



The spectrum of heart defects in the *TRAF7*-related multiple congenital anomalies-intellectual disability syndrome

Elise Pisan^a, Chiara De Luca^b, Francesco Brancati^{b,c}, Rossana Sanchez Russo^d, Dong Li^{e,f,g}, Elizabeth Bhoj^{e,f,g}, Tara Wenger^h, Ashish Marwahaⁱ, Nicole Johnsonⁱ, Claire Beneteau^j, Elise Brischoux-Boucher^k, Gunnar Houge^l, Julie Paulsen^m, Trine Bjørg Hammer^{n,o}, Jakob Ek^o, Daniela Schweitzer^p, Bianca E. Russell^p, Marina Dutra-Clarke^p, Stanley Nelson^p, Emilie D. Douine^p, Rosario I. Corona^p, Tracy Dudding^q, Hannah Thomson^q, Karen Low^r, Newell Belnap^s, Maria Iascone^t, Manuela Priolo^u, Diana Carli^{v,w}, Alessandro Mussa^x, Emilia K. Bijlsma^y, Nathan Kopp^z, Jean-Philippe Jais^{aa}, Jeanne Amiel^{ab,bb,1}, and Christopher T. Gordon^{a,1}

Heterozygous missense variants in *TRAF7* lead to various cancers when somatic and a multiple congenital anomalies-intellectual disability syndrome (MCA-IDS) when germline (1, 2). Variants are predominantly within the WD40 repeats and recurrent, suggesting specific alterations to the protein as the pathomechanism, as opposed to haploinsufficiency. Mishra-Gorur et al. (3) report three novel *TRAF7* variants, p.Val142Met, p.Val442Met, and c.1998+2T>G, identified through analysis of exome data from 2,871 cases with congenital heart defects (CHDs). We disagree with several of the arguments put forward to support the pathogenicity of these variants. All were inherited from healthy parents, while non-penetrance of *TRAF7* variants has not been previously reported in the MCA-IDS. Although absent from ExAC, p.Val142Met is found in 4/192,850 alleles in gnomAD v2 (a larger control dataset), indicating that it is unlikely to be the cause of the severe CHD in the patient in whom it was identified. The authors cite the high missense Z score of *TRAF7* in ExAC as evidence that *TRAF7* is susceptible to haploinsufficiency and therefore that their essential splice site variant is pathogenic through loss-of-function. However, the gnomAD probability of being loss-of-function intolerant of *TRAF7* is 0.02, suggesting good tolerance to heterozygous loss-of-function variants in the general population, and arguing against c.1998+2T>G causing a CHD. One could propose that damaging missense variants in *TRAF7* might increase susceptibility to CHDs, however, based on the frequency of missense variants with CADD score ≥ 26 in gnomAD ($n = 42$ from exomes, 33 from genomes), we estimate that the number of missense variants with the same CADD criterion identified by Mishra-Gorur et al. (2/2,871) is not more than expected in an equivalent-sized sample of controls (methodology of probability determination available on request).

The *TRAF7*-related MCA-IDS is composed of several recurrent features, including a typical facial gestalt, with >50 patients published. Apart from one paper (1), Mishra-Gorur et al. do not cite other studies describing this syndrome prior to 2023 (2, 4–7), including the largest study (45 patients), from 2020 (2). We present 21 new *TRAF7*-related MCA-IDS patients, in whom phenotypes are consistent with those previously published (Table 1). The three patients described in Mishra-Gorur et al. do not have typical clinical associations of the *TRAF7* disorder, and their CHDs are variable; the patient with p.Val142Met has renal cysts and cardiac heterotaxy (suggestive of a ciliopathy), one has an isolated cardiopathy, and the patient with c.1998+2T>G has severe limb defects and truncus arteriosus (neither reported in *TRAF7*-related MCA-IDS). Based on the previous and new cohorts, we refine the

frequencies of cardiac defects in the *TRAF7* MCA-IDS (Table 2). Patent ductus arteriosus (PDA; not found in Mishra-Gorur et al.'s patients) is the most frequent feature, followed by septal and valvular defects.

Although cardiac looping was abnormal upon *traf7* knock-down in zebrafish and *Xenopus* in Mishra-Gorur et al., it was recently reported as normal in *Traf7* knock-out mice (9). These mice display midgestational death due to endothelial dysplasia, which is intriguing given the frequency of PDA (a vessel wall closure anomaly) in the *TRAF7* MCA-IDS.

Author affiliations: ^aLaboratory of embryology and genetics of human malformations, INSERM Unité Mixte de Recherche 1163, Institut Imagine and Université Paris Cité, Paris 75015, France; ^bHuman Genetics, Department of Life, Health and Environmental Sciences, University of L'Aquila, Coppito 67100, L'Aquila, Italy; ^cHuman Functional Genomics Laboratory, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Roma, Rome 00163, Italy; ^dDepartment of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322; ^eCenter for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA 19104; ^fDivision of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; ^gDepartment of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104; ^hDepartment of Pediatrics, School of Medicine, University of Washington, Seattle, WA 98105; ⁱAlberta Children's Hospital, University of Calgary, Calgary, AB T2N 4N1, Canada; ^jService de Génétique Médicale, Centre Hospitalo-Universitaire de Bordeaux, Bordeaux F-33000, France; ^kCentre de Génétique Humaine, Centre Hospitalo-Universitaire de Besançon, Besançon 25000, France; ^lDepartment of Medical Genetics, Haukeland University Hospital, Bergen 5021, Norway; ^mDepartment of Medical Genetics, St. Olavs Hospital, Trondheim University Hospital, Trondheim 7006, Norway; ⁿDepartment of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre, Dianalund 4293, Denmark; ^oDepartment of Genetics, Rigshospitalet, Copenhagen 2100, Denmark; ^pDepartment of Human Genetics, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA 90095; ^qGenetics of Learning Disability Service, Hunter Genetics, Waratah, NSW 2298, Australia; ^rDepartment of Clinical Genetics, St. Michaels Hospital, University Hospitals Bristol and Weston National Health Service Trust, Bristol BS2 8EJ, United Kingdom; ^sCenter for Rare Childhood Disorders, Translational Genomics Research Institute, Phoenix, AZ 85012; ^tMedical Genetics Laboratory, Azienda Socio-sanitaria Territoriale Papa Giovanni XXIII, Bergamo 24127, Italy; ^uUnit of Medical Genetics, Azienda Ospedaliera di Rilievo Nazionale Cardarelli, Naples 80131, Italy; ^vDepartment of Medical Sciences, University of Torino, Torino 10124, Italy; ^wImmunogenetics and Transplant Biology Unit, Città della Salute e della Scienza University Hospital, Torino 10126, Italy; ^xPediatric Clinical Genetics Unit, Ospedale Infantile Regina Margherita, Department of Public Health and Pediatric Sciences, University of Torino, Torino 10126, Italy; ^yDepartment of Clinical Genetics, Leiden University Medical Centre, 2300 RC Leiden, the Netherlands; ^zDepartment of Pathology, Medical College of Wisconsin, Milwaukee, WI 53223; ^{aa}Biostatistics Unit, Université Paris Cité, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris 75015, France; and ^{bb}Service de Médecine Génétique des Maladies Rares, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris 75015, France

Author contributions: E.P. collated patient data; C.D.L., F.B., R.S.R., D.L., E.B., T.W., A. Marwaha, N.J., C.B., E.B.-B., G.H., J.P., T.B.H., J.E., D.S., B.E.R., M.D.-C., S.N., E.D.D., R.I.C., T.D., H.T., K.L., N.B., M.I., M.P., D.C., A. Mussa, E.K.B., and N.K. provided clinical and genetic data; J.-P.J. performed statistical analysis; and J.A. and C.T.G. wrote the paper.

The authors declare no competing interest.

Copyright © 2024 the Author(s). Published by PNAS. This article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

¹To whom correspondence may be addressed. Email: jeanne.amiel@inserm.fr or chris.gordon@inserm.fr.

Published March 11, 2024.

Table 1. Clinical features of a novel cohort of 21 patients with the *TRAF7*-related MCA-ID syndrome

Patient number	1	2	3	4	5	6	7	8	9	10	11
TRAF7 variant (c.)	1013A>T	1111C>T	1288A>G	1288A>G	1564T>C	1564T>C	1570C>T	1603A>G	1673C>T	1717G>T	1873C>T
TRAF7 variant (p.)	D338V	R371W	K430E	K430E	W522R	W522R	R524W	S535G	S558F	V573F	L625F
Inheritance	dn	un	dn	dn	un	i5	dn	dn	dn	dn	un
Age at LC	8 y	5 y	13 y	17 y	46 y	14 y	11 m	19 m	21 y	21 y	43 y
Sex	M	M	M	M	M	M	M	F	M	M	M
Clinical feature											
ND abnormality	+	+	+	+	+	+	+	+	+	+	+
Hypotonia	+			+							
Anomalies on brain scan										+	
Abnormality of facial/skull shape	+						+		+		
Hypertelorism		+		+							+
Blepharo or SPF	+					+			+	+	+
Ptosis	+			+			+		+	+	
Micro- and/or retrognathia			+						+		
Abnormality of external ear	+						+		+		
Hearing impairment			+	+					+		
Abnormality of hands or feet		+	+	+	+	+	+		+	+	+
Scoliosis or kyphosis			+			+				+	
Short stature			+			+				+	
FD or FTT				+							
Cardiac anomalies											
Quadricuspid AV											
Bicuspid AV									+	+	
Coarc of aorta											
ASD											
PFO											
LSVC draining to CS											
PDA							+		+		
VSD									+		
Aortic aneurysm									+		
PAS											

Table 1. (Continued)

Patient number	12	13	14	15	16	17	18	19	20	21
TRAF7 variant (c.)	1873C>T	1873C>T	1958_1959 delinsTT	1964G>A	1964G>A	1964G>A	1964G>A	1964G>A	1964G>A	1964G>T
TRAF7 variant (p.)	L625F	L625F	R653L	R655Q	R655Q	R655Q	R655Q	R655Q	R655Q	R655L
Inheritance	i11	i11	dn	dn	dn	dn	dn	dn	dn	dn
Age at LC	8 y	4 y	7 y	9 y	24 y	10 y	18 m	9 y	18 m	14 m
Sex	M	F	F	F	F	F	M	F	F	F
Clinical feature										
ND abnormality	+	+	+	+	+	+	+	+	+	
Hypotonia			+			+		+	+	
Anomalies on brain scan			+	+	+	+		+	+	
Abnormality of facial/skull shape			+		+			+	+	+
Hypertelorism	+	+			+					+
Blepharo or SPF	+	+	+	+	+		+	+		+
Ptosis			+	+				+	+	
Micro- and/or retrognathia			+			+	+		+	
Abnormality of external ear			+	+	+					+
Hearing impairment			+	+		+		+		
Abnormality of hands or feet	+	+	+			+				+
Scoliosis or kyphosis					+	+			+	
Short stature	+				+	+		+		
FD or FTT			+			+	+	+		+
Cardiac anomalies										
Quadricuspid AV					+					
Bicuspid AV										
Coarc of aorta						+	+			
ASD			+			+		+		
PFO								+		+
LSVC draining to CS								+		
PDA	+		+	+			+	+	+	
VSD						+				+
Aortic aneurysm										
PAS									+	

All *TRAF7* variants were identified by diagnostic testing in independent centers by whole-exome or genome sequencing (or an intellectual disability panel in one case), and patients were recruited to the present study via Genematcher or direct contact with the senior authors. Consent was obtained from the families for genetic analysis and for publication of anonymous data. Due to space limitations, only the most frequent phenotypes (or phenotype categories) are listed here, apart from cardiac anomalies, which are listed in detail. Major neurodevelopmental abnormalities consisted of global developmental delay, intellectual disability, or specific learning disabilities. Major abnormalities of the extremities consisted of contractures and finger/toe deviations. A complete list of HPO terms describing each patient is available from the authors on request. Mutation nomenclature is relative to RefSeq transcript NM_032271.3. LC, last consultation; dn, de novo; un, unknown; i5, inherited from patient 5; i11, inherited from patient 11; ND, neurodevelopmental; blepharo, blepharophimosis; SPF, short palpebral fissures; FD, feeding difficulties; FTT, failure to thrive; AV, aortic valve; coarc, coarctation; ASD, atrial septal defect; PFO, patent foramen ovale; PDA, patent ductus arteriosus; LSVC, left superior vena cava; CS, coronary sinus; VSD, ventricular septal defect; PAS, pulmonary artery stenosis.

Table 2. Cardiac features in patients with the TRAF7-related MCA-ID syndrome

Reference	Tokita 2018 (N = 7)	Castill. 2020 (N = 42)	Acco. 2020 (N = 2)	Papro. 2021 (N = 2)	Chais. 2022 (N = 1)	Malin. 2022 (N = 1)	Colleran 2023 (N = 2)	Mish. 2023 (N = 3)	Present report (N = 21)
PDA (surgically repaired)	4 (2)	24 (10)	1	1	1		2		8 (6)
ASD	1	8		1			1	1	3
PFO		1						1	2
VSD	1	5	1			1			3
AV canal defect		1							
Bicuspid aortic valve	2	6							2
Quadricuspid aortic valve									1
Mitral valve anomalies	2	3							
SV pulmonary stenosis	1	1							
Pulmonary atresia/stenosis	1							2	1
Bicuspid tricuspid valve				1					
Tricuspid atresia								1	
DORV	2					1			
DOLV								1	
L-loop transposition								1	
Truncus arteriosus								1	
Aortic coarctation	1		1						2
Hypoplastic aortic arch		1							
Aortic aneurysm		1							1
Hypoplastic LV	2								
Hypoplastic RV								1	
Univentricle	1							1	
Normal	1	9							6
Unknown		1							3

Only anomalies affecting the four chambers and great arteries are listed. Full details of references can be found in the main text, plus: Colleran *et al* (8). Although Castilla-Vallmánya *et al.*, identified *TRAF7* variants in 45 patients, N = 42 refers to the core cohort of patients harboring variants in the WD40 repeats. PDA, patent ductus arteriosus; ASD, atrial septal defect; PFO, patent foramen ovale; VSD, ventricular septal defect; AV, atrioventricular; DORV, double-outlet right ventricle; DOLV, double-outlet left ventricle; SV, supra-ventricular; LV, left ventricle; RV, right ventricle.

ACKNOWLEDGMENTS. We sincerely thank the families for their participation. This work was supported by the Agence Nationale de la Recherche "Investissements d'Avenir" program (ANR-10-IAHU-01), MSD Avenir (Devo-Decode project), the Philanthropy Department of Mutuelles AXA through the Head and Heart Chair, the NIH National

Center for Advancing Translational Science UCLA Clinical and Translational Science Institute (Grant Number UL1TR001881), and the California Center for Rare Diseases within the Institute of Precision Health at UCLA. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

1. M. J. Tokita *et al.*, De novo missense variants in *TRAF7* cause developmental delay, congenital anomalies, and dysmorphic features. *Am. J. Hum. Genet.* **103**, 154-162 (2018).
2. L. Castilla-Vallmánya *et al.*, Phenotypic spectrum and transcriptomic profile associated with germline variants in *TRAF7*. *Genet. Med.* **22**, 1215-1226 (2020).
3. K. Mishra-Gorur *et al.*, Pleiotropic role of *TRAF7* in skull-base meningiomas and congenital heart disease. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2214997120 (2023).
4. A. Accogli *et al.*, Sinus pericranii, skull defects, and structural brain anomalies in *TRAF7*-related disorder. *Birth Defects Res.* **112**, 1085-1092 (2020).
5. J. Paprocka *et al.*, Case report: Blepharophimosis and ptosis as leading dysmorphic features of rare congenital malformation syndrome with developmental delay—new cases with *TRAF7* variants. *Front. Med. (Lausanne)* **8**, 708717 (2021).
6. S. Chaisrisawadisuk, A. Taranath, J. Azzopardi, M. H. Moore, Multi-suture craniosynostosis in c.1570C>T (p.Arg524Trp) mutated *TRAF7*: A case report. *Childs Nerv. Syst.* **38**, 843-846 (2022).
7. A. Malinowski, E. A. Elsamadicy, S. Turan, Prenatal diagnosis of a germline variant in *TRAF7*: Importance of accessibility to prenatal exome sequencing in cases of structural fetal anomalies. *Clin. Genet.* **102**, 164-165 (2022).
8. J. A. Colleran, E. C. Daykin, C. Hernandez, J. Ray, M. Morand, Novel mosaic *TRAF7* likely pathogenic variant in an African American family. *Am. J. Med. Genet. A* **191**, 1990-1993 (2023).
9. E. N. Tsitsikov *et al.*, *TRAF7* is an essential regulator of blood vessel integrity during mouse embryonic and neonatal development. *iScience* **26**, 107474 (2023).