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Heterozygous Pathogenic Variant in *DACT1* Causes an Autosomal-Dominant Syndrome with Features Overlapping Townes–Brocks Syndrome

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Abstract

A heterozygous nonsense variant was identified in dapper, antagonist of beta-catenin, 1 (*DACT1*) via whole-exome sequencing in family members with imperforate anus, structural renal abnormalities, genitourinary anomalies, and/or ear anomalies. The *DACT1* c.1256G>A;p.Trp419* variant segregated appropriately in the family consistent with an autosomal dominant mode of inheritance. *DACT1* is a member of the Wnt-signaling pathway, and mice homozygous for null alleles display multiple congenital anomalies including absent anus with blind-ending colon and genitourinary malformations. To investigate the *DACT1* c.1256G>A variant, HEK293 cells were transfected with mutant *DACT1* cDNA plasmid, and immunoblotting revealed stability of the DACT1 p.Trp419* protein. Overexpression of *DACT1* c.1256G>A mRNA in *Xenopus* embryos revealed a specific gastrointestinal phenotype of enlargement of the proctodeum. Together, these findings suggest that the *DACT1* c.1256G>A nonsense variant is causative of a specific genetic syndrome with features overlapping Townes–Brocks syndrome.

Keywords

DACT1; Townes–Brocks syndrome; imperforate anus; microtia; renal malformation; genitourinary anomaly

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Townes–Brocks syndrome (TBS) (MIM# 107480) is eponymously named for Dr. Philip Townes and Eric Brocks, who reported the association of imperforate anus, bony abnormalities, and ear defects in a father and several of his children. Autosomal dominant inheritance for this condition was presumed [Townes and Brocks, 1972]. TBS is now characterized by the triad of imperforate anus, dysplastic ears, and thumb malformations. Minor features of the condition include hearing loss, foot malformations, renal impairment with or without renal malformations, genitourinary malformations, and congenital heart disease. Heterozygous mutations in *SALL1* were identified as a genetic etiology of TBS, but as many as 25% of patients with a TBS diagnosis are not identified to have *SALL1* mutations based on sequence analysis and gene-targeted deletion/duplication testing [Kohlhase, 1993; Kohlhase et al., 1998]. Strict clinical criteria are not available for TBS, but *SALL1* testing is generally recommended if a patient displays two or more major features. Here, we present a family with multiple persons affected with symptoms overlapping TBS and identify a novel disease gene for a TBS-related disorder.

The proband (III.4) is a 20-month-old female born to a 29-year-old mother by Cesarean section due to breech positioning (Fig. 1A). Prenatal ultrasounds were normal. The mother was treated with levothyroxine throughout the pregnancy due to a history of hypothyroidism and Graves' disease. No other medications or exposures were noted during the pregnancy. By 1 day of age, the patient was diagnosed with imperforate anus. The patient had an anal opening, which was not patent, but had a rectovaginal fistula, which allowed some stooling. A colostomy was performed at 8 days of age. The patient tolerated breastfeeding and was discharged home shortly after the colostomy procedure. She passed her newborn hearing screen.

At approximately 2 months of age, the patient had an anoplasty procedure. A colostomy takedown procedure was performed at 7 months of age. She tolerated this procedure well, and has not required any manual dilatation. An echocardiogram completed at the age of 1 year was normal. Renal ultrasound was notable for crossed fused renal ectopia, and voiding cystourethrogram was notable for grade II reflux. She has no history of urinary tract infections. Recent audiology examination was normal. Developmentally, she has reached appropriate milestones and has no signs of delays.

On examination, head circumference was at the 21st%, weight was at the 24th%, and length was at the 21st%. She was normocephalic with a closed anterior fontanelle. She had a nondysmorphic facies. She had appropriately spaced eyes with horizontal palpebral fissures. Extraocular movements were normal, and no abnormalities were noted on ophthalmoscope examination. The right ear appeared smaller than the left, and both ears had overfolding of the superior helix and cupping, although more pronounced on the right (Fig. 1D and E). There were no preauricular pits or tags. Nose and mouth examinations were unremarkable; no clefting was noted. Neck was well-developed. Chest was clear and without pectus deformity. Cardiac examination revealed a regular rate and rhythm without a murmur. No scoliosis was appreciated. She had normal Tanner 1 female genitalia. A surgical scar was present at the site of the imperforate anus repair. She had full range of motion in all

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extremities. She had normal palmar creases and nails. No thumb or radius anomalies were noted. She had no clinodactyly or syndactyly. Neurological examination was normal.

The proband's 7-year-old brother (III.2) was born at 36 weeks gestation by normal standard vaginal delivery (Fig. 1A). Prenatal ultrasounds were normal, and imperforate anus and hypospadias were diagnosed shortly after birth. The brother received a colostomy at 2 days of age. Renal sonogram revealed crossed fused renal ectopia, and voiding cystourethrogram revealed grade I reflux. Echocardiogram was normal. At approximately 5 months of age, the brother had a colostomy takedown procedure and hypospadia repair. At 2 years of age, dilated ophthalmology examination was normal. Audiology examinations have been normal. A recent voiding cystourethrogram revealed grade III reflux. The brother has been diagnosed with attention deficit hyperactivity disorder and is being evaluated for a possible diagnosis of Asperger syndrome. He currently receives occupational therapy two times per week to improve his fine motor skills. On brief examination, the brother is noted to have overfolding of the superior helix of his ears bilaterally, although more pronounced on the right. The right ear is also cupped (Fig. 1F and G). Prior chromosome analysis and chromosomal microarray testing were normal.

The mother of the proband, II.2, is a 31-year-old female with a history of spina bifida occulta diagnosed by X-ray during her teen years when she was being evaluated for scoliosis (Fig. 1A). History is also notable for Graves' disease. She has not had an echocardiogram. Recent renal sonogram was normal. Ear examination is notable for cupping bilaterally. She denies history of hearing loss. The mother's half-sister, II.3, is a 37-year-old female with a history of left ear severe microtia (Fig. 1A). She has had a normal renal sonogram. The maternal grandmother of the proband, I.4, is a 59-year-old female with a history of left ear severe microtia, bifid uterus, and Hashimoto's thyroiditis (Fig. 1A). She has had a normal renal sonogram. She was suspected of having TBS; *SALL1* gene sequencing with deletion/duplication analysis was negative.

In an effort to identify a possible causative genetic etiology for the clinical features of the affected family members, the family was enrolled into our Mount Sinai IRB-approved genetics research study (Genetic Studies of Congenital Anomalies) (Supp. Table S1). Written informed consent was obtained, and all investigations were conducted in accordance with the principles of the Declaration of Helsinki.

DNA from persons III.4 and I.4 were processed for whole-exome sequencing (WES) in the Mount Sinai Genomics Core Facility. Individual WES samples were barcoded and pooled with three other samples prior to enrichment with the SureSelect V5 library (Agilent Technologies, Santa Clara, CA). Sequencing was performed on a HiSeq 2500 instrument (Illumina, San Diego, CA) with a 100-bp paired-end protocol. Alignment and variant calling was completed using an in-house GATK-based pipeline. Sequencing of sample III.4 was repeated and data were combined. A total of 231,275,262 reads were generated for sample III.4, and a total of 92,533,684 reads were generated for sample I.4. The targeted mean coverage for sample III.4 was 208.0, and 98.5% of the target had 10x coverage. The targeted mean coverage for sample I.4 was 92.6, and 98.2% of the target had 10X coverage.

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Called variants were filtered with Ingenuity Variant Analysis (Qiagen, Redwood City, CA, http://www.ingenuity.com). A total of 130,755 total variants in 17,338 genes were identified in samples III.4 and I.4. Variants were filtered based on confidence (call quality of 20, passed upstream pipeline filtering, and outside top 3% most exonically variable 100 base windows and/or 3% most exonically variable genes in healthy public genomes included), frequency (variants excluded if frequency was at least 0.5% in the 1000 Genomes Project, NHLBI ESP exomes, or ExAC), predicted deleteriousness (frameshift, in-frame indel, start/stop codon changes, missense changes, splice site loss up to six bases into intron or as predicted by MaxEntScan, and variants listed in HGMD were included), and genetic analysis (heterozygous variants common to both individuals III.4 and I.4 included). This filtering strategy resulted in the identification of 84 variants in 71 genes (Supp. Table S2). All of these variants were considered, and knowledge of gene function was reviewed.

We noted a nonsense variant in *DACT1* (MIM# 607861), or Dapper, antagonist of beta-catenin, 1 that was of particular interest. The proband (III.4) and affected grandmother (I.4) were heterozygous for the *DACT1* c.1256G>A;p.Trp419* variant (Ref-Seq NM 016651.5). This variant has not been reported in dbSNP, 1000 Genomes Project, NHLBI Exome Variant Server, or ExAC. The PHRED-scaled CADD score for this variant is 35 [Kircher et al., 2014]. All of these measures indicate that this variant is potentially deleterious. Additionally, this variant was the top hit during further evaluation of all candidates using Exomiser with hiPHIVE analysis and HPO ontology terms for imperforate anus, crossed fused renal ectopia, microtia, hypospadias, and bicornuate uterus (Exomiser score: 0.898; phenotype score: 0.648; variant score: 0.950) [Smedley et al., 2015]. Sanger sequencing confirmed the heterozygous *DACT1* c.1256G>A genotype in individuals III.4 and I.4. Additional family members were genotyped and *DACT1* c.1256G>A; p.Trp419* variant segregated in the family with the phenotype and was consistent with an autosomal dominant mode of inheritance (Fig. 1A; Supp. Fig. S1). This variant has been submitted to the Leiden Open Variation Database (http://lovd.nl/3.0/).

Human *DACT1* is the ortholog of *Xenopus* Dapper (Dpr) and Frodo proteins, which function in the Wnt-signaling pathway. *Xenopus dapper* and *frodo* are alloalleles located on chromosomes 8L and 8S, respectively, and share 89.8% amino acid identity [Katoh and Katoh, 2005]. In this pathway, extracellular Wnt ligands bind to Frizzled transmembrane receptors to activate Dishevelled (Dsh) [Brott and Sokol, 2005]. *Xenopus* Dact1 was identified by yeast two-hybrid screens to identify Dsh-interacting proteins [Cheyette et al., 2002; Gloy et al., 2002]. *Xenopus* embryos depleted of either Dact1 alloallele by morpholino injection of four-cell embryos have mild anterior abnormalities, whereas simultaneous depletion of both Dact1 proteins leads to dorsally bent embryos lacking head structures, suggesting both proteins function synergistically [Hikasa and Sokol, 2004]. Treatment of Dact1-depleted *Xenopus* embryos with *β-catenin* mRNA partially rescues this abnormal head phenotype [Hikasa and Sokol, 2004]. Injection of Dact1 morpholino into a single blastomere at the eight or 16 cell stage results in an absent neural fold leading to an open neural tube on the injected side. For this neural development phenotype, *β-catenin* mRNA fails to rescue the abnormal phenotype, whereas *dact1* mRNA is suf-ficient [Hikasa and Sokol, 2004]. Thus, *Xenopus* Dact1 functions in both *β*-catenin-dependent and *β*-

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catenin-independent Wnt-signaling pathways [Hikasa and Sokol, 2004; Brott and Sokol, 2005].

Dpr is highly similar to human DACT1 (60% identical) and mouse DACT1 (55% identical) [Cheyette et al., 2002]. Mice homozygous for *Dact1* null alleles have multiple congenital anomalies and most often die within 1 day of birth [Suriben et al., 2009; Wen et al., 2010]. These anomalies include a short tail, absent anus with blind-ending colon, absent bladder, and renal malformations. Renal malformations range from fusion at the midline to complete renal agenesis. ~90% of homozygous *Dact1* mutants have segmental truncation, which most often (73%) is restricted to the tail, but may also involve the sacral, lumbar, or pelvic regions or hindlimbs. 13% of homozygous mice have spina bifida [Suriben et al., 2009]. Additionally, the outlet of the urethra may be absent, and the uterus or vas deferens may have structural abnormalities [Wen et al., 2010]. Human DACT1 is widely expressed in many organ systems, including strong cytoplasmic expression in central nervous system tissues and moderate immunoreactivity in rectum, appendix, most endocrine glands, urinary bladder, seminal vesicle, and most myocytes.

DACT1, isoform 1, contains 836 amino acids (NP 057735) with seven DAPPER homologous (DAPH) domains. In the p.Trp419* variant, three of the seven DAPH domains are missing, including the C-terminal PDZ-binding region (DAPH7), as well as a bipartite nuclear localization signal located at amino acids 610–623 (Fig. 1B). The DAPH2 domain includes a leucine zipper motif, a type of coiled coil domain, which has been shown to participate in homodimerization and heterodimerization with Dact paralogs [Kivimae et al., 2011]. This coiled coil domain is located at amino acids 101–149 in DACT1, and thus present in the p.Trp419* mutant protein. We reasoned that if the mutant protein retains its ability to dimerize, the resulting homodimer with the wild-type protein may be nonfunctional and possibly explain a dominant negative effect. In order to further investigate this hypothesis, we performed functional analysis of wild-type *DACT1*, *DACT1* c. 1256G>A;p.Trp419* (mut), and *DACT1* c.1256G>A;p.Trp419* with additional deletion of the coiled coil domain (mut +   cc).

We first determined whether mutant DACT1 proteins were stable by transfecting human cDNA wild-type *DACT1* ($pCS2^+$-5^3FLAG-wild-type-DACT1), human cDNA c.1256G>A *DACT1* ($pCS2^+$-5^3FLAG-mut-DACT1), human cDNA c.1256G>A +   cc *DACT1* ($pCS2^+$-5^3FLAG-mut+   cc-DACT1), or empty control vector pCDNA3 in HEK293 cells. Cells were harvested after 1 day, and protein was extracted and prepared for immunoblotting. Using a FLAG tag antibody, we detected wild-type DACT1 at ~90 kDa, mutant DACT1 p.Trp419* protein (mut) at ~50 kDa, and mut +   cc protein at ~44 kDa (Fig. 1C). This indicates that these mutant proteins are stable. To also determine whether *DACT1* c.1256G>A mRNA was stable, we isolated mRNA from patient III-2 and control lymphoblasts using the Qiagen miRNeasy Mini Kit and generated cDNA using the Invitrogen SuperScript IV First-Stand cDNA Synthesis Reaction Kit. PCR followed by Sanger sequencing of the cDNA revealed the heterozygous *DACT1* c.1256G>A variant in the affected individual indicating stability of this nonsense mRNA (Supp. Fig. S2).

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To evaluate whether *DACT1* c.1256G>A variant may lead to a gastrointestinal phenotype, we performed functional overexpression studies in *Xenopus*. We injected either human *DACT1* wild-type or c.1256G>A mutant mRNA into one of two blastomeres at the two-cell stage and examined the effects on development of the proctodeum, the ectodermal depression at the caudal end of the embryo that develops into the anal canal. In *Xenopus* tadpoles, the proctodeum develops at the junction between endoderm and ectoderm and is complete by stage 36 (Fig. 2A and B). In agreement with previous studies, we found that injection of wild-type (or mutant) *DACT1* mRNA into *Xenopus* embryos perturbed gastrulation in approximately half of the embryos resulting in abnormal development (Supp. Table S3) [Hikasa and Sokol, 2004; Brott and Sokol, 2005; Shi et al., 2012]. However, in the remaining approximately 50% of embryos that survived with normal morphology at stage 36, we observed defective development of the proctodeum in c.1256G>A mutant (mut), but not wild-type *DACT1*-injected embryos. In embryos injected with *DACT1* c.1256G>A mutant mRNA, the proctodeum did not form properly displaying incomplete closure (Fig. 2C, D, G, and H). This phenotype was dose-dependent, with 55.91% of embryos injected with 1 ng and 69.43% of embryos injected with 2 ng of mutant mRNA (mut) displaying an enlarged proctodeum (Fig. 2K). In contrast, the proctodeum closed properly in embryos injected with wild-type *DACT1* mRNA (Fig. 2E, F, I, and J); the enlarged proctodeum phenotype was observed in 7.39% and 7.38% of embryos injected with 1 or 2 ng wild-type *DACT1*, respectively (Fig. 2K; Supp. Table S3). To further investigate the molecular mechanism underlying the enlarged proctodeum phenotype, we examined whether the coiled coil domain was responsible for generating this dominant negative effect. In embryos injected with mut + cc mRNA, the enlarged proctodeum phenotype was rarely observed; 3.70% and 4.76% of embryos injected with 1 or 2 ng of mut + cc mRNA had this phenotype. These results indicate that the c.1256G>A (mut) variant causes a gastrointestinal developmental defect in a dominant negative manner. Because the presence of the coiled coil domain was necessary for the increased incidence of the enlarged proctodeum phenotype in *Xenopus* embryos, the DACT1 p.Thr419* truncated protein may be functioning in a dominant negative fashion. The coiled coil domain is necessary for DACT protein homodimerization, and the DACT1 p.Thr419* truncated protein may form a nonproductive homodimer with the wild-type allele.

Haploinsufficiency of DACT1 may offer an alternative for some of the features in the affected family members. Although there are cases where knockout mice produce phenotypes similar to human haploinsufficient presentations, we believe our data is more consistent with a dominant negative mechanism. We have shown that the coiled coil domain in the mutant protein is necessary to produce the defective development of the proctodeum in *Xenopus* embryos, and thus the mutant protein is likely functioning in a dominant negative fashion for this phenotype. In agreement with this, heterozygous *Dact1* knockout mice are viable and healthy with no apparent phenotypic defects [Wen et al., 2010].

Here, we report affected family members with variable features of imperforate anus, structural renal anomalies, genitourinary abnormalities, and ear abnormalities who harbor the heterozygous *DACT1* c.1256G>A;p.Trp419* variant. The missense DACT1 variants p.R45W, p.D142G, p.N356K, p.V702G, and p.T808K have previously been identified in stillborn or miscarried Han Chinese fetuses with neural tube defects, and biochemical

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analyses of several of these missense variants revealed altered DACT1 function, but matched parental samples were unavailable, and subsequent segregation studies could not be performed [Shi et al., 2012]. The authors postulate that rare variants in *DACT1* and other planar cell polarity pathway-related genes may contribute to human neural tube defects [Shi et al., 2012]. To the best of our knowledge, this is the first report of a *DACT1* variant segregating with a TBS-like phenotype. Most interestingly, the clinical features of imperforate anus, structural renal anomalies, and structural genitourinary anomalies identified in the presented family overlap with clinical features identified in homozygous *Dact1* knock-out mice [Suriben et al., 2009; Wen et al., 2010].

In conclusion, this is the first report of a *DACT1* variant segregating with a disease phenotype in humans. The features of imperforate anus, structural renal anomalies, genitourinary abnormalities, and ear abnormalities that were present in this family overlap with features of TBS. In fact, individual I.4 in the presented family was diagnosed with TBS, but clinical *SALL1* gene sequencing with deletion and duplication analysis did not reveal a pathogenic variant. Identification of additional affected individuals with *DACT1* mutations is necessary to understand the full spectrum of disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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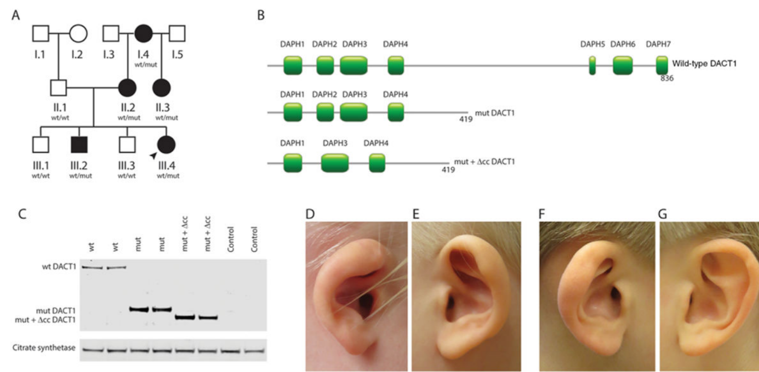

Figure 1.

A: Family pedigree. Squares denote males, circles denote females, and shaded symbols denote affected individuals. The proband is indicated with an arrow. wt represents wild-type and mut represents the *DACT1* c.1256G>A;p.Trp419* mutation. **B**: DACT1 protein structure. Human DACT1, isoform 1 contains 836 amino acids (NP_057735). Seven DAPPER homologous (DAPH) domains, which are conserved between DACT1 and DACT2 are labeled (DAPH1-7). The DAPH2 domain has a leucine zipper motif, a type of coiled coil domain. The coiled coil domain is located from amino acids 101–149. The DAPH3 domain is serine-rich, and the DAPH7 domain has a PDZ-binding region. Additionally, a nuclear export signal motif is located at amino acids 132–141, and a bipartite nuclear localization signal is located at amino acids 610–623. mut + cc DACT1 represents DACT1 p.Trp419* with additional deletion of the coiled coil domain. **C**: The *DACT1* c.1256G>A variant leads to a truncated DACT1 protein (DACT1 p.Trp419*). Western blot analysis revealed stability of the DACT1 p.Trp419* protein. 2.5 μg of human cDNA wild-type *DACT1* ORF clone [pCS2$^+$-5^{3}FLAG-wild-type-DACT1] (wt), human cDNA c.1256G>A *DACT1* ORF clone [pCS2$^+$-5^{3}FLAG-mut-DACT1] (mut), human cDNA c.1256G>A with additional deletion of coiled coil domain *DACT1* ORF clone [pCS2$^+$-5^{3}FLAG-mut+ cc-DACT1] (mut + cc), or empty control vector pCDNA3 (control) were transfected in HEK293 cells, plated in six-well plates, using Lipofectamine 2000. Cells were harvested after 1 day, and immunoblotting was performed using standard conditions. DACT1 was visualized using an anti-FLAG antibody. The experiment was performed in duplicate. **D** and **E**: The right (**D**) and left (**E**) ears of proband III.4 are shown. Cupping and overfolding of the superior helix are noted. **F** and **G**: The right (**F**) and left (**G**) ears of III.2 are shown. The right ear is notable for cupping and overfolding of the superior helix, and the left ear has mild overfolding.

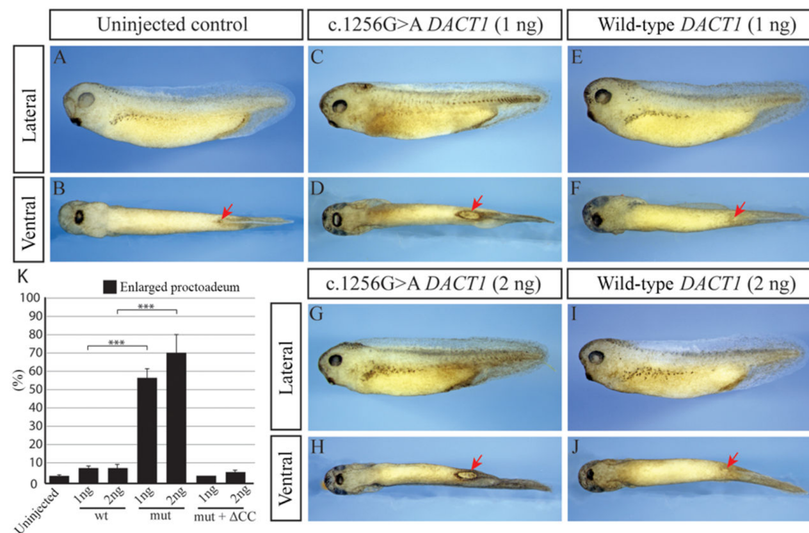

Figure 2.

Overexpression of human wild-type *DACT1*, c.1256G>A *DACT1* (mut), or c.1256G>A + cc *DACT1* (mut + cc) mRNA in *Xenopus laevis* embryos. All injections were performed at the two-cell stage into one blastomere. Injections were traced using Texas Red dextran. **A and B**: Uninjected control embryos at stage 36 showing lateral and ventral views. **C–F**: Embryos injected with 1 ng of either c.1256G>A mutant (mut) or wild-type *DACT1* (wt) mRNA are shown. **G–J**: Embryos injected with 2 ng of either c.1256G>A mutant (mut) or wild-type *DACT1* (wt) mRNA shown. Arrows point to proctodeum in ventral panels. **K**: Bar graph showing percentage of embryos with enlarged proctodeum at different conditions from four (1 ng) and three (2 ng) independent experiments for wt and mut. Two independent experiments were performed for mut + cc mRNA for 1 and 2 ng injections. Error bars represent standard deviation. Significance was calculated using Chi-square test, $^{***}P<0.0005$.

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