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ARTICLE

Atrioventricular canal defect in patients with RASopathies

Maria Cristina Digilio*,[1], Francesca Romana Lepri[1], Maria Lisa Dentici[1], Alex Henderson[2], Anwar Baban[1], Maria Cristina Roberti[1], Rossella Capolino[1], Paolo Versacci[3], Cecilia Surace[1], Adriano Angioni[1], Marco Tartaglia[4], Bruno Marino[3] and Bruno Dallapiccola[1]

Congenital heart defects affect 60-85% of patients with RASopathies. We analysed the clinical and molecular characteristics of atrioventricular canal defect in patients with mutations affecting genes coding for proteins with role in the RAS/MAPK pathway. Between 2002 and 2011, 101 patients with cardiac defect and a molecularly confirmed RASopathy were collected. Congenital heart defects within the spectrum of complete or partial (including cleft mitral valve) atrioventricular canal defect were diagnosed in 8/101 (8%) patients, including seven with a *PTPN11* gene mutation, and one single subject with a *RAF1* gene mutation. The only recurrent mutation was the missense *PTPN11* c.124 A>G change (T42A) in *PTPN11*. Partial atrioventricular canal defect was found in six cases, complete in one, cleft mitral valve in one. In four subjects the defect was associated with other cardiac defects, including subvalvular aortic stenosis, mitral valve anomaly, pulmonary valve stenosis and hypertrophic cardiomyopathy. Maternal segregation of *PTPN11* and *RAF1* gene mutations occurred in two and one patients, respectively. Congenital heart defects in the affected relatives were discordant in the families with *PTPN11* mutations, and concordant in that with *RAF1* mutation. In conclusion, our data confirm previous reports indicating that atrioventricular canal defect represents a relatively common feature in Noonan syndrome. Among RASopathies, atrioventricular canal defect was observed to occur with higher prevalence among subjects with *PTPN11* mutations, even though this association was not significant possibly because of low statistical power. Familial segregation of atrioventricular canal defect should be considered in the genetic counselling of families with RASopathies.
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INTRODUCTION

Noonan syndrome (NS) and related disorders, including Noonan syndrome-multiple lentigines (NS-ML), Noonan-like with loose anagen hair, Cardio-Facio-Cutaneous and Costello syndromes (the so-called Neuro-Cardio-Facio-Cutaneous syndromes or RASopathies) are causally linked to germ line mutations in a number of genes coding transducers and modulatory proteins participating in the RAS-MAP kinase (MAPK) signaling pathway.[1–3] Clinical features include dysmorphic features, congenital heart defects (CHDs), postnatal growth retardation, ectodermal and skeletal defects and variable cognitive deficits. CHDs occur in approximately 60–86% of patients affected by one of these RASopathies, depending on the mutated genes. Pulmonary valve stenosis (PVS) and hypertrophic cardiomyopathy (HCM) are the most common defects displaying a distinct association with the RASopathies.[4,5] Nevertheless, the spectrum of CHDs in NS syndrome is wider, and the family of atrioventricular canal defects (AVCD) is the third most common heart defect.[5–7]

AVCD includes different anomalies of atrioventricular valves and atrial and ventricular septa. In the complete form, a single common atrioventricular valve and an atrial septal defect (ostium primum) confluent with a posterior ventricular septal defect in the inlet portion of the ventricular septum are found. In the partial form, there are two separate right and left atrioventricular valves with a clefted mitral valve, an atrial septal defect (ostium primum), and no ventricular septal communication. Cleft mitral valve is considered the less severe form of AVCD.[8–13] A defect of the extracellular matrix has been considered a likely pathogenic mechanism for the defects in the spectrum of AVCD.[14,15] AVCD is also the most common CHD found in children with Down syndrome and one of the structural heart defects most frequently associated with extracardiac anomalies in the setting of chromosomal and mendelian disorders.[16,17] Distinct anatomic features are found in AVCD associated with NS. In fact, in general this defect is of the partial type, eventually associated with subaortic stenosis, due to accessory fibrous tissue and/or anomalous insertion of the mitral valve with anomalous papillary muscle of the left ventricle.[7,18]

Recent studies have reported discordant figures for the association between AVCD and RASopathies, which was found only in some series,[19–22] and not in others.[23–28] Therefore, the RASopathies are often not considered and probably underestimated among syndromic patients with AVCD. In this study, we analysed clinical, familial and

[1]Department of Medical Genetics, Cytogenetics, Pediatric Cardiology, Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy; [2]Northern Genetics Service, Newcastle upon Tyne Hospitals, UK; [3]Pediatric Cardiology, Department of Pediatrics, Sapienza University, Rome, Italy; [4]Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy
*Correspondence: Dr MC Digilio, Department of Medical Genetics, Bambino Gesù Hospital, IRCCS, Piazza S. Onofrio 4, Rome 00165, Italy. Tel: +39 06 6859 2227; Fax: +39 06 6859 2004; E-mail: mcristina.digilio@opbg.net
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molecular characteristics of AVCD patients with mutations in genes affecting the RAS/MAPK pathway.

SUBJECTS AND METHODS

From 2002 to 2011, 150 patients with a cardiac anomaly (including structural defects, hypertrophic cardiomyopathy and arrhythmias) associated with other clinical features suggesting the RASopathies' spectrum were evaluated. In all the patients, complete physical examination for major and minor anomalies was carried out by two medical geneticists (MCD, BD). Clinical inclusion criteria of van der Burgt[29] for NS, Voron *et al*,[30] for NS-ML syndrome, Kavamura *et al*,[31] for Cardio-Facio-Cutaneous syndrome, Gripp *et al*,[32] for Costello syndrome were used. All patients had cardiological assessment, including chest radiograph, electrocardiogram and two-dimensional color Doppler echocardiography.

Genomic DNA was obtained from circulating leucocytes, and the entire coding sequence of *PTPN11* gene was screened for mutations in all patients by single-strand conformation polymorphism analysis or denaturing high-performance liquid chromatography (DHPLC), as previously reported.[19,23] Fragments with an aberrant migration/elution pattern were sequenced. *PTPN11* mutation-negative samples were successively screened for mutations in the coding region of the *SOS1*, *RAF1*, *KRAS*, *NRAS*, *SHOC2*, *CBL*, *BRAF*, *MEK1*, *MEK2* and *HRAS* genes by DHPLC analysis and bidirectional direct sequencing.

Patients with AVCD were selected, including those with cleft mitral valve, which is the mildest form of AVCD.[6,8,11–13] In cardiac classification, the focus was to the spectrum of AVCDs, and the other cardiovascular anomalies were considered as secondary diagnosis. Mutations found in the present series of patients with AVCD/related defects and in published series of AVCD were reviewed and analysed for possible genotype–phenotype correlations. The proportion of patients in the present series with AVCD and mutations in one of the genes of the RAS/MAPK pathway was compared with that of patients displaying other CHDs.

RESULTS

Pathogenic mutations were found in 101/150 (67%) patients, including 67/101 (66%) in *PTPN11* gene, 9/101 (9%) in *SOS1*, 8/101 (8%) in *RAF1*, 7/101 (7%) in *BRAF*, 5/101 (5%) in *HRAS*, 2/101 (2%) in *SHOC2*, 1/101 (1%) each in *MEK2*, *NRAS* and *CBL*. None of the analysed patients had any *KRAS* gene mutation.

Among the 13 patients exhibiting AVCD or a related heart defect (9% of the total cases), disease causative changes occurred in 8/101 (8%), indicating similar distribution among mutation-positive and mutation-negative subjects (Fisher's exact probability = 0.51). Seven subjects (88% of the eight mutated cases) had a *PTPN11* gene mutation, including six subjects with NS and one with NS-ML. A heterozygous *RAF1* gene mutation was found in one NS patient (12% of the eight mutated cases). No pathogenic mutation in any of the other analysed genes was detected in the remaining five patients (Table 1). Although the observed AVCD distribution among subjects with molecularly confirmed RASopathy was suggestive for a higher prevalence of this defect in individuals with a mutated *PTPN11* allele, this association did not reach statistical significance (7/67 *vs* 1/34; Fisher's exact probability = 0.179).

Cardiac anatomy, clinical diagnosis and molecular details of AVCD patients with mutations are summarized in Table 1. Partial AVCD was diagnosed in six patients, complete AVCD and cleft mitral valve in one each. AVCD was found to be associated with an additional cardiac defect found in 4 (57%) patients, including HCM (two cases) and subvalvular aortic stenosis, PVS, parachute mitral valve in one patient each. Isolated cleft mitral valve was associated with atrial septal defect ostium secondum type in one patient.

Familial segregation of the mutation was documented in 3/8 (38%) families, the mutated allele having been inherited by the affected mother in all cases. CHDs in the affected relatives were discordant in two families with *PTPN11* gene mutations (PVS, atrial septal defect ostium secundum type), whereas it was concordant in the *RAF1* gene family with mutation (cleft mitral valve) (Table 2).

A relatively wide spectrum of mutations was documented in patients with AVCD, the only recurrent mutation being the *PTPN11* c.124 A>G (T42A) substitution. Table 3 summarizes known mutations in AVCD patients with any RASopathy, including two patients with AVCD and *PTPN11* mutation,[22] one patient with cleft mitral valve and *KRAS* mutation,[33] and one with cleft mitral valve and *BRAF* mutation.[34] The patients are counted once, accordingly to the primary diagnosis. The percentage of subjects with AVCD among the patients with molecular diagnosis and CHDs and frequency of the different mutations in the total series of patients with mutations is shown in Supplementary Table 4.

Table 1 Clinical, cardiological and molecular findings in patients with AVCD and any RASopathy

Patient	*Sex*	*Phenotype*	*Cardiac defect*	*Exon*	*Mutation*
PTPN11					
1	M	Noonan syndrome	Partial AVCD, subvalvular aortic stenosis	2	c.124A>G (T42A)
2	F	Noonan syndrome	Cleft mitral valve, atrial septal defect (ostium secundum type)	2	c.124A>G (T42A)
3	F	Noonan syndrome	Partial AVCD	3	c.188A>G (Y63C)
4	M	Noonan syndrome	Partial AVCD	3	c.214G>T (A72S)
5	F	Noonan syndrome	Partial AVCD, PVS	8	c.923A>G (N308S)
6	M	LEOPARD syndrome	Partial AVCD, parachute mitral valve, HCM	12	c.1403C>T (T468M)
7	F	Noonan syndrome	Complete AVCD	13	c.1472C>T (P491L)
RAF1					
8	F	Noonan syndrome	Partial AVCD, HCM	7	c.781 C>T (P261S)
No mutations					
9	F	Noonan syndrome	Partial AVCD	—	—
10	M	Noonan syndrome	Partial AVCD	—	—
11	M	Noonan syndrome	Partial AVCD, subvalvular aortic stenosis	—	—
12	F	Noonan syndrome	Partial AVCD, double mitral orifice, cor tiatriatum-	—	—
13	F	Noonan syndrome	Partial AVCD	—	—

Abbreviations: AVCD, atrioventricular canal defect; F, female; HCM, hypertrophic cardiomyopathy; M, male.

DISCUSSION

AVCD is the third most common CHD in NS, after PVS and HCM,[7,21] and it has been also documented to occur in NS-ML syndrome.[19,35] The first clinical series reported a figure of 15% AVCD in NS.[5–7,16,36,37] In more recent molecular studies, however, AVCD was described only in a few patients, arguing that it might represent a relatively rare complication in RASopathies. It is likely that this discrepancy could be simply a matter of misdiagnosis, as shown by personal experience based on cases originally designated as 'non-Down AVCD syndrome', displaying 'peculiar facial appearance with hypertelorism, epicanthus, depressed nasal bridge and ear anomalies',[38] which later were proved to be affected by NS. Actually, the fact that the association between NS and AVCD is not rare is also evidenced by previous clinical observations from our group, reporting that the prevalence of NS in a consecutive series of patients with non-Down AVCD and situs solitus was corresponding to 11% (22/203 cases).[16]

In the present series, AVCD was diagnosed in 8% of the patients with a molecularly confirmed RASopathy. A mutation in the known genes can be detected in the 62% of patients with AVCD and clinical features consistent with any RASopathy. The majority of these patients were heterozygous for a pathogenic PTPN11 mutation, a single familial case presenting with a RAF1 mutation (Table 1). Although further studies are required to formally confirm such a genotype–phenotype correlation, published data appear also to support this association,[22] with single reports of cleft mitral valve in individuals with KRAS[33] and BRAF[34] mutations.

Mutations in unknown genes of the RAS/MAPK pathway or a clinical misdiagnosis could account for the AVCD syndromic patients in which no pathogenic change was detected in any of the 11 screened genes.

A relatively wide spectrum of *PTPN11* mutations were documented in the present cohort of subjects with AVCD, the only recurrent mutation being c.124 A>G (T42A), which was found in two patients with AVCD, and with cleft mitral valve and atrial septal ostium secundum type, respectively (Table 1). Interestingly, a missense change affecting

Table 2 Clinical and molecular characteristics of families segregating mutations of the RAS/MAPK pathway

Family	*Sex*	*Phenotype*	*Mutated gene*	*Mutation*	*Cardiac anomaly*
Family 1					
Proband (patient 5 in Table 1)	F	NS	PTPN11	N308S	partial AVCD, PVS
Brother	M	NS	PTPN11	N308S	PVS
Mother	F	NS	PTPN11	N308S	Normal heart
Family 2					
Proband (patient 7 in Table 1)	F	NS	PTPN11	P491L	complete AVCD
Brother	M	NS	PTPN11	P491L	ASD ostium secundum
Mother	F	NS	PTPN11	P491L	PVS
Family 3					
Proband (patient 8 in Table 1)	F	NS	RAF1	P261S	partial AVCD, HCM
Mother	F	NS	RAF1	P261S	cleft MV, HCM

Abbreviations: ASD, atrial septal defect; AVCD, atrioventricular canal defect; F, female; HCM, hypertrophic cardiomyopathy; M, male; NS, Noonan syndrome; MV, mitral valve; PVS, pulmonary valve stenosis.

Table 3 CHDs associated with mutations found in AVCD patients with RASopathies

Gene	*Mutation*	*Complete or partial AVCD reviewed*	*Cleft MV*	*PVS*	*ASD*	*HCM*	*VSD*	*Total patients*	*References*
PTPN11									
	T42A	1 (+ SubAoSt)	1 (+ASD,HCM)		3 (1+ IVC and SVC anomaly			5	Present series, 21,48,49
	L43F	1						1	39
	Y63C	1		6	2			9	Present series, 21,50–52
	A72S	1		1 (+ASD)				2	Personal series[a], 50
	N308S	1 (+ PVS)		10 (1+ASD)	1 (+PVS)		1	13	Present series, 21,37,52,53
	Exon 8 (mutation unspecified)	1						1	22
	T468M	1 (+HCM,PMV)		3		9 (2+MVA) (1+SubAoSt) (1+PVS)		13	Present series, 19,21,35,54–56
	P491L	1		1	1			3	Present series
RAF1									
	P261S	1 (+HCM)	1 (+HCM)	1		7 (1+AoSt) (1+ASD) (1+ASD,VSD)		10	Present series, 47,57
BRAF									
	Q257R		1 (+ASD,HCM)	1		1 2 (+PVS,ASD) 1 (+PVS)		6	32,34,58
KRAS									
	V14I		1					1	33

Abbreviations: AVCD, atrioventricular canal defect; MV, mitral valve; PVS, pulmonary valve stenosis; ASD, atrial septal defect; HCM, hypertrophic cardiomyopathy; VSD, ventricular septal defect; SubAoSt, subaortic stenosis; IVC, inferior vena cava; SVC, superior vena cava; PMV, parachute mitral valve; MVA, mitral valve anomaly; AoSt, aortic stenosis.
[a]The patients are counted once, accordingly to the primary diagnosis.

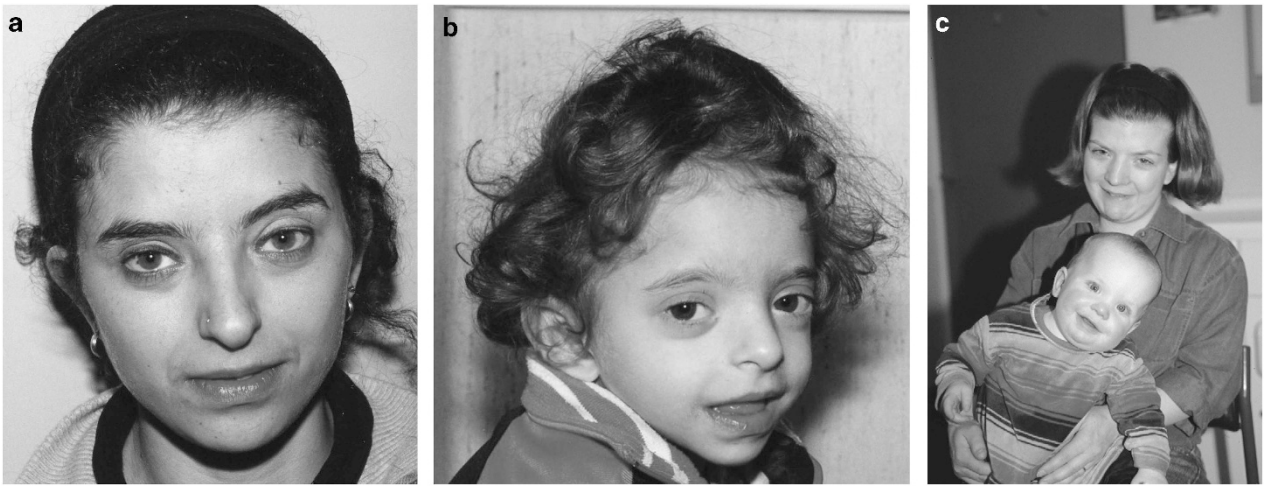

Figure 1 Facial appearance of affected mother and daughter from families 2 (**a**,**b**) and 3 (**c**).

the adjacent leucine residue (L43F) mutation was reported previously in a subject with an apparently nonsyndromic complete AVCD.[39] This observation suggests that mutations predicting to enhance SHP2's binding to signiling partners might represent a functional hot spot for this anatomic defect.[40] Additionally, Krenz *et al*,[41] have shown that *PTPN11* Q79R mutation in exon 3 affects cell proliferation during endocardial cushion development in chick embryos, by increasing signaling via the Ras-MAPK pathway. Based on personal experience, we infer that mutations affecting the PTPN11 N-SH2 domain are in general associated with sporadic AVCD without additional CHDs, whereas familial RASopathies with AVCD are mostly associated with mutations in the PTP-domain, specifically involving exons 8 and 13.

We documented the occurrence of intrafamilial variability of the CHD phenotype, with affected relatives displaying either PVS, atrial septal defect ostium secundum type or a normal heart (Table 2) (see Figures 1a and b). This observation suggests that AVCD-related mutations can result in a wider spectrum of CHDs, and that this issue should be considered in the genetic counseling of familial NS. Concordant segregation of CHDs, however, was also found. In particular, isolated cleft mitral valve was diagnosed in the mother of a child with AVCD, both carrying the *RAF1* P261S mutation (see Figure 1c). HCM was also found in both the cases.

Anatomic peculiarities of AVCD previously reported in patients with NS include the partial-type defect, and the association with subaortic stenosis.[7,18,42] The structural abnormalities causing congenital subaortic stenosis consist of accessory fibrous tissue and/or anomalous insertion of the mitral valve and anomalous left ventricle papillary muscle. Anomalies of mitral valve leaflets and subvalvular mitral apparatus reported in patients with NS are similar to those found in subjects with HCM.[43–45] This is not surprising, considering that myocardial disarray and cardiac hypertrophy are common in NS. Therefore, a perturbed developmental mechanism of left ventricular myocardium and mitral valve should be contemplated in the pathogenesis of CHDs in these patients. Accordingly, mitral valve anomalies associated with AVCD in NS could result from the same pathogenic mechanism responsible for mitral anomalies in HCM. The coexistence of both AVCD and HCM in the same patient affected by RASopathy can lead to the speculation that a defect of the extracellular matrix, known to be pathogenetically associated with AVCD, could be linked also to HCM. Additionally, the association of subvalvular aortic stenosis with HCM may be an aggravating factor in these patients, as it may change the geometry of the outflow tract and promote the development of fibrous abstruction together with the usual shape of the outflow tract in AVCD.

Partial AVCD was the most common type in the present patients. Additional heart defects included subaortic stenosis, HCM, parachute mitral valve and PVS (Table 1). Previous attempts to correlate CHDs and mutations in the RAS/ MAPK pathway have identified possible hot spots for PVS, HCM and atrial septal defects.[21,46] In the present study, although the numbers of patients are low, we found a somewhat distinct association between AVCD with HCM and *PTPN11* T468M and *RAF1* P261S mutations, which had been previously related to HCM. For example, *PTPN11* T468M mutation was reported in NS-ML patients with HCM,[19] whereas the prevalence of HCM appeared significantly higher in NS patients carrying *RAF1* mutations clustering around Ser259 and Ser612.[47] Our patient with AVCD and PVS was heterozygous for *PTPN11* N308S mutation, previously reported in NS with PVS.[21,23]

The echocardiographic follow-up of patients with mutations linked to HCM is highly recommended. In fact, our patient with NS-ML and *PTPN11* Thr468Met mutation developed HCM in childhood, soon after the onset of multiple lentigines. In addition, Pandit *et al*,[47] reported on a 6-year-old girl with *RAF1* P261S mutation and a normal heart, while the same mutation in her mother was associated with HCM with an onset in the young adult age.

In conclusion, AVCD is a part of the phenotypic spectrum of CHDs found in patients with RASopathies, in particular those caused by PTPN11 and RAF1 mutations. Clinical features of NS should be investigated in syndromic patients with AVCD. In general, the spectrum of molecular changes in these patients is rather heterogeneous, the only recurrent mutation being PTPN11 c.124A>G (T42A). Both partial AVCD and left-sided obstructions, PVS and HCM, must be regarded as markers of the RASopathies in which the anatomic type of CHDs appears at time mutation-specific. Finally, the possibility of AVCD familial segregation should be discussed in the genetic counselling of RASopathies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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