrogen bonds seen within the catalytic site hinder the resolution of the ester-sulfate ester, but further experimental studies are needed. For example, mutation correction at a translational level has been found, especially in cases of attenuated presentation (Bunge et al. 1993; Lualdi et al. 2010).

The new mutations Del.Q200_E203 and R294GfsX2 (Del. exon 7) showed markedly distorted interactions within the pocket (Galvis et al. 2014).

Conclusions

This is the first study in South America which performs a molecular analysis of MPSII with three available laboratory methodologies and performs in silico simulations with the identified mutations. In our population, the observed age at diagnosis was similar to what has been reported in the literature. The genotyping study based on 18 samples achieved a sensitivity of 100% when employing three methodologies, but the result of the study will possibly be different if a larger number of samples are included. The frequency of novel mutations (46.15%) is similar to what has been reported elsewhere. The use of bioinformatic tools was of utility and molecular docking allowed for identification of changes at the level of the catalytic site of each mutant. However, given the small sample size, a phenotype-genotype correlation was not feasible; studies with larger groups of patients are needed.

Acknowledgments To patients and their families, MRC-Holland for the donation of the MLPA kit for this research, ACOPEL, and Doctors Gabriel Sierra, Luz Norella Correa, Gustavo Contreras, Sandra Mansilla, and Juan Carlos Prieto. To professors Mauricio Rey, Rita Baldrich, Blanca Schroeder, Henry J Rodríguez, and other members of immunogenetics/biology group at Universidad Nacional de Colombia for their support relating to laboratory work. To Tatiana Vinasco, David Serrano, and Andres Gutierrez for contribution of literature and counseling.

One-Sentence Take-Home Message

Deep genotyping and bioinformatic approaches can lead to a better understanding of Hunter syndrome (MPSII).

Compliance with Ethics Guidelines

All procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent for study participation was obtained from all patients included in the study.

This study included 18 Colombian patients with a diagnosis of MPS II whose familial or legal guardians gave informed consent. The informed consent was previously evaluated and approved by the Ethics Committee of the School of Medicine of the National University of Colombia

(Comité de Etica de la Facultad de Medicina de la Universidad Nacional de Colombia). By Act Number 16 of November 5, 2010, the Ethics Committee approved the study. After a careful review, the Committee determined that the project meets the ethical requirements and complies with Approbatory Concept. Signed: Prof. CARLOS ARTURO GUERRERO, President of the Ethics Committee.

Conflict of Interest

Johanna Galvis, Jannet González, Alfredo Uribe declare that they have no conflict of interest. Harvy Velasco declares that he received an educative grant from Shire.

Details of the Contribution of Individual Authors

Johanna Galvis: Planning of the work; patients' recruitment; clinical data extraction and analysis; genotyping steps at laboratory; bioinformatic simulations; reporting of the results; writing and revision of manuscript. Guarantor 1

Jannet Gonzalez: Bioinformatic simulations. Revision of bioinformatic results

Alfredo Uribe: IDS Enzyme assays results, additional clinical data of patients

Harvy Velasco: Planning of the work; patients' recruitment; clinical data extraction and analysis. Revision of manuscript. Guarantor 2

References

Alves S, Mangas M, Prata MJ et al (2006) Molecular characterization of Portuguese patients with mucopolysaccharidosis type II shows evidence that the IDS gene is prone to splicing mutations. J Inherit Metab Dis 29:743–754

Bondeson ML, Dahl N, Malmgren H et al (1995) Inversion of the IDS gene resulting from recombination with IDS-related sequences is a common cause of the Hunter syndrome. Hum Mol Genet 4:615–621

Brusius-Facchin AC, Schwartz IV, Zimmer C et al (2014) Mucopolysaccharidosis type II: identification of 30 novel mutations among Latin American patients. Mol Genet Metab 111:133–138

Bunge S, Steglich C, Zuther C et al (1993) Iduronate-2-sulfatase gene mutations in 16 patients with mucopolysaccharidosis type II (Hunter syndrome). Hum Mol Genet 2:1871–1875

Bunge S, Rathmann M, Steglich C et al (1998) Homologous nonallelic recombinations between the iduronate-sulfatase gene and pseudogene cause various intragenic deletions and inversions in patients with mucopolysaccharidosis type II. Eur J Hum Genet 6:492–500

Dierks T, Lecca MR, Schlotterhose P, Schmidt B, von Figura K (1999) Sequence determinants directing conversion of cysteine to formylglycine in eukaryotic sulfatases. EMBO J 18:2084–2091

Diez-Roux G, Ballabio A (2005) Sulfatases and human disease. Annu Rev Genomics Hum Genet 6:355–379

Froissart R, Maire I, Millat G et al (1998) Identification of iduronate sulfatase gene alterations in 70 unrelated Hunter patients. Clin Genet 53:362–368

Froissart R, Da Silva IM, Maire I (2007) Mucopolysaccharidosis type II: an update on mutation spectrum. Acta Paediatr Suppl 96:71–77

Galvis J, Gonzalez J, Torrente D, Velasco H, Barreto G (2014) In silico analysis of iduronate 2 sulfatase mutations in Colombian patients with hunter syndrome (MPSII) in: advances in computational biology. Adv Intel Syst Comput 232 (2014):205–212

Gómez A, García-Robles R, Suárez-Obando F (2012) Estimación de las frecuencias de las mucopolisacaridosis y análisis de agrupamiento espacial en los departamentos de Cundinamarca y Boyacá. Biomedica 32:602–609

Gucev ZS, Tasic V, Sinigerska I, Kremensky I et al (2011) Hunter syndrome (Mucopolysacharidosis Type II) in Macedonia and Bulgaria. Prilozi 32(2):187–198

Holt J, Poe MD, Escolar ML (2011) Early clinical markers of central nervous system involvement in mucopolysaccharidosis type II. J Pediatr 159(320–326):e322

Isogai K, Sukegawa K, Tomatsu S et al (1998) Mutation analysis in the iduronate-2-sulphatase gene in 43 Japanese patients with mucopolysaccharidosis type II (Hunter disease). J Inherit Metab Dis 21:60–70

Jones SA, Almassy Z, Beck M et al (2009) Mortality and cause of death in mucopolysaccharidosis type II-a historical review based on data from the Hunter Outcome Survey (HOS). J Inherit Metab Dis 32:534–543

Kato T, Kato Z, Kuratsubo I et al (2005) Mutational and structural analysis of Japanese patients with mucopolysaccharidosis type II. J Hum Genet 50:395–402

Lagerstedt K, Carlberg BM, Karimi-Nejad R, Kleijer WJ, Bondeson ML (2000) Analysis of a 43.6 kb deletion in a patient with Hunter syndrome (MPSII): identification of a fusion transcript including sequences from the gene W and the IDS gene. Hum Mutat 15:324–331

Lau C, Lam CW (2008) Molecular investigations of a novel iduronate-2-sulfatase mutant in a Chinese patient. Clin Chim Acta 392:8–10

Li P, Thompson JN (1996) Detection of four novel mutations in the iduronate-2-sulphatase gene by single-strand conformation polymorphism analysis of genomic amplicons. J Inher Metab Dis 19(1):93–94

Lin SP, Chang JH, Lee-Chen GJ, Lin DS, Lin HY, Chuang CK (2006) Detection of Hunter syndrome (mucopolysaccharidosis type II) in Taiwanese: biochemical and linkage studies of the iduronate-2-sulfatase gene defects in MPS II patients and carriers. Clin Chim Acta 369:29–34

Lissens W, Seneca S, Liebaers I (1997) Molecular analysis in 23 Hunter disease families. J Inher Metab Dis 20:453–456

Lualdi S, Regis S, Di Rocco M et al (2005) Characterization of iduronate-2-sulfatase gene-pseudogene recombinations in eight patients with Mucopolysaccharidosis type II revealed by a rapid PCR-based method. Hum Mutat 25:491–497

Lualdi S, Tappino B, Di Duca M et al (2010) Enigmatic in vivo iduronate-2-sulfatase (IDS) mutant transcript correction to wild-type in Hunter syndrome. Hum Mutat 31:E1261–E1285

Malm G, Lund AM, Mansson JE, Heiberg A (2008) Mucopolysaccharidoses in the Scandinavian countries: incidence and prevalence. Acta Paediatr 97:1577–1581

Martin R, Beck M, Eng C et al (2008) Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome). Pediatrics 121: e377–e386

Miech C, Dierks T, Selmer T, von Figura K, Schmidt B (1998) Arylsulfatase from Klebsiella pneumoniae carries a formylglycine generated from a serine. J Biol Chem 273:4835–4837

Millat G, Froissart R, Maire I, Bozon D (1997) Characterization of iduronate sulphatase mutants affecting N-glycosylation sites and the cysteine-84 residue. Biochem J 326(Pt 1):243–247

Nelson J (1997) Incidence of the mucopolysaccharidoses in Northern Ireland. Hum Genet 101:355–358

Neufeld EF, Muenzer J (2001) The mucopolysaccharidoses. In: Scriver CR (ed) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, NY

Ochiai T, Suzuki Y, Kato T et al (2007) Natural history of extensive Mongolian spots in mucopolysaccharidosis type II (Hunter syndrome): a survey among 52 Japanese patients. J Eur Acad Dermatol Venereol 21:1082–1085

Parkinson EJ, Muller V, Hopwood JJ, Brooks DA (2004) Iduronate-2-sulphatase protein detection in plasma from mucopolysaccharidosis type II patients. Mol Genet Metab 81:58–64

Poorthuis BJ, Wevers RA, Kleijer WJ et al (1999) The frequency of lysosomal storage diseases in The Netherlands. Hum Genet 105:151–156

Rathmann M, Bunge S, Steglich C, Schwinger E, Gal A (1995) Evidence for an iduronate-sulfatase pseudogene near the functional Hunter syndrome gene in Xq27.3-q28. Hum Genet 95:34–38

Rathmann M, Bunge S, Beck M, Kresse H, Tylki-Szymanska A, Gal A (1996) Mucopolysaccharidosis type II (Hunter syndrome): mutation "hot spots" in the iduronate-2-sulfatase gene. Am J Hum Genet 59:1202–1209

Scarpa M, Almassy Z, Beck M et al (2011) Mucopolysaccharidosis type II: European recommendations for the diagnosis and multidisciplinary management of a rare disease. Orphanet J Rare Dis 6:72

Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 30:e57

Schwartz IV, Ribeiro MG, Mota JG et al (2007) A clinical study of 77 patients with mucopolysaccharidosis type II. Acta Paediatr Suppl 96:63–70

Sohn YB, Ki CS, Kim CH et al (2012) Identification of 11 novel mutations in 49 Korean patients with mucopolysaccharidosis type II. Clin Genet 81:185–190

Steen-Bondeson ML, Dahl N, Tonnesen T et al (1992) Molecular analysis of patients with Hunter syndrome: implication of a region prone to structural alterations within the IDS gene. Hum Mol Genet 1:195–198

Sukegawa-Hayasaka K, Kato Z, Nakamura H et al (2006) Effect of Hunter disease (mucopolysaccharidosis type II) mutations on molecular phenotypes of iduronate-2-sulfatase: enzymatic activity, protein processing and structural analysis. J Inherit Metab Dis 29:755–761

Vafiadaki E, Cooper A, Heptinstall LE, Hatton CE, Thornley M, Wraith JE (1998) Mutation analysis in 57 unrelated patients with MPS II (Hunter's disease). Arch Dis Child 79:237–241

Villani GR, Daniele A, Balzano N, Di Natale P (2000) Expression of five iduronate-2-sulfatase site-directed mutations. Biochim Biophys Acta 1501(2–3):71–80

Waldow A, Schmidt B, Dierks T, von Bulow R, von Figura K (1999) Amino acid residues forming the active site of arylsulfatase A. Role in catalytic activity and substrate binding. J Biol Chem 274:12284–12288

Whitley CB, Anderson RA, Aronovich EL et al (1993) Caveat to genotype-phenotype correlation in mucopolysaccharidosis type II: discordant clinical severity of R468W and R468Q mutations of the iduronate-2-sulfatase gene. Hum Mutat 2(3):235–23

Wilson PJ, Morris CP, Anson DS et al (1990) Hunter syndrome: isolation of an iduronate-2-sulfatase cDNA clone and analysis of patient DNA. Proc Natl Acad Sci U S A 87:8531–8535

Wraith JE, Beck M, Giugliani R, Clarke J, Martin R, Muenzer J (2008a) Initial report from the hunter outcome survey. Genet Med 10:508–516

Wraith JE, Scarpa M, Bec M et al (2008b) Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy. Eur J Pediatr 167:267–277

Yatziv S, Erickson RP, Epstein CJ (1977) Mild and severe Hunter syndrome (MPS II) within the same sibships. Clin Genet 11:319–326

Young ID, Harper PS, Newcombe RG, Archer IM (1982) A clinical and genetic study of Hunter's syndrome. 2. Differences between the mild and severe forms. J Med Genet 19:408–411

Zhang H, Li J, Zhang X et al (2011) Analysis of the IDS gene in 38 patients with Hunter syndrome: the c.879G > A (p.Gln293Gln) synonymous variation in a female create exonic splicing. PLoS One 6:e22951

JIMD Reports
DOI 10.1007/8904_2014_378

CASE REPORT

Expanding the Clinical Spectrum of Mitochondrial Citrate Carrier (SLC25A1) Deficiency: Facial Dysmorphism in Siblings with Epileptic Encephalopathy and Combined D,L-2-Hydroxyglutaric Aciduria

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Received: 03 July 2014 / Revised: 15 October 2014 / Accepted: 21 October 2014 / Published online: 23 January 2015
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Abstract Recessive mutations in *SLC25A1* encoding mitochondrial citrate carrier cause a rare inherited metabolic disorder, combined D,L-2-hydroxyglutaric aciduria (D,L-2-HGA), characterized by epileptic encephalopathy, respiratory insufficiency, developmental arrest and early death. Here, we describe two siblings compound heterozygotes for null/missense *SLC25A1* mutations, c.18_24dup (p.Ala9Profs*82), and c.134C>T (p.Pro45Leu). These children presented with classic clinical features of D,L-2-HGA, but also showed marked facial dysmorphism. Additionally, there was prominent lactic acidosis in one of the siblings. Our observations suggest that facial dysmorphism is a previously unrecognized but an important diagnostic feature of SLC25A1 deficiency and expand the clinical phenotype linked to *SLC25A1* mutations.

Communicated by: Francois Feillet, MD, PhD

Competing interests: None declared

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Introduction

Combined D-2- and L-2-hydroxyglutaric aciduria (D,L-2-HGA; OMIM:615182) is a rare inborn error of metabolism characterized by elevated levels of both D-2- and L-2-hydroxyglutaric acids (HG) in body fluids which typically manifests as severe neonatal epileptic encephalopathy, respiratory insufficiency, global hypotonia, lack of developmental progress, and early death. Deficiency of the mitochondrial citrate carrier as a result of recessive mutations in the *SLC25A1* gene was recently recognized as the underlying cause of D,L-2-HGA (Nota et al. 2013). SLC25A1 belongs to the inner mitochondrial membrane transporters of SLC25 family and promotes the efflux of citrate/isocitrate from mitochondria to cytoplasm (Gutiérrez-Aguilar and Baines 2013; Palmieri 2013, 2014). It plays key roles in mitochondrial function, lipid biosynthesis, and inhibition of glycolysis (Catalina-Rodriguez et al. 2012). The origin of elevated D and L enantiomers of 2-HG due to SLC25A1 deficiency is still unclear, but has been attributed to accumulation of citrate and other tricarboxylic acid (TCA) cycle intermediates in the mitochondria including 2-ketoglutarate (2-KG) which in turn is converted to D and L, 2-HG (Kranendijk et al. 2012). We report two siblings with classic clinical features of D,L-2-HGA, and additionally facial dysmorphism expanding the phenotype of SLC25A1 deficiency. Also, there was marked lactic

acidosis in one of the siblings. These observations suggest the importance of testing for SLC25A1 mutations and/or 2-HG enantiomers in patients with facial dysmorphism, encephalopathy, and lactic acidosis.

Case Reports

The patients were siblings born from healthy nonconsanguineous Hispanic parents. Patient 1 was born at full term via cesarean section. Global hypotonia and facial dysmorphism consisting of hypertelorism, broad depressed nasal bridge, micrognathia, and retrognathia were noted at birth. The patient developed encephalopathy and seizures at approximately 24 h of life. The patient had to be intubated for respiratory failure and altered mental status. Subsequently, the patient remained ventilator dependent. Magnetic resonance imaging of the brain showed diffuse atrophy and dilation of both ventricles. The patient died at 2 months of age.

Patient 2 was born at term by spontaneous vaginal delivery and also exhibited hypertelorism, broad depressed nasal bridge, micrognathia, and retrognathia, accompanied by bilateral ptosis and global hypotonia. The patient had respiratory failure and seizures with encephalopathy on day 1 of life. Intubation of this patient for respiratory failure was difficult due to narrow anteriorly placed vocal cords. This patient too remained ventilator dependent. An ultrasound of the head revealed bilateral ventriculomegaly and increase in extra axial space in the right frontal region. Echocardiography showed ventricular and atrial septal defects, patent ductus arteriosus, and bicommissural aortic valve. The patient died at 3 weeks of age.

Molecular and Metabolic Studies

A chromosomal microarray (performed using the Affymetrix Cytoscan HD array: this array consisted of nearly 2.7 million genetic markers incorporating 743,304 single nucleotide polymorphism probes as well as 1,953,246 nonpolymorphic copy number variation probes) for both patients was normal. Blood lactate of patient 1 on day 8 of life was 3.6 mmol/L (reference range 0.6–2.5). Blood lactate of patient 2 was initially 9.8 mmol/L (day 1 of life) and increased to 25 mmol/L on day 5. Plasma amino acids and acylcarnitine profile assays for both patients were normal. Urine organic acid assay for patient 1 showed elevated 2-HG, lactate, and fumarate. Further analysis of the urine for the differentiation of D and L, 2-HG enantiomers by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Struys et al. 2004) showed elevations of both metabolites (Table 1). Initial qualitative urine organic acid analysis for patient 2 did not show an elevation of 2-HG, while further analysis by LC-MS/MS showed mild to modest elevations in four of five urine samples. Urinary citrate was low for patient 2 (Table 1).

Both patients were compound heterozygous for the mutations c.18_24dup (p.Ala9Profs*82) and c.134C>T (p.Pro45Leu) in *SLC25A1*. The mutational status of patient 1 was previously reported in the study by Nota et al., which first identified *SLC25A1* mutations in D,L-2-HGA (Nota et al. 2013). The mother was heterozygous for c.134C>T mutation, while the father was heterozygous for c.18_24dup.

Discussion

Only few cases of mitochondrial citrate carrier deficiency have been reported in the literature thus far (Chaouch et al. 2014; Edvardson et al. 2013; Nota et al. 2013). The hallmark biochemical alteration caused by *SLC25A1* mutations is D,L-2-hydroxyglutaric aciduria, accompanied by higher urinary concentrations of 2-KG and variable elevations of other TCA cycle intermediates (succinate,

Table 1 Urinary concentrations of D and L, 2-hydroxyglutaric acids (2-HG) and citrate

Patient	Day of life	D-2-HG (mmol/mol creatinine)	L-2-HG (mmol/mol creatinine)	Citrate (mmol/mol creatinine)
Control		2.8–17	1.3–19	72–1,449
Patient 1	20	449[a]	110[a]	ND
Patient 2	1	29[a]	40[a]	ND
	3	11	17	ND
	9	61[a]	35[a]	ND
	12	57[a]	30[a]	19
	17	84[a]	38[a]	33

[a] Indicates elevations compared with the reference range. Please note normal concentrations in the sample obtained on day of life 3 for patient 2
ND not determined

fumarate, and malate) (Kranendijk et al. 2012). These patients typically present with severe neonatal epileptic encephalopathy, respiratory insufficiency, hypotonia, and developmental arrest. However, a relatively milder presentation as congenital myasthenia has also been reported (Chaouch et al. 2014).

Our described siblings carried compound heterozygous mutations in *SLC25A1* that generate early truncated (p.Ala9Profs*82) and missense (p.Pro45Leu) alleles. It was shown previously that the patient homozygous for p.Ala9Profs*82 had no detectable SLC25A1 on immunoblot analysis of fibroblasts (Nota et al. 2013). Pro45 residue is highly conserved throughout evolution in all SLC25A1 members, and the 134 C>T transition has an unfavorable score (Pierri et al. 2014). From a structural point of view, it belongs to the region involved in conformational changes of the citrate carrier occurring during the translocation mechanism. Therefore, this substitution is likely to affect the transport rate of citrate (Palmieri and Pierri 2010).

The clinical presentation of these patients is typical for D,L-2-HGA. The hallmark biochemical alteration of increased D and L, 2-HG enantiomers in urine was also present in these children, although not persistently in one of the siblings. The facial dysmorphism present in both siblings is a hitherto unrecognized feature of this disease. One of the siblings had severe lactic acidosis and cardiac malformations. Our findings suggest that in newborns with facial dysmorphism, multiple malformations, epileptic encephalopathy, and lactic acidosis, SLC25A1 deficiency should be suspected. The initial qualitative urine organic assay in the younger sibling was normal during the acute presentation implying that excretion of these metabolites may be intermittent or may potentially be overlooked when using the traditional urinary organic acid screening method. A dedicated analytical procedure that is able to quantify both enantiomers of 2-HG and *SLC25A1* sequencing is required to confirm the diagnosis.

The severity of the clinical findings in these siblings is likely accounted for by the key roles played by SLC25A1 in carbohydrate and lipid metabolism and in promoting mitochondrial function (Catalina-Rodriguez et al. 2012). Citrate is produced predominantly in mitochondria and is oxidized via the TCA cycle and oxidative phosphorylation (OXPHOS). The entry of malate (another TCA cycle intermediate) in exchange for citrate is coupled with the transport of one proton to maintain electroneutrality across the mitochondrial membrane (Palmieri 2013). Thus, the mitochondrial citrate transporter maintains the integrity of the TCA cycle as well as mitochondrial inner membrane potential, the stability of which is linked to proton flux and electron transport chain activity. In the cytoplasm, citrate functions as an allosteric inhibitor of the glycolytic enzyme, phosphofructokinase-1 (Newsholme et al 1977). Therefore, it is likely that when SLC25A1 is severely compromised, glycolysis proceeds unchecked and mitochondrial respiration is impaired leading to lactic acidosis (Fig. 1). The high lactate, global hypotonia, and encephalopathy observed in patients with SLC25A1 deficiency are similar to clinical findings in patients with mitochondrial encephalomyopathies caused by respiratory chain defects.

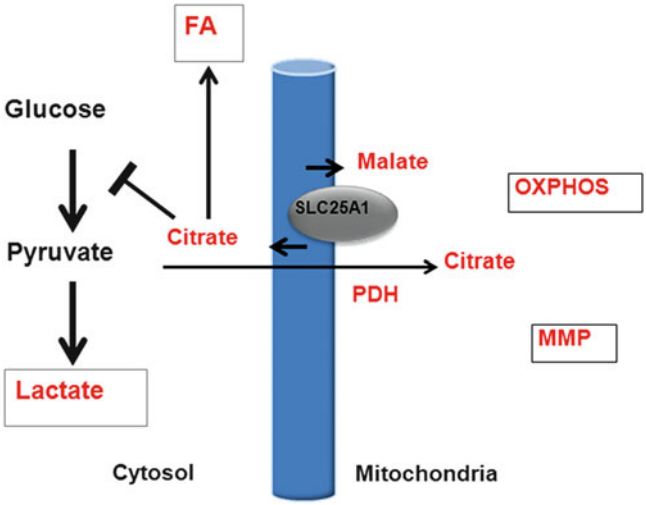

Fig. 1 Cytoplasmic and mitochondrial effects due to SLC25A1-dependent citrate export. See also text for explanation. In the cytoplasm, citrate promotes fatty acid (FA) synthesis. Malate oxidation in the mitochondria generates NADH, which donates its electrons to the electron transport chain thereby promoting oxidative phosphorylation (OXHPHOS). Malate/citrate shuttle is also crucial in stabilizing mitochondrial membrane potential (MMP). Cytoplasmic citrate acts as an allosteric inhibitor of the glycolytic enzyme phosphofructokinase-1. Pyruvate can either be converted to lactate in anaerobic glycolysis or enter the mitochondria where through the action of pyruvate dehydrogenase (PDH) is converted to acetyl coenzyme A (not shown) which then binds with oxaloacetate to form citrate. In red are the major input and output pathways linked to SLC25A1 activity

SLC25A1 also plays a crucial role in lipogenesis (Gnoni et al. 2009; Iacobazzi and Infantino 2014; Watson and Lowenstein 1970). Studies on skin fibroblasts from patients with SLC25A1 deficiency showed low citrate in culture medium (Nota et al. 2013). Similar observation in cell lines suggests that the cytoplasmic pool of citrate is maintained by the mitochondrial citrate carrier (Catalina-Rodriguez et al. 2012). Cytosolic citrate is cleaved by citrate lyase into acetyl-CoA – the main carbon source for fatty acid and cholesterol biosynthesis – and oxaloacetate (Gnoni et al. 2009; Iacobazzi and Infantino 2014; Watson and Lowenstein 1970). Cytoplasmic citrate is a positive allosteric modulator of acetyl-CoA carboxylase, a key enzyme in fatty acid synthesis (Halestrap and Denton 1974; Iacobazzi and Infantino 2014).

The etiology of facial dysmorphism and other developmental malformations is unclear. There is inadequate

phenotypic description of previous patients with D,L-2-HGA in the literature. One patient with D,L-2-HGA reported to have dysmorphism was initially diagnosed as D-2-hydroxyglutaric aciduria (D-2-HGA) (Kranendijk et al. 2012). Inhibition of the mitochondrial citrate carrier in zebrafish showed reduced jaw and small brain (Catalina-Rodriguez et al. 2012) implicating SLC25A1 deficiency in the dysmorphism and developmental defects seen in these patients. It may be secondary to abnormal epigenetic control of nuclear gene expression. Levels of histone acetylation are dependent upon availability of acetyl-CoA which is produced from cleavage of citrate exported from the mitochondria (Wellen et al. 2009). Histone acetylation plays essential roles in cell cycle progression, gene expression, and silencing. Inhibition of the drosophila ortholog of *SLC25A1* resulted in extensive chromosomal breakage, cell cycle arrest, and global reduction in histone acetylation (Morciano et al. 2009). Facial dysmorphism and brain malformations are also recognized features of D-2-HGA (Kranendijk et al. 2012). Accumulation of D-2-HG in both these conditions may be a causative factor as it acts as a competitive inhibitor of 2-KG (a TCA cycle intermediate)-dependent enzymes including histone demethylase and prolyl hydroxylase leading to global alteration in histone and DNA methylation patterns (Xu et al. 2011). Epigenetic dysregulation leading to disruption of embryogenesis is the most plausible mechanism for malformations in D,L-2-HGA. However, it may also be the result of impaired lipogenesis, particularly cholesterol synthesis. Agenesis of corpus callosum and optic nerve hypoplasia, previously reported in a patient with SLC25A1 deficiency (Edvardson et al. 2013), and the dysmorphism observed in our patients are reminiscent of patients with Smith-Lemli-Opitz syndrome and other defects of cholesterol biosynthesis (Herman and Kratz 2012). Interestingly, heterozygous deletion of the entire *SLC25A1* gene occurs in 22q.11.2 microdeletion syndrome which shares many of the facial features of these patients. However, the facial features of the parents of these patients were unremarkable. Loss of single copy of *SLC25A1* by itself is unlikely to be of any major consequences. However, its modifying role cannot be denied. This aspect needs to be further explored.

In summary, our observations suggest that facial dysmorphism and lactic acidosis are important diagnostic markers of SLC25A1 deficiency along with neonatal epileptic encephalopathy, global hypotonia, and D,L-2-HGA. An early diagnosis may be helpful from a therapeutic perspective as citrate therapy was found to reduce the frequency and severity of seizures in a patient with mitochondrial citrate carrier deficiency (Mühlhausen et al. 2014).

Compliance with Ethics Guidelines

This clinical report is a retrospective clinical observation that does not require ethics committee approval at this institution.

There are no prior publications of this manuscript.

The work is not and will not be submitted to any other journal while under consideration by "JIMD."

The authors, Pankaj Prasun, Sarah Young, Gajja Salomons, Andrea Werneke, Yong-hui Jiang, Eduard Struys, Mikell Paige, Maria Laura Avantaggiati, and Marie McDonald, have no potential conflicting or competing interests that could in any way affect the conduct of the study, interpretation of results, or preparation of the manuscript.

The authors, Pankaj Prasun, Sarah Young, Gajja Salomons, Andrea Werneke, Yong-hui Jiang, Eduard Struys, Mikell Paige, Maria Laura Avantaggiati, and Marie McDonald, do not have any funding sources to declare related to the study and to the article preparation.

Dr. Pankaj Prasun was involved in patient care, laboratory interpretation, initial drafting of the manuscript, and revisions of each draft.

Dr. Sarah young was involved in laboratory interpretation and revising the manuscript critically for important intellectual content.

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Dr. Marie McDonald supervised the case report and was involved in patient care, laboratory interpretation, and revising the manuscript critically for important intellectual content.

References

Catalina-Rodriguez O, Kolukula VK, Tomita Y et al (2012) The mitochondrial citrate transporter, CIC, is essential for mitochondrial homeostasis. Oncotarget 3:1220–1235

Chaouch A, Porcellli V, Cox D et al (2014) Mutations in the mitochondrial citrate carrier SLC25A1 are associated with impaired neuromuscular transmission. J Neuromuscul Dis 1:75–90

Edvardson S, Porcelli V, Jalas C et al (2013) Agenesis of corpus callosum and optic nerve hypoplasia due to mutations in SLC25A1 encoding the mitochondrial citrate transporter. J Med Genet 50:240–245

Gnoni GV, Priore P, Geelen MJ, Siculella L (2009) The mitochondrial citrate carrier: metabolic role and regulation of its activity and expression. IUBMB Life 61:987–994

Gutiérrez-Aguilar M, Baines CP (2013) Physiological and pathological roles of mitochondrial SLC25 carriers. Biochem J 454:371–386

Halestrap AP, Denton RM (1974) Hormonal regulation of adipose-tissue acetyl-Coenzyme A carboxylase by changes in the polymeric state of the enzyme. The role of long-chain fatty acyl-Coenzyme A thioesters and citrate. Biochem J 142:365–377

Herman GE, Kratz L (2012) Disorders of sterol synthesis: beyond Smith-Lemli-Opitz syndrome. Am J Med Genet C Semin Med Genet 160C:301–321

Iacobazzi V, Infantino V (2014) Citrate-new functions for an old metabolite. Biol Chem 395:387–399

Kranendijk M, Struys EA, Salomons GS, Van der Knaap MS, Jakobs C (2012) Progress in understanding 2-hydroxyglutaric acidurias. J Inherit Metab Dis 35:571–587

Morciano P, Carrisi C, Capobianco L et al (2009) A conserved role for the mitochondrial citrate transporter Sea/SLC25A1 in the maintenance of chromosome integrity. Hum Mol Genet 18:4180–4188

Mühlhausen C, Salomons GS, Lukacs Z et al (2014) Combined D2-/L2-hydroxyglutaric aciduria (SLC25A1 deficiency): clinical course and effects of citrate treatment. J Inherit Metab Dis 37:775–781

Newsholme EA, Sugden PH, Williams T (1977) Effect of citrate on the activities of 6-phosphofructokinase from nervous and muscle tissues from different animals and its relationships to the regulation of glycolysis. Biochem J 166:123–129

Nota B, Struys EA, Pop A et al (2013) Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. Am J Hum Genet 92:627–631

Palmieri F (2013) The mitochondrial transporter family SLC25: identification, properties and physiopathology. Mol Aspects Med 34:465–484

Palmieri F (2014) Mitochondrial transporters of the SLC25 family and associated diseases: a review. J Inherit Metab Dis 37:565–575

Palmieri F, Pierri CL (2010) Structure and function of mitochondrial carriers – role of the transmembrane helix P and G residues in the gating and transport mechanism. FEBS Lett 584:1931–1939

Pierri CL, Palmieri F, De Grassi A (2014) Single-nucleotide evolution quantifies the importance of each site along the structure of mitochondrial carriers. Cell Mol Life Sci 71:349–364

Struys EA, Jansen EE, Verhoeven NM, Jakobs C (2004) Measurement of urinary D- and L-2-hydroxyglutarate enantiomers by stable-isotope-dilution liquid chromatography-tandem mass spectrometry after derivatization with diacetyl-L-tartaric anhydride. Clin Chem 50:1391–1395

Watson JA, Lowenstein JM (1970) Citrate and the conversion of carbohydrate into fat. Fatty acid synthesis by a combination of cytoplasm and mitochondria. J Biol Chem 245:5993–6002

Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324:1076–1080

Xu W, Yang H, Liu Y et al (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. Cancer Cell 19:17–30

JIMD Reports
DOI 10.1007/8904_2014_379

CASE REPORT

A Korean Case of β-Ureidopropionase Deficiency Presenting with Intractable Seizure, Global Developmental Delay, and Microcephaly

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Received: 11 August 2014 / Revised: 23 October 2014 / Accepted: 29 October 2014 / Published online: 01 February 2015
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Abstract β-Ureidopropionase deficiency (OMIM #613161) is a rare autosomal recessive inborn error of metabolism due to mutations in the *UPB1* gene, which encodes the third enzyme involved in the pyrimidine degradation pathway. A total of 28 cases have been reported, mainly presenting with seizures, microcephaly, and intellectual disabilities. However, 11 of them were asymptomatic cases (Nakajima et al., J Inherit Metab Dis 37(5):801–812, 2014). We report on a 9-year-old female presenting with intractable epilepsy, microcephaly, and global developmental delay. She was homozygous for p.R326Q (c.977G>A) and heterozygous for p.G31S (c.91G>A) in the *UPB1* gene, detected by targeted next-generation sequencing test and subsequently confirmed by biochemical analysis of urine, plasma, and cerebrospinal fluid (CSF) using reversed-phase HPLC, combined with electrospray tandem mass spectrometry. We report a first Korean female case with β-ureidopropionase deficiency.

Communicated by: John Christodoulou, MB BS PhD FRACP FRCPA

Competing interests: None declared

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Introduction

β-Ureidopropionase (β-UP), the enzyme involved in pyrimidine degradation pathway, catalyzes the conversion of *N*-carbamyl-β-alanine and *N*-carbamyl-β-aminoisobutyric acid to β-alanine and β-aminoisobutyric acid, respectively (van Kuilenburg et al. 2012; Yaplito-Lee et al. 2008). The prevalence of β-UP deficiency in Japan has been estimated at 1 in 6,000 (Kuhara et al. 2009). Molecular findings of 13 Japanese β-UP-deficient patients revealed three novel missense mutations (p.G31S, p.E271K, and p.I286T), with all 13 patients showing a p.R326Q mutation and 8 patients being homozygous. The p.R326Q mutation was previously described to abolish the enzyme activity (Nakajima et al. 2014). Two Chinese patients have been reported, both carried the p.R326Q mutation, but ß-UP deficiency has not been reported in Korea up to date (van Kuilenburg et al. 2012; Nakajima et al. 2014).

We report a first Korean case of ß-ureidopropionase deficiency who was homozygous for p.R326Q (c.977G>A) and heterozygous for p.G31S (c.91G>A) in the *UPB1* gene detected by Illumina next-generation sequencer in panel gene test for microcephaly and lissencephaly and Sanger sequencing analysis and the diagnosis was subsequently confirmed by biochemical analysis of the pyrimidine pathway.

Case Report

A 3-month-old female visited the outpatient department after a generalized tonic-clonic seizure without fever. Her family history was nonspecific, and her medical history included a normal vaginal delivery at 41 weeks with a birth weight of 3,140 g (40th percentile), height of 51 cm

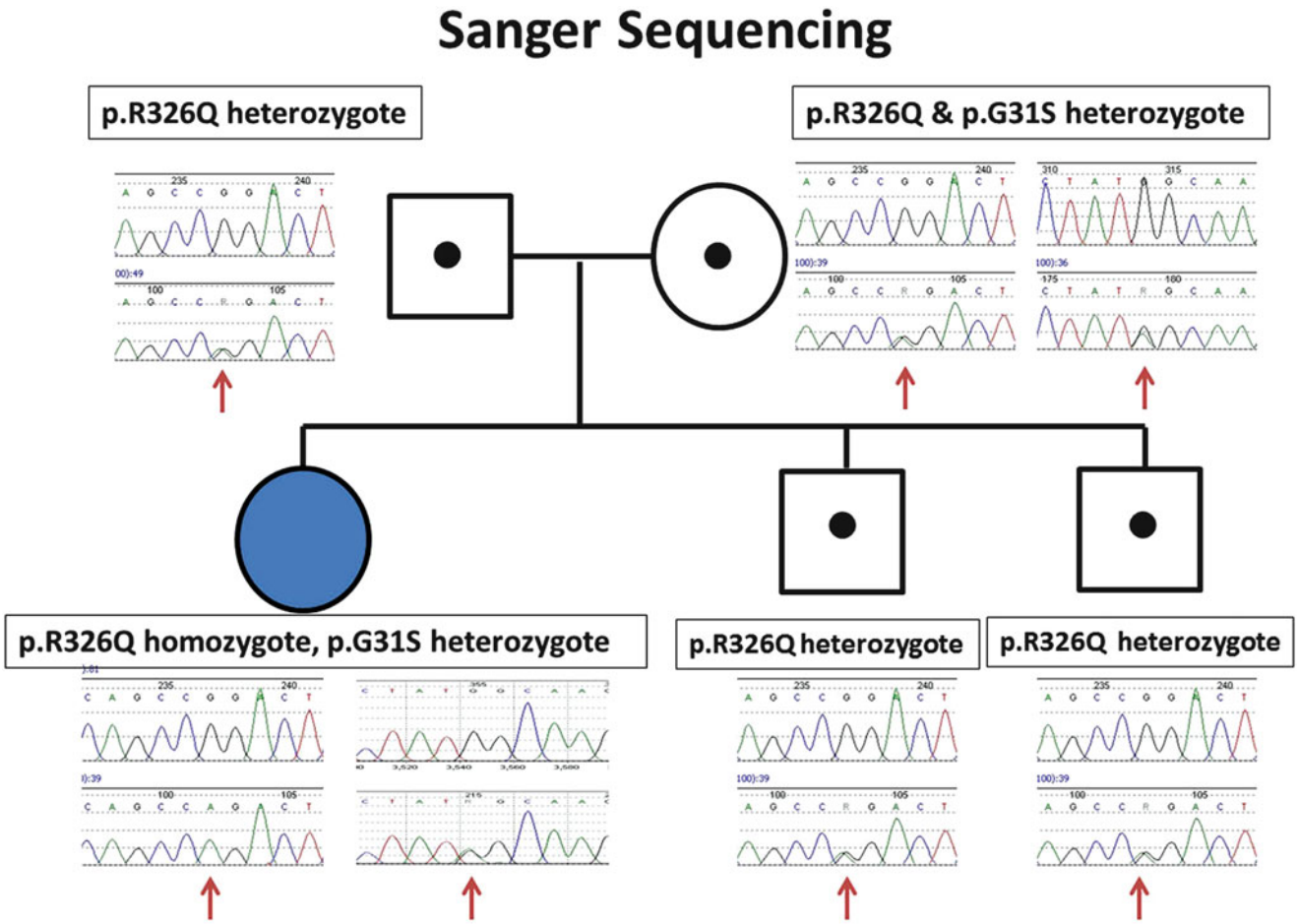

Fig. 1 Sanger sequencing analysis for the *UPB1* gene. Proband was homozygous for p.R326Q (c.977G>A) and heterozygous for p.G31S (c.91G>A) in the *UPB1* gene. All family members were found to be carriers, with heterozygosity for p.R326Q in the father and heterozygosity for both p.R326Q and G31S in the mother and heterozygosity for p.R326Q in two siblings

(70th percentile), and head circumference of 32.5 cm (15th percentile) from non-consanguineous Korean parents. We performed an epilepsy workup with electroencephalography (EEG), brain magnetic resonance image (MRI), electrolyte, thyroid function test, and neonatal tandem mass screening, which were all nonspecific findings. A recurrent seizure was stopped after administration of phenobarbital medication. Development has been nearly normal with mild gross motor delay until the age of 12 months when recurring seizures began and multiple antiepileptic drug medications such as valproic acid, carbamazepine, and clobazam (Sentil®) were administered. Seizures were not well controlled, which required reevaluation and workups that included EEG, brain MRI, and tandem mass screening, but the results did not reveal any specific findings. At the age of 17 months, recurrent seizures continued with poor control, which lead to changes in her medication to valproic acid, topiramate, and pyridoxine. By that time, she showed microcephaly and global developmental delay. At the age of 27 months, we examined the patient using the Korean Bayley Scale of Infant Development-II (K-BSID-II) for developmental delay. Results showed a mental performance age of 4 months and a motor performance age of 6 months. At the age of 3 years, she had an episode of generalized status epilepticus. Further diagnostic workups including an *MeCP2* gene test for Rett syndrome, methylation test for Prader-Willi/Angelman syndrome, and muscle biopsy for mitochondrial disease were all normal. At the age of 4 years, the patient had another episode of intractable epilepsy and subsequently started ketogenic diet with multiple antiepileptic drugs such as topiramate, lamotrigine, and levetiracetam. Seizures were aggravated by a minor upper respiratory infection or gastrointestinal infection. At 8 years of age, neurological development had deteriorated to generalized hypotonia, with inability to speak or sit upright by herself. At that time, she had still intractable epilepsy, but her brain MRI and EEG were nonspecific.

A deoxyribonucleic acid (DNA) sample was sent to the Seattle Children's Hospital Research Institute for analysis by target panel test after IRB approval. Targeted massive

Table 1 Pyrimidine metabolite levels in urine, plasma, and cerebrospinal fluid (CSF) of patient

	Urine (μmol/mmol creatinine)		Plasma (μM)		Cerebrospinal fluid (μM)	
Pyrimidine metabolites	Patient	Reference	Patient	Reference	Patient	Reference
N-carbamyl-β-alanine	248	11.0 ± 9.2	6.81	0.2 ± 0.3	0.90	0.1 ± 0.3
N-carbamyl-β-aminoisobutyric acid	186	1.8 ± 2.3	25.15	0.1 ± 0.2	1.60	0.01 ± 0.04
Dihydrouracil	16	6.3 ± 5.3	0.81	1.3 ± 0.8	1.60	2.1 ± 1.0
Dihydrothymine	47	3.1 ± 2.1	3.58	0.9 ± 0.3	4.30	1.1 ± 0.3
Uracil	4	11.8 ± 9.1	<0.6	0.2 ± 0.4	0.20	0.1 ± 0.2
Thymine	1	0.5 ± 0.6	<0.08	0.05 ± 0.03	<0.1	<0.1

The reference values are depicted as mean ± SD

parallel sequencing was performed using GAIIx instrument (Illumina, San Diego, CA). The exons of a total of 1,461 genes were targeted for various neurological conditions, including seizures, brain development, intellectual disability, and movement disorders (SureSelect, Agilent). Sequence was aligned to hg19 using BWA 0.5.7, and single-nucleotide variants and indels were called using GATK (version 1.6.5). Mean read depth of the targeted exome was calculated with the GATK DepthOfCoverage walker. Annotation of variants was performed with SeattleSeq Annotation 134. Common variants were identified by filtering against the NHLBI Exome Variant Server. Variants identified as possibly disease causing were confirmed by standard polymerase-chain-reaction (PCR) combined with bidirectional Sanger sequencing using standard methods.

The *UPB1* gene for β-UP deficiency was one of the genes included in this panel, which revealed that she was homozygous for p.R326Q (c.977G>A) and heterozygous for p.G31S (c.91G>A) in the *UPB1* gene (confirmed by Sanger sequencing analysis, NM_016327). Her family was subsequently subjected to sequencing analysis, which revealed heterozygosity for p.R326Q in the father and heterozygosity for both p.R326Q and p.G31S in the mother. Her two asymptomatic siblings were heterozygous for p.R326Q (Fig. 1). Biochemical analysis of pyrimidine metabolites in patient samples (serum, urine, and CSF) revealed highly elevated *N*-carbamyl-β-amino acids and moderately elevated levels of dihydrothymine in CSF and plasma, supporting the biochemical diagnosis of β-UP deficiency (Table 1).

At the age of 8 years and 10 months, the patient showed failure to thrive, global developmental delay, and microcephaly. She had body weight of 11.2 kg (<1 percentile), height of 103 cm (<1 percentile), and a head circumference of 44.5 cm (<1 percentile). At 9 years and 1 month of age, she started a restricted purine and pyrimidine diet, which helped reduce her frequency of drop attack seizure and generalized tonic-clonic seizure from 20–30 attacks per day to 1–3 attacks per day. Now, her age is 9 years and 8 months of age; she is still receiving multiple antiepileptic drug medications such as valproic acid, clobazam (Sentil®), zonisamide (Excegran®), and recently added rufinamide (Inovelon®).

Her two siblings, age 5 years and 21 months, show development in the normal range and have no seizure history.

Discussion

Pyrimidine nucleotides are essential for a number of biological processes, such as the synthesis of ribonucleic acid (RNA), DNA, phospholipids, and glycogen and the sialylation and glycosylation of proteins. Metabolic changes affecting the levels of pyrimidines may lead to abnormal neurological activity, as they play an important role in the regulation of the central nervous system (CNS) (van Kuilenburg et al. 2004).

Metabolism of pyrimidine nucleotides is divided into three pathways: biosynthetic, catabolic, and salvage. In the catabolic pathway of pyrimidine nucleosides, uracil and thymine are degraded in three steps: dihydropyrimidine dehydrogenase, dihydropyrimidinase, and β-UP (Saudubray et al. 2012; Kuhara et al. 2009). β-UP is the last enzyme involved in the pyrimidine degradation pathway, catalyzing the conversion of *N*-carbamyl-β-alanine and *N*-carbamyl-β-aminoisobutyric acid to β-alanine and β-aminoisobutyric acid, respectively (van Kuilenburg et al. 2012; Yaplito-Lee et al. 2008).

Clinical presentation of β-UP-deficient patients varies from asymptomatic to severe neurological deterioration (Nakajima et al. 2014). A number of symptoms have been reported, including muscular hypotonia, dystonic movement (Assmann et al. 1998), febrile status epilepticus (Assmann et al. 2006b), congenital anomalies of the urogenital and colorectal systems (Yaplito-Lee et al. 2008), growth retardation, microcephaly, intellectual disability, autism, progressive mood change, abnormal facial morphology such as prominent metopic suture, dolicho-

cephaly, recurrent episodes of desaturation unrelated to seizure activity, recurrent attacks of vomiting and severe dehydration, bilateral microphthalmia, nephrotic syndrome, transient cerebral blindness, abnormal eye movement, abnormal retina pigmentation, severe speech delay, and mild optic atrophy (van Kuilenburg et al. 2012; Nakajima et al. 2014). Our patient's clinical presentations were comparable with other severe cases previously reported such as microcephaly, intractable epilepsy, status epilepticus, and global developmental delay.

Diagnosis of β-UP deficiency is challenging as neurological manifestations and MRI abnormalities such as cortical dysplasia, atrophia cerebri, delayed myelination, vermis hypoplasia, brain stem hypoplasia, callosal body hypoplasia, and subdural hematoma are overall nonspecific. Biochemical analysis for pyrimidine metabolites shows highly elevated levels of *N*-carbamyl-β-amino acids, moderately elevated levels of the dihydrouracil and dihydrothymine, but normal levels of uracil and thymine in urine, plasma, and CSF (van Kuilenburg et al. 2012).

Mutations of the *UPB1* gene can cause β-UP deficiency. Eight missense and three splice site mutations in *UPB1* were reported (van Kuilenburg et al. 2012), and three novel missense mutation and one p.R326Q mutation were identified in 13 patients from 12 unrelated Japanese families (Nakajima et al. 2014). No genotype-phenotype correlation was detected. In Japan, 1.8% of the Japanese population is heterozygous for the p.R326Q mutation and an estimated 1 in 12,500 individuals is homozygous for the p.R326Q mutation. Transient expression of mutant β-UP enzymes in HEK293 cells revealed that the p.R326Q mutation decreased residual activities to less than 1.3%. However, the p.G31S mutation showed a residual activity of 50% (Nakajima et al. 2014). Of note, four asymptomatic neonates were detected during a pilot study of newborns, and the prevalence of a β-UP deficiency in Japan has been estimated to be 1 in 6,000 (Kuhara et al. 2009). In a more recent study, Nakajima et al. reported 13 Japanese β-UP-deficient patients, 4 of which were symptomatic with seizures, motor retardation, mental retardation, and autism, but nine were asymptomatic and remained asymptomatic up to 10 years (Nakajima et al. 2014). Many of these asymptomatic cases were homozygous for the p.R326Q mutation.

Treatment of the ß-UP-deficient patient is symptomatic. Treatment with ß-alanine for more than 1.5 years revealed no clinical improvement (Assmann et al. 2006a). ß-Aminoisobutyric acid supplementation and ß-UP administration for removal of *N*-carbamyl-ß-alanine and *N*-carbamyl-ß-aminoisobutyric acid have not yet been attempted in ß-UP-deficient patients. A pyrimidine-restrictive diet could be an option for treating ß-UP-deficient patients and may help to reduce the seizure frequency as observed in our patient.

In conclusion, ß-UP deficiency should be considered for a possible diagnosis in patients with unexplained symptoms such as intractable epilepsy, progressive neurologic degeneration, and microcephaly; p.R326Q variation in UPB1 gene seems a prevalent one in Far Eastern Asians.

Acknowledgment We would like to thank the Green Cross Laboratories for delivering samples to the Netherlands.

Take-Home Message

We report a first Korean case of ß-ureidopropionase deficiency presenting with intractable seizure, intellectual disability, and microcephaly who was homozygous for p.R326Q and heterozygous for p.G31S in the *UPB1* gene, presenting with typical biochemical analysis results in pyrimidine pathways.

Contribution of Individual Authors

Si Houn Hahn and Valeria Vasta performed genetic study interpret and analyze the results. André B.P. van Kuilenburg and N. G. G. M. Abeling performed biochemical analysis. Si Houn Hahn and André B.P. van Kuilenburg participated in drafting the manuscript. Jun Hwa Lee drafted the manuscript and was the treating pediatricians.

Guarantor for the Article

Jun Hwa Lee

Details of Funding

None

Details of Ethics Approval

The study was approved by the Institutional Review Board of Samsung Changwon Hospital (IRB study #2013-SCMC-058-00).

Conflicting Interests

Jun Hwa Lee declares that he has no conflict of interest.

André B.P. van Kuilenburg declares that he has no conflict of interest.

Valeria Vasta declares that she has no conflict of interest.

N. G. G.M. Abeling declares that he has no conflict of interest.

Si Houn Hahn declares that he has no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Samsung Changwon Hospital, Republic of Korea) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from the parents.

References

Assmann B, Gohlich-Ratmann G, Brautigam C et al (1998) Presumptive ureidopropionase deficiency as a new defect in pyrimidine catabolism found with in vitro H-NMR spectroscopy. J Inherit Metab Dis 21(suppl2):1

Assmann B, Gohlich G, Baethmann M et al (2006a) Clinical findings and a therapeutic trial in the first patient with beta-ureidopropionase deficiency. Neuropediatrics 37:20–25

Assmann BE, Van Kuilenburg AB, Distelmaier F et al (2006b) Beta-ureidopropionase deficiency presenting with febrile status epilepticus. Epilepsia 47:215–217

Kuhara T, Ohse M, Inoue Y, Shinka T (2009) Five cases of beta-ureidopropionase deficiency detected by GC/MS analysis of urine metabolome. J Mass Spectrom 44:214–221

Nakajima Y, Meijer J, Dobritzsch D et al (2014) Clinical, biochemical and molecular analysis of 13 Japanese patients with beta-ureidopropionase deficiency demonstrates high prevalence of the p. 977G>A (p.R326Q) mutation. J Inherit Metab Dis 37 (5):801–812

Saudubray JM, van Berghe G, Walter JH (2012) Disorders of purine and pyrimidine metabolism. In: van Berghe G, Vincent MF, Marie S (eds) Inborn metabolic disease, 5th edn. Springer, Heidelberg, pp 511–515, Chapter 36

van Kuilenburg AB, van Lenthe H, Ratmann GG et al (2000) Confirmation of the enzyme defect in the first case of beta-ureidopropionase deficiency. Beta-alanine deficiency. Adv Exp Med Biol 486:243–246

van Kuilenburg AB, Meinsma R, Beke E et al (2004) beta-Ureidopropionase deficiency: an inborn error of pyrimidine degradation associated with neurological abnormalities. Hum Mol Genet 13:2793–2801

van Kuilenburg AB, Dobritzsch D, Meijer J et al (2012) ß-ureidopropionase deficiency: phenotype, genotype and protein structural consequences in 16 patients. Biochim Biophys Acta 1822:1096–1108

Yaplito-Lee J, Pitt J, Meijer J, Zoetekouw L, Meinsma R, van Kuilenburg AB (2008) Beta-ureidopropionase deficiency presenting with congenital anomalies of the urogenital and colorectal systems. Mol Genet Metab 93:190–194

JIMD Reports
DOI 10.1007/8904_2015_433

RESEARCH REPORT

Erratum to: Growth Hormone Deficiency and Lysinuric Protein Intolerance: Case Report and Review of the Literature

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Received: 06 March 2014 / Revised: 19 August 2014 / Accepted: 03 September 2014
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Erratum to: "Growth Hormone Deficiency and Lysinuric Protein Intolerance: Case Report and Review of the Literature" in JIMD Reports DOI 10.1007/8904_2014_362.

The first and last names of the authors were swapped in the original version. The correct author names are as follows:

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The online versions of the original chapter can be found under DOI 10.1007/8904_2014_362

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