DYRK1A-related intellectual disability: a syndrome associated with congenital anomalies of the kidney and urinary tract

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Purpose: Haploinsufficiency of *DYRK1A* causes a recognizable clinical syndrome. The goal of this paper is to investigate congenital anomalies of the kidney and urinary tract (CAKUT) and genital defects (GD) in patients with *DYRK1A* variants.

Methods: A large database of clinical exome sequencing (ES) was queried for de novo *DYRK1A* variants and CAKUT/GD phenotypes were characterized. *Xenopus laevis* (frog) was chosen as a model organism to assess *Dyrk1a*'s role in renal development.

Results: Phenotypic details and variants of 19 patients were compiled after an initial observation that one patient with a de novo pathogenic variant in *DYRK1A* had GD. CAKUT/GD data were available from 15 patients, 11 of whom presented with CAKUT/GD. Studies in *Xenopus* embryos demonstrated that knockdown of *Dyrk1a*, which is expressed in forming nephrons, disrupts the development of segments of embryonic nephrons, which ultimately

give rise to the entire genitourinary (GU) tract. These defects could be rescued by coinjecting wild-type human *DYRK1A* RNA, but not with *DYRK1A*^{R205*} or *DYRK1A*^{L245R} RNA.

Conclusion: Evidence supports routine GU screening of all individuals with de novo *DYRK1A* pathogenic variants to ensure optimized clinical management. Collectively, the reported clinical data and loss-of-function studies in *Xenopus* substantiate a novel role for *DYRK1A* in GU development.

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INTRODUCTION

The dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family of protein kinases are conserved across species from lower eukaryotes to mammals.¹ DYRK family members are activated by autophosphorylating a tyrosine residue in their activation loop.² *DYRK1A* is the most extensively characterized member of the DYRK family, which in humans, is encoded by the *DYRK1A* gene located in the Down syndrome critical region of chromosome 21.³ A growing body

of literature implicates a strong causal relationship between *DYRK1A* haploinsufficiency and a recognizable syndrome known as *DYRK1A*-related intellectual disability syndrome.^{3–5} In addition to intellectual disability (ID), other frequently occurring features include intrauterine growth restriction (IUGR), difficulty feeding (DF) with failure to thrive (FTT), microcephaly, seizures, dysmorphic facial features, and developmental delays (DD),⁵ while additional phenotypic features are observed less commonly.^{5,6} Although many features of this

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syndrome are well-characterized; the full phenotypic spectrum has yet to be defined. This paper presents a cohort of individuals with de novo (when both parental samples available) *DYRK1A* single-nucleotide variants (SNVs) or small deletions ≤ 10 base pairs and defines congenital anomalies of the kidney and urinary tract (CAKUT) and genital defects (GD) that have not been previously described in patients with *DYRK1A* syndrome. We also provide supporting evidence, using *Xenopus* embryos as a model, that *DYRK1A*, which is expressed in embryonic nephrons, is required for genitourinary (GU) development and that two pathogenic variants of human *DYRK1A* are likely responsible for the CAKUT/GD phenotype. Together, these findings support the investigation of potential CAKUT/GD in the clinical workup of patients with *DYRK1A*-related ID syndrome.

MATERIALS AND METHODS

Study participants

The index patient was seen in the Renal Genetics Clinic (RGC) at Texas Children's Hospital (TCH). Subsequently, patients who had exome sequencing (ES) in a clinical diagnostic laboratory (Baylor Genetics [BG]) were queried for de novo (except P1, P12, and P15) pathogenic (except P10 [likely pathogenic], and P5, P7, and P17, called as variants of uncertain significance [VUS] in the initial report) variants in *DYRK1A*. Inclusion criteria also included (1) variant confirmation using Sanger sequencing, and (2) lack of other variants that could explain the phenotype observed. Exclusion criteria included multiple additional candidate genes that may be related to the phenotype. The final size of our cohort after applying those filters was 19 patients. Clinical phenotype information was collected from initial ES requisition form or contacting referring physicians. The Institutional Review Board at Baylor College of Medicine approved the study protocol for the Protection of Human Subjects. Consent was obtained for clinical genetic testing/exome sequencing from each family participating in this study.

ES and data analysis

ES was performed by previously published methods at BG.⁷⁻⁹ In brief, an Illumina paired-end precapture library was constructed with 1 μ g of DNA, according to the manufacturer's protocol (Illumina Multiplexing_SamplePrep_Guide_1005361_D), with modifications as described in the BCM-HGSC Illumina Barcoded Paired-End Capture Library Preparation protocol.⁷ Four precaptured libraries were pooled and then hybridized in solution to the HGSC CORE design (52 Mb, NimbleGen) according to the manufacturer's protocol (NimbleGen SeqCap EZ Exome Library SR User's Guide Version 2.2), with minor revisions. Sequencing was performed in paired-end mode with the Illumina HiSeq 2000 platform, with sequencing-by-synthesis reactions extended for 101 cycles from each end with an additional cycle for the index read. With a sequencing yield of 12 Gb, 92% of the targeted exome bases were covered to a depth of 20 $\times$ or greater. Illumina sequence analysis was performed with the HGSC Mercury analysis pipeline (<https://www.hgsc.bcm.edu/software/mercury>), which moves data through various analysis tools from the initial sequence generation on the instrument to annotated variant calls (SNVs and intraread indels). Variant interpretation was performed according to the most recent guidelines published by the American College of Medical Genetics and Genomics (ACMG).¹⁰ Accordingly, only variants that met strict criteria were called pathogenic. Sanger sequencing confirmed all variants reported in this paper.

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Whole mount in situ hybridization

Digoxigenin-11-UTP labeled antisense RNA probe for *dyrk1a* was synthesized in vitro from *Xenopus* Genome Collection IMAGE clone 7687837 (ref. ¹¹) using SalI restriction enzyme and T7 polymerase. This clone carries the *Xenopus tropicalis* coding sequence for *dyrk1a*, therefore both *X. tropicalis* and *X. laevis* embryos were stained to ensure proper detection of the target. Embryos were staged, fixed, and stained according to standard procedures^{12,13} using an antidigoxigenin antibody (1:3000, Sigma 11093274910, St. Louis, MO, USA) and BM Purple (Sigma 11442074001).

Xenopus laevis embryos and microinjections

Xenopus eggs were obtained by standard means, placed in 0.3 $\times$ MMR and fertilized in vitro.¹³ Blastula cleavage stages and dorsal versus ventral polarity were determined by established methods.¹² Microinjections were targeted to the V2 blastomere at the eight-cell stage, which provides major contributions to the development of the pronephros.^{12,14,15} Ten nL of injection mix (described below) was injected into embryos. Ten ng of *Dyrk1a* morpholino 5'-TGCATCGT CCTCTTTCAAGTCTCAT-3'¹⁶ or Standard morpholino 5'-CCTCTTACCTCAGTTACAATTATATA-3' was coinjected with 50 pg RNA (either control β -galactosidase, wild-type human *DYRK1A*, *DYRK1A*^{R205*}, or *DYRK1A*^{L245R}) along with 1 ng membrane-RFP RNA¹⁷ as a lineage tracer to verify that the correct blastomere was injected. Details about design and statistical analysis are included in Supplemental Methods.

Immunostaining

Embryos were staged,¹² fixed, and immunostained¹⁸ using established protocols. Proximal tubule lumens were labeled with the antibody 3G8 (1:30, European *Xenopus* Resource Centre, Portsmouth, UK), while the cell membranes of distal and connecting tubules were labeled with antibody the 4A6 (1:5, European *Xenopus* Resource Centre).¹⁹ Rabbit anti-red fluorescent protein (anti-RFP) (1:250, MBL International, Woburn, MA, USA) antibody was used to detect the RFP tracer. Goat antimouse or anti-rabbit conjugated to Alexa Fluor 488 or Alexa Fluor 555 (1:500, Invitrogen, Carlsbad, CA, USA) secondary antibodies were used to visualize antibody staining.

Imaging

Embryos used for in situ hybridization were imaged on a Zeiss AxioZoom V16 with a 1 $\times$ objective, Zeiss 512 color camera

(Zeiss, Oberkochen, Germany), and extended depth of focus processing. Embryos used for immunostaining were scored and photographed using an Olympus SZX16 fluorescent stereomicroscope and Olympus DP71 camera (Olympus, Tokyo, Japan); 3G8/4A6 immunostained kidney images were taken using a Zeiss LSM800 confocal microscope (Zeiss, Oberkochen, Germany). Fixed embryos were cleared with BABB/Murray's clearing solution for confocal imaging (1:2 volume of benzyl alcohol to benzyl benzoate). Images were processed with Adobe Photoshop.

RESULTS

CAKUT/GD identified in patients with *DYRK1A* variants

The index patient was seen in the Renal Genetics Clinic (RGC) for the evaluation of ID, global DD, hypospadias, and congenital chordee. Trio ES (tES) revealed a novel de novo pathogenic p.G168fs single base pair deletion in *DYRK1A*. A subsequent query of the ES database at BG revealed a total of 18 additional individuals with SNVs or deletions ≤ 10 base pairs (as defined in "Materials and Methods.") in *DYRK1A* among approximately 8000 probands. Phenotype and molecular information of these patients are summarized in Table 1 and Fig. 1. Probands were mostly children ranging from 2 to 27 years of age. All 19 of these individuals had neurodevelopmental phenotypes consistent with loss-of-function of *DYRK1A* (MIM 614104). We subsequently contacted all referring physicians to obtain further details regarding the CAKUT/GD phenotypes. However, CAKUT/GD Status of four patients remain unknown. Eleven of fifteen (73%) individuals with available information presented with CAKUT including unilateral renal agenesis (URA) and/or GD including undescended testis, hypospadias, etc. (Table 2). One patient (P6) with URA was identified after this newly acquired association of *DYRK1A* with CAKUT was discussed with the referring physician.²⁰ Probability of loss-of-function intolerance (pLI) score of *DYRK1A* is 1, indicating that this gene is intolerant to loss-of-function variants.²¹

A majority of the variants found in *DYRK1A* are loss-of-function and are found in the kinase domain

Many of the variants found in this cohort are found in the kinase domain (14/17 [82%]), which spans from residues 159 to 479, and 11/17 (65%) are thought to undergo nonsense-mediated decay (NMD), as they result in premature stop codons (<https://nmdpredictions.shinyapps.io/shiny/>) (Fig. 1). Of the remaining six variants, one affects splicing, one escapes NMD (p.S494fs), and four are missense variants. The four missense variants are all found in the kinase domain in four individuals. Of these four individuals with missense variants, P7 was diagnosed with URA (p.L245R), P5 and P10 had normal renal ultrasounds (p.V173F and p.G348R), and P17 has an unknown CAKUT status (R467Q). Because these variants still lead to other *DYRK1A* syndrome features such as ID, they may be important for the catalytic activity or conformational stability of *DYRK1A*. In fact, in a separate *DYRK1A* structural study the R467Q variant was found to be

a part of a network of electrostatic interactions thought to play a role in the stability of the *DYRK1A* protein.²² Additionally, the L245R variant was found to prevent autophosphorylation of *DYRK1A*'s activation loop in HEK293 cells²³ and was shown to be catalytically inactive via an in vitro kinase assay.²⁴ Of the three variants not found in the kinase domain, two are just N-terminal (N151fs, K154fs) and the last is found just C-terminal (to kinase domain) in the PEST domain (S494fs). Although theoretically all variants that are more N-terminal should result in NMD, a majority of the variants reside in the kinase domain for unknown reasons that should be studied further.

Xenopus laevis as a model of GU development

DYRK1A's amino acid sequence is highly conserved among amniotes (<https://www.ncbi.nlm.nih.gov/homologene>). Even though the N- and C-terminal regions diverge in invertebrates, the amino acid sequence of the kinase domains are similar indicating the importance of this protein throughout evolution. To model CAKUT/GD associated with human *DYRK1A* loss-of-function variants using *Xenopus laevis* embryos (hereafter referred to simply as *Xenopus*), we analyzed the conservation of the whole *DYRK1A* protein sequence, focusing on the kinase domain. Human and *Xenopus* *DYRK1A* proteins are 91.3% identical over the entire amino acid sequence (Fig. S1A). Additionally, the kinase domain, which is where a majority of the variants that cause CAKUT/GD (in this study) are found, is 97.5% identical to the human protein (Fig. 1). Importantly, the human and *Xenopus* kinase activation loop sequence, which are essential for the kinase activity of *DYRK1A*, are identical.

Xenopus produces large clutch sizes with hundreds of embryos that develop externally, and unilateral embryo injections allow for tissue-targeted knockdowns that are specific to organs such as the kidney.¹⁴ Their embryonic kidney, the pronephros, can easily be visualized and imaged through a transparent epidermis, and they develop a fully functional kidney in ~56 hours.²⁵ *Xenopus* was chosen because it is an established model of nephron development, and gene expression studies demonstrate that the developing *Xenopus* nephron is anatomically and functionally similar to the mammalian nephron.^{26–28} The embryonic pronephros is the precursor to the mesonephric and metanephric kidney in mammals, and subsequent GU development is dependent upon this structure. Specifically, as the pronephros extends toward the cloaca, the mesonephric nephrons form adjacent to the elongating nephric duct, also known as the Wolffian duct.²⁹ The ureteric bud, which is required for the development of the collecting duct system in mammals, then branches from this duct. Because the Wolffian duct is required for GU development in males and Müllerian duct elongation, which is necessary for normal female anatomy, depends upon the development of the Wolffian duct,^{30,31} the development of the pronephros is critical to both renal and genital development in mammals. Thus, although it is not a well-established model for studying genital formation, the

Table 1 Demographics, molecular data, and phenotype of 19 patients with single-nucleotide variants (SNVs) and small indels (<10 bp) in *DYRK1A* identified by clinical exome sequencing

Patient number	Age (years)	Gender	Ethnicity	Nucleotide change	AA change	Novel variant	DF/ FTT	FD	Microcephaly	Seizures	ID	DD	Renal/ GU	ASD
P1 ^a	2.3	Female	Caucasian	c.452dupT	p.N151fs	-	+	+	+	-	ukn	M,S	+	ukn
P2 ^a	3.3	Male	Caucasian	c.461delA	p.K154fs	-	+	+	+	+	+	M,S	+	+
P3	9.5	Female	Caucasian	c.489_495del	p.L164fs	+	+	+	+	+	+	M,S	+	+
P4	11	Male	Hispanic	c.501delA	p.G168fs	+	-	+	+	+	+	G	+	ukn
P5	2	Male	Caucasian	c.517G>T	p.V173F	+	+	+	+	+	ukn	S	nRUS	ukn
P6 ^a	5.1	Male	Not specified	c.613C>T	p.R205X	-	-	+	+	-	ukn	U	+	+
P7 ^a	7	Female	Vietnamese	c.734T>G	p.L245R	-	-	+	+	-	+	M,S	+	ukn
P8	20.5	Male	Caucasian	c.787C>T	p.R263X	-	+	+	+	+	+	M,S	+	+
P9	5.7	Male	Caucasian	c.986_995del	p.S329fs	+	+	+	+	+	+	G	+	+
P10	9.8	Male	Middle Eastern	c.1042G>A	p.G348R	+	-	+	ukn	+	+	G	nRUS	ukn
P11	13.5	Male	Not specified	c.1098+1G>A	N/A	-	-	+	+	+	ukn	G	ukn	+
P12	27.1	Male	Not specified	c.1162dupG	p.A388fs	-	+	ukn	+	+	+	G	+	ukn
P13	14.8	Female	Not specified	c.1217_1220del	p.K406fs	+	+	ukn	ukn	+	ukn	M	ukn	+
P14	13.8	Female	Not specified	c.1309C>T	p.R437X	-	-	ukn	+	-	+	M,S	ukn	ukn
P15	10.1	Female	Hispanic	c.1309C>T	p.R437X	-	-	+	+	+	ukn	S	nRUS	ukn
P16	5.7	Female	Filipino	c.1309C>T	p.R437X	-	-	+	+	+	+	G	nRUS	ukn
P17	19.6	Female	Caucasian	c.1400G>A	p.R467Q	-	+	+	+	-	+	G	ukn	ukn
P18 ^a	18.5	Male	Caucasian	c.1399C>T	p.R467X	-	-	+	+	+	+	M,S	+	+
P19	9.4	Male	Hispanic	c.1478dupT	p.S494fs	+	ukn	+	+	+	+	M,S	+	+

+ Denotes phenotype observed (see Table 2 for more details)
AA amino acid, ASD autism spectrum disease (HP:0000717), DD developmental delays, DF/FTT difficulty feeding (HP:0011968)/failure to thrive (HP:0001508), FD facial dysmorphism (HP:0001999), G global (HP:0001263), GU genitourinary, ID intellectual disability (HP:0001249), M motor (HP:0001270), nRUS normal renal ultrasound, S speech (HP:0000750), U unspecified, ukn unknown.
^aPublished patients.²⁰

Fig. 1 Congenital anomalies of the kidney and urinary tract (CAKUT) associated with *DYRK1A* variants in patients with *DYRK1A*-related intellectual disability syndrome. Schematic shows the *DYRK1A* protein domains. Shapes, which identify the type of variant (squares = frame shift variants, circles = missense variants, stars = nonsense variants, triangles = splice variants), are positioned where *DYRK1A* patient variants impact the amino acid sequence. Patient variants are labeled by patient number as listed in Tables 1 and 2. Variants that result in CAKUT are red, those that do not result in CAKUT are blue, and those in which the effects on CAKUT status are unknown are black. Protein domains are abbreviated as follows: *DH* DYRK homology box; *HIS* histidine; *NLS* nuclear localization signal; *PEST* proline (P), glutamic acid (E), serine (S), and threonine (T); *Ser/Thr* serine/threonine. Inset shows highly conserved sequence surrounding the activation loop (labeled in orange) of the kinase domain. *GU* genitourinary.

Table 2 Available information about genitourinary (GU) phenotype of patients reported in this study

Case number	SNV	Segregation	Renal or GU phenotype
P1	p.N151fs	Mother negative, father's sample unavailable	Mild unilateral pelviectasis (HP:0010946) and frequent UTIs (HP:0000010)
P2	p.K154fs	De novo	Genital anomalies (HP:0000078)
P3	p.L164fs	De novo	Kidney abnormalities (not specified; HP:0000077)
P4	p.G168fs	De novo	Hypospadias (HP:0000047), micropenis (HP:0000054), and congenital chordee (HP:0000041)
P5	p.V173F	De novo	Renal ultrasound is normal with normal genitalia on exam
P6	p.R205X	De novo	Left renal agenesis (HP:0000122)
P7	p.L245R	De novo	Left renal agenesis (HP:0000122)
P8	p.R263X	De novo	Shawl scrotum (HP:0000049) and history bilateral orchiopexy (HP:0000028)
P9	p.S329fs	De novo	Hypospadias (HP:0000047) and kidney abnormalities (tiny echogenic foci)
P10	p.G348R	De novo	Normal renal ultrasound
P11	c.1098 +1G>A	De novo	Unknown
P12	p.A388fs	Mother negative, father is mosaic	Frequent UTI (HP:0000010)
P13	p.K406fs	De novo	Unknown
P14	p.R437X	De novo	Unknown
P15	p.R437X	Mother negative, father's sample unavailable	Normal renal ultrasound
P16	p.R437X	De novo	Normal renal ultrasound
P17	p.R467Q	De novo	Unknown
P18	p.R467X	De novo	Orchiopexy (HP:0000028) and inguinal hernia (HP:0000023)
P19	p.S494fs	De novo	Bilateral inguinal hernias (HP:0000023) but no renal ultrasound

Bold indicates 11 patients with GU phenotype. This strongly suggests an important role for *DYRK1A* in GU tract development. SNV single-nucleotide variant, UTI urinary tract infection.

Fig. 2 In situ hybridization of *dyrk1a* across developmental stages demonstrates kidney expression in *X. laevis* and *X. tropicalis*. To demonstrate spatial-temporal expression of *dyrk1a* in the kidney, in situ hybridization was performed. Given that the RNA probe was designed against the *X. tropicalis* sequence, both species were analyzed. Pronephric kidney development occurs between stages 12.5 and 40, which is demonstrated in **a–f** for *Xenopus laevis* and **g–l** for *Xenopus tropicalis*. Expression of *dyrk1a* can be visualized in stage 31–40 embryos suggesting Dyrk1a may be important for kidney development. For clarity, insets (**d'** and **j'**) for stage 30/31 tadpole kidneys with 200- μ m scale bars have been added. All other scale bars represent 1000 μ m.

Xenopus pronephros is essential for development of the mesonephric/Wolffian duct and subsequently the Müllerian duct, which are required for GU development.^{32,33}

***dyrk1a* is expressed in the *Xenopus* kidney and in vivo knockdown demonstrates its role in kidney development**

To assess whether *dyrk1a* is expressed in the *Xenopus* kidney, in situ hybridization was performed across developmental stages in both *X. laevis* and *X. tropicalis* (Fig. 2). *dyrk1a* expression is seen in the pronephros in several stages of both *X. laevis* and *X. tropicalis* embryos during kidney development, suggesting that it may be important for nephrogenesis.

To examine *dyrk1a*'s role in renal development in *Xenopus laevis*, an antisense morpholino (MO) that blocks translation was used to knock down endogenous Dyrk1a protein expression. *Xenopus laevis* has two copies of the *dyrk1a* gene because of its allotetraploid genome. Both *dyrk1a* transcripts are targeted by the Dyrk1a MO used in this study (Fig. S1B). Western blot analysis was used to confirm that the MO correctly targets *Xenopus dyrk1a* RNA (Fig. S2B). Knockdown experiments were used to determine whether loss of Dyrk1a function in *Xenopus* results in disruption of kidney development. Embryos were injected in a single V2 blastomere to target a single kidney, leaving the other as an internal control. Knockdown with a Dyrk1a MO resulted in abnormal pronephroi when immunostained with antibodies 3G8 and 4A6, which label the proximal tubules and the distal and connecting tubules (nephric duct), respectively (Fig. 3b). Loss of Dyrk1a primarily affected the proximal and distal tubules, with defects in the connecting tubules (nephric duct) occurring only in embryos with a more severe phenotype.

Variants identified in *DYRK1A*-related ID syndrome fail to rescue Dyrk1a loss-of-function in *Xenopus*

To assess if patient *DYRK1A* variants lead to pronephric anomalies as they do in *Xenopus*, rescue experiments were

carried out upon MO-mediated Dyrk1a knockdown in *Xenopus*. To express human *DYRK1A* in *Xenopus*, three constructs with HA tags were generated: wild-type human *DYRK1A*, a truncating patient variant *DYRK1A*^{R205*}, and a missense patient variant *DYRK1A*^{L245R}. Western blot analysis was used to confirm that the wild-type human *DYRK1A* and *DYRK1A*^{L245R}, ~95 kDa, and the truncated human *DYRK1A*^{R205*} RNA constructs, ~25 kDa, could be successfully expressed in *Xenopus* (Fig. S2C). Overexpression of the rescue dose (50 pg) of either β -galactosidase (β -gal), wild-type *DYRK1A*, *DYRK1A*^{R205*}, or *DYRK1A*^{L245R} RNA demonstrated no gain-of-function phenotype of either *DYRK1A* variant (Fig. S3). The kidney anomalies caused by Dyrk1a knockdown were partially rescued by coinjecting wild-type human *DYRK1A* RNA (Fig. 3c). However, neither *DYRK1A*^{R205*} or *DYRK1A*^{L245R} RNA rescued these anomalies (Fig. 3d, e). Detailed descriptions of how embryos are scored can be found in Fig. S4.

To assess whether Dyrk1a depletion affects kidney function, an assay was performed evaluating edema formation.¹⁸ Edema can be caused by a disruption in the kidneys' ability to excrete excess fluid, but it can also be caused by heart or liver failure. Both the heart and liver arise from dorsal cells in *Xenopus* (Xenbase.org). To prevent knockdown in these tissues, embryos were injected with Dyrk1a MO or standard MO in both ventral cells at the four-cell stage to affect both kidneys. Embryos injected with the Dyrk1a MO suffered from edema and abnormal kidneys, characterized by swelling in the chest cavity due to fluid retention (Fig. 3i) while embryos injected with the standard MO did not (Fig. 3h). This technique suggests that loss of *dyrk1a* affects kidney function in *Xenopus*. Taken together, these data support a role for *DYRK1A* in pronephric development and strongly suggest that the *DYRK1A*^{R205*} and *DYRK1A*^{L245R} variants are responsible for the kidney anomalies observed in these patients.

Fig. 3 Loss of *Dyrk1a* affects kidney development in *Xenopus laevis*. (a'–e') Embryos were unilaterally injected at the 8-cell stage with 10 ng of *Dyrk1a* morpholino (MO) or standard MO (Std MO) along with 50-pg β -gal, wild-type, *DYRK1A*^{R205*}, or *DYRK1A*^{L245R} RNA. Stage 40 tadpoles were stained with kidney antibodies 3G8, which labels the proximal tubules, and 4A6, which labels the distal and connecting tubules. Letters without apostrophes (a–e) represent the injected side, whereas letters with apostrophes (a'–e') represent the uninjected side. (b) Knockdown with a translation-blocking *Dyrk1a* MO disrupts kidney development, which can be partially rescued (c) by coinjecting with wild-type human *DYRK1A* RNA but not (d–e) *DYRK1A*^{R205*} or *DYRK1A*^{L245R} RNA. (a) Coinjection of a standard MO and β -gal serves as a negative control. Scale bars represent 100 μ m. (f) The graph demonstrates a significant difference between embryos injected with either *Dyrk1a* MO + β -gal or *Dyrk1a* MO + *DYRK1A*^{R205*} versus with *Dyrk1a* MO + *DYRK1A* suggesting successful rescue with human *DYRK1A* but not with the nonsense RNA. (g) The second graph demonstrates a significant difference between embryos injected with *Dyrk1a* MO + *DYRK1A* versus with *Dyrk1a* MO + *DYRK1A*^{L245R}, which suggests that the missense RNA also fails to rescue. Significance was established against embryos that had a moderate or severe kidney phenotype (orange bar) and excluded embryos that had a weak phenotype (yellow bar). (f) Asterisk (*) indicates $p < 0.001$ comparing individual experimental groups with standard MO + β -gal. Pound sign (#) indicates $p < 0.006$ comparing *Dyrk1a* MO + *DYRK1A* with *Dyrk1a* MO + *DYRK1A*^{R205*}. (g) Asterisk (*) indicates $p < 0.001$ comparing standard MO + β -gal with *Dyrk1a* MO + β -gal or *Dyrk1a* MO + *DYRK1A*^{L245R}. Pound sign (#) indicates $p < 0.05$ comparing *Dyrk1a* MO + *DYRK1A* with *Dyrk1a* MO + *DYRK1A*^{L245R}. For edema assays, embryos were injected at the 4-cell stage in both ventral cells to target both kidneys while avoiding the dorsal cells fated to become the heart and liver, which can also lead to edema. (h) Embryos injected with the standard MO did not develop edema while embryos injected (i) with the *Dyrk1a* MO did develop edema and also suffered from abnormal kidney formation. (j) The graph demonstrates a significant difference in edema and kidney abnormalities in embryos injected with either standard MO or *Dyrk1a* MO. Asterisk (*) indicates $p < 0.008$ comparing standard MO with *Dyrk1a* MO embryos with edema, defects in one, and defects in both kidneys. Error bars represent standard error. For ease of comparison of (n) and p values across conditions, please refer to Table S1–3.

DISCUSSION

Recent discoveries demonstrate that de novo pathogenic variants in *DYRK1A* cause a syndromic form of ID (OMIM 614104). The findings in the current study indicate that CAKUT/GD should be included as features associated with this syndrome.

CAKUT consist of a heterogeneous clinical spectrum, and how CAKUT arise is largely unknown. Thus, it is important to identify all genes and causal variants involved.³⁴ Strong genetic causality of monogenic disease only accounts for 14% of CAKUT cases,³⁵ and polygenic causes are speculated to occur but are largely unknown.³⁶ Next-generation sequencing,

specifically ES, has improved the discovery of novel causative genes that are important in GU development.^{37–39} Here, we report on a novel genetic contribution of *DYRK1A* to CAKUT/GD. We identified 17 unique variants in *DYRK1A* from clinical ES in 19 unrelated individuals. As summarized in Table 1, microcephaly, ID, DD, and seizures are some of the more common features of this syndrome.

Eleven of fifteen (73% of those with available data) individuals in this study (Table 2) have CAKUT/GD, with 36% having renal anomalies, in addition to other organ involvement. While renal anomalies have been reported previously,²⁰ broadly based phenotyping for CAKUT was not performed in previous studies.

Seven of the *DYRK1A* variants identified in our study were novel as they were absent in ClinVar as well as the gnomAD and ExAC databases. Per inclusion criteria, most of *DYRK1A* variants were de novo and included truncating variants. This suggests a loss-of-function mechanism for variants causing this syndrome, which further supports the findings of another group who proposed reduced kinase function as the cause.²²

In addition, we determined that *dyrk1a* is expressed in the developing *Xenopus* kidney and found that *dyrk1a* knock-down results in abnormal tubules or complete loss of the kidney. This phenotype could be partially rescued by human *DYRK1A* RNA. However, a nonsense (R205*) and a missense (L245R) variant failed to rescue the phenotype, indicating that loss-of-function variants in this gene are likely causative for the observed phenotype in some patients. This suggests that *DYRK1A*'s kinase domain may be important for kidney development. Furthermore, *Dyrk1a* MO was injected into two cells to affect both kidneys, which resulted in edema suggesting that *Dyrk1a* is important for both kidney development and function. Although *Xenopus* is an established model to study kidney development, it has not been commonly used to study GD. However, our findings related to pronephric development are likely relevant to the mammalian GU tract, given the dependence of the formation of the male and female urogenital tract upon the nephric duct and the pronephros.

One limitation of this study is that we were not able to obtain GU information from all patients. Future plans for research include identification and studying the phenotype and underlying variants in a larger number of affected families. Furthermore, signaling pathways involved in *DYRK1A*-related CAKUT/GD should be investigated.

In summary, the phenotype of *DYRK1A*-related ID syndrome is expanded to include CAKUT/GD. Based on our data, we empirically recommend that individuals with *DYRK1A* syndrome undergo a renal ultrasound and a thorough genital physical exam.

SUPPLEMENTARY INFORMATION

The online version of this article (<https://doi.org/10.1038/s41436-019-0576-0>) contains supplementary material, which is available to authorized users.

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DISCLOSURE

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