Sialuria: Ninth Patient Described Has a Novel Mutation in *GNE*

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Abstract Sialuria is a rare autosomal dominant inborn error of metabolism characterized by cytoplasmic accumulation and urinary excretion of gram quantities of free sialic acid due to failure of feedback inhibition of the rate-limiting enzyme in the sialic acid synthesis pathway, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (*GNE*/*MNK*). To date, eight cases had been published worldwide, all with heterozygous missense variants at the allosteric site, specifically at Arginine 294 (formerly 263) and Arginine 297 (formerly 266) of *GNE*. The described cases so far have rather homogeneous clinical features which include developmental delay, mildly coarse features, hepatomegaly and prolonged neonatal jaundice. The apparent rarity of this disorder is hypothesized to be due to the variable and sometimes transient nature of the clinical features and to the absence of routine testing for urinary sialic acids. Here we present the ninth case of sialuria diagnosed in a child investigated because of clinical signs and symptoms and furthermore describe a novel pathogenic variant in the associated gene, *GNE*.

Introduction

Sialuria (MIM 269921) is a rare autosomal dominant, inborn error of metabolism characterized by constitutive overproduction, cytoplasmic accumulation and urinary excretion (>1 g/day) of free sialic acid (Seppala et al. 1991; Hinderlich et al. 2015). This is the result of failure of feedback inhibition of the rate-limiting enzyme in the sialic acid synthesis pathway, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (*GNE*/*MNK*) (Seppala et al. 1999).

The term sialuria is derived from the Greek ‘sialos’ owing to, at least in part, the identification of sialic acids in salivary mucins some 70 years ago (Varki and Schauer 2009). Sialic acids are a group of more than 50 different (Varki and Schauer 2009) negatively charged acetylated nine-carbon sugars derived from neuraminic acid, which decorate glycoproteins on most cell surfaces (reviewed in Varki 2008). They play a myriad of roles in glycobiology, including but not limited to, cellular recognition, binding of the influenza virus (Suzuki 2005) and metastatic cancers (Bull et al. 2014; Chen et al. 2016). The critical role of sialic acids in vivo is epitomized by the early embryonic lethality of the *GNE* loss of function murine model (Schwarzkopf et al. 2002). Despite this, loss of expression in vitro appears to be better tolerated (Abeln et al. 2017). Equally, the murine model overexpressing mutated *GNE* resulting in overexpression of sialic acids perhaps goes some way towards explaining the neurocognitive features seen in patients with sialuria (Kreuzmann et al. 2017).

GNE/*MNK* in humans is encoded by *GNE* gene on 9p13.3. Structurally, *GNE*/*MNK* is a bifunctional enzyme containing both an epimerase and a kinase domain. Importantly, the epimerase domain has an allosteric site known to bind its downstream product and negative

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regulator, cytidine monophosphate (CMP)-sialic acid (Chen et al. 2016). Heterozygous missense variants at the allosteric site, specifically at Arginine 294 and Arginine 297 [NM_001128227.2; NP_001121699.1, hGNE2 protein isoform (formerly Arginine 263 and Arginine 266; NM_005476.5; NP_005467.1, hGNE1 protein isoform)], result in sialuria (Champagne et al. 2016; Kurochkina et al. 2010). Conversely mutations elsewhere in GNE/MNK, which lead to decreased sialylation, have been implicated in neuronal development and hereditary inclusion body/GNE myopathy (Leroy 2004; Cho et al. 2017; Kurochkina et al. 2010).

Furthermore, of the disorders of sialic acid metabolism, the allelic sialic acid storage disorders (SASD) – infantile sialic acid storage disease (ISSD) and its milder version Salla disease – share many clinical features with sialuria (reviewed in Varki 2008). These however are attributable to mutations in *SLC17A5*, which results in defective transport of sialic acids from the lysosomes to the cytosol and thereby in lysosomal accumulation (Adams and Gahl 2003).

Since the first report of a French patient with sialuria in 1968 (Montreuil et al. 1968), there have been a total of eight cases published worldwide (Champagne et al. 2016; Wilcken et al. 1987; Don and Wilcken 1991; Seppala et al. 1991; Krasnewich et al. 1993; Ferreira et al. 1999; Leroy et al. 2001; Enns et al. 2001). Most of these patients have been identified in childhood based on variable and often transient clinical findings and through biochemical tests, which are not routine, making it likely that this is an underrepresentation of the true prevalence. Signs and symptoms of sialuria may include mild coarse facies, prolonged neonatal jaundice, hepatomegaly, microcytic anaemia, frequent upper respiratory tract infections and gastroenteritis, failure to thrive, developmental delay, hypotonia, seizures and delayed bone age. Most recently, a relationship between sialuria and intrahepatic cholangiocarcinoma has also been proposed (Champagne et al. 2016). The previous patient identified through our local services here, who was the second patient with sialuria in the literature (Wilcken et al. 1987), was last seen at age 20 years. She then demonstrated ongoing hepatosplenomegaly, mild distinctive features and moderate developmental delays. Interestingly, the eighth patient in the literature was diagnosed through whole exome sequencing (Champagne et al. 2016), and we envisage that further patients will be identified in this way.

Here we describe a ninth patient with sialuria who was also identified in childhood on the basis of his clinical picture. Furthermore, we also propose a new pathogenic variant in *GNE*.

Materials and Methods

The patient was initially seen at Sydney Children's Hospital, Randwick, NSW Australia. Urine metabolic screen was conducted through The Children's Hospital at Westmead, NSW Australia. Oligosaccharide analysis by thin layer chromatography and sialic acid quantification was carried out by the SA Pathology, National Referral Laboratory Department of Biochemical Genetics North Adelaide, South Australia. Cell fractionation was undertaken by Dr. Tim Wood at Greenwood Genetic Centre, Greenwood, South Carolina, USA. *GNE* mutation analysis was through Prevention Genetics, Marshfield WI. *SLC17A5* mutation analysis was through Medical Neurogenetics, Atlanta, Georgia, USA.

The Case

The patient, a boy, was diagnosed with sialuria at 2 years and 4 months. He was born at 36 weeks and 3 days gestation by normal vaginal delivery to a then G1P1 mother. His parents are non-consanguineous, of European descent, and with pertinent family history. The pregnancy was complicated throughout by nausea. Antenatal ultrasounds were unremarkable. At birth his weight was 2.385 kg (above the 10th centile), length was 48 cm (above the 50th centile) and head circumference was 32 cm (10th centile). He did not require resuscitation. At 2 days of age, he developed unconjugated hyperbilirubinaemia and required phototherapy for 3 days. Jaundice then recurred, and he was readmitted to hospital for a further 4 days of phototherapy. Investigations for recurrent jaundice identified hepatosplenomegaly associated with elevation in plasma alanine aminotransferase (ALT) 199 U/L, aspartate aminotransferase (AST) 242 U/L and gamma-glutamyl transferase (GGT) 765 U/L. Infectious serology for hepatitis A, B and C, as well as toxoplasmosis, cytomegalovirus and herpes simplex, was negative. Jaundice gradually resolved over the first 3 months of life; however his hepatic transaminitis and hepatosplenomegaly persisted.

As an infant he had several viral illnesses, with predilection for upper respiratory tract infections. Although he usually recovered well, during some of these episodes, he experienced febrile convulsions. The frequency of illnesses and seizures generally improved with age. However, he had a further episode of tonic-clonic seizures in the context of a febrile viral illness at 6 years and 3 months of age. This occurred after having 1.5 years seizure-free. The patient was also briefly troubled by obstructive sleep apnoea as an infant and reviewed by an otolaryngologist. It was postulated that his obstructive symptoms were likely

secondary to enlarged tonsils. He did not undergo adenotonsillectomy.

A mildly decreased IgG (3.74 g/L) was noted during routine investigations at 1 year of age. IgM and IgA levels were in the normal ranges. Influenza immunization was recommended. Repeat immunoglobulin levels at 6 years and 10 months of age demonstrated complete normalization – IgG 5.13 g/L, IgA 1.34 g/L and IgM 0.89 g/L. Additionally, iron deficiency anaemia (haemoglobin 123 g/L, mean corpuscular volume 71.8 fL, ferritin 15 µg/L) and dyslipidaemia with elevated cholesterol (5.7 mmol/L) and triglycerides (4.6 mmol/L) were detected on routine bloods at 1 year of age. He was commenced on iron replacement, with variable compliance. Dyslipidaemia was not treated. Repeat blood samples at 6 years and 10 months demonstrated haemoglobin of 131 g/L, mean corpuscular volume 74.6 fL, ferritin 16 µg/L, cholesterol 5.1 mmol/L and triglycerides 1.2 mmol/L.

Developmentally he sat at 6 months, walked at 11 months and had single words as well as a pincer grip by 14 months. At 3 years of age, he underwent a developmental assessment and was diagnosed with mild developmental delay and autism spectrum disorder. His cognitive skills at 5 years and 2 months of age were in the low average range as per The Wechsler Preschool and Primary Scale of Intelligence – 4th edition (WPPSI-IV). Audiology and vision tests were normal. He is now 7 years of age and with ongoing early intervention attends a mainstream primary school. He continues to have restricted, repetitive behaviours with symptoms of anxiety.

Clinical examination at 6 years and 4 months of age revealed softening of his coarse features with persistence of epicanthic folds, flattening of the nasal bridge and posteriorly rotated ears. Tonsils were enlarged bilaterally. His abdomen remained protuberant with appreciable hepatomegaly and a 14 cm liver span. The spleen was approximately 8 cm long. The remainder of his examination was unremarkable.

Diagnosis The patient's diagnosis of sialuria was made when he was 2 years and 4 months of age during ongoing investigations for his persistent transaminitis and hepatosplenomegaly. A paediatric gastroenterologist also reviewed the patient at that time, and there was consideration of a liver biopsy if the extensive battery of testing did not yield a diagnosis. In brief, comparative genomic hybridization (CGH) array was normal as was his amino acid profile, organic acid profile, acylcarnitine profile, very long chain fatty acids profile, lysosomal enzymes, transferrin isoforms, copper, ceruloplasmin and coeliac serology. However, urinary oligosaccharide analysis by thin layer chromatography revealed large amounts of sugar at the position of free sialic acid. Further quantification reported a level of

2,980 µmol of free urinary sialic acid/mmol creatinine. Free sialic acid in cultured skin fibroblasts was also elevated at 34 nmol/mg protein. Cell fractionation was undertaken which demonstrated 76–83% of the sialic acid localizing to the soluble fraction that is the cytosolic component, compared to 32–47% in the control samples. Parental urinary sialic acid levels were normal, as was the urinary sialic acid level in the patient's only sibling.

Genetic testing was also pursued. No variants were reported in sequencing of *SLC17A5*. This was followed by bidirectional Sanger sequencing of *GNE*, whereby the patient was found to be heterozygous for a previously undocumented variant NM_001128227.2: c.250G>C; p.Asp84His in the *GNE* gene (MIM 603824, GeneID 10020). Additionally segregation testing in the patient's parents found this to be a de novo change. A second heterozygous variant c.51+34 T>C (refSNP number in dbSNP: rs7875447) in *GNE* was also identified in the patient. However, this variant is known to be in an intronic region, commonly found, not associated with disease states and therefore benign as per American College of Medical Genetics and Genomics (ACMG) criteria (Richards et al. 2015).

Since diagnosis the patient has undergone yearly review with our local metabolic service. His monitoring included a skeletal survey at 2.5 years of age, which was normal, and routine measurement of his liver function tests. Liver function tests at 6 years and 10 months of age demonstrated persistence of the mild derangement in the transaminases (ALT 194 U/L and AST 89 U/L). In the light of the report by Champagne et al. (2016), a decision has been made to undertake yearly liver ultrasounds for monitoring. His imaging in 2018 again noted hepatosplenomegaly, not associated with any other specific anomaly.

Discussion

Given the apparent rarity of this condition, the identification of a ninth patient with sialuria was felt to be worth publishing. Importantly, although this patient possesses many of the clinical features associated with sialuria and SASDs, the diagnosis was not initially forthcoming lending support to the hypothesis that sialuria may be an under-recognized condition, at least when the diagnosis is based on subtle and sometimes transient clinical findings. However we envisage there is scope for further diagnoses being made through whole genomic sequencing. Indeed since we commenced proceedings for this publication, we have been made aware of a potential tenth case of sialuria worldwide.

Secondly, we report a novel de novo heterozygous missense variant in *GNE* c.250G>C, p.Asp84His

(NM_001128227.2; NP_001121699.1, hGNE2 protein isoform), which results in autosomal dominant sialuria, with biochemical correlate in this patient. Importantly, the wild-type aspartic acid residue is highly conserved among GNE proteins; among the 100 vertebrates in the Multiz alignment, only platypus has a different amino acid residue at this location. Protein structure analysis using HOPE (Venselaar et al. 2010) indicates that substitution of aspartic acid, which carries a negative charge, with histidine, which is neutral and larger, is expected to disrupt the wild-type conformation of GNE.

Further structural information on the effect of this variant can be gauged from the recently published GNE crystal structure by Chen et al. (2016). Aspartic acid 84 as denoted using the newer hGNE2 nomenclature proposed by Huizing et al. (2014) was formerly known as aspartic acid 53 using the older hGNE1 (NM_005476.5; NP_005467.1) nomenclature. The hGNE1 nomenclature has been used to report previous patients with sialuria, as well as in the crystal structure by Chen et al. (2016). The crystal structure provides evidence that aspartic acid 84 (formerly 53) also forms part of the allosteric site or at least is required for correct folding of the site (Fig. 4b of Chen et al. 2016). They demonstrate that the cytosine base of CMP-sialic acid, the negative downstream regulator, is sandwiched between the side chains of aspartic acid 84 (formerly 53) and Valine 293 (formerly 262). This suggests a possible mechanism by which variants affecting aspartic acid 84 may have similar effects to variants affecting Arginine 294 (formerly 263) and Arginine 297 (formerly 266) which are known to cause autosomal dominant sialuria.

Overall, based on the available evidence, the c.250G>C; p.Asp84His variant identified here meets the ACMG criteria (Richards et al. 2015) for PS2 (de novo variant), PM2 (absent from controls), PP2 (low rate of missense variants and where missense variants are a common mechanism of disease), PP3 (in silico support for pathogenicity), PP4 (patient's phenotype including biochemical evidence is highly specific for a condition with a single genetic aetiology) and PM1 (variant affects a critical functional region of the protein which lacks benign variation). We therefore propose that there is sufficient compelling evidence for this novel variant being classified as pathogenic and extending the known sialuria causing mutations in *GNE*.

Finally, with very rare (or rarely recognized) disorders, it is often difficult to be sure of the clinical significance of biochemical or other findings. In the case of sialuria, the clinical findings of described cases have a homogeneity which suggests that they are a real consequence of the disorder. As more cases are discovered primarily by next-generation sequencing rather than primarily by clinical

features, there may be more information about the full picture and prevalence of this condition.

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Take-Home Message

Sialuria: Ninth patient described has a novel mutation in *GNE*.

Contributions of the Individual Authors

Noelia Nunez Martinez, part of the clinical team, wrote the paper, organized all outstanding investigations and approved the submitted version.

Michelle Lipke organized the original investigations, made the diagnosis and approved the submitted version.

Jacqueline Robinson, part of the clinical team, organized the patient follow-up and approved the submitted version.

Bridget Wilcken headed the clinical team, edited the paper and approved the submitted version.

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None of the authors has any competing interest.

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The investigations were all clinically required, and no ethical approval was necessary.

The patient's parents consented to all investigations.

No laboratory animals were involved.

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