Functional characterization of a novel TP53RK mutation identified in a family with Galloway–Mowat syndrome

Ernestine Treimer1,2 | Tugba Kalayci3 | Sven Schumann2 | Ilknur Suer3 | Sara Greco1 | Denny Schanze4 | Michael J. Schmeisser2,5 | Susanne J. Kühl1 | Martin Zenker4

1Institute of Biochemistry and Molecular Biology, Ulm University, Ulm, Germany
2Institute of Anatomy, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany
3Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
4Institute of Human Genetics, University Hospital, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
5Focus Program Translational Neurosciences, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

Abstract
Galloway–Mowat syndrome (GAMOS) is a very rare condition characterized by early-onset nephrotic syndrome and microcephaly with variable neurologic features. While considerable genetic heterogeneity of GAMOS has been identified, the majority of cases are caused by pathogenic variants in genes encoding the four components of the Kinase, endopeptidase, and other proteins of small size (KEOPS) complex, one of which is TP53RK. Here we describe a 3-year-old male with progressive microcephaly, neurodevelopmental deficits, and glomerular proteinuria. He was found to carry a novel homozygous TP53RK missense variant, c.163C>G (p.Arg55Gly), which was considered as potentially disease-causing. We generated a morpholino tp53rk knockdown model in Xenopus laevis showing that the depletion of endogenous Tp53rk caused abnormal eye and head development. This phenotype could be rescued by the expression of human wildtype TP53RK but not by the c.163C>G mutant nor by another previously described GAMOS-associated mutant c.125G>A (p.Gly42Asp). These findings support the pathogenic role of the novel TP53RK variant.

KEYWORDS
disease modeling, KEOPS complex, microcephaly, nephrotic syndrome, TP53RK
Various accompanying features have been described including intrauterine growth restriction, ocular defects, hiatal hernia, camptodactyly, arachnodactyly, talipes equinovarus, cardiac malformations, and facial anomalies. It has long been proposed that the "nephrosis-microcephaly syndromes" constitute a heterogeneous group of disorders (Meyers et al., 1999), but only recently an unexpected genetic heterogeneity of GAMOS has indeed been confirmed: Pathogenic variants in 10 different genes with autosomal or X-linked inheritance have so far been associated with GAMOS (https://www.omim.org/phenotypicSeries/PS251300). The majority of patients with the typical phenotype of GAMOS, however, have a defect in either of the four components of the highly conserved "Kinase, endopeptidase, and other proteins of small size" (KEOPS) complex, encoded by the genes OSGEP, TP53RK, TPRKB, and LAGE3 (Braun et al., 2017). This protein complex is known to participate in various functions related to transcription control (Kisseleva-Romanova et al., 2006), telomere regulation (Downey et al., 2006), and transfer RNA (tRNA) modification (Srinivasan et al., 2011). Knockdown of OSGEP or TP53RK in human podocytes induced defects in the actin cytoskeleton and decreased the migration rate, thereby providing a model for the significance of the KEOPS complex defects in the pathogenesis of nephrotic syndrome (Braun et al., 2017). Morpholino oligonucleotide (MO) knockdown of osgep or tprkb in zebrafish larvae recapitulated a primary microcephaly phenotype and showed early lethality (15–18 days postfertilization). Moreover, CRISPR–Cas9 knockout of Lage3, Osgep, or Tprkb led to significantly decreased brain size in mouse embryos (Braun et al., 2017).

We have recently examined the spatiotemporal expression pattern of osgep, tp53rk and tprkb during early Xenopus development and observed that all three genes were expressed during early embryogenesis and enriched in tissues and organs affected in GAMOS including the developing eye, head, and brain (Treimer et al., 2021). Here we report on a novel TP53RK missense variant identified in homozygosity in a 3-year-old male patient with a GAMOS phenotype, and demonstrate the pathogenetic relevance of this variant using Xenopus embryos as an experimental model.

The patient is the second child of a consanguineous couple (first cousins once removed) originating from Turkey. The boy was born after an uneventful pregnancy at 39 weeks of gestation with a birth weight of 2860 g (SD: −1.2), body length of 49 cm (SD: −0.45), and an occipitofrontal head circumference (OFC) of 33.5 cm (SD: −1.0). Progressive microcephaly was documented: OFC at 3 months: 39 cm (SD: −1.5), at 9 months: 40 cm (SD: −3.96), at 16 months: 41 cm (SD: −4.69); and at 3 years: 41 cm (SD: −5.79). He was first presented at the Istanbul Medical Genetics Department at the age of 16 months. Besides prominent microcephaly and severe developmental delay, he was noted to have facial dysmorphism including a narrow forehead, deep-set eyes, bilateral esotropia, prominent nasal bridge, hypoplastic alae nasi, large ears, and prominent stem of anthelices (Figure 1a–c). Additional findings included arachnodactyly, bilateral clinodactyly, soft skin, feeding difficulty, gastroesophageal reflux, bilateral cryptorchidism, and a hypoplastic scrotum.

Neurologically he first presented with neonatal hypotonia, which evolved later into progressive truncal and peripheral spasticity. Cranial

![Clinical photographs and brain magnetic resonance images (MRIs) of the patient.](https://www.onlinelibrary.wiley.com/doi/10.1002/humu.24472)
MRI at the age of 8 months showed severe cortical atrophy with increased extra-axial cerebrospinal fluid spaces, simplified gyral pattern, thin corpus callosum, diffuse T2-hypointense signal of the thalamus, mild cerebellar atrophy, and a normal brainstem (Figure 1d–i). Electroencephalography revealed 3 Hz spike-and-wave discharges on the centro-parietal region during sleep. No history of clinical seizures was described. Oral therapy with carbamazepine was started for seizure prophylaxis. Combined ocular and cerebral visual impairments were detected by electroretinogram (ERG) and visual evoked potential (VEP) vision tests showing bilateral prolonged mean latencies and attenuated amplitudes. Bilateral ocular axial length measured on T2-weighted MRI was below the 5th percentile for 8 months (19 mm on the right and 19.1 mm on the left, the mean ocular axial length for 8 months: 20.70 mm) (Groot et al., 2022) (Figure 1).

There was no clinical history of edema or renal failure. Proteinuria was first noticed after molecular diagnosis of GAMOS had been established at 2 years of age. Spot urine protein was 860 and 10,720 mg/dl (normal range: 2.8–14.1 mg/dl), spot urine protein/creatinine ratio was 348 and 724 mg/mg at the age of 2 years and 3 years 5 months, respectively, thereby confirming nephrotic proteinuria. Laboratory workup for renal function at that age showed normal serum urea (24.82 mg/dl), creatinine (0.19 mg/dl), electrolytes, and low normal albumin (3.71 g/dl). Urinary system ultrasound revealed normal size, echogenicity, and corticomedullary differentiation of both kidneys at the age of 2 years. Renal biopsy was not found to be indicated. He was started on enalapril, 5 mg per day, but no improvement in urinary protein waste was noted.

Clinical exome sequencing revealed a homozygous TP53RK missense variant: NM_033550.4:c.163C>G (p.Arg55Gly). This variant has not been reported in patients with GAMOS before. In ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), the variant has one entry with the annotation "uncertain significance" (VCV000624197.10). The variant is not listed in gnomAD (https://gnomad.broadinstitute.org/). In silico prediction tools are inconsistent regarding the classification of this variant: some rate the variant as potentially damaging (MutationTaster: 0.9998, SIFT: 0.003, PolyPhen2: 0.795, M-CAP: 0.1762, PROVEAN: −4.55, −5.78, LRT: 0.00), while others rate it as rather tolerated (REVEL: 0.1379, MetaLR: 0.06) (Supporting Information: Table S1). According to The American College of Medical Genetics (ACMG)/The Association for Molecular Pathology (AMP) criteria (Richards et al., 2015), the variant was classified as variant of uncertain significance (VUS).

As tp53rk was found to be expressed during early Xenopus laevis development and enriched in tissues and organs affected in GAMOS including the developing eye, cranial cartilage, and brain (Treimer et al., 2021), we analyzed the defect of Tp53rk in anterior neural development of X. laevis using an antisense-based MO knockdown approach. Initially, we performed an assay to test whether the used Tp53rk MO is suitable for knockdown experiments. Therefore, the respective MO binding site of X. laevis (Xtp53rk MOs-GFP) and the corresponding 5’UTR of human TP53RK (hTP53RK MOs-GFP) was cloned in front of and in frame with GFP (green fluorescent protein) (Supporting Information: Figure S1A). RNA of these constructs was co-injected together with Tp53rk MO and CoMO into two-cell stage embryos. The GFP expression was then monitored in stage 20. Tp53rk MO blocked GFP expression upon Xtp53rk MOs-GFP RNA coinjection, whereas Control MO (CoMO) did not (Supporting Information: Figure S1B), indicating the interference of Tp53rk MO with Xtp53rk MOs-GFP translation. In addition, Tp53rk MO did not block translation of hTP53RK MOs-GFP (Supporting Information: Figure S1B) demonstrating that RNA coding for human TP53RK is suitable for rescue experiments.

To investigate the functional relevance of Tp53rk during early anterior neural development, we injected Tp53rk MO unilaterally into one animal-dorsal blastomere of Xenopus embryos at the eight-cell-stage. Tp53rk MO-injected embryos revealed significantly smaller and deformed eyes (Figure 2A.b and Supporting Information: Figure S2A) on the injected side (Figure 2A.g). Furthermore, we quantitatively measured the eye size and showed a significant smaller eye area upon Tp53rk depletion (Figure 2C) describing a mild microphthalmia phenotype. Vibratome sections confirmed a smaller eye size as well as abnormal eye shape and showed a disturbed lamination (Figure 2A.i). On the Tp53rk MO-injected side of the Xenopus embryo, the retinal pigmented epithelium (RPE) was thickened and disturbed as well as the lens deformed (Figure 2A.j). Coinjection of human full-length TP53RK partially rescued this phenotype indicating the specificity of Tp53rk MO-induced phenotype pointing to a conserved function of Tp53rk across species (Figure 2A.c,C and Supporting Information: Figure S2A).

Besides the abnormal eye, Tp53rk MO-injected Xenopus embryos showed significant differences in the head size (Figure 2B.b and Supporting Information: Figure S2B). To analyze the head phenotype further, we measured the interocular distance, head width and head area of X. laevis embryos (Figure 2D and Supporting Information: Figure S2B,E,F). Tp53rk MO-mediated knockdown showed a significant reduction in the interocular distance, head width, and head area while human full-length TP53RK RNA rescued these phenotypes (Figure 2B.c,D; Supporting Information: Figure S2B,E,F). We also dissected cranial cartilages of CoMO- and Tp53rk MO-injected embryos which were stained with Alcian blue at stage 45/46. Upon Tp53rk depletion structures of the cranial cartilage, in particular the branchial arch cartilage, were reduced in size and deformed (Supporting Information: Figure S2D).

To use this model for the functional evaluation of the TP53RK variant c.163C>G (p.Arg55Gly; R55G) identified in the patient presented here, we introduced this variant into human full-length TP53RK. A previously reported pathogenic variant, c.125G>A (p.Gly42Asp; G42D), served as a positive control. While human full-length TP53RK RNA rescued the described eye and head phenotype (Figure 2A.c,B,c,D; Supporting Information: Figure S2A,B,E,F) both GAMOS-associated variants failed to rescue these phenotypes (Figure 2A.d,e,B.d,e,C,D; Supporting Information: Figure S2A,B,E,F). Taken together, this study demonstrated that the two variants are functionally relevant and support their pathogenic role for GAMOS.

TP53RK encodes for one component of the KEOPS complex, defects of which represent the major cause of GAMOS. While the
FIGURE 2  (See caption on next page)
largest fraction of patients exhibits pathogenic variants of OSGEP. TP53RK pathogenic variants have previously been reported only in a small number of patients from five unrelated families (Supporting Information: Table S2) (Braun et al., 2017; Hyun et al., 2018; Turro et al., 2020). All patients from whom clinical information is available presented primary microcephaly, severe neurodevelopmental deficits including global developmental delay, seizures, and spasticity, as well as early-onset nephrotic syndrome (within the first 4 weeks of life). Cranial imaging findings were variable and included polymicrogyria (2), cerebral atrophy (1), cerebellar hypoplasia (1), bilateral myelination defects (1). Kidney histology was classified as focal-segmental glomerulosclerosis or diffuse mesangial sclerosis (Braun et al., 2017; Hyun et al., 2018). One patient also showed hiatal hernia (Hyun et al., 2018), originally described as a cardinal feature of the syndrome (Galloway & Mowat, 1968). From one of the previously published cases no detailed clinical data are available; the phenotype was classified as neurodevelopmental disorder, but no further information about renal involvement is available (Turro et al., 2020).

In contrast to the other previous observations, kidney involvement (proteinuria) was only recognized quite late at the age of 2 years in the patient presented here with asymptomatic proteinuria. Survival until the age of 3 years 7 months as observed in the patient presented here, has not been reported in previously published cases of TP53RK-related GAMOS. The apparently milder disease severity might reflect gradual differences in the functional incapacity caused by different genetic changes, and the later onset and milder course of renal disease in the patient presented here suggests a higher sensitivity to the KEOPS complex defect in the developing brain compared to the kidney. Notably, late-onset and even absence of glomerular disease until adolescence has already been reported in WDR73-related GAMOS (Vodopiutz et al., 2015). It has been proposed that most GAMOS-related KEOPS complex variants probably have some residual function, as the complete defect of this complex might lead to embryonic lethality (Braun et al., 2017). Slight differences in the residual function may have considerable impact on disease severity. Both variants examined here are located in exon1 of the TP53RK gene and affect the protein kinase domain of TP53RK protein. The previously reported variant c.125G>A (p.Gly42Asp) changes a glycine residue of the very conserved consensus motif $^{43}$KQGA$^{45}$ of TP53RK (Li et al., 2021). Alterations in this consensus motifs may disrupt the G-loop conformation and affect ATP binding (Steinberg, 2018). Besides its possible impact on the sterical integrity of the kinase domain, the novel variant c.163C>G (p.Arg55Gly) affects a residue that is known to create a salt bridge between TP53RK R55 and TPRKB D163 (Li et al., 2021). The substitution of arginine by glycine at codon 55 may therefore weaken interactions and stability within the KEOPS complex. Although the inability of both examined variations to rescue the knockdown phenotype generally supports their functional relevance, limitations of this study are regarding the lack of experimental data demonstrating precisely the way how the mutants affect TP53RK function and the small number of mutants analyzed. More research is needed to elucidate the molecular mechanisms in more detail and to decipher possible genotype-phenotype correlations.

In conclusion, we could confirm the significance of the functional integrity of the KEOPS complex for the developing eye and head in a Xenopus model. In this model, MO knockdown of Tp53rk caused smaller head and eye size as well as ocular abnormalities in Xenopus embryos, which could be rescued by the human wildtype TP53RK construct but not by constructs carrying the c.163C>G variant and a previously reported pathogenic variant c.125G>A. These results strongly support the pathogenic role of the TP53RK variant c.163C>G (p.Arg55Gly) identified in the patient presented here, and indicate the usefulness of the experimental model X. laevis to evaluate the functional relevance of variants of uncertain significance in TP53RK or in other GAMOS-associated human gene variations.

**AUTHOR CONTRIBUTIONS**
Tugba Kalayci undertook patient assessment. Ilknur Suer and Tugba Kalayci analyzed the exome data. Susanne J. Kühl, Martin Zenker, and Michael J. Schmeisser conceived and planned the study. Susanne J. Kühl and Ernestine Treimer designed the Xenopus experiments in detail. Ernestine Treimer, Sara Greco, and Denny Schanze performed the experiments. Susanne J. Kühl and Ernestine Treimer interpreted the Xenopus data. Susanne J. Kühl, Martin Zenker, Michael J. Schmeisser, Sven Schumann, and Ernestine Treimer discussed the

**FIGURE 2** Tp53rk MO injection leads to an eye and head phenotype that is rescued by human wildtype (wt), but not mutant TP53RK RNA. (A) Unilateral knockdown of Tp53rk results in microphthalmia (b; black arrowheads) compared with Control MO (CoMO) (a) during Xenopus laevis eye development. While coinjection of human full-length TP53RK RNA rescued the eye phenotype (c), TP53RK RNAs with variants identified in affected individuals (c.125G>A, p.Gly42Asp; G42D; c.163C>G, p.Arg55Gly; R55G) failed to rescue the Tp53rk MO-mediated eye phenotype (d and e). The lateral views (f–j) of the embryos show the eye in more detail and the sections (k–o) specify the lamination of the eye. Green arrowheads point to the disturbed and thickened retinal pigmented epithelium (RPE), black arrowheads to the externally directed and deformed lenses. Representative embryos are shown. (B) Tp53rk depletion leads to microcephaly on the MO injected side (b; black arrowheads) in X. laevis embryos, while CoMO-injected embryos show no abnormal phenotype (a). Coinjection of human full-length TP53RK RNA rescued the head phenotype (c), while TP53RK RNA with variants identified in affected individuals (c.125G>A, p.Gly42Asp; G42D; c.163C>G, p.Arg55Gly; R55G) failed to rescue the Tp53rk MO-mediated head phenotype (d and e). Representative embryos are shown. (C) Statistical evaluation of the eye area as illustrated in (A,f) (red dashed line: injected eye). (D) Statistical evaluation of the head width as illustrated in (B,a). Error bars indicate standard errors of the means. CoMO, control morpholino oligonucleotide; GFP, green fluorescent protein; hTP53RK, human TP53RK; inj., injected; MO, morpholino oligonucleotide; n, number of independent experiments; N, number analyzed embryos in total; n.s., non-significant; RPE, retinal pigmented epithelium; Tp53rk, Tp53 regulating kinase; uninj., uninjected. n.s. $\leq 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. 

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**Supporting Information**

Table S2. Clinical Data of Affected Individuals

<table>
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<th>Head Size</th>
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<td>M</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>Normal</td>
</tr>
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**Aviary Information**

The Xenopus laevis used in the study were maintained according to the guidelines of the local animal care committee.
data. Martin Zenker, Tugba Kalayci, and Ernestine Treimer wrote the manuscript. All authors commented on the manuscript and approved the final draft.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ORCID
Ernestine Treimer http://orcid.org/0000-0003-4930-1827
Martin Zenker http://orcid.org/0000-0003-1618-9269

REFERENCES
gnomAD. https://gnomad.broadinstitute.org/
OMIM. https://www.omim.org/

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.