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Letter to the Editor

Analysis of *CRELD1* as a candidate 3p25 atrioventicular septal defect locus (*AVSD2*)

The genetics of atrioventricular septal defect (AVSD) are complex. Most cases are sporadic though up to 20% are thought to have a genetic basis. *AVSD* genes have been localized by their association with cytogenetic aberrations, e.g. trisomy 21 and 8p22-p23 and 3p25-p26 deletion syndromes. In a family with autosomal dominantly inherited, isolated AVSD, the *AVSD1* locus was mapped to chromosome 1p31-p21 (1). Recently inactivating mutations were found in the *GATA4* transcription factor (at 8p22–23) in three kindreds with familial cardiac septal defects (2, 3). About one-third of 3p- patients have cardiac defects, characteristically an AVSD, and an association between the presence of congenital heart disease and the centromeric extent of 3p25-pter deletions mapped an AVSD locus (*AVSD2*) at 3p25 (4–6). Recently, *CRELD1* was proposed as the 3p25 *AVSD* susceptibility gene (7). *CRELD1* encodes a novel cell adhesion molecule that is expressed during cardiac development (8). Robinson et al. (7) investigated 50 subjects with an isolated (n = 35) or a complex (n = 15, 11 with heterotaxy) AVSD for *CRELD1* mutations (7). No unequivocal inactivating mutations were detected, but three cases (6%, two with isolated partial AVSD and one with partial AVSD and heterotaxy) had a missense substitution (R329C, T311I and R107H) that was not detected in at least 300 control chromosomes. The pathogenic significance of these changes was unclear, as none of the missense substitutions were associated with familial disease or were proven to arise *de novo* in an isolated case. To further investigate the role of *CRELD1* in AVSD pathogenesis, we have (a) investigated a panel of 3p- patients with and without an AVSD to establish *CRELD1* deletion status and (b) analyzed a cohort of 49 sporadic AVSD cases for *CRELD1* mutations.

Analysis of *CRELD1* status in 3p- patients

We initially examined the location of *CRELD1* with respect to the 3p25 deletion breakpoint in 12 subjects with 3p- syndrome (Table 1). *CRELD1* maps telomeric to *VHL* and was deleted in all five cases with an AVSD (P1 to P5). However, *CRELD1* was also deleted in four patients without congenital heart disease (P6, P7, P8 and P9). In addition, multiplex ligation-dependent probe amplification (MLPA) analysis demonstrated a deletion of CRELD1, FANCD2, GHRL, IRAK2 and VHL in a child with a reciprocal translocation 3; 13 translocation [t(3;13) (p26;q21.2)]. However this deletion was not associated with congenital heart disease.

Mutation analysis of CRELD1

CRELD1 mutation analysis in 49 sporadic AVSD cases (14 with isolated complete AVSD, six with complete AVSD and another cardiac anomaly, 27 with isolated partial AVSD and two with partial AVSD and another cardiac anomaly) revealed six sequence variants (five novel). Three of five novel sequence variants detected in AVSD cases were also detected in normal controls (Table 2). Two variants were detected in AVSD cases but not in normal controls. Firstly, an exon 10: c.1194G > A variant was detected in a subject with a complete AVSD but not in 200 normal chromosomes. However, the c.1194G > A substitution does not change the CRELD1 protein sequence (A398A) and is not predicted to create a cryptic splice site [NETGENE (http://www.cbi.pku.edu.cn/mirror/GenomeWeb/nuc-geneid.html)]. Secondly, an exon 5 missense substitution (P162A, c.484C > G) was identified in a subject with a partial isolated AVSD and a 47, XXX karyotype (Fig. 1). The c.484C > G variant was not detected in 200 control chromosomes. Family studies of seven members of this family demonstrated that the variant was also present in the subject's mother and maternal grandmother. Neither of these had a history of congenital heart disease.

We identified a *CRELD1* missense substitution in one of 49 subjects with sporadic AVSD.

Table 1. Location of *CRELD1* in reference to deletion breakpoints in 3p- patients (patients P1–P10 included in our previous report) (6).

Marker	P1[a]	P2[a]	P3[a]	P4[a]	P5[a]	P6	P7	P8	P9	P10	P11	P12
Telomere												
CALL (CHL1)	–	–	–	–	–	–	–	–	–	–	–	–
D3S1297	nd	NI	NI	NI	NI	NI	–	NI	NI	nd	nd	nd
D3S1304	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd
D3S18	nd	–	nd	–	–	nd	nd	–	nd	–	nd	nd
D3S3691	–	NI	nd	NI	NI	NI	–	nd	NI	nd	NI	NI
D3S1597	–	–	–	NI	–	NI	–	–	NI	nd	NI	+
CRELD1	–	–	–	–	–	–	–	–	–	+	+	+
FANCD2	–	–	–	–	–	–	–	–	+	+	+	+
VHL	–	–	–	–	–	–	–	–	+	+	+	+
IRAK/D3S1317	–	–	–	–	–	–	–	–	+	+	+	+
GHRL	–	–	–	–	–	–	–	–	+	+	+	+
D3S601	–	nd	–	–	–	nd	nd	+	nd	nd	nd	nd
D3S1038	–	–	–	–	–	–	–	+	+	+	nd	nd
D3S3611	–	–	NI	nd	nd	–	–	NI	+	nd	+	+
Centromere												

nd, not done; NI, not informative; P1–P12, patients with 3p- syndrome; –, deleted; +, retained.
[a]Patients with AVSD.
Results shown are the combined results of polymorphic markers analysis, fluorescent in situ hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA). The smallest region of overlap for 3p- deletions associated with AVSD is centromeric to D3S3611 between D3S3594 and D3S1263 (6).

Table 2. Novel SNPs detected in *CRELD1*. In addition a known polymorphism was detected in intron3: IVS3-39A > G (39nt upstream Ex4). Nomenclature for the description of sequence variations is according to den Dunnen (9).

SNP	Location	Aa change	Frequency in AVSD cases	Frequency in control subjects
c.588C > A	Ex5	G196G (silent)	0:48	1:100
c.945G > A	Ex9	P315P (silent)	1:48	2:100
c.1194G > A	Ex10	A398A (silent)	1:48	0:100
IVS9 + 23G > T	IVS9, (23nt downstream Ex9)	–	1:48	1:100

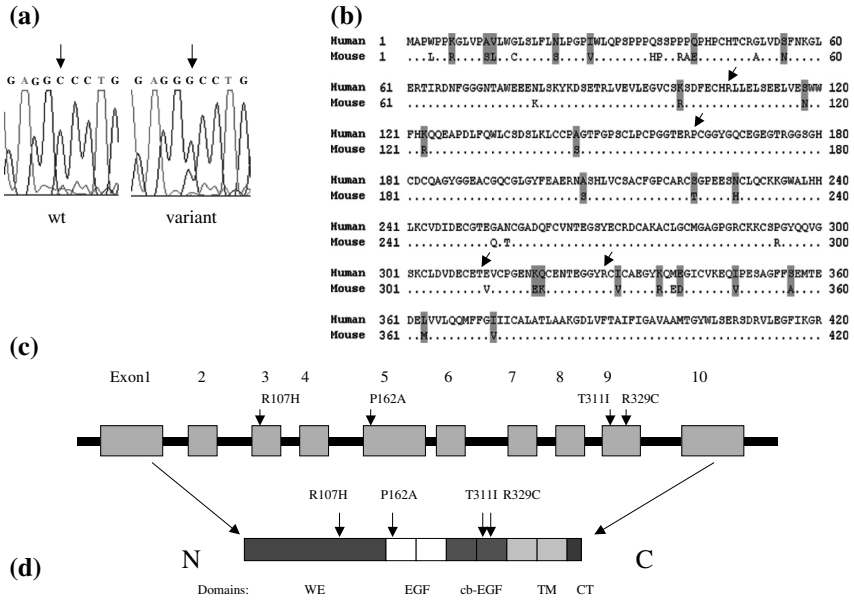

Fig. 1. (a) *CRELD1* missense sequence variant c.484C > G (P162A) in a sporadic AVSD patient. Wild type (left) and variant (right) sequence traces are shown. (b) Human and mouse protein sequences showing conservation of P162, R107, T311 and R329. (c) *CRELD1* gene structure and location of the P162A missense substitution and the three missense substitutions reported (7). (d) Diagram of CRELD1 protein as presented (7) with approximate positions of amino acids changes. The domains are described below diagram: cb-EGF, calcium binding EGF-like; CT, carboxy terminal; EGF, EGF-like; TM, type III transmembrane; WE, rich in tryptophan and glutamic acid.

Combining our data with those already reported by Robinson et al. (7), *CRELD1* missense substitutions (that are not present in at least 200 control chromosomes) have been detected in four of 99 subjects with AVSD. For both of the missense substitutions, for which family studies have been undertaken, other relatives harbouring the substitution have not had evidence of AVSD. Thus, if rare *CRELD1* missense substitutions are implicated in the pathogenesis of AVSD, they must be incompletely penetrant. While incomplete penetrance is a feature of the familial AVSD1 locus, incomplete penetrance for cardiac involvement has not been described in 3p- patients with centromeric deletions (4–6). In our 3p- patients, *CRELD1* mapped outside the smallest region of overlap for 3p deletions associated with cardiac defects centromeric to D3S3611 (Table 1) between D3S3594 (AFMA337XG5) and D3S1263 (AFM079YG5) (6), and we identified five patients without congenital heart disease in whom *CRELD1* was deleted (four 3p- cases and a translocation case). Thus, while a subset of sporadic AVSD cases may have rare CRELD1 missense variants, *CRELD1* deletions are unlikely to account for AVSD in children with terminal deletions of 3p.

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