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# Association of Familial Fanconi Syndrome with a Novel *GATM* Variant

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Fanconi syndrome is a disorder of the proximal renal tubule. Recently, advanced genetic analysis technology has revealed that several genes cause familial Fanconi syndrome. We identified a family with autosomal dominant Fanconi syndrome and chronic kidney disease with a novel *glycine amidinotransferase* (*GATM*) variant. Case 1 was a 57-year-old Japanese woman. Her father and two siblings had Fanconi syndrome or chronic kidney disease. She presented to our hospital at the age of 34 years with recurrent glucosuria. Her height and weight were 151 cm and 46.6 kg, respectively. Laboratory tests showed glucosuria, hypophosphatemia, hypouricemia, and normal renal function. Her serum creatinine level gradually increased over the following next two decades, and she developed end-stage renal disease. Case 2, the daughter of Case 1, was a 26-year-old woman. Her height and weight were 151 cm and 37.5 kg, respectively. Glucosuria was detected at the age of 13 years, which led to a referral to our hospital. Urinalysis showed low-molecular-weight proteinuria. She was diagnosed with Fanconi syndrome. At the age of 26 years, she had glucosuria, low-molecular-weight proteinuria, hypouricemia, and normal renal function. Genetic testing of both cases revealed a novel missense variant in *GATM*. The heterozygous missense variants in *GATM* have been reported to cause familial Fanconi syndrome, which manifests early in life and progresses to renal glomerular failure by mid-adulthood. The novel *GATM* variant detected in our cases was suspected to be associated with the development of Fanconi syndrome. *GATM* variants should be tested in patients with idiopathic Fanconi syndrome.

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## Introduction

Fanconi syndrome, a disorder of the proximal renal tubule, causes hypokalemia, generalized aminoaciduria, glucosuria, phosphaturia, uricosuria, proximal renal tubular acidosis, and low-molecular-weight proteinuria. This disorder may be hereditary or acquired. Fanconi syndrome in children usually presents with a comorbid genetic disorder, particularly cystinosis. Acquired Fanconi syndrome may be caused by various drugs, multiple myeloma, or amyloidosis. Cases with unclear etiologies are diagnosed as idiopathic Fanconi syndrome. Fanconi syndrome is rarely familial. However, without a verified family history, familial Fanconi syndrome may not be accurately diagnosed and treated as an isolated case.

Recently, advanced genetic analysis technology revealed that several genes [*SLC34A1L* (Magen et al. 2010), *EHHADH* (Klootwijk et al. 2014), *HNF4A* (Stanescu et al. 2012; Hamilton et al. 2014), and *NDUFAF6* (Hartmannová et al. 2016)] caused familial Fanconi syndrome. Reichold et al. (2018) showed that the heterozygous missense variants in *glycine amidinotransferase (GATM)* cause familial Fanconi syndrome, manifesting early in life and progressing to renal glomerular failure in middle adulthood. Only

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five variants in *GATM* responsible for Fanconi syndrome have been reported (Reichold et al. 2018; Seaby et al. 2023), and the epidemiology and clinical features of the disease have not been elucidated yet. Herein, we present a family with autosomal dominant Fanconi syndrome and chronic kidney disease (CKD) that had a novel missense variant in *GATM*, which was identified in two affected individuals.

## Case Presentation

*Case 1*

The mother (II-2, Fig. 1) was a 57-year-old Japanese woman. She had no known abnormalities at birth and in the neonatal period. Her father (I-1) and two siblings (II-3 and II-4) had Fanconi syndrome or CKD. At 34 years of age, she presented to our hospital due to recurrent detection of glucosuria during her annual check-up. Her height was 151 cm and weight was 46.6 kg. Laboratory tests revealed glucosuria, hypophosphatemia (serum phosphate level of 1.9 mg/dL), and hypouricemia (serum uric acid level of 2.0 mg/dL), but there was no indication of hyperglycemia. She was diagnosed with Fanconi syndrome. At that time, she had a normal renal function [estimated glomerular filtration rate (GFR) was 74.6 ml/min/1.73 $m^2$]. Her serum creatinine level gradually increased in the following two decades (Fig. 2), and she developed end-stage renal disease (ESRD) and will soon begin undergoing peritoneal dialysis.

*Case 2*

Her daughter (III-3, Fig. 1) was a 26-year-old woman. She was born with a birth weight of 2,550 g at the 37th gestational week. Her height was 151 cm and weight was 37.5 kg. At 13 years of age, glucosuria was detected during routine annual urinary screening, prompting referral to our hospital. Urinalysis also showed low-molecular-weight proteinuria (urine beta 2-microglobulin level of 3,260 $\mu$g/L). She was also diagnosed with Fanconi syndrome. At 26 years of age, she still had positive urinary glucose, and the urine beta 2-microglobulin, serum phosphate, serum uric acid, and serum creatinine levels were 10,520 $\mu$g/L, 2.8 mg/dL, 2.2 mg/dL, and 0.66 mg/dL (estimated GFR was 89.7 ml/min/1.73 $m^2$), respectively.

*Genetic analysis*

Based on the family history, the probands were suspected of having a genetic predisposition. Genetic analysis was performed on Case 1 and 2 with written informed consent. All investigations, including genetic studies, were approved by the Institutional Review Board of Kobe University Graduate School of Medicine (approval number 301) and conducted according to the principles of the Declaration of Helsinki.

Genomic DNA was isolated from the peripheral blood cell. Targeted sequencing for 123 known renal disease genes, including *GATM*, *EHHADH*, *SLC34A1*, *SLC2A2*, *BCS1L,* and *HNF4A,* were performed as described previously (Taniguchi et al. 2021). The results revealed that Case 1 and 2 shared a novel heterozygous missense variant in *GATM*, i.e., NM_001482.2: c.888T > A (p.Phe296Leu) (Fig. 3a). This variant was not registered in gnomAD v3.1.2 (https://gnomad.broadinstitute.org/), ToMMo 8.3KJPN (https://jmorp.megabank.tohoku.ac.jp). Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), or ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Although the variant was classified as "variant of uncertain significance" according to the ACMG guidelines (PM2, PP3, PP4), it was predicted to be DELETERIOUS by SIFT (score 0.00) and probably damaging by PolyPhen2 (score 0.982). Thus, we considered these variants to likely to be related to disease occurrence.

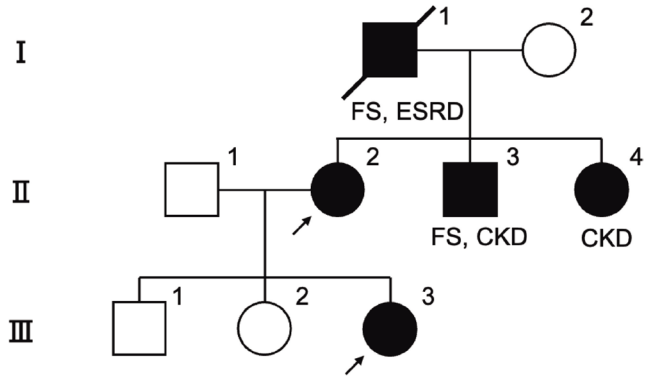


Fig. 1. Family pedigree.
The probands are indicated with an arrow. CKD, chronic kidney disease; ESRD, end-stage renal disease; FS, Fanconi syndrome.

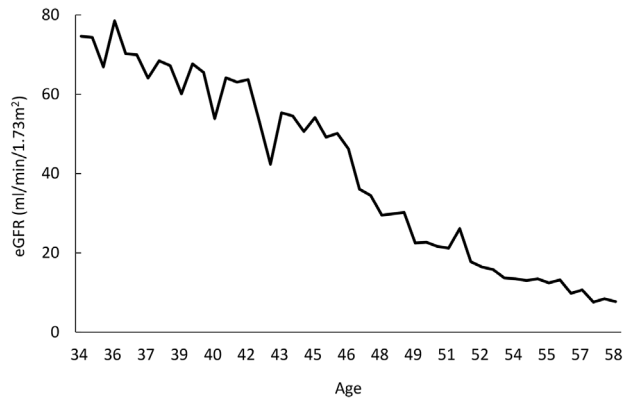


Fig. 2. Estimated glomerular filtration rate of Case 1 (II-2).
Her renal function constantly declined and, in her sixth decade of life, her condition progressed to end-stage renal disease. eGFR, estimated glomerular filtration rate.

## Discussion

In this report, we identified a novel *GATM* missense variant in a familial cohort of patients diagnosed with Fanconi syndrome. *GATM* encodes the mitochondrial enzyme glycine amidinotransferase (GATM) (Humm et al. 1997a). GATM catalyzes the transfer of a guanidino group

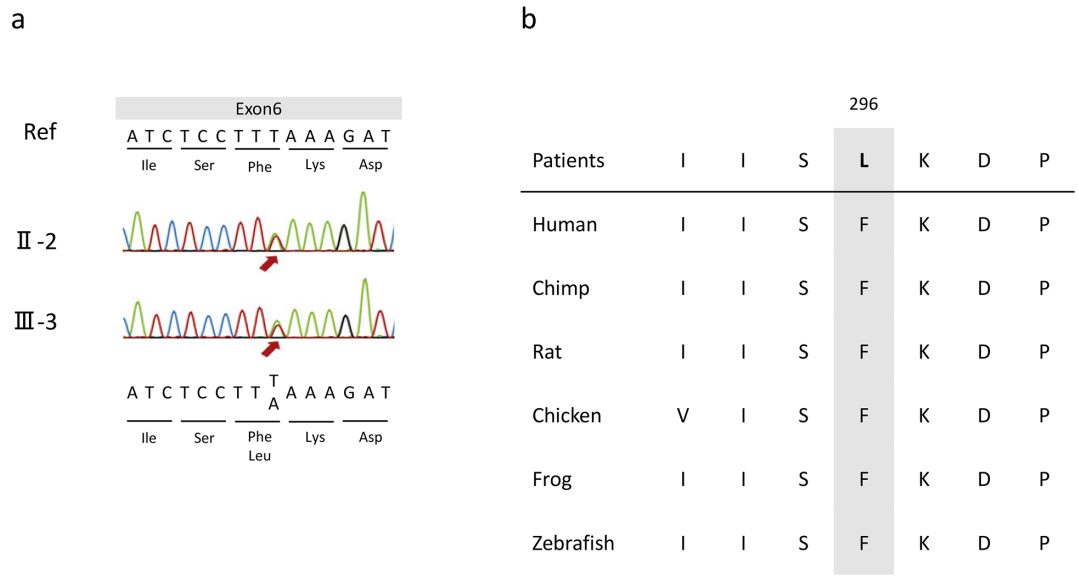


Fig. 3. Heterozygous variant in *GATM*.
(a) Genomic DNA sequence analysis. Heterozygous variants in *GATM* were detected in two probands, as revealed by the target sequences. II-2, Case 1; III-3, Case 2. (b) The p.Phe296 amino acid was extremely conserved in various species.

from L-arginine to glycine, thereby forming guanidino acetic acid, the precursor of creatine (Humm et al. 1997b; Wyss and Kaddurah-Daouk 2000). Recessive loss-of-function variants of *GATM* cause "cerebral creatine deficiency syndrome (OMIM: 612718)," a rare inborn error of creatine metabolism characterized by severe neurologic impairment (Item et al. 2001). Reichold et al. (2018) showed that the four monoallelic missense variants in *GATM* (p.Pro320Ser, p.Thr336Ala, p.Thr336Ile, and p.Pro341Leu) result in renal Fanconi syndrome with progressive kidney failure but without extra-renal features. Seaby et al. (2023) identified a novel heterozygous *GATM* variant (p.Arg322Pro) in a family with Fanconi syndrome.

Wild-type GATM isolated from the human kidney forms a dimer (Gross et al. 1986). *In silico* analysis by Reichold et al. (2018) investigated the effect of the disease causing variant on the GATM protein structure. They reported that these variants may result in the formation of an interaction site that can enable GATM to form linear multimers. Mutant GATM overexpression in renal proximal tubule cells resulted in abnormal and elongated mitochondria containing GATM-positive fibrillary aggregates, similar to the deposits observed in the proximal tubules of patients undergoing renal biopsies. All known variants in *GATM* causing Fanconi syndrome were highly conserved across various species and are located within a small region of exons 6 and 7. The p.Phe296 amino acid was also extremely conserved in species (Fig. 3b) and located within exon 6.

Fanconi syndrome with *GATM* variant is characterized by childhood onset, a gradual decline in renal function during late adolescence or adulthood, and progression to ESRD in the third to sixth decades. In our case, the daughter's glucosuria was first detected at 13 years of age during routine annual urinary screening for all elementary and junior high school students in Japan. The results of her urine analysis in the previous year had been normal, indicating that Fanconi syndrome in this case was childhood onset rather than infantile onset. The mother's renal function declined over two decades. The clinical courses of our two probands are similar to those reported previously (Reichold et al. 2018; Seaby et al. 2023). Reichold et al. (2018) reported that biopsy samples from their patients demonstrated interstitial fibrosis and drastically enlarged mitochondria containing pathogenic GATM protein aggregates. Mutant GATM enables the formation of linear multimers, resulting in fibrillary aggregation within the mitochondria. Mutant GATM overexpression in proximal tubule cells impaired mitochondrial turnover and led to increased production of reactive oxygen species, initiation of an inflammatory response, release of profibrotic factors, and increased cell death (Reichold et al. 2018). These findings suggest that an increase in mitochondrial mutant GATM leads to the development of acquired Fanconi syndrome and slow progression to ESRD. Only 5 variants in *GATM* causing Fanconi syndrome have been detected from 6 families. The correlation between genotype and phenotype in cases with *GATM* variants remains unclear. GATM expression is regulated through a negative feedback mechanism involving creatine in rat kidney (McGuire et al. 1984). Oral intake of creatine may suppress the production of mutant GATM. Creatine supplementation is a potential treatment for the progression of renal dysfunction caused by Fanconi syndrome with *GATM* variant.

The limitations of our investigation were the lack of structural studies, histological examinations, and genetic testing of unaffected members. Although molecular dynam-

ics simulations of the mutant GATM were not performed, *in silico* analyses indicated the pathogenicity of this variant. Since no renal biopsy was performed, the etiology of the maternal renal failure remains uncertain. No findings were suggestive of glomerulonephritis or any systemic disease. We attempted to analyze other family members to obtain true segregation data but failed to secure cooperation.

In conclusion, we report a novel missense variant in *GATM*, c.888T > A (p.Phe296Leu), in a family with autosomal dominant Fanconi syndrome and CKD. On the basis of the characteristic phenotype, rarity of the variant, and *in silico* prediction, we considered that this variant likely causes the disease. Most patients with *GATM* variants develop ESRD. Genetic diagnosis may enable the prediction of long-term prognosis and early introduction of specific therapy in the future. *GATM* variants should be routinely tested in cases of familial or idiopathic Fanconi syndrome, even in those without renal failure.

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## Author Contributions

Ryota Suzuki, Atsushi Kondo, and Kandai Nozu performed genetic analysis. Yuki Nakamura, Hitoshi Mikami, and Jun Soma were responsible for the resources. Hiroki Kudo wrote the original draft of the manuscript. Izaya Nakaya supervised the project. All authors agreed to the submission of the final manuscript.

## Conflict of Interest

The authors declare no conflict of interest.

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