Received: 8 August 2017 | Revised: 22 November 2017 | Accepted: 22 December 2017

DOI: 10.1111/chd.12578

ORIGINAL ARTICLE

WILEY Congenital Heart Disease

Analysis of *DICER1* in familial and sporadic cases of transposition of the great arteries

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Funding information
This work was funded by Alex's Lemonade Stand Foundation: https://www.alexslemonade.org/

Abstract

Objective: We previously identified a pathogenic germline *DICER1* variant in a child with transposition of the great arteries who was a member of a family with DICER1 syndrome. In view of a report linking *Dicer1* knockout in murine cardiomyocytes to cardiac outflow defects, we investigated the involvement of DICER1 in transposition of the great arteries.

Design: We used Fluidigm access array followed by next generation sequencing to screen for variants in the coding exons, their exon/intron boundaries and the 3′ untranslated region of *DICER1* in patient DNA.

Cases: Germline DNA was collected from 129 patients with either sporadic or familial forms of transposition of the great arteries from two sites in Australia and Italy.

Results: Most cases (85%) did not have any germline *DICER1* variants. In the remaining 15% of cases, we identified 16 previously reported variants (5 synonymous, 6 intronic, and 5 missense) and 2 novel variants (1 intronic and 1 missense). None of the identified variants were predicted to be pathogenic.

Conclusions: Here, we report that neither likely pathogenic nor pathogenic variants in *DICER1* appear to play a major role in transposition of the great arteries.

KEYWORDS
DICER1, fluidigm, next-generation sequencing, transposition of the great arteries

1 | INTRODUCTION

DICER1 is an endoribonuclease that plays an essential role in modulating the expression of genes by producing mature microRNAs (miRNA), which are small, single stranded RNA molecules that bind to and thereby inhibit target mRNAs. *DICER1*-related diseases are referred to collectively as DICER1 syndrome and result from germline pathogenic and likely pathogenic variants in individuals with rare childhood cancers such as: pleuropulmonary blastoma, cystic nephroma, Sertoli-Leydig cell tumor, embryonal rhabdomyosarcoma, and other rare tumors.[1] Several years ago, we identified a pathogenic germline *DICER1* variant (c.2117-1G > A, in intron 13 at the junction with exon 14, predicted to result in p.Gly706Aspfs*8) in a child with transposition of the great arteries (TGA), associated with a bicuspid pulmonary valve, an atrial septal defect and a patent ductus arteriosus.[1] Later, at the age of 18, he developed a solitary nodule in the left lobe of the thyroid gland. Two years later, he was found to have further nodules and cysts in the same lobe. Other heterozygotes for this pathogenic variant in the family also had phenotypes consistent with DICER1 syndrome.[1]

TGA is a cyanotic congenital heart defect (CHD) characterized by ventriculo-arterial discordance and represents 5% to 7% of CHD.[2] It is often accompanied by other structural changes that allow mixing of

Abbreviations: CHD, congenital heart defect; miRNA, microRNAs; TGA, Transposition of the great arteries.

oxygenated and deoxygenated blood; although there have been studies looking for the genetic causes of TGA, data so far have been inconclusive.[3] Saxena and Tabin previously reported cardiac outflow defects in mice with a conditional knock-out of Dicer in the developing murine heart.[4] In light of this, observation of this phenotype in a patient with a pathogenic variant in *DICER1* prompted us to screen for *DICER1* variants in additional familial and sporadic cases with TGA.

2 | METHODS

We screened 129 germline DNA samples from children with sporadic (n = 91) or familial (n = 38) forms of TGA for *DICER1* variants using targeted capture with a Fluidigm access array followed by next-generation sequencing and confirmatory Sanger sequencing.[5]

Variants were visualized against read alignments using Integrative Genomic Viewer (IGV)$_6$ version 2.3 (Broad Institute, Cambridge, Massachusetts). The mean and median coverage calculations were done using GATK DepthOfCoverage (Broad Institute).[7]

Eighty-two cases were referred to our study from The Heart Centre for Children, The Children's Hospital at Westmead, Westmead, Australia, and forty-seven from the Department of Medical Genetics, Bambino Gesù Pediatric Hospital, Rome, Italy.

Details of the cases studied are shown in Tables 1–3. All patients signed an Institutional Review Board-approved consent form to participate in the study.

3 | RESULTS

No *DICER1* variants were detected in 110 cases (85%), of which 77 cases were sporadic and 33 cases were familial. Seventy-one of these sporadic TGA cases had malformation of outflow tracts and 6 had a functional single ventricle (Table 1). Of the 33 familial cases, 22 had malformation of outflow tracts including 3 pairs of cousins where one of the cousins had malformation of outflow tracts and the other had either tetralogy of Fallot, or atrial septal defect or ventricular septal defect, respectively; 6 had one functional ventricle, and 3 had heterotaxy (Table 2). Nineteen individuals carried one or more variants in *DICER1* including 5 synonymous, 7 intronic and 6 missense variants. A list of the individual variants and the associated physical manifestation for each carrier, where known, is presented in Table 3. As was the case for individuals with no variants detected, most cases presented with malformation of outflow tracts. All samples were sequenced to at least 1000x median coverage (except one sample at 644x), and 90% of the samples at 2000x median coverage. The scaled CADD[8] and REVEL[9] scores are listed in Table 3.

TABLE 1 *DICER1* negative sporadic[a] cases of TGA, n = 77

TGA clinical info	No. of cases	Extra-cardiac features	Genetic tests (other than *DICER1* sequencing)
Functional single ventricle n = 6	1	Asthma	None
	1	Asplenia	Normal karyotype and FISH[b]
	4	None[c]	None
Malformatin of outflow tracts n = 71	19	None[c]	None
	1	None[c]	Normal karyotype
	1	None[c]	Normal karyotype
	3	None[c]	Normal karyotype and FISH[d]
	1	Heterozygous for cystic fibrosis	None
	1	Congenital talipes equinovarus, divergent strabismus	None
	1	Capillary hemangioma on left neck and face	None
	1	Hip dysplasia	None
	1	Delay in gross motor skills	None
	1	Tongue tie, asthma, autosomal dominant polycystic kidney disease, mild speech delay	None
	1	Congenital asplenia, gut malrotation, bowel obstruction secondary to adhesions	Agilent (Santa Clara, California) SurePrint G3 ISCA Targeted Microarray. No abnormality
	1	Delayed receptive and expressive language skills	Balanced reciprocal translocation involving chr 3 and 6. No FISH abnormality[d]
	1	Speech delay	Normal karyotype and FISH[d]
	1	Possible liver mass (intrahepatic)	Normal karyotype, normal FISH at the TUPLE1 (VCFS) locus
	1	Subarachnoid hemorrhage on left hemisphere with small right parietal white matter infarction.	Normal karyotype and FISH[d]
	1	Intermittent right eye exotropia	Normal karyotype and FISH[b]
	35	Not known	Not known

Abbreviations: FISH, fluorescent in situ hybridization; HIRA, histone regulator A; ISCA, International Standards for Cytogenomics Arrays; TBX1, T-Box1; TUPLE1, TUP1-like enhancer of split protein; VCFS, velocardiofacial syndrome; 22q11.2, long arm of chromosome 22.

[a]Sporadic TGA was defined as any individual without any family history of CHD.

[b]At the HIRA (VYSIS,[e] TUPLE1) locus.

[c]TGA cases with no extra-cardiac features could be referred to as isolated TGA.

[d]At the HIRA (VYSIS, TUPLE1) or TBX1 (22q11.2) loci.

[e]Abbott Molecular, Des Plaines, Illinois.

TABLE 2 *DICER1* negative familial[a] cases of TGA, n = 33

TGA clinical info	No. of cases	Extra-cardiac features	Genetic tests (other than *DICER1* sequencing)
Functional single ventricle n = 6	2	None[b]	None
	1	None[b]	Normal karyotype, normal FISH[c]
	1	Annular pancreas, duodenal atresia	Normal karyotype
	1	Functional asplenia	None
	1	Cerebral palsy, jejunal, and sigmoid atresia, microcephaly	None
Heterotaxy n = 3	1	Central liver with left sided stomach and spleen. Malposition of the superior mesenteric vein.	None
	1	Hip dysplasia, congenital asplenia, duodenal atresia, tracheal stenosis	None
	1	Congenital asplenia, situs ambigous, midline liver, right sided stomach	Normal karyotype, normal FISH[c] Agilent SurePrint G3 Targeted Microarray—normal
Malformation of outflow tracts n = 22	7	None[b]	None
	1	None[b]	Normal karyotype, normal FISH at the HIRA (VCFS) locus
	1	Delayed speech development	None
	1	Delay in receptive and expressive language skills	None
	1	Delayed expressive language at 2 years	None
	1	Multiple intestinal atresia, situs inversus	Normal karyotype
	1	Hypoxic ischemic encephalopathy secondary to shoulder dystocia, global developmental delay	Normal karyotype, normal FISH at the TUPLE1 (VCFS) locus
	1	Mild encephalopathy resulting from early acidosis and hypoxemia	Normal karyotype, Normal FISH at the HIRA (VYSIS, TUPLE1) locus
	1	None[b]	Agilent aCGH 60K targeted array—normal
	3	None[b]	Normal karyotype, normal FISH[c]
	1	Iron deficiency anemia	none
	3[def]	NA	NA
Tetralogy of Fallot	1[e]	NA	NA
Atrial septal defect	1[f]	NA	NA

Abbreviations: aCGH, array comparative genomic hybridization; FISH, fluorescent in situ hybridization; HIRA, histone regulator A; NA, not available; TBX1, T-Box1; TUPLE1, TUP1-like enhancer of split protein1; VCFS, velocardiofacial syndrome; 22q11.2, long arm of chromosome 22.

[a]Familial TGA was defined as any individual with immediate or extended family history of CHD.

[b]TGA cases with no extra-cardiac features could be referred to as isolated TGA.

[c]At the HIRA (VYSIS, TUPLE1) or TBX1 (22q11.2) loci.

[d], [e], and [f] are 3 pairs of cousins ([d]one cousin with malformation of outflow tracts and the other cousin with ventricular septal defects [included in Table 3]).

We listed the extra-cardiac features in our TGA cases and the genetic testing done, where available, in Tables 1–3.

4 | DISCUSSION

The full spectrum of phenotypes associated with pathogenic germline variants in *DICER1* is still being defined, and newly associated phenotypes such as pituitary blastoma[5] and macrocephaly[10] are still emerging. As such, it is important to fully explore all possible associations. Here, no likely pathogenic or pathogenic variants in *DICER1* were observed among 129 patients affected by TGA. Extra-cardiac malformations are common in patients with congenital heart disease.[11] Furthermore, 16.5% of total cardiovascular MRI studies in children (145 studies) have noncardiovascular findings, thus proving one estimate of the prevalence of comorbid conditions in pediatric cardiovascular disease.[12] We did not detect any evidence of the presence of DICER1 syndrome in the TGA patients (Tables 1–3).

Two of the variants observed (Table 3), c.307 + 13T > C and c.4886C > T, were novel intronic and missense variants, respectively. The c.4886C > T variant is predicted to result in a protein with an amino acid change from serine to leucine, (p.S1629L) at position 1629. The algorithms SIFT (Sorting Intolerant from Tolerant)[13] and Polyphen 2 (Polymorphism Phenotyping-2)[14] predicted this variant to be "tolerated and benign," respectively. The G93E variant along with the missense at codon Y1835S and T60I in the transcript are all classified as variants of unknown significance (VUS_{15}). The variants R1599Q and C1153Y are both predicted to be tolerated by SIFT and benign by Polyphen 2, their REVEL and CADD scaled scores are lower than 0.75 and 30, respectively. S1629L is a novel variant that is tolerated by SIFT and benign by Polyphen 2, respectively. Most predictions for the missense variants varied from possibly damaging to benign by Polyphen 2, but all variants identified were predicted to be tolerated by SIFT (Table 3). For the missense variants we considered REVEL scores lower than 0.75 and CADD scaled scores lower than 30 to be likely benign.[16] In our

TABLE 3 *DICER1* variants in TGA study

Variant		Clinical information/genetic tests			Predictions				MAF
Missense n = 6	N	TGA (sporadic/familial)	Extra-cardiac features	Genetic tests	REVEL	CADD (scaled)	SIFT	Polyphen 2	ExAC (MAF/count)
c.1278A > G p.E426E rs878855242***	1[a]	Malformation of outflow tracts (sporadic)	None[b]	None	NA	0.352	NA	NA	Not found
c.1935G > A p.P645P rs61751177	4	Ventricular septal defect[c] (familial, n = 1)	NA	NA	NA	16.49	NA	NA	0.0095/1148
		Malformation of outflow tracts (sporadic n = 3)	None[b]	None	NA				
c.2337A > G p.T779T rs747210633	1	Malformation of outflow tracts (familial)	None[b]	None	NA	2.59	NA	NA	0.00003/4
c.2718C > T p.R906R rs370692165	1	Malformation of outflow tracts (sporadic)	NA	NA	NA	NA	NA	NA	0.00008/10
c.5145C > T p.L1715L rs139500905	1	Malformation of outflow tracts (sporadic)	None[b]	None	NA	11.22	NA	NA	0.0015/179
Intronic n = 7									
c.307 + 13T > C	1[a]	Malformation of outflow tracts (sporadic)	None[b]	None	NA	NA	NA	NA	Novel
c.574-5G > A rs368253792	1	Malformation of outflow tracts (sporadic)	hypokalemia	None	NA	1.77	NA	NA	0.00006/7
c.1377-4T > G rs192490028	1	Heterotaxy (sporadic)	mild congenital tracheomalacia, congenital intestinal malrotation	None	NA	4.98	NA	NA	0.0033/401
c.1377-4T>G rs192490028	1	Malformation of outflow tracts (sporadic)	NA	NA	NA	4.98	NA	NA	0.0033/401
c.2040 + 29T > C rs370866625	1[d]	Malformation of outflow tracts (familial)	Seborrhoeic dermatitis, asthma	Translocation, normal FISH at HIRA (VYSIS, TUPLE1)[e]	NA	2.57	NA	NA	0.0002/19
c.3093 + 149_3093 + 153delGTTTT rs575610432	1	Malformation of outflow tracts (sporadic)	None[b]	None	NA	NA	NA	NA	0.0008/4***
c.5364 + 18C > T rs777415635	1	Malformation of outflow tracts (sporadic)	NA	NA	NA	1.16	NA	NA	0.00007/8
c.5527 + 19A > G rs765497219	1[d]	Malformation of outflow tracts (familial)	Seborrhoeic dermatitis, asthma	Translocation, normal FISH at HIRA (VYSIS, TUPLE1)[e]	NA	1.16	NA	NA	0.000008/1
Missense n = 6									
c.179C > T p.T60I rs587778228	1	Malformation of outflow tracts (familial)	None[b]	None	0.32	15.46	Tolerated	Benign	0.00005/6
c.278G > A p.G93E rs776219930	1	Malformation of outflow tracts (sporadic)	NA	NA	0.39	23.7	Tolerated	Possibly damaging	0.00003/4
c.3458G > A p.C1153Y rs762999390	1[d]	Malformation of outflow tracts (familial)	Seborrhoeic dermatitis, asthma	Translocation, normal FISH at HIRA (VYSIS, TUPLE1)[e]	0.085	7.8	Tolerated	Benign	0.000008/1
c.4796G > A p.R1599Q rs569615549	1	Malformation of outflow tracts (sporadic)	Early onset occipital epilepsy (Panayiotopoulos syndrome), speech delay	Normal karyotype, normal FISH at the HIRA (VYSIS, TUPLE1) or TBX1 (22q11.2) loci	0.046	20.8	Tolerated	Benign	0.0002/23

(Continues)

TABLE 3 (Continued)

Variant		Clinical information/genetic tests			Predictions				MAF
Missense n = 6	N	TGA (sporadic/familial)	Extra-cardiac features	Genetic tests	REVEL	CADD (scaled)	SIFT	Polyphen 2	ExAC (MAF/ count)
c.4886C > T p.S1629L	1	Malformation of outflow tracts (familial)	None[b]	None	NA	NA	Tolerated	Benign	novel
c.5504A > C p.Y1835S rs747510783	1	Malformation of outflow tracts (sporadic)	NA	NA	0.68	25.7	Tolerated	Possibly damaging	0.00006/7

Abbreviations: chr 4, chromosome 4; FISH, fluorescent in situ hybridization; HIRA, histone regulator A; MAF, minor allele frequency; NA, not available; TBX1, T-Box1; TUPLE1, TUP1-like enhancer of split protein1; VCFS, velocardiofacial syndrome; 22q11.2, long arm of chromosome 22.

***From 1000 Genomes.

[a]Individual with 2 variants.

[b]TGA cases with no extra-cardiac features could be referred to as isolated TGA.

[c]Cousin of an individual with malformation of outflow tracts (Table 2).

[d]Individual with 3 variants.

[e]FISH to 4q sub-telomere and Nucleolar Organising Region (NOR) show translocation of NOR to the long arm of chr4. Satellited chromosome regarded as heteromorphism with no expected clinical effect.

study, all missense variants found have a REVEL value lower than 0.75 and all CADD scaled scores are lower than 30 (Table 3). In addition, the ones with CADD scores above 15 had no other significant pathogenicity flags. Notably, we did not identify any variants predicted to result in a truncated protein. Thus far, most disease-associated germline pathogenic variants in *DICER1* are predicted to truncate the protein.[17] Our results suggest that TGA is not likely to be caused by *DICER1* pathogenic variants in humans and that occurrence of TGA in our patient carrying a pathogenic germline *DICER1* variant may have been coincidental.

We recognize that we may have missed deletions/duplications as we did not investigate our sample set for large duplications/deletions via multiplex ligation-dependent probe amplification (MLPA).

This study did not address the potential of finding deleterious variants in *DICER1* in other tissues (somatic mosaicism).[18]

5 | CONCLUSION

Here, we report that TGA does not appear to be part of the DICER1 syndrome. The genetics underlying predisposition to TGA remain enigmatic[2] and it is likely that whole genome approaches in a large series of cases will be required to identify causal variants and genetic modifiers.

ACKNOWLEDGMENTS

This work was funded by Alex's Lemonade Stand Foundation. We thank Dr. Catherine Choong, Perth, Australia, for drawing our attention to the possible connection between TGA and DICER1, and for providing updated clinical information on the patient in question. We also thank François Plourde, Montreal, for his help in coordinating reception of the DNA samples. We would like to thank Nancy Hamel and Mona Wu, Montreal, for their help reviewing the manuscript. We also thank Dr. Jiannis Ragoussis, McGill University and Genome Quebec Innovation Centre, Montreal, for his assistance with the bioinformatics analyses.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

The manuscript was reviewed and edited by all authors, who commented on and approved the final version. All authors read and approved the final manuscript.

Nelly Sabbaghian Analyzed and validated the results and wrote the manuscript.

William D. Foulkes co-wrote the manuscript with Nelly Sabbaghian and oversaw the study.

Maria C. Digilio provided the samples, Gillian M. Blue, and David S. Winlaw provided the samples and the results of the additional genetic tests and extra-cardiac features.

Timothée Revil provided help with the bioinformatics analyses.

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How to cite this article: Sabbaghian N, Digilio MC, Blue GM, Revil T, Winlaw DS, Foulkes WD. Analysis of *DICER1* in familial and sporadic cases of transposition of the great arteries. *Congenital Heart Disease*. 2018;13:401–406. https://doi.org/10.1111/chd.12578