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A homozygous truncating *ETV4* variant in a Nigerian family with congenital anomalies of the kidney and urinary tract

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Abstract

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most prevalent cause of chronic kidney disease that manifests in children. To date ~23 different monogenic causes have been implicated in isolated forms of human CAKUT, but the vast majority remains elusive. In a previous study, we identified a homozygous missense variant in E26 transformation-specific (ETS) Variant Transcription Factor 4 (*ETV4*) causing CAKUT via dysregulation of the transcriptional function of ETV4, and a resulting abrogation of GDNF/RET/ETV4 signaling pathway. This CAKUT family remains the only family with an *ETV4* variant reported so far. Here, we describe one additional CAKUT family with a homozygous truncating variant in *ETV4* (p. (Lys6*))

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CONFLICT OF INTEREST

Friedhelm Hildebrandt is a cofounder and Scientific Advisory Committee (S.A.C.) member and holds stock in Goldfinch-Bio. All other authors declare that they have no competing financial interests.

SUPPORTING INFORMATION

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

that was identified by exome sequencing. The variant was found in an individual with isolated CAKUT displaying posterior urethral valves and renal dysplasia. The newly identified stop variant conceptually truncates the ETS_PEA3_N and ETS domains that regulate DNA-binding transcription factor activity. The variant has never been reported homozygously in the gnomAD database. To our knowledge, we here report the first CAKUT family with a truncating variant in *ETV4*, potentially causing the isolated CAKUT phenotype observed in the affected individual.

Keywords

congenital anomalies of the kidney and urinary tract; ETV4; exome sequencing; GDNF/RET signaling; kidney development

1 | INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) comprise a large spectrum of congenital malformations involving the kidney (e.g. renal agenesis, hypodysplasia) and the urinary tract (e.g., vesicoureteral reflux [VUR], ureteropelvic junction obstruction [UPJO], posterior urethral valves [PUV]; Ichikawa et al., 2002). CAKUT represents the most common birth defect (~3–6 per 1000 live births) and the main cause of chronic kidney disease in children (~50%; Becherucci et al., 2016; Pohl et al., 2002). Several lines of evidence in humans and mouse models indicate that CAKUT is often caused by recessive or dominant variants in single (monogenic) genes (van der Ven et al., 2018). To date, genetic variants in 174 genes have been described to cause CAKUT in humans, explaining 13%–20% of CAKUT cases (Bekheirnia et al., 2017; Kohl et al., 2021; Seltzsam et al., 2022; van der Ven et al., 2018). Genetic research in CAKUT is complicated by variable expressivity of the phenotype, incomplete penetrance, and a distinct heterogeneity.

E26 transformation-specific (ETS) Variant Transcription Factor 4 (*ETV4*, also known as *PEA3* or *E1AF*) belongs to the ETS transcription factor family (Cooper et al., 2014). *ETV4* is involved in the GDNF/RET signaling pathway, which is essential for migration of progenitor cells during renal branching morphogenesis during kidney development (Lu et al., 2009; Riccio et al., 2016). It is a known cause of CAKUT in mice and zebrafish (Lu et al., 2009; Marra & Wingert, 2016). In a previous study, we identified a homozygous missense variant in *ETV4* (c.1244G > A; p.(Arg415His)) in an individual with CAKUT causing right-sided VUR Grade II (Chen et al., 2016). Using electrophoretic mobility shift assay and a cell-based luciferase reporter assay loss of DNA binding affinity and transcription activation of the *ETV4* mutant was demonstrated. Furthermore, *ETV4* knockdown cell lines showed impaired cell migration, which could not be rescued by the mutant, but the wild-type *ETV4*, indicating pathogenicity of the identified variant. This remains the only CAKUT family with a biallelic *ETV4* variant reported so far. To test whether there are additional families harboring *ETV4* variants, we examined exome sequencing (ES) data from 731 families with CAKUT. Here, we describe one additional CAKUT family with a likely causative biallelic truncating variant in *ETV4*.

2 | CASE REPORT

2.1 | Case presentation

Individual B583-21 was a male of Nigerian origin who was diagnosed with PUV and renal dysplasia (Figure 1a and Table 1). He was born to a healthy 30-year-old mother (B583-12; Figure 1a). His father was absent throughout the period of care and not available for clinical and genetic examination (Figure 1a). According to the index individual, there are no other family members with CAKUT, and his parents were reported not to be consanguineous. Individual B583-21 was first seen at the age of 22 years presenting with a fever in the course of a urinary tract infection (UTI) and with increased urine output. Other complaints were diurnal enuresis, poor urinary stream, insufficient weight gain, and lethargy. On the day of initial presentation, weight was 61 kg and height 171 cm (body mass index: 20.9 kg/m^2). Laboratory findings revealed a serum creatinine level of 11 mg/dL, anemia, leukocytosis, and proteinuria. During the initial renal ultrasound examination, individual B583-21 was found to have renal dysplasia and bilateral hydroureteronephrosis (images not available). A following voiding cystourethrogram showed a thickened trabeculated bladder and blockage of the urethra compatible with a presumptive diagnosis of PUV (Figure 1b,c). Subsequent medical treatment included antibiotic therapy of the UTI. Valve ablation was performed to incise the PUV, and urinary drainage was ensured via suprapubic diversion. Anticholinergics were given to control poorly compliant bladder function. Also, due to poor renal function, individual B583-21 was started on dialysis. Over the next 3 years, he was repeatedly hospitalized (>3) due to UTIs/urosepsis, anemia-related and chronic-kidney-disease-related complications. B583-21 was lost to follow-up about 3 years after his first presentation and appeared to continue care at a secondary facility. He deceased at the age of 25 due to progression to end-stage renal disease and progressive bladder dysfunction.

2.2 | ES analysis revealed a homozygous truncating variant in *ETV4*

To identify a potential genetic cause for the individual's phenotype, we performed duo ES analysis in individual B583-21 and his mother B583-12. Given the parents' unaffected status in regard to CAKUT, we hypothesized a recessive mode of inheritance. Homozygosity mapping revealed only 4.2 Mb of homozygosity, confirming the nonconsanguinity of the parents (Figure 1d). We detected a homozygous nonsense variant in B583-21 (c.16A > T; p.(Lys6*)), which was not located in a homozygosity peak (Figure 1e and Table 1). The variant has neither been reported homozygously in the gnomAD v2.1.1 database in the African/African American population nor in the total allele number of 184,778 (Table 1). Of note, no homozygous loss of function alleles are listed for *ETV4* in gnomAD. The variant was inherited from the healthy mother (B583-12), who was heterozygous for the allele (Figure 1a and Table 1). Paternal DNA was not available for genetic testing, leaving it inconclusive whether individual B583-21 carries both alleles (c.16A > T; p.(Lys6*)) or a heterozygous allele in association with a deletion of the homologous allele. Subsequent exome coverage analysis on the gene *ETV4* for the proband and his mother was performed, which revealed similar coverage in both. This means that there is only a low possibility of deletion of the homologous *ETV4* allele in B583-21. Therefore, it can be assumed that the identified variant (c.16A > T; p.(Lys6*)) is present homozygously in individual B583-21 (Figure S1). The p.(Lys6) amino acid resides in the ETS_PEA3_N domain, implicated in

transactivation and inhibition of DNA binding (Figure 1e). The newly incorporated stop gain at this early position of the protein leads to an almost complete loss of protein length and thus protein structure (Figure 1e,f). This includes the transcriptional and DNA-binding activity of ETV4. In addition to the homozygous truncating variant in *ETV4*, we also detected a homozygous inframe deletion in *BRCA1* (c.1846_1848delTCT; p.(Ser616del)) in B583-21, which was present in a heterozygous state in his mother B583-12 (Table S1). The variant was not present homozygously in the gnomAD database in 282,534 total samples (Table S1).

The patient's DNA was screened for potentially deleterious variants in the 23 known isolated and 16 known syndromic CAKUT-causing genes without results, followed by an additional analysis for variants in the 135 genes, in which variants are known to cause syndromes with facultative CAKUT, if mutated (Seltzsam et al., 2022). Furthermore, we examined our worldwide cohort of 822 individuals from 731 families with CAKUT for variants in *ETV4* and its close paralog *ETV5* but were not able to detect any further allele carriers.

3 | DISCUSSION

In this study, we discovered a homozygous nonsense variant in *ETV4* in an individual with isolated CAKUT. The variant leads to an early stop gain at the amino acid position p.(Lys6) in the ETS_PEA3_N domain, likely resulting in a complete loss of the protein (Figure 1e,f). *ETV4* is a transcription factor, that acts downstream of the GDNF/RET signaling pathway, which is important for ureteric bud growth and branching (Riccio et al., 2016). Null mice for *Etv4* show a high proportion of renal anomalies, including renal agenesis and renal hypoplasia (Lu et al., 2009). Previously published functional data from our research group demonstrated the pathogenicity of the first described biallelic variant in *ETV4* in an individual with CAKUT (Chen et al., 2016). The reported missense variant in *ETV4* caused loss of DNA binding property and a loss-of-function effect in cell migration assays (Chen et al., 2016). Therefore, we hypothesize that the biallelic early truncating variant identified in *ETV4* causes the CAKUT phenotype seen in B583-21, the most likely mechanism being an impairment of the GDNF/RET/ETV4 signaling pathway. As previously discussed by Chen et al. (2016) neither the first individual B24-21 with a homozygous variant in *ETV4* nor the here described individual B583-21 display extrarenal manifestations, such as nervous system or limb malformations, which have been described in *Etv4* knockout mice (Chen et al., 2016; Lu et al., 2009). This observation strengthens our hypothesis that this discrepancy may be explained by a redundancy of *ETV4* with its close paralog *ETV5* or other ETS proteins and/or alternative mechanisms of *ETV4* that work independently of the DNA-binding property (Chen et al., 2016).

Lu et al. (2015) previously discovered the allele p.(Lys6*) in an individual with breast adenocarcinoma by screening their cohort of 4034 individuals affected by one of 12 different cancer types for 624 candidate cancer-associated genes by ES (Lu et al., 2015). However, due to a lack of functional data supporting the role of *ETV4* in the causation of cancer, it remains elusive whether *ETV4* is involved in the formation of breast adenocarcinoma.

Ellis et al. (2000) screened a cohort of 110 affected females with breast cancer for potential causative variants in *BRCA1*, which revealed the allele p.(Ser616del) in a 27-year-old woman with breast cancer (Ellis et al., 2000). Variants in *BRCA1* are a known cause of autosomal recessive Fanconi Anemia, complementation group S (MIM no. 617883), and autosomal dominant or multifactorial inherited familial breast-ovarian cancer, type 1 (MIM no. 604370). None of these reported phenotypes for variants in *BRCA1* involve CAKUT. Additionally, the variant was rated multiple times as benign in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Therefore, we did not consider this variant to be causative for the individual's CAKUT phenotype.

Animal models and previously published functional data by our group demonstrate the role of *ETV4* in the causation of CAKUT. By the discovery of a second CAKUT family with a biallelic variant in *ETV4*, we confirm variants in *ETV4* as a likely monogenic cause of human isolated autosomal recessive CAKUT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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.

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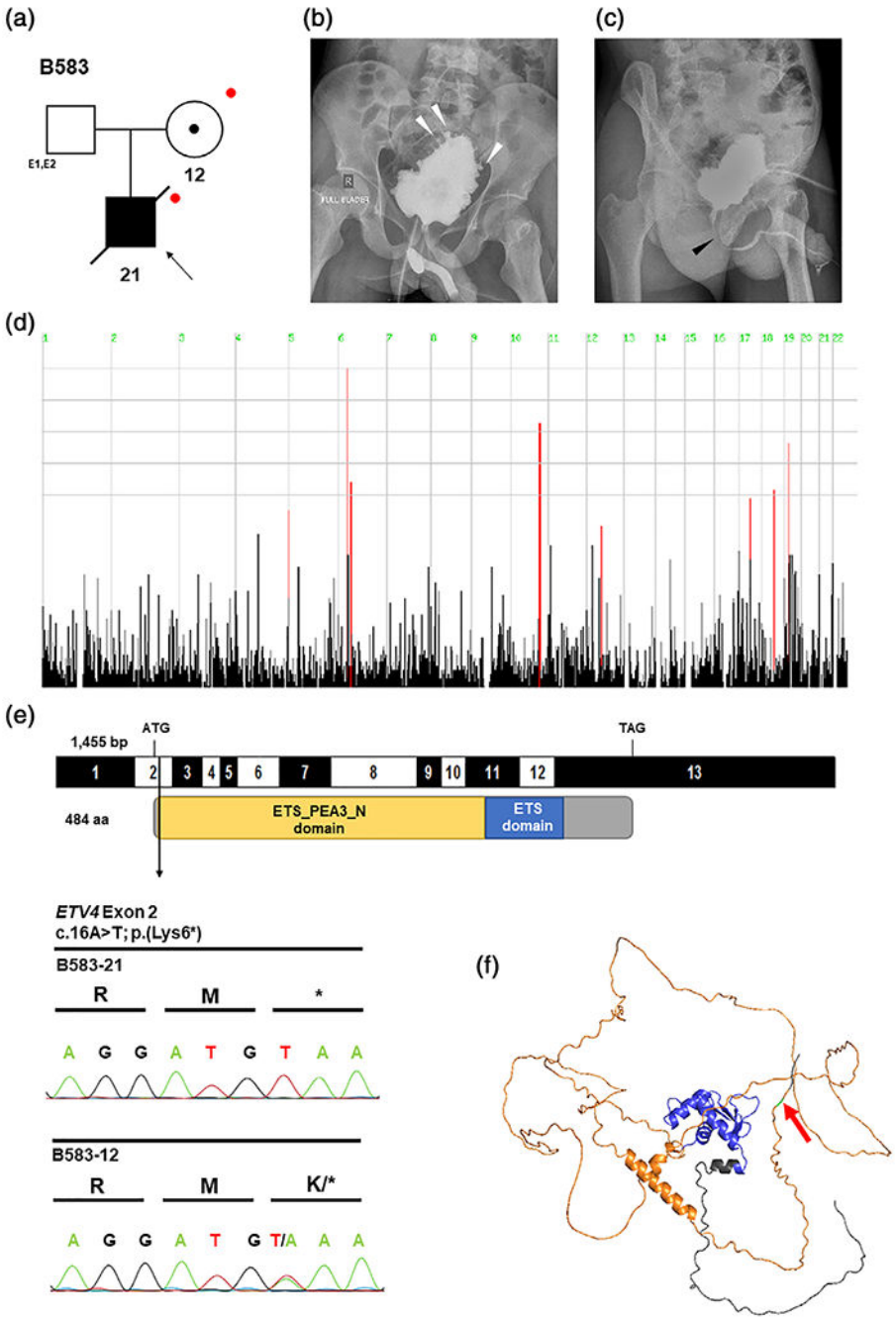

FIGURE 1.

Identification of a biallelic truncating variant in E26 transformation-specific (ETS) Variant Transcription Factor 4 (*ETV4*) in family B583 with isolated Congenital anomalies of the kidney and urinary tract. (a) Pedigree of index family B583. Squares represent males, circles females, black shading indicates the affected individual, and white shading the unaffected parents. The black arrow points to the proband B583-21. Red dots highlight individuals included in exome sequencing analysis. The diagonal line denotes the deceased individual. E1, unavailable for clinical evaluation; E2, unavailable for genetic testing. (b)

Voiding cystourethrography of individual B583-21 shows the trabeculated bladder (white arrowheads). (c) Voiding cystourethrography shows the nonpassage of the urethra for the contrast agent caused by posterior urethral valves (black arrowhead). (d) Homozygosity mapping depicts a homozygosity of 4.2 Mb and confirms the reported nonconsanguinity of the parents in family B583. (e) The exon (black and white), ETS_PEA3_N protein domain (orange), and ETS protein domain (blue) structures of human *ETV4* cDNA are shown. Positions of start codon (ATG) and stop codon (TAG) are indicated. The arrow indicates the location of the *ETV4* variant in relation to exons and the protein domain. Chromatograms obtained by direct sequencing of PCR products revealed a homozygous substitution of A for T in Exon 2 of the *ETV4* gene in B583-21, and a heterozygous variant in the individual's mother B583-12. (f) The predicted molecular model of *ETV4* which was obtained from AlphaFold Protein Structure Database (ID AF-P43268-F1) is depicted. The changed amino residue p.(Lys6) is indicated (red arrow). The ETS_PEA3_N protein domain is shown in orange and the ETS protein domain is marked in blue.

TABLE 1

A homozygous truncating variant in *ETV4* in a Nigerian male individual with isolated CAKUT.

Family-Indivi-dual	Gene	hg19 position	Nucleotide change	Amino acid change	Exon	gnomAD v2.1.1 allele frequency[a]	SNP ID	Ethnicity gender	CAKUT	Segregation
B583-21	*ETV4*	chr17:41622970	c.16A > T	p.(Lys6*)	2/13	0/84/184,778 African/African American population: 0/77/17,106	rs75202146	Nigerian M	Renal dysplasia PUV	Inherited heterozygously from healthy mother B583-12; no data available from father

Note: Transcript accession number for *ETV4* NM_001079675.5.

Abbreviations: M, male; PUV, posterior urethral valves.

[a]Variant frequencies listed for homozygous/heterozygous/total alleles detected in the population.