KI REPORTS
KIReports.org
TRANSLATIONAL RESEARCH

Parental Whole-Exome Sequencing Enables Sialidosis Type II Diagnosis due to an *NEU1* Missense Mutation as an Underlying Cause of Nephrotic Syndrome in the Child

Reza Maroofian[1,6], Isabel Schuele[2,6], Maryam Najafi[3], Zeineb Bakey[2,3], Abolfazl Rad[3,4], Dinu Antony[2,3], Haleh Habibi[5] and Miriam Schmidts[2,3]

[1]Genetics Research Centre, Molecular and Clinical Sciences Institute, St George's, University of London, London, UK; [2]Center for Pediatrics and Adolescent Medicine, University Hospital Freiburg, Freiburg University, Faculty of Medicine, Freiburg, Germany; [3]Genome Research Division, Human Genetics Department, Radboud University Medical Center Nijmegen and Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands; [4]Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran; and [5]Genetic Counselling Center, Hamadan University of Medical Science, Daneshgah-e-Bu Ali Sina, Hamedan, Iran

Introduction: Monogenetic renal diseases, including recessively inherited nephrotic syndromes, represent a significant health burden despite being rare conditions. Precise diagnosis, including identification of the underlying molecular cause, is especially difficult in low-income countries and/or if affected individuals are unavailable for biochemical testing. Whole-exome sequencing (WES) has opened up novel diagnostic perspectives for these settings. However, sometimes the DNA of affected individuals is not suitable for WES due to low amounts or degradation.

Methods: We report on the use of parental WES with implementation of specific stepwise variant filtering to identify the underlying molecular cause of the childhood-onset nephrotic syndrome as nephrosialidosis resulting from a mutation in *NEU1*.

Results: Sequencing both parents enabled a nephrosialidosis diagnosis in the deceased child. To date, only 16 other cases of nephrosialidosis have been reported in the literature, with only 1 genetically confirmed case. After we reviewed the clinical information of all reported cases, we found that most patients presented with proteinuria, which started at between 2 and 3 years of age. Renal pathology showed mainly focal segmental glomerulosclerosis (FSGS)with vacuolated cells, and steroid treatment was always unsuccessful. Hepatomegaly was present in nearly all cases, whereas corneal clouding and a cherry red spot on the macula was observed in only approximately 50% of cases. Fourteen of 16 previously reported cases were no longer alive at the time of reporting.

Conclusions: Our findings demonstrate the power of parental WES to diagnose rare genetic diseases, such as childhood-onset nephrotic syndrome. We further provide a comprehensive overview of the clinical course of nephrosialidosis and raise awareness of this ultra-rare condition as an underlying cause of FSGS.

Kidney Int Rep (2018) **3,** 1454–1463; https://doi.org/10.1016/j.ekir.2018.07.015
KEYWORDS: nephrotic syndrome; *NEU1*; sialidosis; whole-exome sequencing

Rare diseases are defined as affecting <1 in 2000 individuals of the population; however, together, they represent a significant disease burden. Extreme genotypic heterogeneity significantly hampers the diagnostic pathway, and in areas of the world with limited medical and technical support, most families are left without a definitive diagnosis. Death of the affected individual with no possible further biochemical testing causes additional diagnostic obstacles. Recent development of next-generation sequencing (NGS) technologies, especially WES, has changed the rare disease landscape dramatically, including the findings for renal phenotypes, and more specifically, nephrotic syndromes. Currently, WES also allows a molecular

Correspondence: Miriam Schmidts, Center for Pediatrics and Adolescent Medicine, University Hospital Freiburg, Freiburg University, Faculty of Medicine, Freiburg, Germany. E-mail: miriam.schmidts@uniklinik-freiburg.de

[6]RM and IS contributed equally to the paper.

Received 24 June 2018; revised 16 July 2018; accepted 23 July 2018; published online 29 July 2018

diagnosis for cases with limited clinical information or laboratory workup, and is not significantly inferior to gene panels with regard to coverage[1] while offering much broader discovery possibilities for genes.

Nephrotic syndrome can occur as steroid-sensitive or steroid resistant disease (SRNS); the latter is a frequent cause of chronic kidney disease and renal failure, especially in the early decades of life. Histologically, FSGS is commonly noted in SRNS. Exome sequencing and other NGS technologies, such as gene panel sequencing, have recently extended the number of genes found to be causative to >30, and the genetic diagnosis rate has likewise increased to approximately 30%.[2]

Sialidosis (Online Mendelian Inheritance in Man [OMIN]# 256550) is a rare lysosomal storage disease inherited in an autosomal-recessive fashion. It was first described clinically as a phenotype entity in 1968 by Spranger and Wiedemann,[3] and as lipomucopolysaccharidosis or mucolipidosis in 1970, and neuraminidase deficiency in affected individuals was detected in fibroblasts as early as 1977. Biallelic loss of function mutations in the gene encoding for neuraminidase, *NEU1*, was identified as a cause by Bonten *et al.* nearly 20 years later.[4,5] However, due to the paucity of the phenotype and the diagnosis often being made clinically and biochemically via testing of the enzyme activity (e.g., in patient fibroblasts and urine analyses), only a few molecularly confirmed cases have been published to date. *NEU1* encodes for an enzyme, α-neuraminidase, which forms a high molecular weight protein together with cathepsin A; both enzymes together function as sialidase. Therefore, dysfunction of *Neu1* results in accumulation of sialylated glycoproteins/peptides and oligosaccharides in lysosomes and their excretion into the urine due to defective degradation of these products. Urine analysis can be helpful for diagnosis, although cases without urine abnormalities have been reported in the literature, and detection of the sialylated products can be expensive and labor intensive.[6,7] The observed different disease severities have resulted in division into the milder type I phenotype, also referred to as "cherry red spot–myoclonus syndrome" in which clinical signs usually only become obvious in adulthood, and the more severe type II, which is congenital or has infantile onset. Individuals affected by sialidosis type II exhibit typical storage disease features, such as coarse face and hepatomegaly. Developmental delay is also usually observed.[8] Nephrosialidosis was already recognized as a part of the phenotypic spectrum of sialidosis II in 1978; however, only 16 cases have been described in the literature to date.[9–25]

Here, we describe the implementation of parental WES to define the underlying molecular cause of nephrotic syndrome in a deceased child. Unexpectedly, this led to a sialidosis type II diagnosis. Nephrosialidosis is an ultra-rare disorder; therefore, it lacks comprehensive information about the clinical course. We provide a summary about all of the cases published to date. Our results serve as an example for using parental WES for molecular diagnosis of genetic renal disorders when patient DNA is not suitable for WES.

METHODS

Human DNA

Parental consent was given for genetic studies as part of the clinical diagnostic workup with approval from the local ethics committee. Genomic DNA was extracted from ethylenediamine tetraacetic acid blood using a standard commercial kit (Qiagen, Germantown, MD). DNA amounts were quantified using Bioanalyzer (Agilent, Santa Clara, CA), and DNA integrity was confirmed by running 500 ng of DNA on an ethidiumbromide-containing 1% agarose gel.

Whole-Exome Sequencing

WES was performed as previously described.[26,27] In brief, 5 μg of DNA from both parents were sent to Novogene Ltd (Hong Kong, China), where WES was performed using the Agilent Sure Select Human whole exome kit V.6 (Agilent Technologies, Santa Clara, CA) for enrichment. Sequencing was undertaken on a PE 150 HiSeq machine (Illumina, San Diego, CA). Read alignment and variant calling were performed with GATK (genome analysis toolkit) using default parameters with the human genome assembly hg19 (GRCh37) as reference. The obtained raw sequencing data were in the range of 12 to 13 GB; the raw depth achieved was 257× to 266×, average depth on target was 126× to 144×, coverage of targeted regions was >99%, targeted regions covered with a depth of at least 4 were >99%, targeted regions covered with a depth of at least 10× were 98.6% to 99.1%, targeted regions covered with a depth of at least 20× were 96.5% to 97.2%, and targeted regions covered with a depth of at least 50× were 93.1% to 94.8%. Splice sites were defined within 10 bp next to intron-exon/exon-intron boundaries.

Variant Filtering

A stepwise filtering procedure was implemented, starting with filtering out any variants with a minor allele frequency ≥1% in control databases (Exome Aggregation Consortium, Genome Aggregation Database, dbSNP, the 1000 Genomes Project human polymorphism database and National Heart, Lung and Blood Institute Exome Sequencing Project; see Supplementary Web Resources). In the second step, only protein-coding non-synonymous, stop gain,

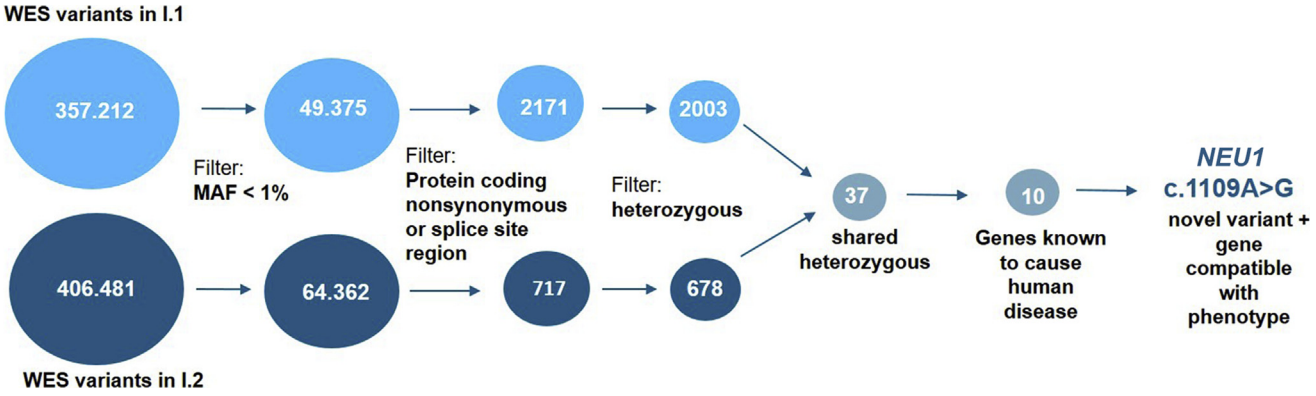

Figure 1. Variant filtering strategy. Visualization of the specific stepwise approach implemented to identify rare deleterious variants carried by both parents in a heterozygous state. MAF, minor allele frequency; WES, whole-exome sequencing.

frameshift or splice variants, as well as intronic splice site variants were retained. Remaining variants were then filtered for variants that occurred in both parents in a heterozygous state. Variants in genes known to cause a human phenotype when mutated, as well as novel variants (no reported frequency in any of the public databases), were subsequently prioritized. See Figure 1 for details.

Phenotypic Features Used for Variant Priorization

Initial phentoypic filtering was performed for variants in genes previously associated with any human phenotype. Secondary variant priorization was performed for variants in genes associated with a phenotype that was either congenital in nature or that occurred during childhood and involved the kidneys, including impaired renal function as determined by blood creatinine measurements, proteinuria, hematuria, abnormal renal ultrasound results (including hyperechogenic kidneys, renal cysts, and smaller or larger than normal kidneys), and abnormal renal biopsy results in pathology (including a diagnosis of fibrosis, glomerulosclerosis, tubular casts, tubular atrophy, hypercellularity, and reported renal or urinary tract malformations).

Sanger Sequencing

Polymerase chain reactions (PCR) reactions were performed using 50 μg of genomic DNA using a standard touchdown PCR protocol with 35 cycles. PCR protocol and primer sequences are available upon request.

RESULTS

Clinical Description

A 9-month Iranian female infant born as the first offspring of first-cousin parents presented to the local pediatric services with onset of edema in the lower

Table 1. Final variant filtering for heterozygous variants shared between both parents

Gene	Variant	Gene previously implicated in human disease	Variant not previously identified in population databases
ASCL5	NM_001270601: c.503G>A:p.R168H		+
EBF1	NM_182708: c.1499C>A:p.P500H		+
ENOX1	NM_001127615: c.447A>T:p.L149F		+
CENPJ	NM_018451: c. 1960G>A:p.A654T	Seckel syndrome type 4, primary microcephaly	
CLCN2	NM_001171088: c. 1930C>T:p.R644W	Leucencephalopathy with ataxia	
FAH	NM_000137: c. 692T>C:p.M231T	Tyrosinemia type 1	
HPR	NM_020995: c. 518T>C:p.V173A		+
KANK1	NM_153186:c. 1588A>T:p.T530S	Spastic quadriplegic cerebral palsy	
MFSD6L	NM_152599:c. 687G>C:p.K229N		+
MYHA4	NM_017533: c. 2385A>G:p.I795M		+
NEU1	NM_000434: c.1109A>G; p.Tyr370Cys	Sialidosis	+
ORA4K1	NM_001004063: c. 185T>C:p.L62S		+
PADI4	NM_012387:c.A926G:p.Y309C	Susceptibility to rheumatoid arthritis	
PRG4	NM_001127710: c.C1756T:p.P586S	Camptodactyly-arthropathy-coxa vara-pericarditis syndrome	
SOX10	NM_006941: c. 820G>A:p.G274S	PCWH syndrome, Waardenburg syndrome	
TACR3	NM_001059: c. 824G>A:p.W275X	Hypogonatropic hypogonadism	
ZNF141	NM_003441: c. 207G>C:p.K69N and NM_003441: c. 212T>C:p.V71A	Polydactyly, postaxial, type A6	

PCWH, peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome, and Hirschsprung disease.

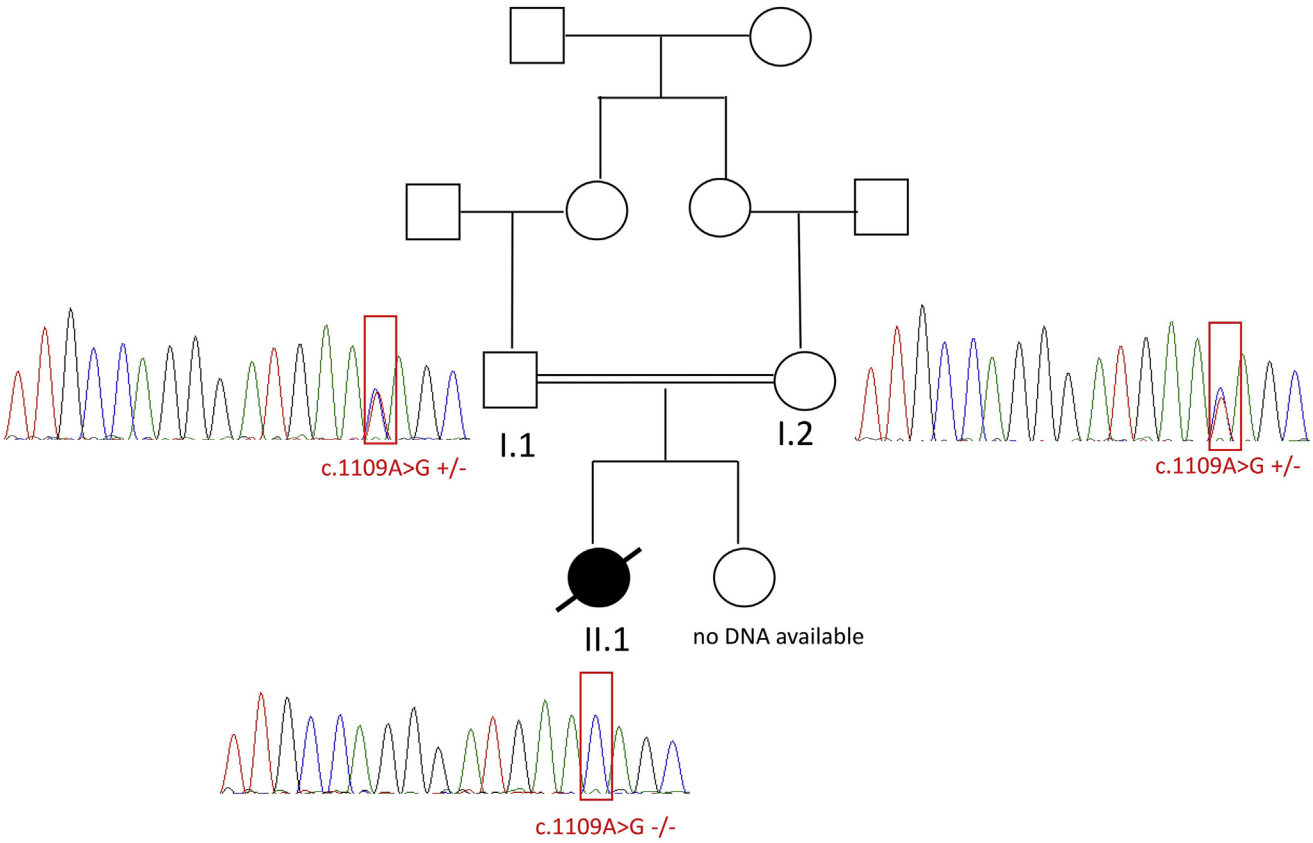

Figure 2. Family pedigree and *NEU1* mutation segregation confirmation by Sanger sequencing. As suggested by whole-exome sequencing, both parents carry c.1109A/G in a heterozygous state, whereas the affected child was homozygous for the mutation. During our study, a healthy sibling was born; however, no DNA was available for testing.

body. She had generalized skin mottling at the time of admission, and organomegaly was noted. Urine analysis revealed nephrotic range proteinuria. Facial dysmorphism was present in the form of relatively course facial features and low-set ears. The extended clinical history revealed bilateral hearing loss, as well as a history of seizures and developmental delay (e.g., delayed sitting). Skeletal radiographic survey revealed no signs of skeletal dysplasia, but did reveal bilateral hip dysplasia and brachycephaly. Chest x-ray showed her heart was mildly enlarged and the presence of right upper lobe pneumonia. Brain magnetic resonance imaging without contrast showed bilateral mild frontotemporal atrophy. An ophthalmology consultation did not take place.

Due to clinically evident nephrotic syndrome, steroid treatment was started, and a renal biopsy was performed; however, only 4 glomeruli were identified in the sample. Glomerular epithelial cells were enlarged, containing large amounts of foamy or granular cytoplasm. Pronounced thickening of the basement membrane was noted, but no signs of fibrosis or vasculitis were found. Tubule cells appeared polygonal, enlarged and likewise showed granular to foamy cytoplasm. Protein debris was observed inside the tubules. Subsequently, peritoneal dialysis was started; however, as her clinical state declined, she was transferred to the pediatric intensive care unit where she passed away after 5 days due to cardiovascular failure with severe blood pressure fluctuations.

Genetic Workup Using Parental WES

To establish a molecular diagnosis, in light of further planning for the family, we decided to perform WES in the parents due to insufficient DNA amounts available from the patient. More than 300,000 variants were reported for each parent in either the homozygous or heterozygous state. Subsequent variant filtering was performed for each parental data set as shown in Figure 1, based on a variant frequency of $<1\%$ in available databases, including the Exome Aggregation Consortium, Genome Aggregation Database, dbSNP, the 1000 Genomes Project human polymorphism database, and National Heart, Lung and Blood Institute–Exome Sequencing Project (see Supplementary Web Resources), as well as protein coding changes and splice site regions. We then proceeded to filtering for variants that occurred in both parents in the heterozygous state, assuming the child was homozygous for the disease-causing variant because of the

Table 2. Summary of previously reported nephrosialidosis cases

Patient	This report	1	2	3	4	5	6	7
Author	NA	Maroteaux/Le Sec *et al.*[9,10]	Maroteaux *et al.*[9]	Aylsworth *et al.*[11]	Matsuda *et al.*[13]	Shimada/Okada *et al.*[14,a]	Beck *et al.*[15,b]	Yamano *et al.*[16,a]
Yr of Publication	NA	1978	1978	1980	1983	1983/1984	1984	1986
Gender	Female	Male	Female, sister P1	Male	Male	Female	Female	Male
Origin	Iran	France[c]	France[c]	Caucasian	Japan	Japan	Germany	Japan
Clinical presentation								
Onset of symptoms	9 mo	4 mo, psychomotor retardation	First week, hepatomegalie	Birth, bilateral hydroceles	1 yr 5 mo, facial dysmorphic signs, abdominal distension	3 mo, psychomotor retardation, coarse face, dysostosis multiplex	Prenatal, hydrops fetalis	3 days before birth, ascites
Onset of proteinuria	9 mo	2 yr	19 mo	20 mo	19 mo	No proteinuria	birth (mild)	Hypoproteinemia, no proteinuria
Treatment w. Steroids	Yes with no effect	No	No	Yes without success	NR	No	No	No
Hepatosplenomegaly	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NR
Skeleton								
Facial dysmorphic signs	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dysostosis multiplex/ osteoporosis	NR	Yes	Yes	Yes	Yes	Yes	NR	NR
Nervous system								
Psychomotor retardation	Yes	Yes	Yes	Yes	Yes	Yes	NR	NR
Other	Mild fronto-temporal atrophy, seizures		Muscular hypotonia	Growth failure				
Eyes								
Corneal clouding/ cataracts	NR	Yes	Yes	**No**	No	Yes	NR	Yes
Macular cherry-red spot	NR	NR	NR	No	Yes	No	NR	Yes
Other								
Hernia	No	Yes	NR	Yes	Yes	NR	NR	NR
Ascites/edema	Yes	Yes	NR	Yes	NR	NR	Yes	Yes
Other	Bilateral hip dysplasia, bialteral hearing loss			Frequent upper respiratory tract infections		Frequent upper respiratory tract infections	Petechia	
Course	Died aged 9 mo	Died at the age of 4.5 yr	NR	NR	NR	Died of unknown cause at 7 mo	Died at the age of 6 mo	Died of respiratory failure at 56 days
Diagnostics								
Deficiency of α neuraminidase in lymphocytes/ fibroblasts	Not tested	Yes	Yes	Yes and mild depression of beta-galactosidase	Yes	Yes and depression of beta-galactosidase	Yes	Yes
Vacuolated lymphocytes	Not tested	Yes	Yes	NR	Yes	NR	NR	Yes
Renal pathology: FSGS/vacuolated cells	Yes	Yes	NR	NR	Yes	Yes	Yes	Yes
Genetic diagnosis	Yes: *NEU1 c.1105A>G*	No	No	No	No	No	No	No

BMT, bone marrow transplant; FSGS, focal segmental glomerulosclerosis; HD, hemodialysis; NA, not applicable; NR, not reported; PD, peritoneal dialysis.
[a]Patients did not show proteinuria but abnormalities in the biopsy/autopsy of the kidney similar to those found in other nephrosialidose.
[b]A short summary of the table is displayed in Supplementary Table S1.
[c]Patient origin unknown, country where the treating hospital is located.

Table 2. (Continued)

Patient	8	9	10	11	12	13	14	15	16
Author	Kelly/Roth *et al.*[17–19]	Kashtan *et al.*[20]	Toyooka *et al.*[16]	Kanaka *et al.*[21]	Tylki-Szymanska *et al.*[12]	Ovali *et al.*[22] [b]	Schiff *et al.*[23]	Caciotti *et al.*[24]	Chen *et al.*[25]
Yr of Publication	1977,1981/1988	1981/1989	1993	1993	1996	1998	2005	2009	2011
Gender	Female	NR	Male	Male	Male	Female	Female	Female	Male
Origin	USA	USA[c]	Japan	Switzerland	Poland	Turkey[c]	France[c]	NR	China
Clinical presentation									
Onset of symptoms	Birth, facial dysmorphic signs	6 mo, macrocephaly	1 yr 7 mo	NR	Birth, hydrops fetalis	Prenatal, hydrops fetalis	1.5 mo failure to thrive, vomiting	NR	12 mo, edema of scrotum
Onset of proteinuria	8 yr	2 yr	19 mo	6.5 yr	3.5 yr	Birth (mild)	25 mo	NR	2 yr
Treatment w. Steroids	No	Yes without success	No	No	Yes without success	No	No	NR	Yes without success
Hepatosplenomegaly	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NR
Skeleton									
Facial dysmorphic signs	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NR
Dysostosis multiplex/ osteoporosis	NR	NR	Yes	NR	NR	NR	Yes	Yes	NR
Nervous system									
Psychomotor retardation	Yes	NR	Yes	NR	Yes	NR	Yes	Yes	NR
Other	Growth retardation	Growth retardation			Hyrocephalus		Growth failure		
Eyes									
Corneal clouding/ cataracts	Yes	Yes	NR	Yes	Yes	No	NR	NR	NR
Macular cherry-red spot	No	NR	Yes	Yes	Yes	No	NR	NR	No
Other		Optic nerve degeneration							
Hernia	NR	Yes	NR	NR	Yes	Yes	NR	NR	NR
Ascites/edema	Yes	NR	NR	NR	Yes	Yes	Yes	Yes	Yes
Other	Joint restriction, gingival hypertrophy, cardiomegaly	Chronic otitis media		Severe recurrent epistaxis with massive loss of blood	Gingival hyperplasia			Petechia, teleangi-ectases	
Course	PD followed by HD at 9 yr, died from sepsis at 9 yr 8 mo	Died at the age of 9 yr	Died of multiple organ failure at 8 yr	Died of cardiac failure at 8.5 yr	Died at the age of 4 yr	Died at 27 days	BMT (9 mo), HD (6 yr), at 11 yr of age poor general condition	Died at the age of 1 yr	Died at the age of 2.5 yr
Diagnostics									
Deficiency of α neuraminidase in lymphocytes/ fibroblasts	Yes	Yes	Yes	Yes	Yes and depression of beta-galactosidase	No but elevated levels of sialic acid in urine	Yes	Yes	Yes
Vacuolated lymphocytes	NR	NR	NR	Yes	NR	Yes	NR	NR	NR
Renal pathology: FSGS/vacuolated cells	Yes	Yes	Yes	NR	NR	NR	Yes	NR	Yes
Genetic diagnosis	No	No	No	No	No	No	No	Yes: *NEU1* c.807 + 1G>A	No

consanguinity of the parents. This resulted in 37 shared heterozygous variants. We prioritized variants in genes known to cause a human phenotype when mutated (technically, a Mendeliome analysis), as well as other variants, which we defined as novel because there was no reported frequency in the public databases (e.g., Exome Aggregation Consortium or the Genome Aggregation Database) or variants that occurred in genes previously linked to human disease.

Among these variants, we prioritized variants that fulfilled both criteria. Ten genes contained variants shared between both parents, which were previously implicated in human disease (*CENPJ, CLCN2, FAH, KANK1, NEU1, PADI4, PRG4, SOX10, TACR3, ZNF141*). Variants in 8 genes (*ASCL5, EBF1, ENOX1, HPR, MFSD6L, MYHA4, NEU1, ORA4K1*) were not present in population databases (Table 1). Combining both filtering steps resulted in only 1 variant: *NEU1* NM_000434: c. 1109A>G, p.Y370C (see Supplementary Figure S1 for the variant location in the binary alignment map [BAM] files). Fifty *NEU1* mutations identified as causative for sialidosis I/II (*OMIM 256550)* were reported to date in the Human Gene Mutation database (www. http://www.hgmd.cf.ac.uk/ac/index.php), among which was the variant we identified, as originally reported by Bonten *et al*. in 2000.[5] Furthermore, the phenotype in our case, with coarse facial features, organomegaly, neurological problems, and foamy cytoplasm found in the renal biopsy material, matched the phenotypic criteria of nephrosialidosis, which was reported in <20 cases in the literature. Subsequent Sanger sequencing confirmed the affected individual carried the *NEU1* missense variant in a homozygous state (see Figure 2 for pedigree and sequencing results). The affected nucleotide was highly conserved (phyloP 4. 56), and so was the amino acid among species, including that in *Mus musculus* (mouse), *Danio rerio* (zebrafish), and *Xenopus tropicalis* (frog). Tyrosine and cysteine are physiochemically distant, and the variant was predicted as disease-causing (score 1.0) by mutation taster prediction software (www.mutationtaster.org; see Supplementary Web Resources). According to the guidelines, the identified variant shows strong evidence for pathogenicity (PS1 variant) and was classified as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) variant classification rules. *NEU1* function and the most severely affected organs in sialidosis are outlined in Figure 3.

Clinical and Molecular Features of Previously Reported Nephrosialidosis Cases

Nephrosialidosis represents an ultra-rare disorder and therefore clinical descriptions are sparse. We screened PubMed for previous reports and found 16 cases, which are summarized in detail in Table 2 and a short summarizing description in Supplementary Table S1. Only 1 of these cases had been molecularly confirmed. The main onset of proteinuria was at age 2 to 3 years, with few earlier or later cases. Four of 16 patients received steroid treatment with no improvement. Renal pathology revealed mainly FSGS with vacuolated cells. Extrarenal manifestations were found in all reported patients, with most presenting with hepatomegaly, whereas corneal clouding and a cherry-red spot on the macula was only observed in approximately 50% of cases. Most patients showed extrarenal symptoms before the onset of renal manifestations. The disease course was unfavorable in most cases, with 14 of 16 cases no longer alive at the time of reporting. It is unknown if the remaining 2 cases survived or died at a later stage.

CONCLUSIONS

Sialidosis is a rare autosomal, recessively inherited disorder that results in lysosomal accumulation of sialyated protein products and excretion of such products in the urine. Diagnosis is usually made based on clinical criteria, including classical storage disorder symptoms, such as facial dysmorphism and organomegaly. Cherry red spots at ophthalmological examination and neurological symptoms in combination with biochemistry analyses for sialyated products in the urine and/or in tissue samples (e.g., fibroblasts) can also be used for diagnosis. However, such analyses may not be available in countries with low socioeconomic status. In our case, the patient came to the attention of medical services due to nephrotic syndrome, and as a result of rapid deterioration of her health with a fatal outcome, a diagnosis could not be made before her death. However, the family was desperate to understand what caused her death and to ascertain knowledge for further family planning. We suspected a storage disease because of the phenotype of the infant, which included coarse facial features, organomegaly, neurological problems, and foamy cytoplasm found in the renal biopsy material. However, because of the family's consanguinity, and therefore, a possibility of 2 recessive disorders segregating (storage disorder and nephrotic syndrome) within the family, we decided to perform WES instead of targeted gene analyses. The number of possibly causative genes was extensive, and WES was the most economic test ($450 USD per sample, including bioinformatics) with the best chances to identify the underlying genetic cause. Due to the unavailability of sufficient high-quality DNA for the index case, we sequenced both parents instead, because we previously successfully identified a causative

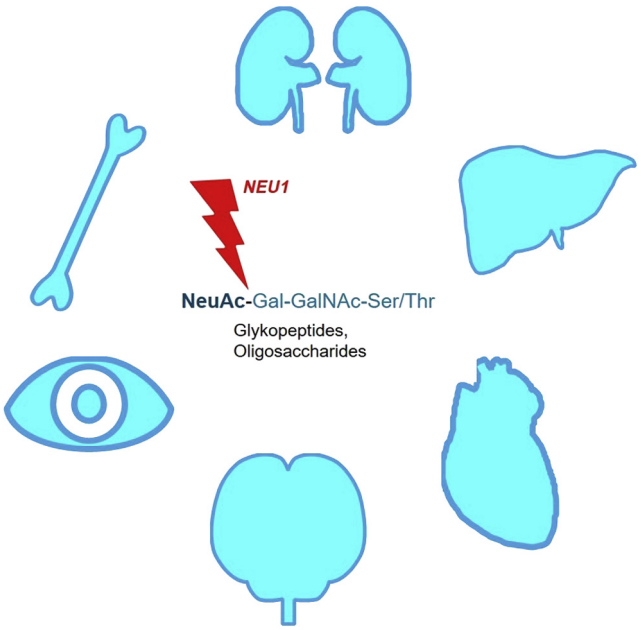

Figure 3. Schematic of *NEU1* function and organ involvement in sialidosis. *NEU1* (α-neuraminidase) functions together with cathepsin as sialidase, removing sialic acid from glycoproteins and/or peptides and oligosaccharides. Accumulation of sialylated products in lysosomes causes progressive organ dysfunction, including the kidneys, liver, heart, brain, eyes, and bones.

recessive gene using parental samples and checked those samples for a shared heterozygous disease-causing variant.[28] This allowed us to save the patient's small amounts of DNA for Sanger sequencing. Although our method is more effective in consanguine families because filtering for a shared variant is possible, it is also feasible for nonrelated parents by filtering for heterozygous variants in the same genes. We applied a filtering cascade that technically contained a Mendeliome analysis. Mendeliome NGS sequencing represents a targeted WES approach in which only genes known to cause human disease are included instead of the genome-wide approach in WES. Numerous medico-biological companies offer this sequencing approach, and some genetics centers apply this method for variant detection rather than WES as the first-line method. With regard to cost, it is usually not significantly cheaper, despite the fact that less sequence reads are required, because sample preparation process and time are equal to WES but less DNA input is required (~200 ng compared with several micrograms for WES). This is still more than that used for a single PCR with subsequent Sanger sequencing, in which 25 to 50 ng are sufficient. Therefore, in our case, we opted not to attempt targeted WES but instead used parental samples for WES. However, it can be an interesting approach for cases in which only 500 ng or 1 μg of DNA is available. Although an advantage of targeted WES is less bioinformatic data usage, which allows easier and faster data analysis, the disadvantage is that due to rapid discovery of novel human disease-causing genes, targeted exome kits are never "up to date"; therefore, recently published data on genes are not covered. Furthermore, by default, targeted WES does not offer the possibility of novel gene identification. In contrast, targeted WES may offer slightly better coverage of known disease-causing genes due to optimizations and less genes to be covered by the sequencing method; however, we achieved a depth of 4× for 99%, 10× for 98.6% to 99.1%, 20× for 96.5% to 97.2%, and 50× for 93.1% to 94.8% of targeted regions, with 99% of targeted regions covered, which is usually sufficient.

The histological changes reported for the renal biopsy in our case are not unique to storage disorders. Some vacuolization of visceral epithelial cells also occurs with other forms of nephrotic syndrome, such as FSGS, and minimal change disease, but not to the same extent, and usually with empty vacuoles.[25] In contrast to some previously reported nephrosialidosis cases, no sclerosis was found in our biopsy, and the glomerular basement membrane likewise appeared normal. As pointed out by Chen *et al.*,[25] Denys-Drash Syndrome (OMIM *194080*) can also present with dysmorphic facial features, neurodevelopmental delay, and nephrotic syndrome, although usually congenitally and foamy podocytes have also likewise been described in Alport syndrome (OMIM *301050*). In addition, action myoclonus renal failure syndrome caused by *SCARB2* mutations presents with overlapping features compared with nephrosialidosis (e.g., progressive neurological diseases, FSGS, nephrotic syndrome, and renal failure). Histology can show vacuolar cytoplasm and granular deposits within the renal tubules.[29] Therefore, these conditions should be considered in the differential diagnosis and rendering of WES, rather than considering hypothesis-driven, single-gene sequencing to be more efficient.

Hepatomegaly seems to be the most common extrarenal feature in nephrosialidosis patients, whereas pathognomonic signs (e.g., a cherry red macular spot or corneal clouding) occur only in approximately 50% of cases. In most previously reported cases, extrarenal symptoms occurred well before renal manifestations; however, in areas of the world with difficult to access medical care or with limited diagnostic possibilities (e.g., our case), nephrotic range proteinuria can be the first symptom that brings a patient to medical attention. As for the previously reported 16 nephrosialidosis cases, only 1 was confirmed on a molecular level; putative genotype-phenotype correlations cannot be

made. It remains unclear why some patients develop renal disease, whereas others do not.

In summary, our findings underline the power of WES for molecular diagnosis in rare diseases. Our findings indicate that primary WES analysis is a reliable diagnostic measure for a relatively cheap price, especially in areas of the world where elaborate biochemical analyses are not available. Parental sequencing should be attempted if there is not enough DNA or only low-quality DNA is available from the index case. Furthermore, nephrosialidosis should be considered as a differential diagnosis in patients with steroid resistant nephrotic syndrome with dysmorphic facial features and developmental delay.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

MS acknowledges funding from Radboudumc and RIMLS Nijmegen (Hypatia tenure track fellowship), the "Deutsche Forschungsgemeinschaft" (DFG CRC1140 KIDGEM) and the European Research Council (ERC StG TREATCilia, grant No 716344).

AUTHOR CONTRIBUTIONS

RM was involved in study conception and manuscript preparation. AR, DA, MN, IS, and ZB performed sample preparation and data analysis. HH was involved in patient recruitment. MS conceived and designed the study and wrote the manuscript. All authors have contributed to the manuscript, and read and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Table S1. Short summarizing description of previously reported nephrosialidosis cases

Figure S1. Integrative genomics viewer (IGV) screenshot visualizing the heterozygous *NEU1* mutation detected in whole-exome sequencing (WES) data of both parents.

Web Resources.

Supplementary material is linked to the online version of the paper at www.kireports.org.

REFERENCES

1. LaDuca H, Farwell KD, Vuong H, et al. Exome sequencing covers >98% of mutations identified on targeted next generation sequencing panels. *PLoS One*. 2017;12:e0170843.
2. Lovric S, Ashraf S, Tan W, Hildebrandt F. Genetic testing in steroid-resistant nephrotic syndrome: when and how? *Nephrol Dial Transplant*. 2016;31:1802–1813.
3. Spranger JW, Wiedemann HR. The genetic mucolipidoses. *Neuropadiatrie*. 1970;2:3–16.
4. Bonten E, van der Spoel, Fornerod M, et al. Characterization of human lysosomal neuraminidase defines the molecular basis of the metabolic storage disorder sialidosis. *Genes Dev*. 1996;10:3156–3169.
5. Bonten EJ, Arts WF, Beck M, et al. Novel mutations in lysosomal neuraminidase identify functional domains and determine clinical severity in sialidosis. *Hum Mol Genet*. 2000;9:2715–2725.
6. Van Pelt J, Kamerling JP, Bakker HD, Vliegenthart JF. A comparative study of sialyloligosaccharides isolated from sialidosis and galactosialidosis urine. *J Inherit Metab Dis*. 1991;14:730–740.
7. Piraud M, Pettazzoni M, Menegaut L, et al. Development of a new tandem mass spectrometry method for urine and amniotic fluid screening of oligosaccharidoses. *Rapid Commun Mass Spectrom*. 2017;31:951–963.
8. Lowden JA, O'Brien JS. Sialidosis: a review of human neuraminidase deficiency. *Am J Hum Genet*. 1979;31:1–18.
9. Maroteaux P, Humbel R, Strecker G, et al. A new type of sialidosis with kidney disease: nephrosialidosis. I. Clinical, radiological and nosological study [in French]. *Arch Fr Pediatr*. 1978;35:819–829.
10. Le Sec G, Stanescu R, Lyon G. A new type of sialidosis with kidney disease: nephrosialidosis. II. Anatomic study [in French]. *Arch Fr Pediatr*. 1978;35:830–844.
11. Aylsworth AS, Thomas GH, Hood JL, et al. A severe infantile sialidosis: clinical, biochemical, and microscopic features. *J Pediatr*. 1980;96:662–668.
12. Tylki-Szymanska A, Lugowska A, Czartoryska B. Neuraminidase deficiency presenting as a nephrosialidosis: the first case detected in Poland. *Acta Paediatr Jpn*. 1996;38:529–532.
13. Matsuda A, Yokoi T, Maeda T, Tniguchi N. A case of nephrosialidosis. *Acta Pediatr Jpn*. 1983;7:2317–2322.
14. Yamano T, Shimada M, Sugino H, et al. Ultrastructural study on a severe infantile sialidosis (beta-galactosidase-alpha-neuraminidase deficiency). *Neuropediatrics*. 1985;16:109–112.
15. Beck M, Bender SW, Reiter HL, et al. Neuraminidase deficiency presenting as non-immune hydrops fetalis. *Eur J Pediatr*. 1984;143:135–139.
16. Toyooka K, Fujimura H, Yoshikawa H, et al. Nephrosialidosis: ultrastructural and lectin histochemical study. *Acta Neuropathol*. 1993;86:198–205.
17. Kelly TE, Graetz G. Isolated acid neuraminidase deficiency: a distinct lysosomal storage disease. *Am J Med Genet*. 1977;1:31–46.
18. Roth KS, Chan JC, Ghatak NR, et al. Acid alpha-neuraminidase deficiency: a nephropathic phenotype? *Clin Genet*. 1988;34:185–194.
19. Kelly TE, Bartoshesky K, Harris DJ, et al. Mucolipidosis I (acid neuraminidase deficiency). Three cases and delineation of the variability of the phenotype. *Am J Dis Child*. 1981;135:703–708.
20. Kashtan CE, Nevins TE, Posalaky Z, et al. Proteinuria in a child with sialidosis: case report and histological studies. *Pediatr Nephrol*. 1989;3:166–174.
21. Kanaka C, Herschkowitz N, Weber JW, et al. Mucocutaneous bleeding, a rare but severe complication in nephrosialidosis. *Eur J Pediatr*. 1994;153:703–704.

22. Ovali F, Samanci N, Guray A, et al. Congenital sialidosis. *Turk J Pediatr*. 1998;40:447–451.

23. Schiff M, Maire I, Bertrand Y, et al. Long-term follow-up of metachronous marrow-kidney transplantation in severe type II sialidosis: what does success mean? *Nephrol Dial Transplant*. 2005;20:2563–2565.

24. Caciotti A, Di Rocco M, Filocamo M, et al. Type II sialidosis: review of the clinical spectrum and identification of a new splicing defect with chitotriosidase assessment in two patients. *J Neurol*. 2009;256:1911–1915.

25. Chen W, Yang S, Shi H, et al. Histological studies of renal biopsy in a boy with nephrosialidosis. *Ultrastruct Pathol*. 2011;35:168–171.

26. Schmidts M, Hou Y, Cortes CR, et al. TCTEX1D2 mutations underlie Jeune asphyxiating thoracic dystrophy with impaired retrograde intraflagellar transport. *Nat Commun*. 2015;6:7074.

27. Schmidts M, Vodopiutz J, Christou-Savina S, et al. Mutations in the gene encoding IFT dynein complex component WDR34 cause Jeune asphyxiating thoracic dystrophy. *Am J Hum Genet*. 2013;93:932–944.

28. Onoufriadis A, Shoemark A, Munye MM, et al. Combined exome and whole-genome sequencing identifies mutations in ARMC4 as a cause of primary ciliary dyskinesia with defects in the outer dynein arm. *J Med Genet*. 2014;51:61–67.

29. Balreira A, Gaspar P, Caiola D, et al. A nonsense mutation in the LIMP-2 gene associated with progressive myoclonic epilepsy and nephrotic syndrome. *Hum Mol Genet*. 2008;17:2238–2243.