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Arrhythmogenic right ventricular cardiomyopathy type 1 (ARVD1): confirmation of locus assignment and mutation screening of four candidate genes

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Arrhythmogenic right ventricular cardiomyopathy type 1 (ARVD1) is an autosomal dominant disorder characterised by progressive degeneration of right ventricular myocardium, arrhythmias and risk of sudden death. By linkage analysis, we previously mapped the involved gene to chromosome 14q24.3. In the present study we report on linkage analysis of one additional and unrelated family, which enabled to confirm previous locus assignment. Another family is reported, in which genetic and clinical data suggest linkage to the same locus. Direct sequencing of DNA from individuals belonging to established ARVD1 families failed to detect causative mutations in exonic sequences of four genes (POMT2, TGF β 3, KIAA1036 and KIAA0759) expressed in the heart and which defects could possibly induce plasma membrane instability or apoptosis, key features of ARVD pathogenesis.

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Introduction

Arrhythmogenic Right Ventricular Dysplasia (ARVD) is a cardiomyopathy characterised by progressive degeneration and fibrous-fatty replacement of right ventricular myocardium,¹ by arrhythmias with a left branch block pattern² and by increased risk of sudden death in juveniles.^{1,3} Several forms of dominant arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVD, OMIM 107970) were identified so far: ARVD1 (14q24.3),⁴ ARVD2 (1q42),⁵ ARVD3 (14q11-q12),⁶ ARVD4 (2q32),⁷ ARVD5 (3p23),⁸ ARVD6 (10p12-p14)⁹ and ARVD7 (10q22).¹⁰ Mutations in the gene encoding the cardiac ryanodine receptor were recently detected in patients affected with ARVD2.¹¹ Attempts to

identify genes involved in other dominant ARVDs were so far unsuccessful.^{12–15}

Here we report on linkage analysis of a novel family affected with ARVD, which enabled to confirm previous chromosomal localisation of ARVD1. We report as well on another novel family probably belonging to the same clinical-genetic entity and on mutation screening of four candidate genes.

Materials and methods

Families

Two independent ARVD families were investigated at the Department of Cardiology of the University of Padua (Italy). Informed consent was obtained from all the family members participating in the study. Diagnostic criteria were according to McKenna *et al.*¹⁶ Peripheral blood samples were collected from ARVD family members in EDTA-coated tubes and genomic DNA was prepared from nucleated blood cells, according to the salting out method. In total 35 individuals were studied, including 15 affected subjects.

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Haplotype and linkage analysis

PCR primers sequences for the different genetic markers were obtained from The Genome Database (<http://gdbwww.gdb.org/gdb/gdbtop.html>). The following markers of ARVD1 locus were used for haplotyping and linkage studies: D14S994, D14S1036, D14S61, D14S42, D14S279, D14S983, D14S59, D14S254, D14S1020, D14S74 and D14S127. Two additional polymorphic markers (GenBank/EMBL Data Libraries accession No. AJ422208 and AJ422209, respectively), identified between D14S74 and D14S127, were used to further refine the critical region.

The following markers were used to assess linkage to other known ARVD loci: D1S2800, D1S2850, D1S2670, D1S2785, D1S304, D1S2842 for ARVD2 critical region; D14S252, D14S257, D14S70 for ARVD3 critical region; D2S152, D2S364, D2S118, D2S117, D2S72, D2S2358 for ARVD4 critical region; D3S3610, D3S2403, D3S3613, D3S2338, D3S3659, D3S1293 for ARVD5 critical region; D10S547, D10S570, D10S191, D10S1653 for ARVD6 critical region; D10S605, D10S201, D10S1777, D10S1786, D10S1696, D10S1686, D10S1744, D10S215 for ARVD7 critical region.

The analysis of the microsatellite markers were performed as described elsewhere.⁴ Two-point lod scores between ARVD and the marker loci were calculated by the MLINK programme of the LINKAGE package (vs 5.02). The disease was assumed as autosomal dominant with both 70 and 95% penetrance and with a frequency of 0.01 in the population. Equal allele frequencies for the markers tested were assumed. FASTMAP programme was used to perform multipoint linkage analysis.

Bioinformatic analysis of the ARVD1 critical region

The sequence of the entire critical region was obtained from the working draft of the human genome sequence (<http://genome.ucsc.edu>). Annotated known genes, expressed-sequence tags (ESTs) and cDNAs were searched for in this genomic sequence. Their expression in the human heart tissue was established according to Unigene (<http://www.ncbi.nlm.nih.gov/UniGene/Hs.Home.html>) and STACK (<http://juju.egenetics.com/stackpack/webblast.html>) databases.

Reconstruction of the genomic structure of candidate genes

The genomic structures of POMT2, TGF β 3, KIAA1036 and KIAA0759 were reconstructed by direct comparison of their full length cDNAs with the sequence of human chromosome 14 genomic clones, retrieved from the GenBank/htgs database (<http://www.ncbi.nlm.nih.gov>) by BLASTN search.

Prediction of additional gene structure/protein elements of KIAA1036 and KIAA0759

DNA sequence and the translated amino acid sequences were compared with the nonredundant nucleotide and

protein sequence databases using BLASTN and BLASTP, respectively.¹⁷ Protein motif and profile searches were performed with ScanProsite (<http://www.expasy.ch/tools/scnpsite.html>) and ProfileScan (<http://www.isrec.isb-sib.ch>). Transmembrane domains were predicted using TM-pred (<http://www.ch.embnet.org>). The prediction of protein sorting signals and of protein localization sites in cells was carried out with PSORT (<http://psort.nibb.ac.jp>).

RT-PCR

For RT-PCR analysis, PCR amplification was performed with cDNA (from human adult myocardium) as template, by using the following primers. Primer KIAA1036F, 5'-TCGGACCCTAATTCACCTTA-3'; KIAA1036R, 5'-TCCCTGTGTGATTGTACTGC-3'; KIAA0759F, 5'-ATTGGAAGTATCGCTCGTG-3'; KIAA0759R, 5'-TCTGGTAAAGCTGATGGGAG-3'; TGF β 3F, 5'-GTGGAAGCCATTAGGGGACA-3'; TGF β 3R, 5'-ATCCGCTTAGAGCTGGGGT-3'; POMT2F, 5'-ATCAGGGCCTACGCTTCTCA-3'; POMT2R, 5'-GTCCACAGAATGCCTGTCA-3'.

PCR experiments were carried out in a reaction buffer (12.5 μ l) containing 2 μ l of cDNA derived from 5 μ g total RNA, 5 pMol oligonucleotide primers, 1 μ l DMSO and 0.2 U of Taq DNA polymerase (ExperTeam). An aliquot of the amplified products was subjected to 2% agarose gel electrophoresis and stained by ethidium bromide. Amplified products were visualised by UV light. RT-PCR products were then subjected to direct DNA sequencing analysis.

Mutation screening

PCR primers flanking the exons of each candidate gene for amplification from genomic DNA for mutation screening were designed by PRIMER3 (<http://www-genome.wi.mit.edu>). The sequences of primers are listed in Table 4. Mutation screening was performed by direct sequencing of both strands. Each exon was amplified from patient genomic DNA, purified (PCR Product Pre-Sequencing kit; USB) and sequenced using the BIG DYE dideoxy-terminator chemistry (Perkin Elmer) on an ABI 377 DNA sequencer (PE Applied Biosystems). Chromas 1.5 software (Technelysium) and LASERGENE package computer programmes (DNASTAR) were used to edit, assemble and translate sequences.

Results

Clinical data

A family (Fam. 112) from Northern Italy, showing recurrence of ARVD cases and unrelated to those described in the original article⁴ was investigated. Clinical features, summarized in Table 1A, fitted a 'classical' form of ARVD.

Linkage analysis

Pedigree of family 112 is shown in Figure 1A, along with haplotypes corresponding to part of chromosomal region 14q24.3-q31.1. Two point lod scores for 11 microsatellite markers are shown in Table 2. Maximum lod scores at $\theta=0$

Table 1 ARVD Patient belonging to family 112 (1A) and family 119 (1B) considered in the present study
A

Type of criterion	Familial history Minor	Repolarization abnormalities Minor	Depolarization/ conduction abnormalities		Right ventricular kinetic alterations		Type of ventricular arrhythmias Minor	Numbers of criteria (M or m)
			Major	Minor	Major	Minor		
Patient/age								
II 2/ 60	+	+	—	+	—	+	—	4 m
II 3/ 62	+	+	—	—	+	—	—	1M, 2 m
II 5/ 59	+	+	—	+	—	+	—	4 m
II 9/ 67	+	+	—	+	—	+	—	4 m
III 1/ 29	+	—	—	+	—	+	PVCs	4 m
III 3/ 24	+	+	—	+	+	—	—	1M, 3 m
III 4/ 24	+	+	—	+	+	—	Sustained VT	1 M, 3m
III 11/ 38	+	—	—	+	—	+	PVCs	4 m
III 14/ 29	+	+	—	+	—	+	—	4 m

Type of criterion	Familial history Minor	Repolarization abnormalities Minor	Depolarization/ conduction abnormalities		Right ventricular kinetic alterations		Type of ventricular arrhythmias Minor	Numbers of criteria (M or m)
			Major	Minor	Major	Minor		
Patient/age								
II 2/63	+	+	+	+	+	+	PVCs	2M, 5 m
II 4/57	+	+	+	+	+	+	Sustained VT	2M, 5 m
II 6/60	+	+	—	—	+	—	PVCs	1M, 3 m
III 1/30	+	+	—	—	—	+	PVCs	4 m
III 2/36	+	+	—	—	+	—	NSVT	1M, 3 m
III 3/32	+	+	—	—	—	+	PVCs	4 m

NOTE: M: major criterion, m: minor criterion; PVC: premature ventricular complex; NSVT: non sustained VT; VT: ventricular tachycardia

(assuming 95% of penetrance) were obtained with marker D14S254 ($Z=4.41$), and marker D14S983 ($Z=4.06$). The 'affected only' lod score resulted close to the level of significance (2.71 for markers D14S983 and D14S59, at $\theta=0$).

Multipoint linkage analysis (peak lod score $Z=5.20$) suggested that the most likely location of ARVD1 is between D14S279 and D14S283 (data not shown). Unfortunately, no recombinants were detected in the family.

An additional family (Family 119, Figure 1B), from South Germany, was investigated because patients showed clinical features consistent with the hypothesis of ARVD1 (Table 1B).

Preliminary haplotype investigation, by means of a panel of markers for each of the ARVD loci identified so far, showed that segregation was consistent only with the hypothesis of association of the disease to ARVD1 locus. Haplotype sharing analysis was performed on affected members of Family 119 and on their relatives, by using the same microsatellite markers tested in Family 112. The at-risk haplotype of Family 119 resulted differently from that detected in Family 112.

In family 119, affected individuals II, 6 and III, 2 showed recombinations between markers D14S74 and D14S127 and between D14S1036 and D14S61, respectively (Figure 1B). Small size of family 119 failed to produce significant lod

scores by linkage analysis. However, it is worth noticing that a lod score of 1.51 was obtained for marker D14S59.

Mutation screening of candidate genes

Mutation screening on coding sequences of four candidate genes (POMT2, TGF β 3, KIAA0759 and KIAA1036) included in the ARVD1 critical region was attempted. Mutations in three of the selected genes could possibly induce plasma membrane instability or apoptosis, which are presently believed as primary steps in ARVD pathogenesis^{4,16–18}; the fourth gene was considered because it is expressed in heart, although its function is still unknown.

After genomic structure of these genes was determined by gapped BLAST comparison of cDNA sequences with genomic DNA sequences available in GenBank, PCR primers were designed. RT-PCR on total RNA extracted from normal human heart demonstrated that all these genes are expressed in cardiac tissue (data not shown). Data about the four candidate genes are summarized in Table 3.

PCR primers (Table 4), designed to flank each exon, were used to screen genomic DNA from two ARVD1 patients, belonging to the two ARVD1 families identified so far in our laboratory (Fam 100 of the previous study and Fam 112, this study) and from one healthy control subject. POMT2, TGF β 3, KIAA1036 and KIAA0759 coding sequences

Figure 1 Pedigree of family 112 and 119. Affected subjects fulfilled the classic clinical criteria for ARVD. Haplotypes are indicated where DNA was available. Boxed haplotypes represent the at-risk chromosome. In family 119, the affected individuals II,6 and III,2 show key recombinations between markers D14S1036 and D14S61 and between D14S74 and D14S127. Thus, the most likely interval for a localisation of the disease gene is between the genetic markers D14S1036 and D14S127.

Table 2 Two-point lod scores for different values of recombination fraction, for 11 markers of the chromosome 14 in the family 112 with recurrence of ARVD/C, reported in Figure 1A. Lod scores were calculated for two different values of penetrance (a: 70%; b: 95%)

Marker		Recombination fraction							θ_{max}	Z max
		0.00	0.01	0.05	0.10	0.20	0.30	0.40		
D14S994	a	2.86	2.82	2.65	2.43	1.90	1.27	0.54	0.00	2.86
	b	1.81	1.90	2.08	2.10	1.82	1.31	0.63	0.10	2.10
D14S1036	a	2.88	2.84	2.66	2.41	1.87	1.23	0.53	0.00	2.88
	b	2.52	2.54	2.52	2.39	1.96	1.36	0.64	0.01	2.54
D14S61	a	1.99	1.97	1.87	1.72	1.35	0.92	0.44	0.00	1.99
	b	2.27	2.26	2.17	2.02	1.63	1.15	0.61	0.00	2.27
D14S42	a	0.20	0.20	0.19	0.18	0.14	0.10	0.05	0.00	0.20
	b	0.26	0.26	0.25	0.24	0.20	0.14	0.07	0.00	0.26
D14S279	a	0.40	0.39	0.36	0.33	0.24	0.16	0.08	0.00	0.40
	b	0.50	0.50	0.47	0.43	0.35	0.24	0.13	0.00	0.50
D14S983	a	3.57	3.51	3.25	2.92	2.21	1.44	0.61	0.00	3.57
	b	4.06	4.00	3.72	3.35	2.58	1.72	0.79	0.00	4.06
D14S59	a	3.56	3.50	3.26	2.94	2.26	1.50	0.65	0.00	3.56
	b	3.93	3.87	3.61	3.28	2.55	1.73	0.82	0.00	3.93
D14S254	a	3.90	3.84	3.58	3.23	2.49	1.65	0.73	0.00	3.90
	b	4.41	4.34	4.06	3.69	2.88	1.97	0.94	0.00	4.41
D14S1020	a	0.28	0.27	0.26	0.24	0.19	0.12	0.06	0.00	0.28
	b	0.26	0.27	0.26	0.25	0.21	0.15	0.08	0.01	0.27
D14S74	a	3.35	3.29	3.05	2.74	2.07	1.34	0.57	0.00	3.35
	b	3.76	3.70	3.44	3.10	2.37	1.58	0.72	0.00	3.76
D14S127	a	1.84	1.81	1.67	1.49	1.11	0.69	0.26	0.00	1.84
	b	2.14	2.10	1.95	1.76	1.34	0.87	0.36	0.00	2.14

Table 3 General information of candidate ARVD1 genes submitted to screening for mutations

Gene (Base Position)	Flanking markers	Genomic Size (Kb)	No. of exons	Domains and activity
POMT2 (75785446– 75829352)	D14S983 D14S59	43.9	21	POMT2 encodes an O-mannosyltransferase, catalyzing the first step in O-glycosylation. Protein O-mannosylation plays an essential role in a number of physiologically important processes, among which is maintenance of cell wall integrity. Four domains present as a triplet in O-mannosyltransferases (MIR domain) are found also in the N-terminals of Ryanodine receptors (RyRs) and inositol 1, 4, 5-triphosphate receptors (IP3Rs). O-mannosyltransferases transfer mannose from DOL-P-mannose to ser or thr residues on proteins [19]. The presence of an uncommon O-linked mannosyl glycan was found in bovine peripheral nerve and rabbit skeletal muscle α -dystroglycans [20,21], an extracellular peripheral membrane glycoprotein binding to a transmembrane glycoprotein, β -dystroglycan. The α -dystroglycan– β -dystroglycan complex acts as a transmembrane linker between the extracellular matrix and intracellular cytoskeleton [22].
TGF β 3 (74466566– 74489613)	D14S61 D14S270	23	7	TGF β is a multifunctional cytokine. The protein is an homodimer, disulfide-linked. TGF β is known to modulate several tissue development and repair processes, including cell differentiation, cell cycle progression, cellular migration, cell adhesion, and extracellular matrix production. The different effects of TGF β are mediated by the TGF β receptors and cell surface-binding proteins [23]. TGF β is a potent growth inhibitor and inducer of apoptosis [24].
KIAA0759 (75270912– 75291491)	D14S42 D14S279	25.6	10	The amino acid sequence of the predicted protein product is 83% identical and 86% similar to the Mus musculus unknown protein AAH19148 and 42% identical and 56% similar to the hypothetical human protein XP034232. The hydropathy analysis of the protein sequence indicated the presence of a hydrophobic region that could form a transmembrane domain. KIAA0759 was found to contain an RGD motif. The sequence Arg-Gly-Asp (RGD), found in fibronectin, is crucial for its interaction with its cell surface receptor, an integrin [25–26] and it was also found in the sequences of a number of other proteins (some forms of collagens, fibrinogen, vitronectin, von Willebrand factor (VWF), snake disintegrins, and slime mold discoidins), where it has been shown to play a role in cell adhesion. Function of this gene is unknown.
KIAA1036 (75270912– 75291491)	D14S42 D14S279	20.6	7	No significant sequence homology was identified using the BLAST server. No obvious structural features or motifs were found. Several potential phosphorylation and N-glycosylation sites are present. Sequence analysis with the TM-pred program showed no significant transmembrane helices. Function of this gene is unknown.

Table 4 Oligonucleotide primers used for PCR amplification and DNA sequencing of the exons of POMT2, TGF β 3, KIAA1036 and KIAA0759 genes

Gene	Exon No.	Product (pb)	Forward primer (5'→3')	Reverse primer (5'→3')	Annealing Temp (°C)
POMT2	1	296	GTACCCTCGGGCCAATCAG	GAGAGTCCGAGCTGCGTC	65
		314	CCATCACTCACCAGATGTGC	GCTGGTCAGACAAAGTGTGC	60
	2	250	AGAAGCCCCAAATCCAAAT	ACCATGCTTTATGAAGGCCA	60
	3	297	CACCACCCAAAGTCCCTTTA	GGAGGGAAGTTCAGAGGACA	60
	4	236	AGCTACATTACGGAGTGGC	TGAGTCCAGTAGGTGCGTGA	60
	5	212	AATTTTGGAGTTGCCACAGC	GCCCTTCTCTTACCTCGCTT	60
	6	331	GAGACAGAGAAGGCCACCAG	TCCAAGTCTGAGAACTGCCC	65
	7	297	CCACAGGCTTAGGAGCAAAA	TCTCTCTCCTCTTGCCATT	60
	8	210	TTTCCAATCAACTCCCAGG	CCTGGGAAATCACACTTTCA	60
	9	223	GTGAACTGTCATGGCGAACA	GGACAGCCTCTGGCACTGT	60
	10	197	CTTAAGGAGGGCTCAGAGGG	GCCAGCGTGTCTGATAGTGA	65
	11	253	CCATTGACCAGAGCCTCATC	TGTTTACCAGTAGGCCCTTGA	60
	12	226	AGCCAAGGCCCATAACTTTT	TCCTGACAGTTGACTCTGAACA	60
	13	277	AAGGAAATCAGGACCCTCG	CGTTTTCTCTAAAGCCCTCA	60
	14	190	TAAATGCTTCCCTCCAGAGC	TCTGGTGGATTTCACGTCTTC	60
	15	217	TTGTGATCCAAATTCATGGC	ATGTGGACACCATGGACAGA	60
	16	193	CTCCCTGCTCTTGTTCTTGG	CCACATTCTGTGACGCCCTC	60
	17	206	GATGAGAACGCAGCCACTCT	GACCATCTTCTCCCTCTAA	65
	18	256	GTGGTAAACGCAAGGATGG	GAGAGTGGCTGCGTTCTCA	60
	19	296	CAAGGAGCAGAGTCACTCCC	GGACCAGAGCCTGGAATCTC	60
	20	218	CTGAGCCCCACTGGGAG	CTGTAGCCCAGAAGTCACCC	65
	21	246	GTCCAGTACTGCTTCCACGC	AATGGAATTCAAAGCCCTC	65
TGF β 3	1	301	CTCCCAGCTCCAGTTCAGAC	AGAAGAGGGTGGAGCCATT	65
		307	GTAAAGGGCCAGGACCTGAT	TGGTTTTCTCCCTCCTTCT	65
	2	281	TATGGCAAAGGAACAGCTT	GCACACTGTTCTGCATGTC	65
	3	326	TGTCTCTTCCCTCATTTC	CATGGCATTCAAGAACCACT	60
	4	233	CTTTTGGGGCAAAGCAGATT	CCCTATAGGAGCAGAGGGCC	68
	5	319	ACCCTCTGTTGAGTGTGGCT	GGGCTCTGCTCTCTTCAGAC	60
	6	307	AACAAGTGGTCTGGTCGGTC	CATGAAAGGACTCCAAGGGA	62
KIAA0759	1	285	TGTTGCTTGTGTGTTTCCC	CTGAGACTTGGCCTTCCAC	62
		325	CCCTTCTTCTCCCTTTAG	GAGAGAGAACCACCACTGCC	58
	2	234	GGGCACGGTAGTCGTGAG	GAGCTGAGGGAGGACCTG	65
		423	CTCTTTAGCAATGGCTCTTGC	GAGAATGCTCGCTTCAGAGGA	62
	3	393	AAGGCTCATCCAGTTCTCT	TGGGCTTATGTTTATGGG	62
		373	TTCTAAGAGCACTGGGCAAC	TGAGTCAGGAGTGGAGATGG	58
	4	205	ATGGGGTCTTCAGGAGAGAG	CTAGAGGGTGGGGGTAC	58
		352	TGCTAACGCCTGAATCTCTC	GGGCTTAGTGTGCTACTGC	58
	5	360	ATCTGGGCCAGCTTGACAT	CATACACTGGGACTGCTGGT	62
		270	GTCGTTGAGACACCAGCTTT	CCAAAACATGGGCTGTTACT	58
KIAA1036	6	258	TGACCAATATCAGGAATGAGG	TATGCTGGAAGTTGCTTGGT	58
		210	TGGGAATCTCTCTTACACCT	TAGGGTGAAAGGTTCTGAT	58
	7	308	GCTACCAATGCCTGACCTA	AGAAAAAGTGATCTAGGGAAAA	58
		300	TTTCCACAGTCTCTGATCCA	AGGACTCTGCCACTGCCT	62
	1	404	CCTCTTGTGAGCCAGTTGTT	CCTAGACTGTGCTATCACCC	58
		168	ACTGGAAGCTCCAAGGTGA	GACCTTGACCAGAGAGGAT	58
	2	166	AGAGGGTCAACCTCCTCAG	TTTCAAGAAGCTCCAAGCC	55
		194	TCCTTCTCACATTCCTCTG	AGCTGAGGCCCAGAGAAG	58
	3	497	CGTGCTTCTCCTGGCTCT	CAATCCTCACATCGCCTCT	58
		240	CTGGAAGGAGAGGTTGGG	CGATGAGAGAGGAAAGGAAAA	58
	7	269	CCATCTCCCTAACAGACCAC	GGCTGAGCTGGAACACACT	58

were screened for mutations by sequencing exons and intron-exon boundaries. Although single-nucleotide substitutions were detected in these genes, no ARVD1-specific sequence alterations were identified (Table 5).

Discussion

In one novel family fulfilling diagnostic criteria for ARVD and unrelated to those previously described, significant

linkage to ARVD1 was detected. This is the first evidence confirming the early locus assignment. It is interesting to notice that no additional linkage data were reported so far for any of the other ARVD forms, if we excluded ARVD2²⁷, which involved gene was recently identified in our laboratory.¹¹

An additional family was investigated in which haplotype analysis excluded linkage to any of known ARVD

Table 5 Single-nucleotide polymorphisms in the ARVD1 candidates genes

Transcript	Location	Position	Polymorphism	Coding change
KIAA0759			No polymorphism	
KIAA1036	Exon2	729	TA(T/C)ATCAG	None (Tyrosine)
KIAA1036	Intron	/	CCA(C/T)AGGC	
POMT2	Exon 13	1587	AAA(A/G)GAC	None (Arginine)
TGF β 3	Exon 1	234	GCC(G/A)TCCC	5'UTR

loci, except ARVD1. However, the small size of the family impeded to obtain significantly positive lod scores by linkage analysis. The at-risk haplotype in this family appeared different from that detected in Family 112. This is not surprising, due to the different geographical origin of the two families. If we would accept that the disease in family 119 is linked to ARVD1 locus, recombinations detected in this family (between disease and D14S1036 and D14S127) would narrow the ARVD1 critical region to approximately 7.9 Mb.

The critical interval for ARVD1 is very large. Nevertheless, we attempted to screen for ARVD1-causing mutations coding sequences of four genes (POMT2, TGF β 3, KIAA1036 and KIAA0759), after having proved that they are expressed in the heart. These genes were selected on the basis of the possible role of their mutations in ARVD pathogenesis, as suggested by identification of pathogenic mutations in the gene encoding cardiac Ryanodine receptor (RYR2/ARVD2) and in the Plakoglobin gene (JUP, involved in Naxos disease, an autosomal recessive form of ARVD). Actually, mutations in three selected genes might produce plasma membrane instability or apoptosis, which are presently believed to be primary steps in ARVD pathogenesis^{4, 16–18}, while fourth gene was considered because it is placed in the region corresponding to the maximum location score, it is expressed in the heart tissue and its function is still unknown.

Mutation screening of the coding regions and of intron-exon boundaries of all these genes revealed only non pathogenic sequence variants. However, the presence of intragenic deletions or of regulatory mutations in one of these genes cannot be ruled out at this moment.

Restriction of the critical ARVD1 region is essential for the progress of the investigation aiming at the identification of the involved gene. Hopefully, two research initiatives on ARVD, recently launched by European Community and by US National Health Institute, should succeed in reaching this goal soon.

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