Analysis of *DICER1* in familial and sporadic cases of Transposition of the Great Arteries

Nelly Sabbaghian[1], M.Cristina Digilio[2], Gillian M Blue[3], David S.Winlaw[3], William D Foulkes[1,4]

[1]Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, Montreal, Quebec, Canada

[2]Department of Medical Genetics, Bambino Gesù Pediatric Hospital, Rome, Italy

[3]Heart Centre for Children, The Children's Hospital at Westmead, Westmead, New South Wales 2145, and University of Sydney, Australia

[4]Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Quebec, Canada

Nelly Sabbaghian: nelly.sabbaghian@mail.mcgill.ca

M.Cristina Digilio: mcristina.digilio@opbg.net

Gillian M Blue: gillian.blue@health.nsw.gov.au

David S.Winlaw: david.winlaw@health.nsw.gov.au

Corresponding author is Dr William D Foulkes: william.foulkes@mcgill.ca

Abstract 148 words

Background: *DICER1* plays a major role in development and in generating mature microRNAs that are important in gene expression. We screened for *DICER1* mutations in a family with DICER1 syndrome and we discovered a pathogenic mutation in a child with transposition of the great arteries (TGA). In view of a report linking *DICER1* knock-out in murine cardiomyocytes to cardiac outflow defects, we investigated the involvement of DICER1 in TGA.

Findings: We screened 129 germline DNA samples from children with either sporadic or familial forms of TGA for *DICER1* mutations using a Fluidigm access array, followed by next-generation sequencing. We identified 16 previously reported variants (5 synonymous, 6 intronic, and 5 missense) and 2 novel variants (1 intronic and 1 missense). We did not find any apparent pathological mutation in our cohort.

Conclusion: Here we report that *DICER1* mutations do not appear to play a major role in TGA.

Keywords

TGA, DICER1

Findings

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 mutations 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 deleterious germline *DICER1* mutation (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 [2]]. 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 mutation-carrying persons in the family also had phenotypes consistent with the DICER1syndrome [2]. Saxena and Tabin had reported cardiac outflow defects in mice with a conditional knock-out of Dicer in the developing murine heart [3]. These two observations prompted us to screen for *DICER1* mutations in familial and sporadic cases with TGA. TGA is a cyanotic congenital heart defect (CHD) characterized by ventriculo-arterial discordance and represents 5 to 7% of CHD [4]. It is often accompanied by other structural changes that allow mixing of oxygenated and de-oxygenated blood, although there have been studies looking for the genetic causes of TGA, data so far have been inconclusive[5] .

We screened 129 germline DNA samples from children with sporadic (n = 91) or familial (n = 38) forms of TGA for *DICER1* mutations using a Fluidigm access array, followed by next-generation sequencing and confirmatory Sanger sequencing [6]. Eighty-two cases were from Australia and 47 were from Italy. Details of the cases studied are shown in Supplementary Table 1. All patients signed an IRB-approved consent form.

No *DICER1* variants were detected in 110 cases. Nineteen individuals had one or more variants for a total of 5 synonymous, 7 intronic and 6 missense variants (Supplementary Table 2). c.307+13T>C and c.4886C>T are novel intronic and missense variants, respectively. c.4886C>T results in a protein with an amino acid change at position 1629, from serine to proline, (p.S1629L). SIFT (Sorting Intolerant from Tolerant)[7] and Polyphen 2 (Polymorphism Phenotyping-2)[8] predicted this variant to be “tolerated and benign”, respectively. Predictions for the other missense variants varied from possibly damaging to benign by Polyphen 2, but all variants identified were predicted to be tolerated by SIFT (supplementary table 2). No definitively damaging mutations in *DICER1* were found. In particular, we did not identify any mutations predicted to result in a truncated protein. Thus far, most disease-associated germline mutations in *DICER1* are predicted to truncate the protein [1].

This study suggests that TGA is not caused by *DICER1* mutations in humans. The full spectrum of phenotypes associated with *DICER1* mutations is still being defined, and newly-associated phenotypes such as pituitary blastoma[6] and macrocephaly [9] are still emerging. As such, it is important to fully explore all possible associations. Here we report that TGA does not appear to be part of the DICER1 syndrome. The genetics of TGA remain enigmatic [4] and it is likely that whole genome approaches in a large series of cases will be required to identify causal variants and genetic modifiers.

List of abbreviations

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

Declarations

Ethics approval and consent to participate

All patients signed an IRB-approved consent form to participate in the study.

Consent for publication

Not Applicable.

Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NS analyzed and validated the results, and wrote the manuscript. WDF wrote the manuscript with NS, and oversaw the study. MCD, GMB, and DSW provided the samples. 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.

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Supplementary Table 1: *DICER1* screening results in 47 Italian and 82 Australian germline DNA samples with clinical information.

Case ID	Variant	Status	Clinical Info	Country of Origin
1	negative	Sporadic	Malformation of outflow tracts	Italy
2	negative	Sporadic	Malformation of outflow tracts	Italy
3	negative	Sporadic	Malformation of outflow tracts	Italy
4	negative	Sporadic	Malformation of outflow tracts	Italy
5	negative	Sporadic	Malformation of outflow tracts	Italy
6	negative	Sporadic	Malformation of outflow tracts	Italy
7	negative	Sporadic	Malformation of outflow tracts	Italy
8	negative	Sporadic	Malformation of outflow tracts	Italy
9	c.5504A>C p.Y1835S rs747510783	Sporadic	Malformation of outflow tracts	Italy
10	negative	Sporadic	Malformation of outflow tracts	Italy
11	negative	Sporadic	Malformation of outflow tracts	Italy
12	negative	Sporadic	Malformation of outflow tracts	Italy
13	negative	Sporadic	Malformation of outflow tracts	Italy
14	c.1935G>A p.P645P rs61751177	Sporadic	Malformation of outflow tracts	Italy
15	c.278G>A p.G93E rs776219930	Sporadic	Malformation of outflow tracts	Italy
16	negative	Sporadic	Malformation of outflow tracts	Italy
17	c.5364+18C>T rs777415635	Sporadic	Malformation of outflow tracts	Italy
18	negative	Sporadic	Malformation of outflow tracts	Italy
19	negative	Sporadic	Malformation of outflow tracts	Italy
20	negative	Sporadic	Malformation of outflow tracts	Italy
21	c.1377-4T>G rs192490028	Sporadic	Malformation of outflow tracts	Italy
22	negative	Sporadic	Malformation of outflow tracts	Italy
23	negative	Sporadic	Malformation of outflow tracts	Italy
24	negative	Sporadic	Malformation of outflow tracts	Italy
25	negative	Sporadic	Malformation of outflow tracts	Italy
26	c.2718C>T p.R906R rs370692165	Sporadic	Malformation of outflow tracts	Italy
27	negative	Sporadic	Malformation of outflow tracts	Italy
28	negative	Sporadic	Malformation of outflow tracts	Italy
29	negative	Sporadic	Malformation of outflow tracts	Italy
30	negative	Sporadic	Malformation of outflow tracts	Italy
31	negative	Sporadic	Malformation of outflow tracts	Italy
32	negative	Sporadic	Malformation of outflow tracts	Italy
33	negative	Sporadic	Malformation of outflow tracts	Italy
34	negative	Sporadic	Malformation of outflow tracts	Italy
35	negative	Sporadic	Malformation of outflow tracts	Italy
36	negative	Sporadic	Malformation of outflow tracts	Italy
37	negative	Sporadic	Malformation of outflow tracts	Italy
38	negative	Sporadic	Malformation of outflow tracts	Italy
39	negative	Sporadic	Malformation of outflow tracts	Italy
40	negative	Sporadic	Malformation of outflow tracts	Italy
41	negative	Sporadic	Malformation of outflow tracts	Italy
42	negative	Familial	Malformation of outflow tracts*	Italy
43	c.1935G>A p.P645P rs61751177	Familial	Ventricular Septal Defect*	Italy
44	negative	Familial	Malformation of outflow tracts#	Italy
45	negative	Familial	Tetralogy of Fallot#	Italy
46	negative	Familial	Malformation of outflow tracts^	Italy
47	negative	Familial	Atrial Septal Defect^	Italy

48	negative	Sporadic	Malformation of outflow tracts	Australia
49	c.5145C>T p.L1715L rs139500905	Sporadic	Malformation of outflow tracts	Australia
50	negative	Sporadic	Malformation of outflow tracts	Australia
51	negative	Sporadic	Malformation of outflow tracts	Australia
52	negative	Sporadic	Malformation of outflow tracts	Australia
53	negative	Sporadic	Functional single ventricle	Australia
54	negative	Sporadic	Malformation of outflow tracts	Australia
55	negative	Sporadic	Malformation of outflow tracts	Australia
56	negative	Sporadic	Functional single ventricle	Australia
57	negative	Sporadic	Malformation of outflow tracts	Australia
58	c.574-5G>A rs368253792	Sporadic	Malformation of outflow tracts	Australia
59	negative	Familial	Malformation of outflow tracts	Australia
60	negative	Familial	Functional single ventricle	Australia
61	c.1935G>A p.P645P rs61751177	Sporadic	Malformation of outflow tracts	Australia
62	c.179C>T p.T60I rs587778228	Familial	Malformation of outflow tracts	Australia
63	negative	Familial	Functional single ventricle	Australia
64	negative	Sporadic	Functional single ventricle	Australia
65	c.1377-4T>G rs192490028	Sporadic	Heterotaxy	Australia
66	negative	Familial	Functional single ventricle	Australia
67	negative	Sporadic	Functional single ventricle	Australia
68	negative	Sporadic	Functional single ventricle	Australia
69	negative	Familial	Malformation of outflow tracts	Australia
70	negative	Sporadic	Malformation of outflow tracts	Australia
71	negative	Sporadic	Malformation of outflow tracts	Australia
72	negative	Familial	Malformation of outflow tracts	Australia
73	negative	Sporadic	Malformation of outflow tracts	Australia
74	negative	Familial	Malformation of outflow tracts	Australia
75	negative	Familial	Malformation of outflow tracts	Australia
76	negative	Familial	Malformation of outflow tracts	Australia
77	negative	Familial	Malformation of outflow tracts	Australia
78	negative	Sporadic	Malformation of outflow tracts	Australia
79	negative	Familial	Malformation of outflow tracts	Australia
80	negative	Familial	Malformation of outflow tracts	Australia
81	negative	Sporadic	Malformation of outflow tracts	Australia
82	c.2337A>G p.T779T rs747210633	Familial	Malformation of outflow tracts	Australia
83	c.2040+29T>C rs370866625 c.3458G>A p.C1153Y rs762999390 c.5527+19A>G rs765497219	Familial	Malformation of outflow tracts	Australia
84	negative	Familial	Heterotaxy	Australia
85	negative	Familial	Malformation of outflow tracts	Australia
86	negative	Sporadic	Malformation of outflow tracts	Australia
87	negative	Sporadic	Malformation of outflow tracts	Australia
88	negative	Familial	Functional single ventricle	Australia
89	negative	Sporadic	Malformation of outflow tracts	Australia
90	negative	Sporadic	Malformation of outflow tracts	Australia
91	c.3093+149_3093+153delGTTTT rs575610432	Sporadic	Malformation of outflow tracts	Australia
92	negative	Familial	Malformation of outflow tracts	Australia
93	negative	Sporadic	Functional single ventricle	Australia
94	negative	Sporadic	Malformation of outflow tracts	Australia
95	c.1935G>A p.P645P rs61751177	Sporadic	Malformation of outflow tracts	Australia
96	negative	Sporadic	Malformation of outflow tracts	Australia
97	negative	Familial	Malformation of outflow tracts	Australia
98	negative	Sporadic	Malformation of outflow tracts	Australia

99	negative	Sporadic	Malformation of outflow tracts	Australia
100	negative	Familial	Functional single ventricle	Australia
101	negative	Sporadic	Malformation of outflow tracts	Australia
102	negative	Sporadic	Malformation of outflow tracts	Australia
103	negative	Familial	Malformation of outflow tracts	Australia
104	negative	Sporadic	Malformation of outflow tracts	Australia
105	negative	Familial	Functional single ventricle	Australia
106	negative	Sporadic	Malformation of outflow tracts	Australia
107	negative	Sporadic	Malformation of outflow tracts	Australia
108	negative	Familial	Malformation of outflow tracts	Australia
109	negative	Sporadic	Malformation of outflow tracts	Australia
110	negative	Familial	Malformation of outflow tracts	Australia
111	negative	Familial	Heterotaxy	Australia
112	c.4796G>A p.R1599Q rs569615549	Sporadic	Malformation of outflow tracts	Australia
113	negative	Familial	Heterotaxy	Australia
114	negative	Sporadic	Malformation of outflow tracts	Australia
115	c.307+13T>C c.1278A>G p.E426E rs878855242	Sporadic	Malformation of outflow tracts	Australia
116	negative	Sporadic	Malformation of outflow tracts	Australia
117	negative	Sporadic	Malformation of outflow tracts	Australia
118	negative	Sporadic	Malformation of outflow tracts	Australia
119	negative	Familial	Malformation of outflow tracts	Australia
120	negative	Sporadic	Malformation of outflow tracts	Australia
121	negative	Sporadic	Malformation of outflow tracts	Australia
122	c.4886C>T p.S1629L	Familial	Malformation of outflow tracts	Australia
123	negative	Sporadic	Malformation of outflow tracts	Australia
124	negative	Familial	Malformation of outflow tracts	Australia
125	negative	Familial	Malformation of outflow tracts	Australia
126	negative	Sporadic	Malformation of outflow tracts	Australia
127	negative	Sporadic	Malformation of outflow tracts	Australia
128	negative	Familial	Malformation of outflow tracts	Australia
129	negative	Sporadic	Malformation of outflow tracts	Australia

*, # and ^ are 3 pairs of cousins among the familial Italian cohort families

Supplementary Table 2: Summary of *DICER1* Variants and Predictions

Variant	n	Clinical information	Prediction		Minor allele frequency (MAF)
Synonymous n=5			**SIFT**	**Polyphen2**	**ExAC(MAF/count)**
c.1278A>G p.E426E rs878855242***	1*	Malformation of outflow tracts (sporadic)	NA	NA	Not found
c.1935G>A p.P645P rs61751177	4	1 Familial 3 Malformation of outflow tracts (Sporadic)	NA	NA	0.0095/1148
c.2337A>G p.T779T rs747210633	1	Malformation of outflow tracts (Familial)	NA	NA	0.00003/4
c.2718C>T p.R906R rs370692165	1	Sporadic	NA	NA	0.00008/10
c.5145C>T p.L1715L rs139500905	1	Malformation of outflow tracts (Sporadic)	NA	NA	0.0015/179
Intronic n=7					
c.307+13T>C	1*	Malformation of outflow tracts (Sporadic)	NA	NA	novel
c.574-5G>A rs368253792	1	Malformation of outflow tracts (Sporadic)	NA	NA	0.00006/7
c.1377-4T>G rs192490028	2	1 heterotaxy sporadic, 1 Familial	NA	NA	0.0033/401
c.2040+29T>C rs370866625	1**	Malformation of outflow tracts (Familial)	NA	NA	0.0002/19
c.3093+149_3093+153delGTTTT rs575610432	1	Malformation of outflow tracts (Sporadic)	NA	NA	0.0008/4***
c.5364+18C>T rs777415635	1	Sporadic	NA	NA	0.00007/8
c.5527+19A>G rs765497219	1**	Malformation of outflow tracts (Familial)	NA	NA	0.000008/1
Missense n=6					
c.179C>T p.T60I rs587778228	1	Malformation of outflow tracts (Familial)	Tolerated	Benign	0.00005/6
c.278G>A p.G93E rs776219930	1	Sporadic	Tolerated	Possibly damaging	0.00003/4
c.3458G>A p.C1153Y rs762999390	1**	Malformation of outflow tracts (Familial)	Tolerated	Benign	0.000008/1
c.4796G>A p.R1599Q rs569615549	1	Malformation of outflow tracts (Sporadic)	Tolerated	Benign	0.0002/23
c.4886C>T p.S1629L	1	Malformation of outflow tracts (Familial)	Tolerated	Benign	novel
c.5504A>C p.Y1835S rs747510783	1	Sporadic	Tolerated	Possibly damaging	0.00006/7

* individual with 2 variants

** individual with 3 variants

***from 1000 genomes

NA: not applicable