Molecular Genetics and Metabolism 118 (2016) 92–99

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

New observation of sialuria prompts detection of liver tumor in previously reported patient

Neena L. Champaigne [a,*], Jules G. Leroy [a], Priya S. Kishnani [b], Jochen Decaestecker [c], Edwin Steenkiste [c], Alka Chaubey [a], Jiarui Li [d], Chris Verslype [e], Jo Van Dorpe [f], Laura Pollard [a], Jennifer L. Goldstein [b], Louis Libbrecht [f], Monica Basehore [a], Nansheng Chen [d], Heping Hu [g], Tim Wood [a], Michael J. Friez [a], Marjan Huizing [h], Roger E. Stevenson [a]

[a] Greenwood Genetic Center, Greenwood, SC, USA
[b] Duke University Medical Center, Division of Medical Genetics, Durham, NC, USA
[c] Delta General Hospital, Department of Gastroenterology & Hepatology, Department of Pathology, Roeselare, Belgium
[d] Simon Fraser University, Department of Molecular Biology and Biochemistry, Burnaby, British Columbia, Canada
[e] University Hospital Leuven, Department of Hepatology, Leuven, Belgium
[f] Ghent University Hospital, School of Medicine, N. Goormaghtigh Institute for Pathology, Ghent, Belgium
[g] Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Department of Hepatobiliary Medicine, Shanghai, China
[h] Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 12 April 2016
Accepted 12 April 2016
Available online 16 April 2016

Keywords:
Sialuria
Sialic acids
UDP acetylglucosamine-2-epimerase
Hepatomegaly
Intrahepatic cholangiocarcinoma

ABSTRACT

Sialuria, a rare inborn error of metabolism, was diagnosed in a healthy 12-year-old boy through whole exome sequencing. The patient had experienced mild delays of speech and motor development, as well as persistent hepatomegaly. Identification of the 8th individual with this disorder, prompted follow-up of the mother-son pair of patients diagnosed over 15 years ago. Hepatomegaly was confirmed in the now 19-year-old son, but in the 46-year-old mother a clinically silent liver tumor was detected by ultrasound and MRI. The tumor was characterized as an intrahepatic cholangiocarcinoma (IHCC) and DNA analysis of both tumor and normal liver tissue confirmed the original *GNE* mutation. As the maternal grandmother in the latter family died at age 49 years of a liver tumor, a retrospective study of the remaining pathology slides was conducted and confirmed it to have been an IHCC as well. The overall observation generated the hypothesis that sialuria may predispose to development of this form of liver cancer. As proof of sialuria in the grandmother could not be obtained, an alternate cause of IHCC cannot be ruled out. In a series of 102 patients with IHCC, not a single instance was found with the allosteric site mutation in the *GNE* gene. This confirms that sialuria is rare even in a selected group of patients, but does not invalidate the concern that sialuria may be a risk factor for IHCC.

Synopsis: Sialuria is a rare inborn error of metabolism characterized by excessive synthesis and urinary excretion of free sialic acid with only minimal clinical morbidity in early childhood, but may be a risk factor for intrahepatic cholangiocarcinoma in adulthood.

1. Introduction

Sialuria (also called 'French type sialuria: MIM 269921) is a rare autosomal dominant inborn error that results in overproduction of free sialic acid (*N*-acetyl-neuraminic acid, Neu5Ac). It was first observed and delineated in a French patient [1,2] and has since been reported in only four other unrelated single children [3–6] and in a mother-and-son pair in a fifth family [7] (Table 1).

Sialuria is characterized metabolically by excessive urinary excretion of free sialic acid (>1 g/day), the consequence of failing feedback-inhibition of epimerase enzymatic activity in the rate limiting bifunctional enzyme, uridine diphosphate (UDP)-*N*-acetylglucosamine (GlcNAc) 2-epimerase/*N*-acetylmannosamine (ManNAc) kinase (GNE:

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CA, carbohydrate antigen; CMP, cytidine monophosphate; CK, cytokeratin; DMB, dihydrochloride; EM, electromagnetic; FFPE, formalin-fixed, paraffin-embedded; GGC, Greenwood Genetic Center; GlcNAc, *N*-acetylglucosamine; GNE, UDP-GlcNAc 2-epimerase/ManNAc kinase enzyme; *GNE*, gene encoding the UDP-GlcNAc 2-epimerase/ManNAc kinase enzyme; GT, glutamyl transpeptidase; HCC, hepatocellular carcinoma; H&E, hematoxylin and eosin; HPLC, high pressure liquid chromatography; IHCC, intrahepatic cholangiocarcinoma; ISSD, infantile free sialic acid storage disorder; LOH, loss of heterozygosity; ManNAc, *N*-acetylmannosamine; MG, mosaic copy gain; MIM, Mendelian Inheritance in Man; MIP, molecular inversion probe; ML, mosaic copy loss; MOHL, mosaic loss of heterozygosity; MRI, magnetic resonance imaging; OFC, occipitofrontal head circumference; SD, standard deviation; UDP, uridine diphosphate.

* Corresponding author at: Greenwood Genetic Center, 106 Gregor Mendel Circle, Greenwood, SC 29646, USA.
E-mail address: neena@ggc.org (N.L. Champaigne).

http://dx.doi.org/10.1016/j.ymgme.2016.04.004
1096-7192/

Table 1
Reported sialuria patients.

#	Gender	*GNE* mRNA Variant[a]	GNE Protein Variant[b]	refSNP number in dbSNP[c]	Inheritance	Age in original report	Estimated Current Age (2015)	References
1	Male[4]	c.788G>T	p.R263L	rs121908623	Unknown	3 y	Deceased[d]	[2,1]
2	Female	c.798C>T	p.R266W	rs121908621	Unknown	2 y	30 y	[3,11]
3	Male	c.797G>A	p.R266Q	rs121908622	Unknown	10 mo	27 y	[10,13,11]
4	Male	c.788G>T	p.R263L	rs121908623	Unknown	4.5 y	27 y	[5,11]
5	Female	c.797G>A	p.R266Q	rs121908622	Unknown	7.5 y	24 y	[6]
6	Male	c.797G>A	p.R266Q	rs121908622	*Familial*	4.5 y	19.5 y	[7]; Patient 2 in current report
7	Female	c.797G>A	p.R266Q	rs121908622	Unknown	34 y	47 y	[7]; Patient 3 in current report
8	Male	c.797G>A	p.R266Q	rs121908622	*De novo*	12 y	14 y	Patient 1 in current report

Abbreviations: y, years; mo, months.
[a] GenBank Accession number NM_005476.5 (*GNE* mRNA Variant 2). This GenBank Accession number was used for reporting al previous sialuria variants. Note that recent GNE nomenclature changes based on the longest mRNA variant have been proposed and may be used to report future sialuria variants [22].
[b] GenBank Accession number NP_005467 (hGNE1 protein isoform).
[c] As reported in dbSNP http://www.ncbi.nlm.nih.gov/SNP/.
[d] Patient died at age 30 (car accident).

EC 5.1.3.14/EC 2.7.1.60) by the downstream intermediate compound, cytidine monophosphate (CMP)-sialic acid [8–10].

The *GNE* gene (9p13.3) encodes the bifunctional GNE enzyme, which contains a small, but still incompletely outlined allosteric site, the binding site for CMP-sialic acid that loses its wild-type function in the heterozygous mutant genotype of sialuria [11,12]. Consequently, a heterozygous missense variant in the allosteric site of GNE in individuals with sialuria results in loss of feedback inhibition, causing overproduction, cytoplasmic accumulation and urinary excretion of large amounts of free sialic acid.

The clinical onset of sialuria in infancy is hardly recognizable because of the inconsistent, nonspecific and rather subtle features that may include mild neuromotor and cognitive developmental delay, hypotonia, slightly coarse facies, recurrent respiratory infections, transient mild anemia and equivocal or mild hepatomegaly [13,14]. Moreover, assaying urinary free sialic acid is not a routine laboratory test. Therefore, individuals with sialuria may go undiagnosed. Increased use of exome sequencing for diagnostic purposes may lead more frequently to making the diagnosis.

The purpose of this report is dual. First, we report on a newly diagnosed eighth patient with sialuria, identified by exome sequencing. Second, evaluation of the new patient prompted follow-up attention to the previously reported family, which found markedly increased hepatomegaly in the son (patient 2 in this report) and was of critical value to the diagnosis and treatment of intrahepatic cholangiocarcinoma (IHCC) in his affected mother (patient 3 in this report). The hepatic findings in the only adult with proven sialuria may also explain the nature and cause of the IHCC with fatal outcome at age 49 years in the maternal grandmother (patient 4 in this report).

2. Materials and methods

2.1. Patients

Patient 1 and his family were clinically examined at Greenwood Genetic Center (Greenwood, SC, USA). Patient 1 was also enrolled in the "Genomic Study of Medical Development or Congenital Problems of unknown Etiology in Pediatric Patients" at Duke University Medical Center (Division of Medical Genetics, Durham, NC, USA).

Patients 2, 3 and 4 were clinically examined at Ghent University Hospital and School of Medicine (Ghent, Belgium). Hepatic analysis for these patients was performed at Delta General Hospital (Department of Gastroenterology & Hepatology, Department of Pathology, Roeselare, Belgium) and University Hospital Leuven (Department of Hepatology, Leuven, Belgium).

The Institutional Review Boards of each respective center approved the described studies and the patients and/or their parents gave written informed consent.

2.2. Pathology

The liver biopsy of patient 2 and the liver resection specimen of patient 3 were processed for light microscopy and immunohistochemistry according to standard procedures. For patient 2, material was fixed in glutaraldehyde for electron microscopy. Hematoxylin and eosin (H&E) sections from a small incisional biopsy of patient 4, the mother of patient 3, prepared in 1989 were available for review; additionally, on this limited amount of tumor tissue immunohistochemistry for cytokeratin 7 (CK7) and cytokeratin 19 (CK 19) was performed, using standard methods.

2.3. Biochemical assays

Urinary sialic acid was measured according to Hommes et al. [15]. Briefly, free sialic acid was derivatized with 1.2-diamino-4.5-methylenedioxybenzene dihydrochloride (DMB). The fluorescent complex was separated by high pressure liquid chromatography (HPLC) using a ZORBAX column SB-C18 (4.6 × 100 mm × 3.5 OD). The amount of free sialic acid was quantitated using a standard curve. Urinary creatinine was calculated using the Jaffe method and final free sialic acid concentration was expressed as nmol sialic acid per mg creatinine.

2.4. GNE mutation analysis

DNA was extracted from peripheral blood samples from patient 1, his parents and two unaffected siblings. Exome sequencing was performed on all samples. To capture the coding regions, the 65-Mb Illumina TruSeq Exome Enrichment Kit (Illumina, San Diego, CA) was used. Sequencing was performed on the Illumina HiSeq 2000 platform (Illumina). Primers were designed to amplify the region of the *GNE* gene containing the c.797G>A (p.R266Q) alteration. Sequencing was performed by the Sanger dideoxy method using the Big Dye Terminator Cycle Sequencing Kit v3.1 on a ABI 3730xl automated sequencer (Applied Biosystems, Life Technologies, Foster City, CA). Data were collected and analyzed using Sequencher 4.5 DNA sequence assembly software (Gene Codes Corporation, Ann Arbor, MI).

2.5. Oncoscan assays

DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) liver tissues from patient 3, both from the tumor tissue and adjacent normal tissue. The Oncoscan® assays (Affymetrix Inc., Santa Clara, CA) were performed on the normal and tumor liver DNA samples following the protocol recommended by the manufacturer. Briefly, the DNA was annealed with molecular inversion probes (MIP) that detect both the copy number and somatic mutations of specific genes at 58 °C overnight