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Mutations in *WDR4* as a new cause of Galloway-Mowat syndrome

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Abstract

Introduction: Galloway-Mowat syndrome (GAMOS) is a phenotypically heterogeneous disorder characterized by neurodevelopmental defects combined with renal-glomerular disease, manifesting with proteinuria. To identify additional monogenic disease causes, we subjected three affected siblings of an Indian family with GAMOS to mutation analysis.

Methods: We performed whole exome sequencing (WES), linkage analysis, and homozygosity mapping.

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Author contributions

- Prof. Hildebrandt conceptualized and designed the study, coordinated and supervised data analysis, and reviewed and revised the manuscript.
- Dr. Braun spearheaded the project, collected and primarily analyzed the data, performed whole exome sequencing analysis, connected genetic data and phenotypic data, drafted the initial manuscript, and reviewed and revised the manuscript.
- Ms. Shril, Ms. Laricchia, and Dr. Lek designed the data analysis pipeline, performed whole exome sequencing, SNP genotyping, analyzed the data, and reviewed and revised the manuscript.
- Dr. Sinha, Dr. Meena, and Dr. Bagga collected clinical data, performed data analysis, gathered follow-up information, evaluated the clinical data in the context of the genetics data, and reviewed and revised the manuscript.
- Dr. Schneider, Dr. Tan, Dr. Ashraf, Dr. Hermle, Dr. Jobst-Schwan, Dr. Widmeier, Dr. Majmundar, Dr. Daga, Dr. Warejko, Dr. Nakayama, Mr. Schapiro, Dr. Chen, Dr. Airik, Dr. Rao, Ms. Schmidt, Ms. Hoogstraten, and Ms. Hugo contributed to data collection, performed analysis of whole exome sequencing data and clinical data, improved the data analysis pipeline, and critically reviewed the manuscript.
- All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Results: Applying established criteria for variant filtering, we identify a novel homozygous splice site mutation in the gene *WDR4* as the likely disease-causing mutation in this family. In line with previous reports, we observe growth deficiency, microcephaly, developmental delay, and intellectual disability as phenotypic features resulting from *WDR4* mutations. However, the newly identified allele additionally gives rise to proteinuria and nephrotic syndrome, a phenotype that was never reported in patients with *WDR4* mutations.

Conclusion: Our data expand the phenotypic spectrum of *WDR4* mutations by demonstrating that, depending on the specific mutated allele, a renal phenotype may be present. This finding suggests that GAMOS may occupy a phenotypic spectrum with other microcephalic diseases. Furthermore, *WDR4* is an additional example of a gene that encodes a tRNA modifying enzyme and gives rise to GAMOS, if mutated. Our findings thereby support the recent observation that, like neurons, podocytes of the renal glomerulus are particularly vulnerable to cellular defects resulting from altered tRNA modifications.

Keywords

Mendelian diseases; variable phenotypic expressivity; rare syndromic diseases; whole-exome sequencing; Galloway-Mowat syndrome

INTRODUCTION

Galloway-Mowat syndrome (GAMOS) is a rare, clinically heterogeneous condition characterized by the combined presence of various anomalies of the central nervous system (CNS) and renal-glomerular disease (Galloway & Mowat, 1968; Vodopiutz et al., 2015). CNS abnormalities can either manifest at birth (primary microcephaly) or only become apparent over time (secondary microcephaly) (Colin et al., 2014). Besides a reduction in head circumference (the defining feature of microcephaly), CNS manifestations of GAMOS may also involve other features such as abnormal cerebral gyration or cerebellar atrophy (Rosti et al., 2016). Clinically, affected patients may present with developmental delay, psychomotor retardation, various degrees of intellectual disability, and propensity to seizures (Vodopiutz et al., 2015). The hallmark feature of renal GAMOS is proteinuria, thus indicating podocytes of the renal glomerulus as the primarily affected cell type in the kidney (Colin et al., 2014). Like CNS manifestations, renal features of GAMOS may also be variable regarding age of onset and severity, ranging from congenital nephrotic syndrome with rapidly declining kidney function to slowly progressive proteinuria (Colin et al., 2014). Correspondingly, the presentation on renal histology may vary from merely showing mild signs of initial podocyte injury, i.e. podocyte foot process effacement, to demonstrating signs of progressive and irreversible glomerular sclerosis (Colin et al., 2014). Typically, the renal disease is refractory to treatment and progresses to end-stage renal failure, thus requiring renal replacement therapy (dialysis) or renal transplantation for survival (Colin et al., 2014). Besides renal and neurological manifestations, additional clinical features can be present. These may include facial dysmorphism, skeletal anomalies, and various degrees of growth retardation (intrauterine and/or postnatally) (Meyers, Kaplan, & Kaplan, 1999). GAMOS is inherited in an autosomal-recessive manner. To date, mutations in six different genes have been identified as causing this distinct phenotype, if mutated. These are *WDR73* (Colin et

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al., 2014; Jinks et al., 2015; Rosti et al., 2016; Vodopiutz et al., 2015), and *LAGE3* (Braun et al., 2017), *OSGEP* (Braun et al., 2017; Edvardson et al., 2017), *TP53RK* (Braun et al., 2017), and *TPRKB* (Braun et al., 2017), which encode subunits of the evolutionarily highly conserved KEOPS complex (Hecker et al., 2008; Mao et al., 2008), as well as *NUP107*, which may cause a GAMOS-like renal-neurological phenotype on an allelic basis (Alazami et al., 2015; Rosti et al., 2017).

The gene *WDR4* encodes the protein 'tRNA (guanine-N(7)-)-methyltransferase non-catalytic subunit WDR4'. This enzyme is required for a specific posttranscriptional modification of tRNA, namely the 7-methylguanosine modification (Alexandrov, Martzen, & Phizicky, 2002). Recently, two independent research teams have described mutations in *WDR4* as causing microcephaly with intellectual disability and severe growth retardation (Shaheen et al., 2015; Trimouille et al., 2018). Interestingly, the three described families displayed significant variation regarding the severity of the phenotype (Trimouille et al., 2018). We here perform whole-exome sequencing in a family with GAMOS and identify a homozygous mutation in the gene *WDR4* as a novel pathogenic allele. Our data expands the phenotypic spectrum of *WDR4* mutations by demonstrating for the first time that nephrotic syndrome may result from certain mutated alleles.

MATERIALS AND METHODS

Study participants.

Following informed consent, we obtained pedigree information, clinical data, and blood samples from individuals with proteinuria or nephrotic syndrome (onset < 25 years of age) including families with extra-renal manifestations such as microcephaly. We obtained approval for human subjects research from the Institutional Review Boards of the University of Michigan and the Boston Children's Hospital. Patient enrollment started in 1998, is still ongoing, and includes numerous collaborating centers worldwide. The patient cohort is ethnically diverse, but families from regions with frequent consanguinity were preferentially chosen for whole-exome sequencing.

Whole exome sequencing.

Whole-exome sequencing (WES) and data processing were performed by the Genomics Platform at the Broad Institute of Harvard and MIT (Broad Institute, Cambridge, MA, USA). Exome sequencing (>250 ng of DNA, at >2 ng/μL) was performed using Illumina exome capture (38-Mb target). Single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) were jointly called across all samples using the Genome Analysis Toolkit (GATK) HaplotypeCaller. Default filters were applied to SNP and indel calls using the GATK Variant Quality Score Recalibration approach. Lastly, variants were annotated using the Variant Effect Predictor. For additional information, please refer to the supplementary section 1 in the Exome Aggregation Consortium (ExAC) study (Lek et al., 2016). The variant call set was uploaded on to seqr (https://seqr.broadinstitute.org) and analysis of the entire WES output was performed. Remaining calls were ranked for their predicted pathogenicity based on the following criteria (Lovric, Ashraf, Tan, & Hildebrandt, 2016; MacArthur et al., 2014; Vivante & Hildebrandt, 2016): a) protein-truncating or obligatory

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splice site mutations *vs.* missense mutations or in-frame deletions/insertions, b) evolutionary conservation, c) minor allele frequency in control databases (gnomAD)(Lek et al., 2016), d) chemical difference between wildtype and altered amino acid residue, e) web-based mutation analysis prediction tools (SIFT (Kumar, Henikoff, & Ng, 2009), PolyPhen-2 (Adzhubei et al., 2010), MutationTaster (Schwarz, Cooper, Schuelke, & Seelow, 2014)). We used the splice prediction module embedded in Alamut® Visual (Rouen, France) to predict the expected impact of the identified variant. This in silico prediction program is based on the 'Human Splicing Finder (HSF)' algorithm (Desmet et al., 2009) and on the algorithm MaxEntScan (Yeo & Burge, 2004).

Homozygosity mapping.

Homozygosity mapping was calculated based on whole exome sequencing data. In brief, aligned BAM files were processed using Picard and SAMtools4 as described by other groups (Li et al., 2009). Single nucleotide variant calling was performed using Genome Analysis Tool Kit (GATK) (Van der Auwera et al., 2013). The resulting VCF files were used to generate homozygosity mapping data and visual outputs using the program Homozygosity Mapper (Seelow, Schuelke, Hildebrandt, & Nurnberg, 2009).

Web Resources.

Exome Aggregation consortium (ExAC), http://exac.broadinstitute.org; Genome Aggregation Database (gnomAD), http://gnomad.broadinstitute.org;

HGMD Professional 2016.3, https://portal.biobase-international.com/hgmd;

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org;

Homozygosity Mapper, http://www.homozygositymapper.org/;

Seqr genomics platform, https://seqr.broadinstitute.org/;

Polyphen2, http://genetics.bwh.harvard.edu/pph2;

Sorting Intolerant From Tolerant (SIFT), http://sift.jcvi.org;

MutationTaster http://www.mutationtaster.org

RESULTS

An obligatory splice site mutation in *WDR4* causes recessive GAMOS

We performed whole exome sequencing (WES) in three of four children of an Indian family (B1028) who displayed a combined renal-neurological phenotype. To identify the most probable disease-causing mutation, we used the following criteria for variant filtering (Lovric et al., 2016; MacArthur et al., 2014; Vivante & Hildebrandt, 2016): 1) We excluded all variants that did not change the amino-acid sequence or affected canonical splice sites (defined as ± 6 nucleotides surrounding the exon-intron boundary). 2) We excluded variants that were reported in the homozygous state or with a minor allele frequency greater than 0.1% in a control cohort (ExAC browser). 3) We postulated a recessive mode-of-inheritance

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and therefore focused on biallelic variants (homozygous or compound heterozygous). Using this strategy, we identified a homozygous mutation in the gene *WDR4* as the only shared variant between the three siblings' datasets. Later, we confirmed that the mutated allele was also present in the homozygous state in the fourth sibling who had a similar phenotype, but was not subjected to WES (Suppl. Fig. 1). The family was not reported to be consanguineous. However, homozygosity mapping detected one homozygous peak region on chromosome 21 that was shared between all three siblings (Suppl. Fig. 2). The *WDR4* locus (red circle) was positioned within this maximum NPL (nonparametric lod score) peak on chromosome 21. The identified mutation resulted in a nucleotide exchange at an obligatory splice site (c.454-2A>C, NM_018669.5). This position is 100% conserved in the canonical sequence of mammalian splice sites. Specifically, the mutation affected the second nucleotide at the universally conserved acceptor splice site of intron 4. Unfortunately, it was not possible to obtain a fresh RNA sample from any of the affected children in order to experimentally demonstrate the impact of this variant on splicing of the encoded transcript. However, in silico analysis (Desmet et al., 2009; Yeo & Burge, 2004) assigned severe scores to this variant and predicted a high likelihood for it to alter splicing *in vivo*. The reported variant is extremely rare in the general population. This is reflected by its variant frequency of 0.017% (1 heterozygous allele out of 62,560 sequenced alleles, no homozygous alleles) in the ExAc browser.

Intrafamilial variation of the clinical phenotype

All four children displayed microcephaly, global developmental delay, and variable degrees of intellectual disability. While all four attended regular schools, they demonstrated impaired scholastic performance and teachers noted that they were slow in understanding as compared to peers. A formal assessment of the oldest child (B1028-22) at an age of 12 years measured a social quotient of 60 on the Vineland Social Maturity Scale, rated as mild intellectual disability. However, the two younger siblings (−21 and −24) demonstrated more pronounced intellectual disability. Brain MRI, which was performed in one child (B1028-21), did not demonstrate any structural anomalies of the cerebrum, cerebellum or abnormalities in cerebral gyration patterns (Fig. 1). None of the four children had a history of seizures. A conductive hearing deficiency due to chronic otitis media was noted in one sibling (B1028-22), but none of the other children showed any signs of hearing impairment. Growth retardation was a prominent feature in all four children. Measurements ranged from Z-scores (standard deviation scores) of −8 (B1028-21) to −4 (B1028-24) for weight, and from −6 (B1028-21) to −5 (B1028-24) for height (Table 1). Unfortunately, data on intrauterine growth retardation could not be retrieved for the children. Furthermore, two of four children demonstrated clinodactyly (Fig. 1). In addition, there was mild hypothyroidism with TSH-levels ranging from 6-14 mIU/ml in the three older children. The youngest brother was euthyroid at the time of this publication.

The three older siblings furthermore demonstrated renal involvement, specifically nephrotic range proteinuria (Table 1). Interestingly, there was a certain variation in the severity of the renal phenotype and only one child (B1028-21) developed the complete clinical picture of nephrotic syndrome including edema and hypalbuminemia. A renal biopsy was performed in two children (B1028-21 and −22). It demonstrated focal-segmental glomerulosclerosis on

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light-microscopy and podocyte foot process effacement on transmission electron microscopy (TEM), both features of podocyte injury (Fig. 1). At the time of this publication, i.e. at ages ranging from 4 to 15 years, renal function was preserved with serum creatinine values ranging between 0.3-0.5 mg/dl in all four children (Table 1). There were no signs of anemia and serum-calcium/–phosphorus levels were within the normal range. Immunosuppressive treatment was initiated in one sibling (B1028-21). While he showed no clinical response to mono-therapy with corticosteroids, he responded partially to a combined treatment regimen of corticosteroids and calcineurin inhibitors (tacrolimus). Specifically, there was a reduction in proteinuria and edema, as well as a normalization of the serum albumin level after 4 months of combined treatment. Since the penetrance of the endocrine and renal phenotype appeared to age-dependent, the youngest sibling, who was euthyroid and showed no signs of proteinuria at the time of this publication, may still develop those phenotypic features over time.

DISCUSSION

Recently, two independent publications reported recessive mutations in the gene *WDR4* in patients with microcephalic primordial dwarfism (Shaheen et al., 2015) and microcephaly with growth retardation (Trimouille et al., 2018), respectively. In line with those reports, all affected children of family B1028 displayed microcephaly, global developmental delay, and severe growth retardation. Interestingly, the severity of the neurological phenotype varied significantly between the different families; ranging from children who did not show any developmental progress and failed to achieve developmental milestones (Shaheen et al., 2015) to children who were able to attend regular schools, even though their scholastic performance was described as impaired when compared to peers (this report). The first two reports described patients who carried the same missense allele, once in the homozygous state and once as a compound heterozygous mutation in combination with a truncating allele. Strikingly, the neurological phenotype caused by two recessive missense alleles was more severe than the one caused by a combined missense-nonsense compound heterozygous mutation, suggesting that genotype-phenotype correlation is very complex and hard to predict in patients with *WDR4* mutations. As the exact impact of the reported splice site variant on the translated protein is difficult to predict, it remains uncertain how this newly identified mutation may fit into the genotype-phenotype spectrum of *WDR4* mutations. Furthermore, tissue-specific differences in splicing of the encoded transcript may add to the complexity of the situation.

In our newly identified family, three children developed hypothyroidism, an endocrine disorder that was not present in any of the previously described cases. However, one previous publication also reported endocrine anomalies, specifically growth hormone deficiency and hypogonadotropic hypogonadotropism. Furthermore, we detected proteinuria, indicative of a disruption of the renal-glomerular filter, as a novel feature that expands the phenotypic spectrum of *WDR4* mutations. As kidney involvement was not reported in any of the previously described families (Shaheen et al., 2015; Trimouille et al., 2018), we thereby identify a new monogenic cause of a syndromic form of nephrotic syndrome. In conclusion, the available genetic data on *WDR4* mutations suggest that, depending on the specific mutated allele, the resulting phenotype may include CNS

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anomalies/intellectual disability of variable severity, growth retardation, different endocrine anomalies, and nephrotic syndrome. For some genetic causes, such as *WDR4*, GAMOS may thus occupy a phenotypic spectrum with other microcephalic diseases. Due to the broad phenotypic heterogeneity observed in patients with mutations of *WDR4* and due to the late onset of the renal phenotype, we would recommend a regular screening for proteinuria in all patients with *WDR4* mutations. Two major factors support this strategy: a) screening for proteinuria is an inexpensive, non-invasive test that is broadly available and b) an early intervention (best supportive care) can significantly delay the progression of kidney disease in patients with nephrotic syndrome.

Recently, mutations in genes of the KEOPS complex were described in more than 30 unrelated patients with GAMOS (Braun et al., 2017; Edvardson et al., 2017). The KEOPS complex is an evolutionarily highly conserved protein complex that catalyzes a specific posttranscriptional modification of tRNA (Perrochia, Crozat, et al., 2013; Perrochia, Guetta, Hecker, Forterre, & Basta, 2013; Srinivasan et al., 2011). By identifying mutations in *WDR4* in patients with GAMOS, we thus recognize the second entity of this disease that can be attributed to an alteration in a tRNA-modifying enzyme. Our data thereby emphasize the relevance of this recently described pathogenic mechanism in GAMOS. Neurons and podocytes of the renal glomerulus appear to be two cell types that are particularly vulnerable to the cellular consequences of altered tRNA modifications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CNS	central nervous system
ExAC	Exome Aggregation Consortium
GAMOS	Galloway-Mowat syndrome
NPL	nonparametric lod score

SNP	Single nucleotide polymorphism
WES	whole exome sequencing

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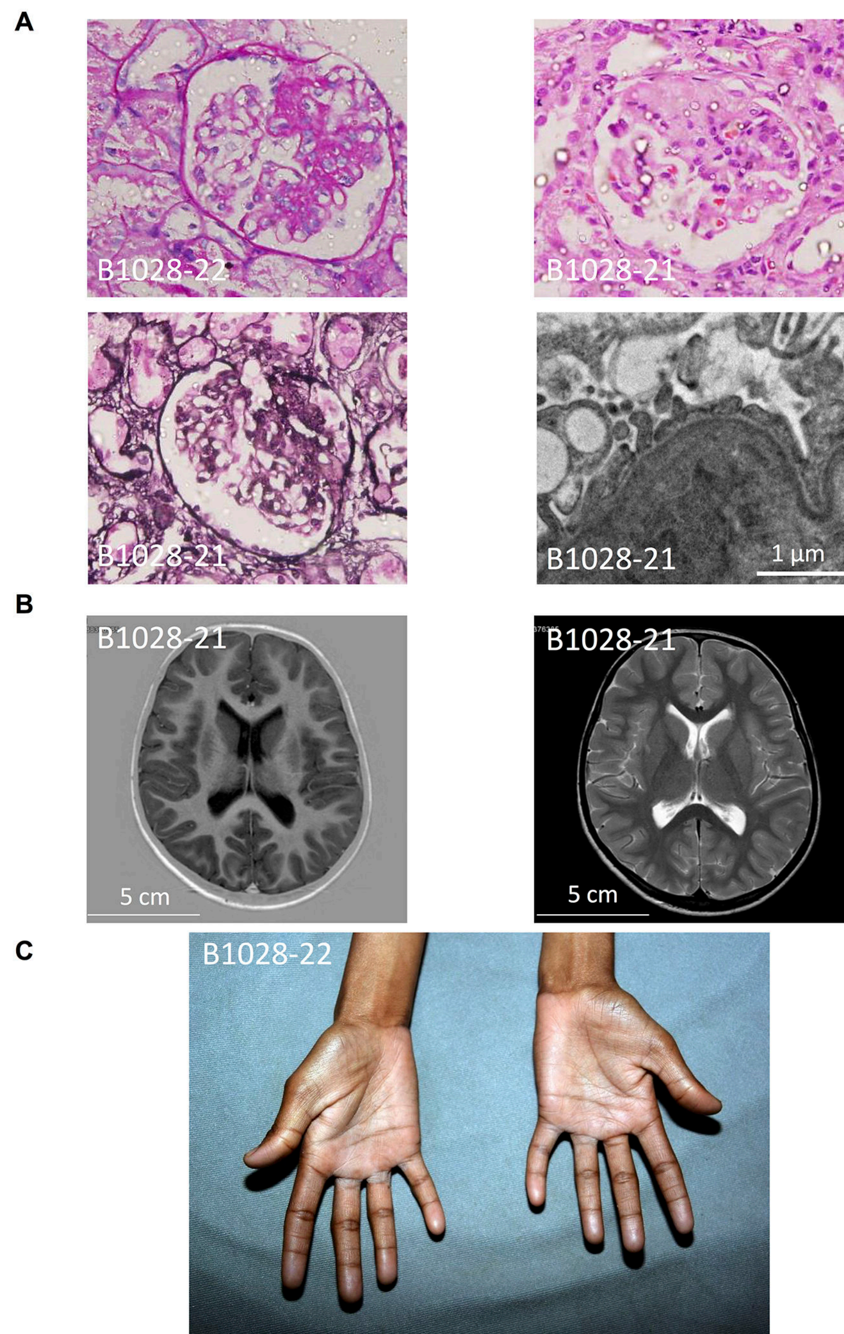

Figure 1. Clinical features of children with a homozygous mutation of *WDR4*.

A) Renal biopsy of patients B1028–22 and B1028–21 demonstrating focal-segmental glomerulosclerosis on light-microscopy and podocyte foot process effacement on transmission electron microscopy (TEM).

B) Brain Magnetic Resonance Imaging (MRI) in patient B1028-21 demonstrating no structural brain anomalies.

C) Clinical photograph of individual B1028–22 demonstrating clinodactyly of the fifth finger of both hands.

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Table 1.

Clinical parameters of 4 siblings with a homozygous mutation in the gene *WDR4* (c.454-2A>C, NM_018669.5)

B1028-Individual	Age [years]	Weight [kg] (Z score)	Height [cm] (Z score)	Severity of intellectual disability	Renal function		Serum-protein [mg/dl]	Serum-albumin [mg/dl]	Urine protein [Dipstick]
					BUN [mg/dl]	Creatinine [mg/dl]			
−22	15	18.9 (−6Z)	122 (−5Z)	Moderate	22	0.3	6.1	3.6	3 +
−23	13	15.1 (−7Z)	105 (−6Z)	Mild	31	0.2	6.2	4.2	2 +
−21	11	13 (−8Z)	98 (−6Z)	Severe	41	0.3	5.5	2.9	3 +
−24	4	7 (−4Z)	69 (−5Z)	Severe	24	0.3	5.6	3.2	negative

BUN, blood urea nitrogen.