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A New Locus for Arrhythmogenic Right Ventricular Dysplasia on the Long Arm of Chromosome 14

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Familial arrhythmogenic right ventricular cardiomyopathy or dysplasia (ARVD) is an idiopathic heart muscle disease with an autosomal-dominant pattern of transmission, characterized by fibro-fatty replacement of the right ventricular myocardium and ventricular arrhythmias. Recently, linkage to the chromosome 14q23–q24 (locus D14S42) has been reported in two families. In the present study, three unrelated families with ARVD were investigated. According to strict diagnostic criteria, 13 of 37 members were considered to be affected. Linkage to the D14S42 locus was excluded. On the other hand, linkage was found in the region 14q12–q22 in all three families (cumulative two-point lod score is 3.26 for D14S252), with no recombination between the detected locus and the disease gene. With multipoint linkage analysis, a maximal cumulative lod score of 4.7 was obtained in the region between loci D14S252 and D14S257. These data indicate that a novel gene causing familial ARVD (provisionally named ARVD2) maps to the long arm of chromosome 14, thus supporting the hypothesis of genetic heterogeneity in this disease.

INTRODUCTION

Arrhythmogenic right ventricular dysplasia (ARVD) is an idiopathic heart muscle disorder, anatomically characterized by fatty or fibro-fatty infiltration of the right ventricular myocardium (Thiene *et al.*, 1988; McKenna *et al.*, 1994). From a clinical point of view, the onset of ARVD is usually characterized by ventricular arrhythmias of right ventricular origin and sometimes by symptoms of right heart failure (Pinamonti *et al.*, 1992). Depolarization and repolarization changes, such as inverted T waves in right precordial leads, epsilon waves, and late potentials on signal-averaged electrocardiograms, are frequently found. Structural and functional alterations of the right ventricle can be detected by echocardiography, angiography, magnetic resonance imaging, or radionuclide scintigraphy. These alterations can be localized (dyskinetic bulges, segmental dilatation, or hypokinesia) or diffuse. The gold standard for diagnosis of ARVD is the histological demonstration of a fatty or fibro-fatty substitution of the right ventricular myocardium (McKenna *et al.*, 1994).

At present, the incidence of the disease is unknown and is probably underestimated. The etiology is also unknown. However, in about 30% of cases, a familial occurrence with autosomal-dominant inheritance has been reported (McKenna *et al.*, 1994; Nava *et al.*, 1988; Miani *et al.*, 1993). Clinical studies have shown a reduced penetrance (Nava *et al.*, 1990) and a delayed age of onset (Thiene *et al.*, 1990; Camerini *et al.*, 1994) in familial ARVD. The variability of penetrance and clinical expression indicates genetic heterogeneity (Nava *et al.*, 1990), suggesting that different genes are likely to be responsible for the disease phenotype.

Recently, in a large four-generation kindred and in another small family, the disease was found to be linked to the polymorphic marker D14S42, which maps to region 14q23–q24 (Rampazzo *et al.*, 1994). To test the hypothesis of genetic heterogeneity, we performed linkage analysis in three unrelated kindreds with familial ARVD.

MATERIALS AND METHODS

Clinical evaluation. Three unrelated families of different ethnic origins (Italy, Slovenia, and Belgium) underwent clinical and genetic

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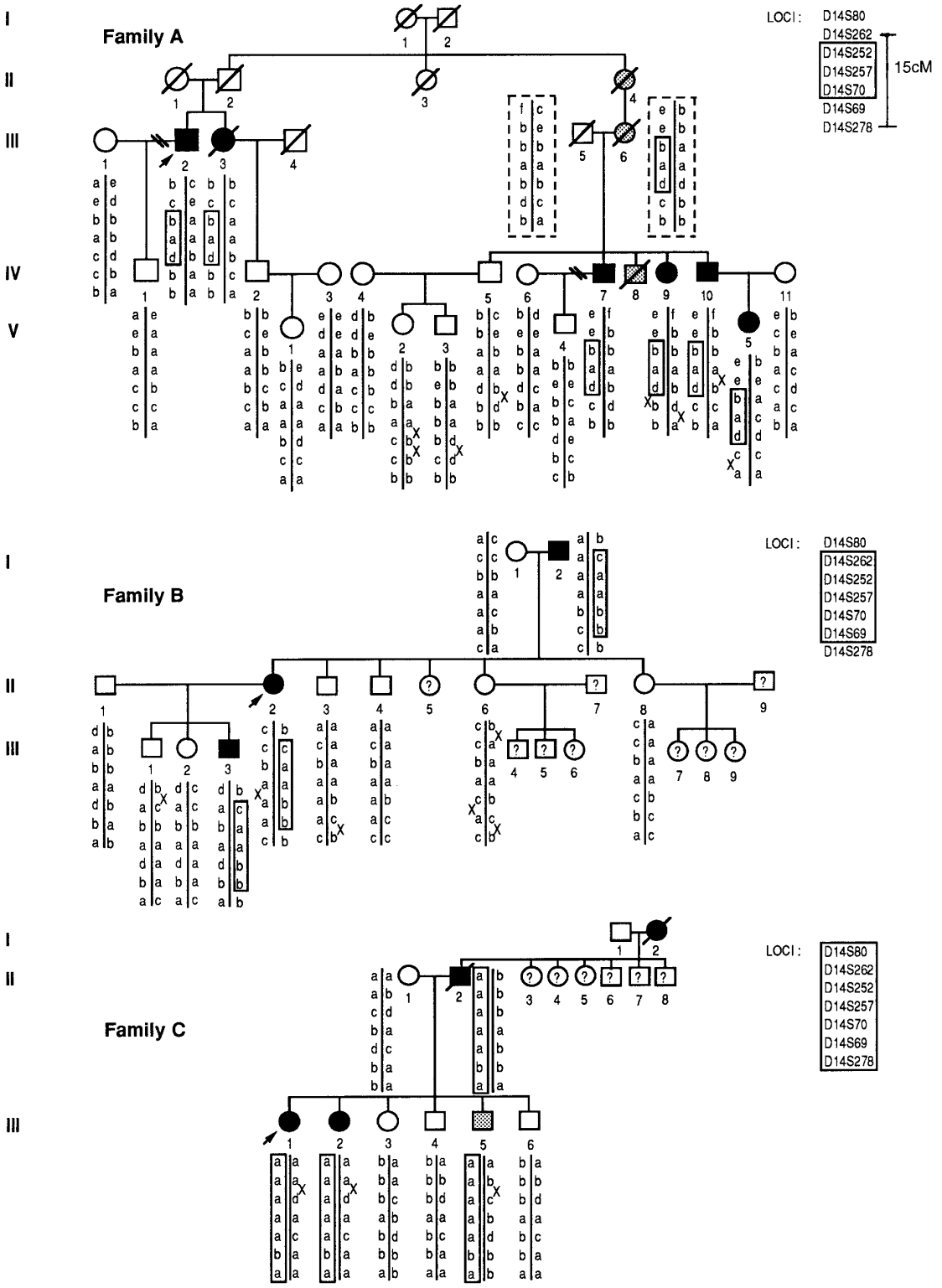

FIG. 1. Pedigrees with haplotype data of the three analyzed families. Males are represented by squares, females by circles, and dead family members by symbols with diagonals. The symbols are shaded black for individuals with defined ARVD and gray for individuals of uncertain clinical diagnosis. Individuals with a question mark inside the symbol were not included in this study. Markers cosegregating with the disorder are boxed. Haplotypes of individuals III-5 and III-6 of family A (dashed line) were inferred. Recombination events in the disease-associated chromosome are indicated by an X between the recombinant markers. Paternity analysis excluded individuals III-1, IV-1, IV-6, and V-4 of family A from the linkage study. The proband in each family is identified by an arrow.

TABLE 1

Clinical Features of the Affected Members with Familial ARVD

	Age[a]	Sex	Clinical findings	ECG[b]	VENTR. ARRH.	SAECG[c]	RV ABN[d]	RV pathology	ESC/ISFC criteria[e] Major	ESC/ISFC criteria[e] Minor	Final diagnosis
Family A											
III-2	49	M	Syncope, RHL, LHF[f]	AF,[g] LAH[h]	VT[i] (LBBB[j] type)	LP[k]	Severe HYPO[l] + DIL[m] + B[n]	Fibro-fatty infiltration (EMB[o])	3	2	ARVD
III-3	51	F	RHF	AF, 3°AVB,[p] T-in RPL,[q] EW[r]	VT (LBBB type)		Severe HYPO + DIL + B	Fibro-fatty infiltration (autopsy)	3	3	ARVD
VI-7	49	M	Asymptomatic	EW	VEB[s]	LP	HYPO + DIL + B		3	1	ARVD
IV-9	36	F	Syncope, LHF, RHF	AF, 1°-3°AVB	VT (LBBB type)		Diffuse HYPO + DIL + B	Fatty infiltration (EMB)	3	1	ARVD
IV-10	29	M	Syncope	SSS,[t] 1°AVB, EW	VT (LBBB type)	LP	Diffuse HYPO + DIL + B	Hypertrophy, endoc. fibrosis (EMB)	3	2	ARVD
V-5	19	F	Asymptomatic	T-in RPL	VEB, VT (LBBB type)	Normal	Mild HYPO + DIL		1	4	ARVD
Family B											
I-2	80	M	Asymptomatic	Prolonged QRS (V1-V3)			DIL + HYPO + B		2	1	ARVD
II-2	20	F	Syncope	Normal	VT (LBBB type)	LP	DIL + HYPO + B	Mild hypertrophy (EMB)	1	3	ARVD
III-3	12	M	Asymptomatic	Normal	VEB	Normal	DIL + HYPO + B		1	2	ARVD
Family C											
II-7	63	M	Syncope	AF, AT,[u] LAH	VT (LBBB type)		HYPO + DIL + B + FAT[v]		1	2	ARVD
III-1	33	F	Syncope, aborted SD[w]	T-in RPL	VT (LBBB type)	LP	Diffuse HYPO + DIL + B + FAT		1	4	ARVD
III-2	17	F	Asymptomatic	T-in RPL		Normal	HYPO + B		1	2	ARVD
III-5	29	M	Palpitations	Normal	Not documented	LP	Normal		0	2	Unknown

[a]Age at first symptom; [b]ECG, electrocardiogram; [c]SAECG, signal-averaged ECG; [d]RV ABN, right ventricular abnormalities (echocardiography, angiography, magnetic resonance imaging); [e]according to McKenna *et al.*, 1994; [f]RHF, right heart failure; LHF, left heart failure; [g]AF, atrial fibrillation; [h]LAH, left anterior hemiblock; [i]VT, ventricular tachycardia; [j]LBBB, left bundle branch block; [k]LP, late potentials; [l]HYPO, hypokinesis; [m]DIL, dilatation; [n]B, bulge; [o]EMB, endomyocardial biopsy; [p]AVB, atrioventricular block; [q]T-in RPL, inverted T wave in right precordial leads; [r]EW, epsilon waves; [s]VEB, ventricular ectopic beats; [t]SSS, sick sinus syndrome; [u]AT, atrial tachycardia; [v]FAT, fatty infilatration; [w]SD, sudden death.

studies beginning in 1991 (Fig. 1). The three kindred were selected from a group of six families with ARVD because of their sufficient informativity, without any other preselection criteria. Thirty-seven members were clinically evaluated with electrocardiography using supplementary chest leads (V4R, V3R, V7, and V8) and with M-mode, 2D, and Doppler echocardiography (Pinamonti *et al.*, 1992). The evaluation was completed by signal-averaged electrocardiography according to current recommendations (Breithardt *et al.*, 1991) in 16 subjects, by magnetic resonance in 9, by left and/or right ventricular angiography in 8, and by right endomyocardial biopsy in 5. Postmortem examination was performed in 1 case. For the definition of the diagnosis and of the clinical status, the criteria proposed by the ARVD ESC/ISFC task force (McKenna *et al.*, 1994) were strictly applied. In summary, the diagnosis was based on the analysis of six main groups of diagnostic criteria: (1) global and/or regional dysfunction and structural alterations; (2) tissue characterization of walls; (3) repolarization abnormalities; (4) depolarization/conduction abnormalities; (5) arrhythmias; and (6) familial history. In each group, major and minor criteria were considered: the diagnosis of ARVD depended on the presence of two major criteria from different diagnostic groups, of one major plus two minor criteria, or of four minor criteria. The clinical status of the affected and unaffected relatives was examined periodically during the follow-up. The phenotypic data were interpreted without knowledge of genotype. The age of the unaffected key members was over 20 years.

Genetic analysis. DNA samples from all available family members were extracted according to the salting out procedure (Miller *et al.*, 1988), either from fresh blood samples or from continuous B-lymphocyte cell lines immortalized with the Epstein–Barr virus (Neitzel 1986). In the case of one deceased patient (family A III-3, Fig. 1), DNA was extracted from a paraffin-embedded tissue sample (Jackson *et al.*, 1991).

Polymorphic microsatellite sequences (CA repeats) with high percentages of heterozygosity (Jordan *et al.*, 1990; Wang and Weber, 1992; Weissenbach *et al.*, 1992; Gyapay *et al.*, 1994) were amplified using the polymerase chain reaction (PCR) technique.

PCR amplifications were carried out in 50 μl of a solution containing Tris–HCl, 10 m*M* (pH 8.0); KCl, 50 m*M;* $MgCl_2$, 1.5 m*M;* gelatin 0.01%; each dNTP, 200 m*M;* each primer, 0.1 μ*M;* 200 ng of template DNA; and 2.5 units of *Taq* DNA polymerase (Perkin Elmer, Roche Molecular Systems, Branchburg, NJ), with 40 repetitions of the following cycle: 30 s at 94°C, 30 s at the annealing temperature, and 30 s at 72°C in the Eppendorf Master Cycler 5330. Annealing temperatures varied from 52 to 62°C, according to the different primer pairs. PCR products were resolved by a long run on a 10–12% polyacrylamide native gel and visualized by ethidium bromide staining. All the oligonucleotides were synthesized by the ICGEB oligonucleotide synthesis service on an Applied Biosystems 380B synthesizer.

Linkage analysis. All polymorphisms were scored without knowledge of phenotypic data and by two independent observers. Two-point and multipoint lod scores were calculated using the LINKAGE software package Version 5.2 (Lathrop *et al.*, 1984; Lathrop and Lalouel, 1985) on a SUN SPARC Server 10-401 computer. For the calculation, the existence of an autosomal-dominant ARVD gene with a gene frequency of 0.0001, an equal female and male recombination rate, and equal marker allele frequencies were assumed. In all events, the results were not significantly affected by modifications of allele frequency calculated from families (Boehnke, 1991) at these loci. Family members under 18 years, with the exception of those found to be affected (family B, III-3, and family C, III-5; Table 1 and Fig. 1), were excluded from the study, to avoid the low penetrance in this age group (10%, based on our clinical observations). For the

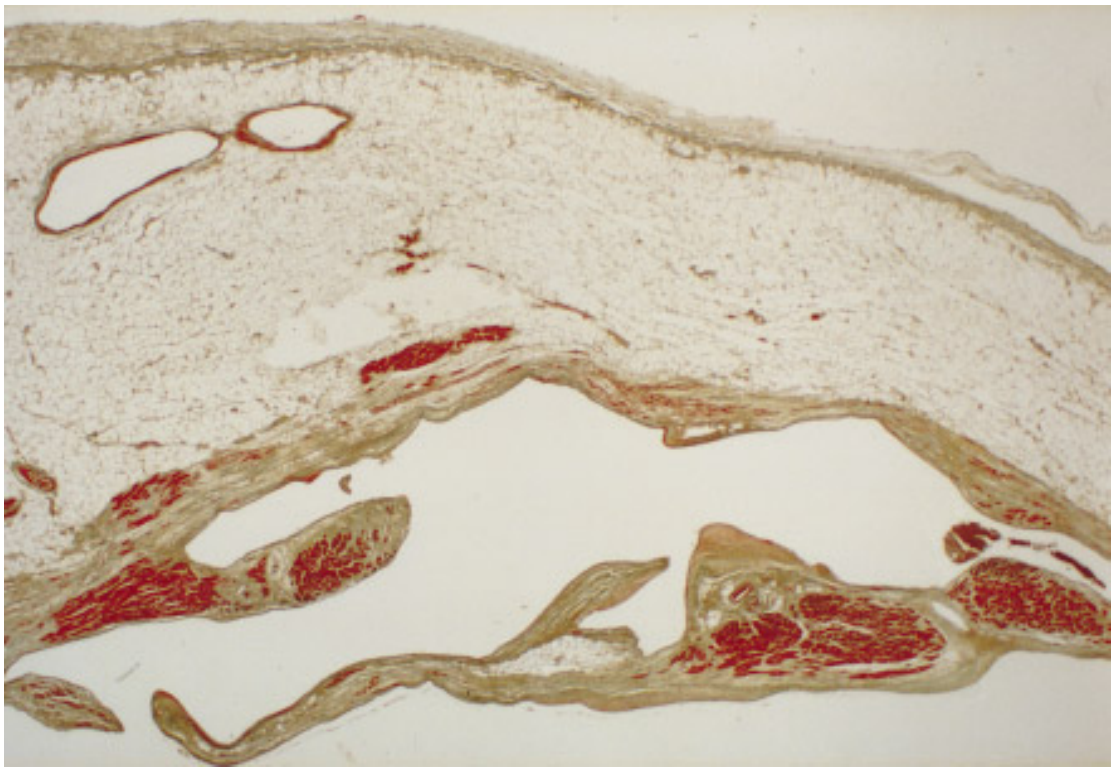

FIG. 2. Histological section of the right ventricular free wall from patient III-3 (family A). The muscle component is replaced by fatty tissue and by a thin layer of subendocardial connective tissue. Residual myocardial cells are present within the trabeculae (Azan; original magnification ×2.5).

lod score calculations, the penetrance was assumed to be 100%; however, to test the stability of the obtained lod values, the maximum two-point lod scores were recalculated, varying the penetrance from 100 to 90%. The calculations of the two-point lod scores were performed at recombination fractions of 0, 0.01, 0.05, 0.1, 0.2, 0.3, and 0.4 between each marker and the putative disease gene. The SIMLINK computer program (Ploughman *et al.*, 1989) and the HOMOG program (Ott, 1991) were used, respectively, to verify the appropriate linkage power and to test the homogeneity of the series of families analyzed.

RESULTS

Clinical Analysis

Three unrelated families of different ethnic origins with familial transmission of ARVD were analyzed in this study.

According to the criteria of the ESC/ISFC task force for the diagnosis of ARVD (McKenna *et al.*, 1994), 13 family members were considered to be affected; of these, 1 young subject (family A, V-5) developed signs of ARVD during the follow-up. The clinical status of another individual (family C, III-5), showing minor abnormalities that were not sufficient for a definitive diagnosis, was considered as unknown for the purpose of genetic analysis. In all families, ARVD was inherited as an autosomal-dominant trait with age-related penetrance (Fig. 1).

The clinical features of the 13 patients are summarized in Table 1. The mean age at the first symptom was 37, ranging from 12 to 80 (family B, I-2 and III-3, respectively), thus demonstrating the variability of expression. No significant sex difference was detected. The majority of patients suffered from symptoms related to complex ventricular arrhythmias at presentation. Patient III-3 of family A had isolated right heart failure. Five cases were asymptomatic and were detected incidentally during the familial screening. The left ventricular cavity was not significantly enlarged in any patient. However, cases III-2 and IV-9 of family A developed signs of left ventricular involvement, with reduced ejection fraction (43 and 51%, respectively).

Transmural fibro-fatty replacement of the right ventricular myocardium was demonstrated at autopsy in patient III-3 of family A (Fig. 2). The presence of relevant fibro-fatty substitution was found at endomyocardial biopsy in patients III-2 and IV-9 of family A. Histology showed aspecific changes in two other cases (family A, IV-10, and family B, II-2). Fatty infiltration was identified by nuclear magnetic resonance imaging in two affected family C members (II-7 and III-1).

Linkage Analysis for the ARVD1 Locus (14q23–q24)

Linkage analysis was performed on 33 members of the three families: 14 from family A, 11 from family B, and 8 from family C (Fig. 1). Four subjects from family A (III-1, IV-1, IV-6, and V-4) were excluded

TABLE 2

Cumulative and Pedigree-Specific Lod Scores Obtained at Different Values of Recombination Fraction (θ) with Two-Point Linkage Analysis for Loci D14S71 (Gyapay *et al.*, 1994), D14S42 (Jordan *et al.*, 1990), D14S76, D14S61, and D14S74 (Gyapay *et al.*, 1994)

		θ					
Loci		0.00	0.01	0.02	0.03	0.04	0.05
D14S71	Cumulative	−Inf.[a]	−3.97	−2.81	−2.17	−1.72	−1.37
	Family A	−2.24	−0.06	0.21	0.35	0.44	0.51
	Family B	−Inf.	−1.11	−0.81	−0.65	−0.53	−0.44
	Family C	−Inf.	−2.80	−2.21	−1.87	−1.63	−1.44
D14S42	Cumulative	−Inf.	−0.99	−0.70	−0.54	−0.43	−0.34
	Family A	0.12	0.12	0.11	0.11	0.10	0.10
	Family B	−Inf.	−1.11	−0.81	−0.65	−0.53	−0.44
	Family C	0.00	0.00	0.00	0.00	0.00	0.00
D14S76	Cumulative	−2.62	−0.47	−0.20	−0.05	0.05	0.11
	Family A	−2.62	−0.47	−0.20	−0.05	0.05	0.11
	Family B	0.00	0.00	0.00	0.00	0.00	0.00
	Family C	0.00	0.00	0.00	0.00	0.00	0.00
D14S61	Cumulative	−Inf.	−7.05	−5.30	−4.30	−3.61	−3.08
	Family A	−7.17	−2.33	−1.75	−1.41	−1.18	−1.01
	Family B	−Inf.	−1.92	−1.34	−1.02	−0.80	−0.63
	Family C	−Inf.	−2.80	−2.21	−1.87	−1.63	−1.44
D14S74	Cumulative	−Inf.	−0.62	−0.33	−0.16	−0.05	0.03
	Family A	0.18	0.19	0.20	0.21	0.21	0.22
	Family B	−Inf.	−0.81	−0.53	−0.37	−0.26	−0.19
	Family C	0.00	0.00	0.00	0.00	0.00	0.00

[a] Minus infinity.

from the linkage study according to the results of a paternity test.

Family members were first examined with a group of five microsatellite markers spread in a region of about 5 cM (Gyapay *et al.*, 1994; Murray *et al.*, 1994; Cox Matise *et al.*, 1994) corresponding to the region of the locus D14S42 (Jordan *et al.*, 1990), the first identified ARVD locus (ARVD1) (Rampazzo *et al.*, 1994). The results of two-point linkage analysis are shown in Table 2, where the locus order is based on the Généthon map (Gyapay *et al.*, 1994). Two-point linkage analysis led to the exclusion of linkage with the ARVD1 locus in our families with marker D14S61. D14S42 (PIC 0.65) (Jordan *et al.*, 1990) was not informative. Results did not change significantly by varying the penetrance from 90 to 100%. Multipoint linkage analysis (data not shown) confirmed the absence of linkage with the ARVD1 region. HOMOG analysis rejected the hypotheses of linkage and of heterogeneity.

The definitive evidence against linkage with the D14S42 locus derived from the haplotype analysis, demonstrating several recombinants in the three ARVD families. A critical recombination occurred in the first generations of family A, in which affected members II-2 and II-3 were shown to carry a different allele than that in the other affected relatives. Other recombinants were found in family A (IV-2), in family B (III-1 and II-3), and in family C (II-5 and III-6).

Linkage of ARVD to 14q12–q22

Ten microsatellite markers with a heterozygosity index greater than 0.75 and located every 10–15 cM were used to analyze chromosome 14. Linkage to locus MYH7 (14q11–q12), corresponding to the localization of the cardiac β myosin heavy chain gene, was excluded. Since a positive result was obtained for locus D14S70, genotyping was performed with six additional markers (Gyapay *et al.*, 1994) in this region (Fig. 1). With two-point linkage analysis, positive lod scores were obtained for three microsatellite markers (Table 3): AFM207xb12 (locus D14S252), AFM224yb8 (D14S257), and AFM191ve1 (D14S70), mapping approximately to chromosome 14q12–q22 and spanning a region of 11 cM. Significant cumulative lod scores (3.26 for D14S252 and 3.07 for D14S70) were detected at a recombination fraction of 0, indicating complete linkage. Lod score values also remained significantly positive ($\geq$3) after reducing penetrance to 85%, as well as after changing allele marker frequencies. Changes in the affection status when performing the sensitivity test (Hodge and Greenberg, 1992) did not affect the lod values.

In accordance with the results of two-point linkage analysis, haplotype analysis detected an obligate recombination event between the disease gene and locus D14S69 for individual IV-9 of family A. Another obligate recombination event occurred between ARVD and locus D14S262, as shown by the different alleles in individuals III-2 and III-3 compared to that in the other affected relatives (IV-7, VI-8, and IV-10), thus setting the boundaries for the disease gene locus. No recombination event was observed within this region.

To determine the most likely location for the disease locus, multipoint linkage analysis was performed using five adjacent loci. Multipoint linkage analysis confirmed the findings of two-point and haplotype analyses by placing the disease gene between loci D14S252 and D14S70, with a maximum multipoint lod score of 4.7 (Fig. 3). These data suggest that the novel gene for ARVD is located between the loci D14S262 and D14S69, in a 15-cM region of chromosome 14, at a distance of about 30 cM from the previously reported ARVD gene (Rampazzo *et al.*, 1994) (Fig. 4).

DISCUSSION

Recently, a linkage study performed in two families with ARVD suggested that the disease gene maps to region q23–q24 of chromosome 14 and is closely associated to the marker D14S42 (Rampazzo *et al.*, 1994). In this report, we present the evidence of a novel locus, ARVD2, localized to the long arm of chromosome 14 in region q12–q22.

TABLE 3

Cumulative and Pedigree-Specific Lod Scores Obtained at Different Values of Recombination Fraction (θ) with the Two-Point Linkage Analysis for the Loci D14S262, D14S252, D14S257, D14S70, and D14S69 (Gyapay *et al.*, 1994)

		θ						
Loci		0.00	0.01	0.05	0.10	0.20	0.30	0.40
D14S80	Cumulative	−Inf.	−1.32	0.47	0.98	1.02	0.65	0.29
	Family A	−2.73	−0.58	0.01	0.17	0.17	0.05	−0.05
	Family B	−Inf.	−1.92	−0.63	−0.17	0.13	0.16	0.09
	Family C	1.20	1.18	1.09	0.98	0.72	0.44	0.15
D14S262	Cumulative	−1.27	−0.93	1.40	1.43	1.14	0.71	0.20
	Family A	−3.37	−1.14	−0.50	−0.27	−0.10	−0.03	−0.01
	Family B	1.20	1.18	1.09	0.98	0.72	0.44	0.15
	Family C	0.90	0.89	0.81	0.72	0.52	0.30	0.09
D14S252	Cumulative	3.26	3.20	2.96	2.64	1.96	1.23	0.50
	Family A	1.16	1.13	1.03	0.90	0.63	0.36	0.12
	Family B	0.90	0.89	0.84	0.77	0.61	0.44	0.24
	Family C	1.20	1.18	1.09	0.98	0.72	0.44	0.15
D14S257	Cumulative	0.38	0.37	0.32	0.26	0.15	0.07	0.02
	Family A	0.38	0.37	0.32	0.26	0.15	0.07	0.02
	Family B	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Family C	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D14S70	Cumulative	3.07	3.02	2.78	2.48	1.83	1.18	0.52
	Family A	0.97	0.95	0.85	0.73	0.50	0.30	0.13
	Family B	0.90	0.89	0.84	0.77	0.61	0.44	0.24
	Family C	1.20	1.18	1.09	0.98	0.72	0.44	0.15
D14S69	Cumulative	−Inf.	−0.92	0.28	0.63	0.71	0.51	0.21
	Family A	−Inf.	−2.40	−1.09	−0.60	−0.21	−0.07	−0.02
	Family B	1.51	1.48	1.37	1.23	0.92	0.58	0.23
	Family C	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D14S278	Cumulative	−Inf.	−3.15	−1.20	−0.49	0.02	0.17	0.14
	Family A	−Inf.	−1.23	−0.59	−0.37	−0.20	−0.12	−0.06
	Family B	−Inf.	−1.91	−0.61	−0.12	0.22	0.29	0.20
	Family C	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Using stringent clinical and genetic criteria, we studied three families of various descent (Italian, Slovenian, and Belgian) with ARVD. The clinical assessment was based on the criteria recently stated by the ARVD ESC/ISFC task force (McKenna *et al.*, 1994). All families had an autosomal-dominant pattern of transmission.

The results obtained from the genetic study clearly indicate that the previously identified ARVD1 locus is not linked to the disease in these families. In fact, marker D14S42 (which has been linked to the disease) as well as other markers in the same region (corresponding to loci D14S71, D14S76, D14S61, and D14S74) yield clearly negative lod scores (incidentally, the latter group of markers was also negative even in the study of Rampazzo *et al.*, 1994).

By screening the whole of chromosome 14, a novel localization for the disease gene was detected between loci D14S262 and D14S69 (maximum combined lod score is 4.7), without any apparent recombination event in all these families. This new locus (ARVD2) is located approximately 30 cM centromeric to ARVD1.

A founder effect, with a high concentration of cases in a limited geographic area, was hypothesized in the case of the ARVD1 gene (Rampazzo *et al.*, 1994). However, our data, showing a homogeneous linkage in families with different ethnic origins, suggest that the ARVD2 gene can account for the disease in various populations.

The possibility of genetic heterogeneity is not surprising in familial ARVD and was previously hypothesized in clinical studies, according to the large variability of phenotypic expression (McKenna *et al.*, 1994; Nava *et al.*, 1990). Likewise, genetic heterogeneity has been found by molecular genetic studies in other myocardial disorders. In familial hypertrophic cardiomyopathy, mutations in chromosomes 1q3 (cardiac troponin T gene) (Watkins *et al.*, 1993; Thierfelder *et al.*, 1994), 11p13–q13 (Carrier *et al.*, 1993), and 15q2 (α-tropomyosin gene) (Thierfelder *et al.*, 1993, 1994) have been identified, in addition to more than 15 different mutations in the cardiac β myosin heavy chain gene on chromosome 14. In addition, the genes responsible for another arrhythmic syndrome, the long QT syndrome,

have been mapped to chromosome 11 (Keating, 1992) and very recently also to chromosomes 3 and 7 (Jiang *et al.*, 1994).

Since not all the markers located in the chromosome 14 candidate region have been physically mapped so far (Murray *et al.*, 1994), the location of the ARVD2 gene is still approximate. However, the exclusion of linkage to loci MYH7 (14q11–q12) and D14S42 (14q23–q24) and the genetic mapping of D14S69 and D14S262 (Gyapay *et al.*, 1994) enable us to locate the ARVD2 gene to 14q12–q22 (Fig. 4). This region of chromosome 14 is relatively poor in identified genes, and none of them seems to be an obvious candidate gene (Murray *et al.*, 1994; Billingsley *et al.*, 1994). Among them, the glycogen phosphorylase gene is related to the glycogen storage disease type VI, leading to a storage heart disease quite different from ARVD. β-Spectrin and α-actinin, other possible candidate genes, were mapped to different regions (14q22–q24). It is expected that the extension of the analysis to other families and the development of new markers flanking the ARVD2 locus will facilitate a positional cloning strategy to identify the gene and eventually to detect the mutations causing the disease.

Among young people, ARVD represents one of the first causes of sudden and unexpected death, which can even be the presenting event (Thiene *et al.*, 1988). Due to the age-related penetrance of the disease, in particular the gene-carriers of young age can appear completely normal at clinical examinations, while being at risk of life-threatening arrhythmias. During the follow-up, we observed the development of ARVD in a 19-year-old patient, previously examined and considered to be unaffected (family A V-5, Fig. 1). In this respect, the identification of polymorphic markers linked to the ARVD genes may be of great clinical value for the presymptomatic diagnosis of gene-carrier status and the prevention of major complications. Moreover, the identification of the specific gene abnormalities will make possible the development of new therapeutic strategies, based on the understanding of the molecular mechanisms that control the cardiac structure and function.

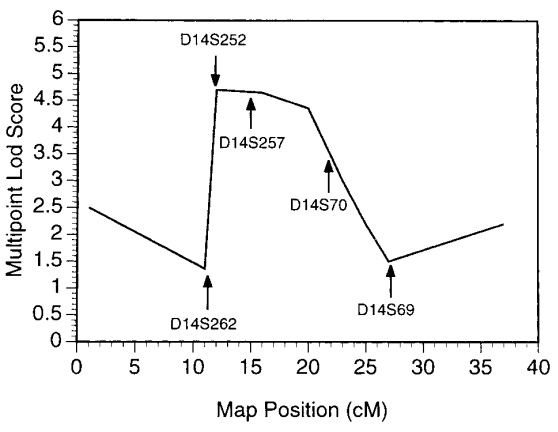

FIG. 3. A cumulative multipoint lod score curve derived from four-point analysis using the five markers indicated. Maximum lod score was obtained between loci D14S252 and D14S257.

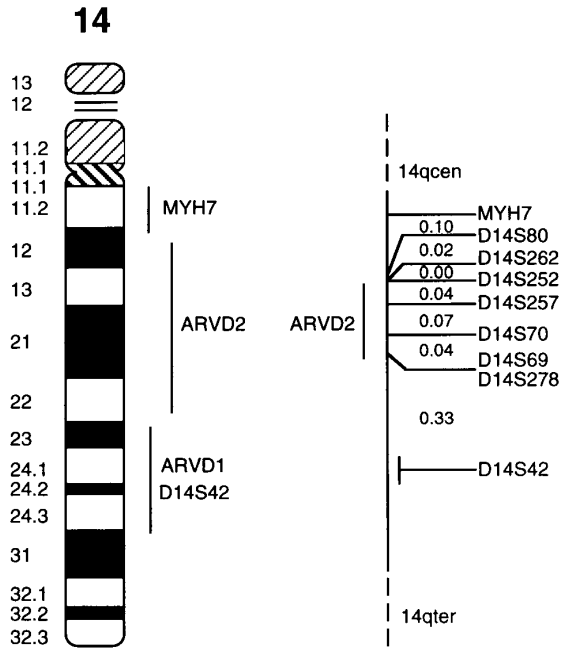

FIG. 4. Physical and genetic maps (left and right, respectively) of the chromosome 14q region containing the ARVD2 locus. In the genetic map, the sex-average recombination fractions are shown between adjacent markers. The ARVD2 locus lies between markers D14S262 and D14S69, physically located in the region 14q12–14q22. Distances are given in centimorgans, based on the Généthon 1994 data (Gyapay *et al.*, 1994).

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Note added in proof. A further ARVD locus on Chromosome 1q42–q43 has recently been described by other authors (Rampazzo *et al.*, 1995).

REFERENCES

Billingsley, G. D., Cox, D. W., Duncan, A. M. V., Goodfellow, P. J., and Grzeschik, K. H. (1994). Regional localization of loci on chromosome 14 using somatic cell hybrids. *Cytogenet. Cell Genet.* **66:** 33–38.

Breithardt, G., Cain, M. E., el-Sherif, N., Flowers, N. C., Hombach, V., Janse, M., Simson, M. B., and Steinbeck, G. (1991). Standards for analysis of ventricular late potentials using high-resolution or signal-averaged electrocardiography. A statement by a Task Force Committee of the European Society of Cardiology, the American Heart Association, and the American College of Cardiology. *Circulation* **83:** 1481–1488.

Boehnke, M. (1991). Allele frequency estimation from data on relatives. *Am. J. Hum. Genet.* **48:** 22–25.

Camerini, F., Di Lenarda, A., Perkan, A., Pinamonti, B., Sinagra,

G., and the Heart Muscle Disease Study Group (1994). Right ventricular dysplasia/cardiomyopathy: What is the evolution of the disease? *In* "Prognosis and Treatment of Cardiomyopathies and Myocarditis. Cardiomyopathy Update 5" (M. Sekiguchi and P. J. Richardson, Eds.), pp. 281–292, Univ. of Tokyo Press, Tokyo.

Carrier, L., Hengstemberg, C., Beckmann, J. S., Guicheny, P., Dufour, C., Bercovici, J., Dausse, E., Berebbi-Bertrand, I., Wisnewsky, C., Pulvenis, D., Fetler, L., Vignal, A., Weissenbach, J., Hillaire, D., Feingold, J., Bouhour, J.-B., Hagege, A., Desnos, M., Isnard, R., Duborg, O., Komajda, M., and Schwartz, K. (1993). Mapping a novel gene for familial hypertrophic cardiomyopathy to chromosome 11. *Nature Genet.* **4:** 311–313.

Cox Matise, T., Perlin, M., and Chakravarti, A. (1994). Automated construction of genetic linkage maps using expert system (Multi Map): A human genome linkage map. *Nature Genet.* **6:** 384–390.

Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M., and Weissenbach, J. (1994). The 1993–94 Généthon human genetic linkage map. *Nature Genet.* **7:** 246–339.

Hodge, S. E., and Greenberg, D. A. (1992). Sensitivity of lod scores to changes in diagnostic status. *Am. J. Hum. Genet.* **50:** 1053–1066.

Jackson, D. P., Hayden, J. D., and Quirke, P. (1991). Extraction of nucleic acid from fresh and archival material. *In* "PCR. A Practical Approach" (M. J. McPherson, P. Quike, and G. R. Taylor, Eds.), pp. 29–50, IRL Press, Oxford.

Jiang, C., Atkinson, D., Towbin, J. A., Splawski, I., Lehmann, M. H., Li, H., Timothy, K., Taggart, R. T., Schwartz, P. J., Vincent, G. M., Moss, A. J., and Keating, M. T. (1994). Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. *Nature Genet.* **8:** 141–147.

Jordan, S. A., McWilliam, P., O'Briain, D. S., and Hunphries, P. (1990). Dinucleotide repeat polymorphism at the D14S42 locus. *Nucleic Acids Res.* **19:** 1171.

Keating, M. (1992). Linkage analysis and long-QT syndrome. Using genetics to study cardiovascular disease. *Circulation* **85:** 1973–1986.

Lathrop, G. M., and Lalouel, J. M. (1985). Easy calculation of lod scores and genetic risk on small computers. *Am. J. Hum. Genet.* **36:** 460–464.

Lathrop, G. M., Lalouel, J. M., Julier, C., and Ott, J. (1984). Strategies for multilocus linkage analysis in humans. *Proc. Natl. Acad. Sci. USA* **81:** 3443–3446.

McKenna, W. J., Thiene, G., Nava, A., Fontaliran, F., Blomstrom-Lundqvist, C., Fontaine, G., and Camerini, F. (1994). Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Br. Heart J.* **71:** 215–218.

Miani, D., Pinamonti, B., Bussani, R., Silvestri, F., Sinagra, G., and Camerini, F. (1993). Right ventricular dysplasia: A clinical and pathological study of two families with left ventricular involvement. *Br. Heart J.* **69:** 151–157.

Miller, S. A., Dykes, D. D., and Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16:** 1215.

Murray, J. C., Beutow, K. H., Weber, J. L., Ludwigsen, S., Scherpbier-Heddema, T., Manion, F., Quillen, J., Sheffield, V. C., Sunden, S., and Duyk, G. M. (1994). A comprehensive human linkage map with centimorgan density. *Science* **265:** 2049–2054.

Nava, A., Canciani, B., Thiene, G., Scognamiglio, R., Buja, G., Martini, B., Daliento, L., Fasoli, G., Stritoni, P., and Dalla Volta, S. (1990). Analyse du mode de trasmission de la dysplasie ventriculaire droite. *Arch. Mal. Coeur* **83:** 923–928.

Nava, A., Thiene, G., Canciani, B., Scognamiglio, R., Daliento, L., Buja, G., Martini, B., Stritoni, P., and Fasoli, G. (1988). Familial occurrence of right ventricular dysplasia: A study involving nine families. *J. Am. Coll. Cardiol.* **12:** 1222–1228.

Neitzel, H. (1986). A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum. Genet.* **73:** 320–326.

Ott, J. (1991). "Analysis of Human Genetic Linkage," Johns Hopkins Univ. Press, Baltimore, MD.

Pinamonti, B., Sinagra, G., Salvi, A., Di Lenarda, A., Morgera, T., Silvestri, F., Bussani, R., and Camerini, F. (1992). Left ventricular involvement in right ventricular cardiomyopathy. *Am. Heart J.* **123:** 711–724.

Ploughman, L. M., and Boehnke, M. (1989). Estimating the power of a proposed linkage study for a complex genetic trait. *Am. J. Hum. Genet.* **44:** 543–551.

Rampazzo, A., Nava, A., Danieli, G. A., Buja, G., Daliento, L., Fasoli, G., Scognamiglio, R., Corrado, D., and Thiene, G. (1994). The gene for arrhythmogenic right ventricular cardiomyopathy maps to chromosome 14q23–q24. *Hum. Mol. Genet.* **3:** 959–962.

Rampazzo, A., Nava, A., Erne, P., Eberhard, M., Vian, E., Slomp, P., Tisio, N., Thiene, G., and Danieli, G. A. (1995). A new locus for arrhythmogenic right ventricular cardiomyopathy (ARVD2) maps to chromosome 1q42–q43. *Hum. Mol. Genet.* **4:** 2151–2154.

Thiene, G., Nava, A., Corrado, D., Rossi, L., and Pennelli, N. (1988). Right ventricular cardiomyopathy and sudden death in young people. *N. Engl. J. Med.* **318:** 129–133.

Thiene, G., Nava, A., Angelini, A., Daliento, L., Scognamiglio, R., and Corrado, D. (1990). Anatomoclinical aspects of arrhythmogenic right ventricular cardiomyopathy. *In* "Advances in Cardiomyopathies" (G. Baroldi, F. Camerini, and J. F. Goodwin, Eds.), pp. 397–408, Springer-Verlag, Berlin/Heidelberg.

Thierfelder, L., MacRae, C., Watkins, H., Tomfohrde, J., Williams, M., McKenna, W. J., Bohm, K., Noeske, G., Schlepper, M., Bowcock, A., Vosberg, H. P., Seidman, J. G., and Seidman, C. E. (1993). A familial hypertrophic cardiomyopathy locus maps to chromosome 15q2. *Proc. Natl. Acad. Sci. USA* **90:** 6270–6274.

Thierfelder, L., Watkins, H., MacRae, C., Lamas, R., McKenna, W., Vosberg, H. P., Seidman, J. G., and Seidman, C. E. (1994). α-Tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: A disease of the sarcomere. *Cell* **77:** 701–712.

Wang, Z., and Weber, J. L. (1992). Continuous linkage map of human chromosome 14 short tandem repeat polymorphism. *Genomics* **13:** 532–536.

Watkins, H., MacRae, C. A., Thierfelder, L., Chou, Y.-H., Frenneaux, M., McKenna, W. J., Seidman, J. G., and Seidman, C. E. (1993). A disease locus for familial hypertrophic cardiomyopathy maps to chromosome 1q. *Nature Genet.* **3:** 333–337.

Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, P., Vaysseix, G., and Lathrop, M. (1992). A second-generation linkage map of the human genome. *Nature* **359:** 794–801.