



# A novel biallelic loss-of-function variant in *DAND5* causes heterotaxy syndrome

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**Abstract** The majority of heterotaxy cases do not obtain a molecular diagnosis, although pathogenic variants in more than 50 genes are known to cause heterotaxy. A heterozygous missense variant in *DAND5*, a nodal inhibitor, which functions in early development for establishment of right–left patterning, has been implicated in heterotaxy. Recently, the first case was reported of a *DAND5* biallelic loss-of-function (LoF) variant in an individual with heterotaxy. Here, we describe a second unrelated individual with heterotaxy syndrome and a homozygous frameshift variant in *DAND5* (NM\_152654.2:c.197del [p.Leu66ArgfsTer22]). Using an in vitro assay, we demonstrate that the *DAND5* c.197del variant is unable to inhibit nodal signaling when compared with the wild-type expression construct. This work strengthens the genetic and functional evidence for biallelic LoF variants in *DAND5* causing an autosomal recessive heterotaxy syndrome.

[Supplemental material is available for this article.]

## CASE PRESENTATION

We present a case of a 5-yr-old girl with clinical features of heterotaxy or situs ambiguus, asplenia, recurrent infections, secondary pulmonary hypertension, and developmental delay. She was born at term, by natural vaginal delivery, to a G4P3 25-yr-old mother and weighed 7 lb at birth. Prenatally, she was noted to have a complex congenital heart disease indicating signs of heterotaxy syndrome. An echocardiogram done at birth confirmed this diagnosis with findings of levocardia, unbalanced complete atrioventricular canal defect (classified as Rastelli type A), patent ductus arteriosus (PDA), bicuspid aortic valve and abnormal blood vessel morphology including interrupted inferior vena cava with azygous continuation, bilateral superior vena cava without bridging vein, and partial anomalous pulmonary venous return. She spent 4 wk in the neonatal intensive care unit and was transferred to a rehabilitation center.

At 7 mo of age, she underwent multiple hospital admissions for recurrent fevers and respiratory infections. Her clinical history was concerning for primary ciliary dyskinesia. She was seen by immunology, and her infectious disease workup was uninformative apart from low T-cell count. Her echocardiogram at this time showed valvular pulmonary stenosis and signs of cardiomyopathy including moderate biventricular hypertrophy with decreased left

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**Ontology term:** abdominal situs inversus

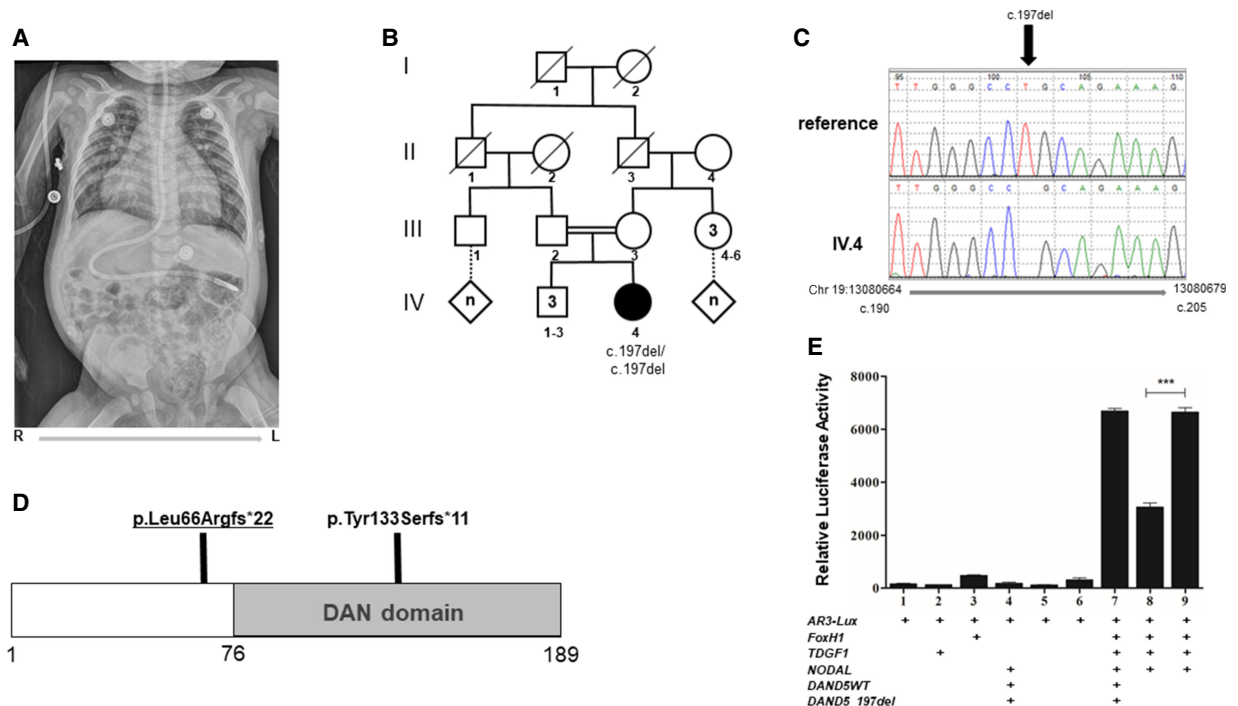
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ventricular systolic function, indicating heart failure. She underwent surgical correction of her atrioventricular canal defect and PDA. Further imaging of chest by computed tomography (CT) scan, radiography, and abdominal ultrasound showed left-sided isomerism, intestinal malrotation, transverse liver, and polysplenia (Fig. 1A). She was noted to be less than the fifth percentile for height and weight post surgery and was placed on a nasogastric feeding tube. She developed laryngeal granulation secondary to prolonged intubation, which was surgically excised. At 11 mo of age, she had surgical correction of her intestinal malrotation. At 13 mo of age, she developed pulmonary hypertension that was considered secondary to her complex congenital heart disease and was managed with sildenafil. She was also noted to have developmental delay at this time, with delayed speech and fine motor milestones. She had multiple hearing evaluations because of her speech delay, which have shown her hearing to be within normal limits. Currently, she is able to use a few short three- to four-word sentences but has trouble with expressing her needs. She receives speech and occupational therapy and is followed by cardiology, immunology, and developmental specialists.

Clinical Genetics was consulted when she was 7 mo of age. During the physical examination, she was noted to have generally coarse facies, hypertelorism, and bilateral epicanthal folds, but no gross dysmorphism was observed. The parents (III.2, III.3) are a



**Figure 1.** (A) Frontal radiograph of the chest and abdomen at 8 mo of age showing heterotaxy, transverse liver, right-sided stomach with duodenal feeding tube, and cardiomegaly. (B) Pedigree showing the family of the affected individual. (C) Sanger sequencing confirmed the *DAND5* homozygous variant in the proband. The genomic (hg19/GRCh37) and the cDNA coordinates are marked below the electrophoregram. (D) Localization of the two homozygous frameshift variants on the *DAND5* protein; the variant seen in the current study is underlined. (E) A luciferase assay performed on HEK293T cells with a reporter gene under the control of three activin-responsive element promoters, pAR3-lux, which is transcriptionally activated strictly by Nodal together with FOXH1 and TDGF1, was used. The results showed the expression vector with the *DAND5* c.197del variant does not inhibit Nodal signaling when compared with wild-type *DAND5* construct. For this over-expression assay, the effect of the different genes altogether and the individual effect for each one is shown. Bars indicate standard deviation of all three experiments of four replicates. Significance: (\*)  $P < 0.01$ , t-test.

consanguineous couple (first cousins; their fathers are brothers II.1, II.3) of Gambian descent (Fig. 1B). They had three other healthy sons, and the family history was negative for birth defects, intellectual disability, recurrent infections, seizures, or genetic or metabolic disorders.

## TECHNICAL ANALYSIS

### Clinical Exome Sequencing and Analysis

For the exome sequencing (ES) testing, written informed consent was obtained for the individual. DNA was extracted from a peripheral blood specimen (Singleton ES). Exome sequencing library was prepared from genomic DNA using Agilent SureSelectXT (Human All Exon v.5 + UTRs) capture kit. Paired-end sequencing was performed on the Illumina HiSeq 2500 platform. The sequence data were aligned and annotated using Nextgene (version 2.3; Softgenetics, LLC). Variant filtering and annotation were performed using a proprietary pipeline developed by the Division of Personalized Genomic Medicine at Columbia University (Wang et al. 2016; Ganapathi et al. 2022). Variants were assessed for pathogenicity following ACMG standards and guidelines (Richards et al. 2015).

The remaining methods are provided in the [Supplemental Material](#).

## VARIANT INTERPRETATION

A karyotype, microarray, and singleton clinical ES were performed in 2017. Her karyotype was noted as 46,XX, and the chromosomal microarray showed a normal female chromosome complement along with multiple long contiguous stretches of homozygosity (>3 Mb) suggestive of common ancestry consistent with the family history.

ES for the proband identified a homozygous rare variant (hg19/GRCh37, Chr 19:13080670-CT-C, c.197del [p.Leu66ArgfsTer22]) in *DAND5* [MIM 609068] coding exon 1 of two (Table 1). This variant is in a large region of homozygosity on Chromosome 19, as reported by the chromosomal microarray. The c.197del variant was confirmed by Sanger sequencing (Fig. 1C) and was absent in gnomADv2 and v3, TOPMED Bravo, and GME variome population databases. It was reported as a variant of uncertain significance (VUS) in a gene of uncertain significance (GUS).

The predicted protein truncation due to c.197del (p.Leu66ArgfsTer22) variant will disrupt the *DAND5* prior to its DAN domain (Fig. 1D) and therefore can ablate the DAND5–

**Table 1.** Biallelic *DAND5* loss-of-function (LoF) variants identified in individuals with a clinical diagnosis of heterotaxy syndrome including the case from this study, along with relevant population frequencies and classification

Case	Genomic coordinates	Ref	Alt	HGVS cDNA	HGVS protein	Variant classification	Allele frequency		
							gnomAD (v2.1.1)	gnomAD (v3.1)	TOPMED
Bolkier et al. 2022	19:12973461(GRCh38) 19:13084275(GRCh37)	T	TCT	c.396_397dup	p.Tyr133SerfsTer11	Likely pathogenic <sup>b</sup>	0.000007954 (2 hets, 0 hom)	0.00001972 (3 hets, 0 hom)	0.00001889 (5 hets, 0 hom)
This study	19:12969856(GRCh38) 19:13080670 (GRCh37) <sup>a</sup>	CT	C	c.197del	p.Leu66ArgfsTer22	VUS in GUS	Absent	Absent	Absent

The Refseq transcript used for annotation is NM\_152654.2.

(VUS) Variant of uncertain significance, (GUS) gene of uncertain significance, (het) heterozygotes, (hom) homozygotes, (VAF) variant allele fraction.

<sup>a</sup>VAF: 0.92, 62/67 total reads.

<sup>b</sup>Bolkier et al. 2022.

NODAL binding interaction and subsequently the inhibition of NODAL. In order to test this hypothesis, we used a Nodal-dependent luciferase assay in HEK293T cells by transfecting expression vectors containing either the human *DAND5* wild-type cDNA (*DAND5*WT) or the cDNA with c.197del variant (*DAND5*\_197del) (Cristo et al. 2017a). Other expression vectors that were transfected included pAR3-lux, which contains three copies of the activin response element (ARE) upstream of a luciferase reporter and is specifically activated by nodal signaling, NODAL, CRIPTO, which is a Nodal coreceptor necessary for the proper activation of nodal signaling, and FOXH1, the major transcriptional transducer of nodal signaling (Yan et al. 2002). Overexpression of the c.197del variant was unable to inhibit nodal and resulted in a complete loss of function when compared with the wild-type protein (Fig. 1E).

*DAND5* encodes a member of the Cerberus/DAN subfamily; in mouse and chicken models, *Dand5* was shown to bind Nodal and inhibit the Nodal signaling pathway essential for left to right patterning (Marques et al. 2004; Tavares et al. 2007). The majority of *Cer12* (*DAND5* ortholog) knockout mice were found to have heterotaxy and left ventricular cardiac hyperplasia; 35% had a severe form that resulted in neonatal death (Marques et al. 2004; Araújo et al. 2014).

During early embryogenesis, the left–right asymmetry is established by the unidirectional leftward flow of extracellular fluids across the ciliated epithelium of the left–right organizer (LRO). Ion channels PKD2 and PKD1L1 function in the sensing of this flow resulting in the reduction of *Dand5* mRNA on the left side, which in turn relieves the repression of Nodal. Recently, *DAND5*, along with *PKD1L1* [MIM 609721], *MMP21* [MIM 608416], *C1orf127* [MIM 619700], and a novel gene *CIROP* [MIM 619703], were shown to be an essential genetic module to establish left–right asymmetry in humans (Szenker-Ravi et al. 2022). Additionally it was shown that *Cirop* is needed for the asymmetric *Dand5* expression. Consistent with their central role in left–right patterning, biallelic loss-of-function (LoF) variants in *PKD1L1*, *MMP21*, and *CIROP* cause autosomal recessive heterotaxy in humans.

To date, *DAND5* does not have an established human Mendelian disease association. Cristo et al. (2017a,b) reported a heterozygous variant (p.Arg152His) in two individuals with laterality defects. However, currently, this heterozygous variant is seen at a relatively higher allele frequency along with several homozygotes in the gnomADv2 and v3 population databases (v2, allele frequency = 0.01145, 3239/282870 alleles, 37 homozygotes; v3, allele frequency = 0.01064, 1618/152134 alleles, 14 homozygotes). Additionally, the *DAND5* gene is not constrained for LoF and missense variants in the human population (gnomADv2, pLi = 0, missense Z = –0.36). Given these findings, currently the evidence for autosomal dominant heterotaxy caused by *DAND5* haploinsufficiency or heterozygous LoF variants is limited. For the two previously reported affected individuals with p.Arg152His variant (Cristo et al. 2017a,b), the possibility of an undetected causative variant in another heterotaxy gene cannot be excluded.

At the Genetics Clinic, this family was lost to follow-up. Hence, the limitations of this study include the lack of genotypes for the parents, three reported healthy siblings, and their unavailability for thorough phenotypic evaluation. The parents who are potential heterozygotes for the *DAND5* LoF variant were also reported to be normal, although they did not undergo imaging studies to rule out situs defects or evaluation for subclinical manifestations. Furthermore, for the proband, details on the disease course and updated current phenotypic features are missing.

## SUMMARY

Recently, a homozygous frameshift variant in *DAND5* (NM\_152654.2, c.396\_397dup [p.Tyr133SerfsTer11]) was reported in an individual with heterotaxy and congenital heart defect (Bolkier et al. 2022). Here, with an additional case of a homozygous frameshift variant,

we add to the genetic and functional evidence for biallelic LoF variants in *DAND5* causing an autosomal recessive heterotaxy syndrome.

## ADDITIONAL INFORMATION

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### Database Deposition and Access

The variant has been submitted to the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar>) under accession number VCV001704287.1.

### Ethics Statement

This study was approved by the Columbia University Institutional Review Board (protocol no.: AAAR1159). Written informed consent was obtained from the family for clinical exome sequencing.

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### Author Contributions

M.G. and C.M.B. analyzed and interpreted the data and drafted and critically reviewed the manuscript. P.A., K.W., C.R.-S., and A.I. provided the clinical data and critically reviewed the manuscript. F.C., J.M.I., and J.A.B. performed the experiments and drafted and critically reviewed the manuscript. V.J. conceived the study, interpreted the data, and critically reviewed the manuscript.

### Competing Interest Statement

The authors have declared no competing interest.

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### Referees

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## REFERENCES

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- Araújo AC, Marques S, Belo JA, 2014. Targeted inactivation of Cerberus like-2 leads to left ventricular cardiac hyperplasia and systolic dysfunction in the mouse. *PLoS ONE* **9**: e102716. doi:10.1371/journal.pone.0102716
- Bolkier Y, Barel O, Marek-Yagel D, Atias-Varon D, Kagan M, Vardi A, Mishali D, Katz U, Salem Y, Tirosh-Wagner T, et al. 2022. Whole-exome sequencing reveals a monogenic cause in 56% of individuals with laterality disorders and associated congenital heart defects. *J Med Genet* **59**: 691–696. doi:10.1136/jmedgenet-2021-107775
- Cristo F, Inácio JM, de Almeida S, Mendes P, Martins DS, Maio J, Anjos R, Belo JA. 2017a. Functional study of DAND5 variant in patients with congenital heart disease and laterality defects. *BMC Med Genet* **18**: 77. doi:10.1186/s12881-017-0444-1
- Cristo F, Inácio JM, Rosas G, Carreira IM, Melo JB, de Almeida LP, Mendes P, Martins DS, Maio J, Anjos R, et al. 2017b. Generation of human iPSC line from a patient with laterality defects and associated congenital heart anomalies carrying a *DAND5* missense alteration. *Stem Cell Res* **25**: 152–156. doi:10.1016/j.scr.2017.10.019
- Ganapathi M, Thomas-Wilson A, Buchovecky C, Dharmadhikari A, Barua S, Lee W, Ruan MZC, Soucy M, Ragi S, Tanaka J, et al. 2022. Clinical exome sequencing for inherited retinal degenerations at a tertiary care center. *Sci Rep* **12**: 9358. doi:10.1038/s41598-022-13026-2

- Marques S, Borges AC, Silva AC, Freitas S, Cordenonsi M, Belo JA. 2004. The activity of the Nodal antagonist *Cerl-2* in the mouse node is required for correct L/R body axis. *Genes Dev* **18**: 2342–2347. doi:10.1101/gad.306504
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17**: 405–424. doi:10.1038/gim.2015.30
- Szenker-Ravi E, Ott T, Khatoo M, de Bellaing A M, Goh WX, Chong YL, Beckers A, Kannesan D, Louvel G, Anujan P, et al. 2022. Discovery of a genetic module essential for assigning left–right asymmetry in humans and ancestral vertebrates. *Nat Genet* **54**: 62–72. doi:10.1038/s41588-021-00970-4
- Tavares AT, Andrade S, Silva AC, Belo JA. 2007. Cerberus is a feedback inhibitor of Nodal asymmetric signaling in the chick embryo. *Development* **134**: 2051–2060. doi:10.1242/dev.000901
- Wang Y, Lichter-Konecki U, Anyane-Yeboah K, Shaw JE, Lu JT, Östlund C, Shin JY, Clark LN, Gunderson GG, Nagy PL, et al. 2016. A mutation abolishing the ZMPSTE24 cleavage site in prelamin A causes a progeroid disorder. *J Cell Sci* **129**: 1975–1980. doi:10.1242/jcs.187302
- Yan YT, Liu JJ, Luo Y EC, Haltiwanger RS, Abate-Shen C, Shen MM. 2002. Dual roles of Cripto as a ligand and coreceptor in the nodal signaling pathway. *Mol Cell Biol* **22**: 4439–4449. doi:10.1128/MCB.22.13.4439-4449.2002



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<b>Supplementary Material</b>	<a href="http://molecularcasestudies.cshlp.org/content/suppl/2022/11/09/mcs.a006248.DC1">http://molecularcasestudies.cshlp.org/content/suppl/2022/11/09/mcs.a006248.DC1</a>
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