Original Article

Autosomal Recessive Atrial Dilated Cardiomyopathy With Standstill Evolution Associated With Mutation of *Natriuretic Peptide Precursor A*

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Background—Atrial dilatation and atrial standstill are etiologically heterogeneous phenotypes with poorly defined nosology. In 1983, we described 8-years follow-up of atrial dilatation with standstill evolution in 8 patients from 3 families. We later identified 5 additional patients with identical phenotypes: 1 member of the largest original family and 4 unrelated to the 3 original families. All families are from the same geographic area in Northeast Italy.

Methods and Results—We followed up the 13 patients for up to 37 years, extended the clinical investigation and monitoring to living relatives, and investigated the genetic basis of the disease. The disease was characterized by: (1) clinical onset in adulthood; (2) biatrial dilatation up to giant size; (3) early supraventricular arrhythmias with progressive loss of atrial electric activity to atrial standstill; (4) thromboembolic complications; and (5) stable, normal left ventricular function and New York Heart Association functional class during the long-term course of the disease. By linkage analysis, we mapped a locus at 1p36.22 containing the *Natriuretic Peptide Precursor A* gene. By sequencing *Natriuretic Peptide Precursor A,* we identified a homozygous missense mutation (p.Arg150Gln) in all living affected individuals of the 6 families. All patients showed low serum levels of atrial natriuretic peptide. Heterozygous mutation carriers were healthy and demonstrated normal levels of atrial natriuretic peptide.

Conclusions—Autosomal recessive atrial dilated cardiomyopathy is a rare disease associated with homozygous mutation of the *Natriuretic Peptide Precursor A* gene and characterized by extreme atrial dilatation with standstill evolution, thromboembolic risk, preserved left ventricular function, and severely decreased levels of atrial natriuretic peptide. **(*Circ Cardiovasc Genet*. 2013;6:27-36.)**

Key Words: atrial cardiomyopathy ■ atrial natriuretic factor ■ atrial standstill ■ genetics ■ *Natriuretic Peptide Precursor A* gene

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Idiopathic atrial dilatation (AD) with disproportionately enlarged atria in the absence of other cardiac or hemodynamic abnormalities and atrial standstill (AS) with loss of electric and mechanical activity can occur as independent entities[1–8] or combined together.[4,9–11] AS can also be associated with Ebstein anomaly,[12] dilated cardiomyopathy,[13] myocarditis,[14,15] amyloidosis,[16] or muscle dystrophies such as Emery–Dreifuss and Limb-Girdle muscular dystrophy.[17,18] Moreover, AS has been reported in families with autosomal dominant Brugada syndrome,[19] in dilated cardiomyopathy with catecholaminergic polymorphic ventricular tachycardia,[20] and in dilated cardiomyopathy with Charcot-Marie-Tooth type 2 axonal neuropathy.[21] The diagnosis may be incidental or coincide with the occurrence of atrial arrhythmias.[4]

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AD and AS can be sporadic or familial, either autosomal dominant or recessive.[2,3,5,9] The genetic bases of idiopathic AD are unknown, whereas idiopathic AS has been associated with combined heterozygous mutations of *SCN5A* and *Connexin40* genes in 2 unrelated families in which only 1 of 5 members with AS also showed AD.[6,7] AS also has been associated with a recessive mutations of *SCN5A* gene in 10 children from 7 families with congenital sick sinus syndrome with evolution from sinus bradycardia to AS in 5; data about atrial dilation in these 5 patients are not available. Four had a congenital heart defect.[22]

In 1983, we described idiopathic AD and AS in 3 families from Northeast Italy,[9] and we later identified 2 siblings

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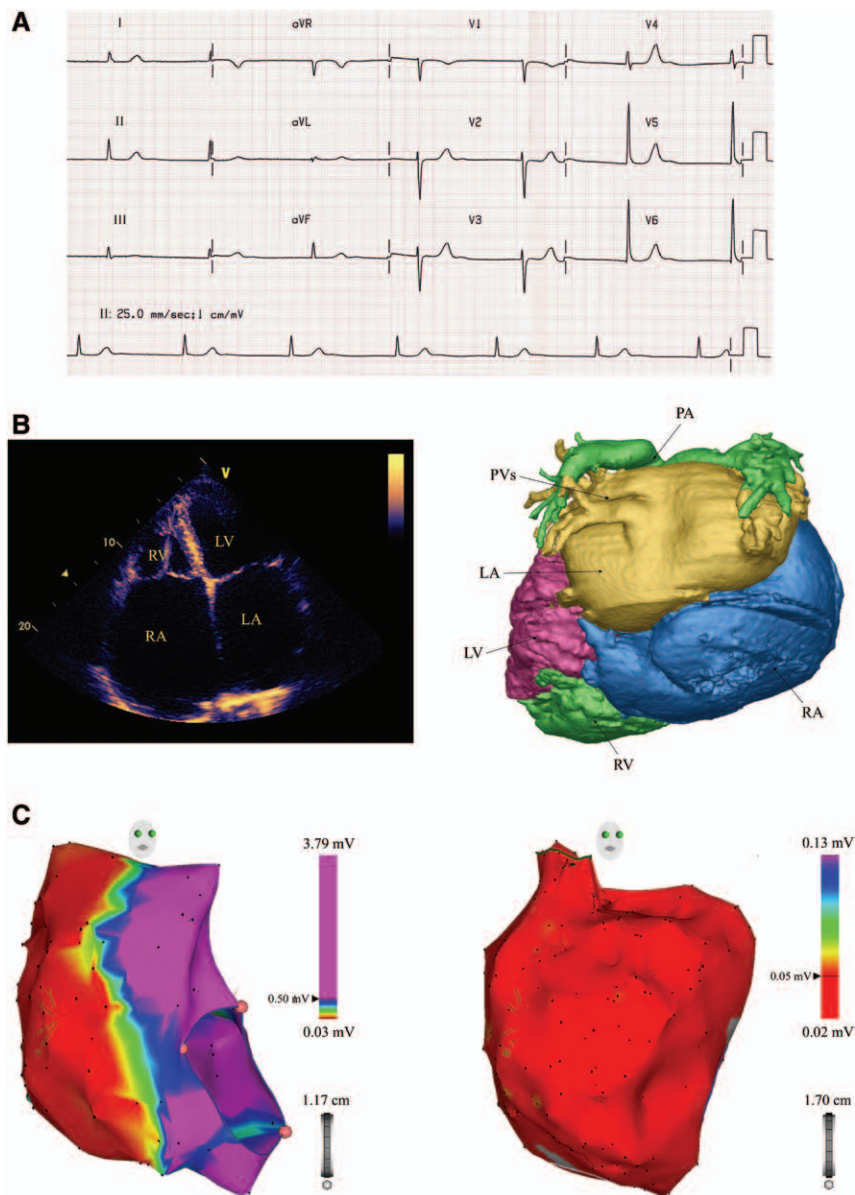
Figure 1. Phenotype. (**A**) Surface ECG with complete atrial standstill: bradycardic (39 bpm) junctional rhythm without atrial activity and narrow QRS (patient A:V:1). (**B**) Giant atria are shown by ultrasound examination and by 3-dimensional cardiac tomography (3DCT) imaging. On the **left**, the apical 4-chamber view of the patient A:IV:5. On the **right**, 3DCT reconstruction of the cardiac chambers[24] of patient A:V:1. Colored inner surfaces of the districts are shown in right posterior view. Right atrial (RA) and left atrial (LA) volumes are 744 and 426 mL, respectively; left (LV) and right ventricular (RV) volumes are 138 mL and 252 mL, respectively (see online-only Data Supplement Video). Pulmonary arteries (PA) and pulmonary veins (PVs) are shown at the top. (**C**) Scars in the RA are shown by 3D voltage mapping (right anterior projection) in patients D:IV:2 (at the left) and A:V:1 (at the right) with Brady–Tachy syndrome and complete atrial standstill, respectively. In the former, the scar is localized at lateral wall, whereas in the latter, the scar is diffused (red color indicates voltages <0.05 mV).

and 2 unrelated apparently sporadic patients with identical phenotypes in 3 additional families. During a long-term follow-up, we monitored the evolving phenotypes, patterned the natural history of the idiopathic AD with progression to AS, and identified a genetic association of the disease with a homozygous mutation in the *Natriuretic Peptide Precursor A* (*NPPA*) gene.

Methods

Patients

The clinical series consists of 13 patients diagnosed with idiopathic AD with AS (Figure 1) from 6 families at the Santa Chiara Hospital of Trento (Table 1). During 37 years of follow-up, all 13 patients and their family members underwent serial clinical, echocardiographic, electrophysiological, laboratory, and instrumental monitoring.

At echocardiographic examination, AD was graded as moderate (34–39 mL/m²), severe (≥40 mL/m²),[23] and giant (>80 mL/m²). Before 1999, the atrial size was semiquantitatively evaluated. Three patients underwent multislice tomography with 3-dimensional reconstruction of the cardiac chambers.[24] All patients underwent at least 1

endocavitary electrophysiological study at the time of diagnosis or at pacemaker implantation. Complete AS was defined as absence of atrial electric activity on surface ECG, with junctional bradycardia, absence of atrial activity in endocavitary recordings, no response to stimulation during electrophysiological study, and absence of A wave by echocardiography.[9,14] Partial AS was defined as absence of atrial electric activity on surface ECG, but with still irregular junctional rhythm and only localized absence of endocavitary atrial activity and no response to stimulation. Carto electroanatomic mapping was performed in 5 patients (Biosense-Welbster, Diamond Bar, CA): the areas displaying electric atrial activity <0.05 mV at bipolar mapping were considered scars.[25] Coronary angiography and endomyocardial biopsy of the right ventricle were performed in 2 patients.[26] In 6 patients, we performed fine needle biopsy of abdominal fat to exclude systemic amyloidosis. All patients underwent serial neurological evaluation, with electromyography and nerve conduction velocity analysis in 7.

Families and Controls

Five of the 13 patients died before receiving genetic testing. The 8 living patients and relatives received genetic counseling. Overall, 85 members of the 6 families underwent clinical evaluation and blood

Table 1.   Clinical Data of the 13 Affected Patients

Family	Pedigree No.	Sex	First Diagnosis, y	Duration Follow-up, y	First Diagnosis Symptoms	NYHA at 1st/Last Observation	Heart Rhythm at 1st/Last Observation	PM, y	Emb., y	Hypert.
A	IV:5	M	34	37	Dyspnea	II/II	Complete atrial standstill	40	No	No
A	IV:10	M	48	8 (56 y*)	Dyspnea	II/II	Complete atrial standstill	48	No	No
A	V:1	F	38	31	Palpitations	II/II	Partial→complete atrial standstill	53	40	Yes
A	V:3	F	40	27	Palpitations	I/II	Partial→complete atrial standstill	53	56	No
B	III:1	F	31	31	Palpitations	I/II	BTS→complete atrial standstill	37	50	No
B	III:4	M	38	25 (63 y*)	Palpitations	I/II	BTS→complete atrial standstill	47	46 - 63	No
B	III:6	M	36	20 (56 y*)	Stroke	II/II	Complete atrial standstill	36	36	No
C	III:2	F	58	9 (67 y*)	Dyspnea	III/II	Complete atrial standstill	59	67	No
C	III:6	F	51	13 (64 y*)	Dyspnea	III/II	Complete atrial standstill	51	No	No
D	IV:1	F	41	9	Palpitations	I/I	BTS→partial atrial standstill	No	No	No
D	IV:2	M	42	4	No symptoms	I/I	BTS→complete atrial standstill	43	No	Yes
E	III:1	M	34	4	Palpitations	I/I	BTS→partial atrial standstill	34	37	No
F	III:2	F	36	5	Palpitations	I/I	BTS→partial atrial standstill	No	No	No

BTS indicates Brady–Tachy syndrome; Emb., embolism; Hypert., hypertension; NYHA, New York Heart Association Class; and PM, pacemaker.

*Died (years of age).

sampling for genetic studies. Figure 2 shows the pedigrees of the 6 families including 64 of the overall 85 family members; 21 healthy relatives (all members of the 5th and 6th generations of family A) are not reported in the figure. On the basis of family pedigrees and clinical screening of the family members, we hypothesized an autosomal recessive inheritance of the disease.

Because the origin of the 6 families is from the same geographic area, we enrolled 192 healthy adults, unrelated up to 4 generations, all with local origin and still resident in the same area, with the aim of exploring the clinical and genetic profile of a representative control population. These subjects accepted to participate in the population study call launched by the local authorities.

An additional 200 adult individuals randomly selected from a sample of 2000 healthy control subjects from the national ground constitute a further geographically unrelated control group for genetic analysis (Table 2). Family members and control subjects provided written informed consent for clinical and genetic testing. The local ethical committees of the 2 centers in which the study was performed approved the study.

Extraction of Genomic DNA and Genotyping

Genomic DNA was isolated from the blood samples using DNA isolation Kit (Maxwell 16 Blood Purification kit, Promega, Madison, WI), quantified by spectrophotometric readings (optical density=260″), diluted to 40 ng/μL, and used for polymerase chain reaction amplification. For genome scan, we used fluorescently labeled short tandem repeats markers (Applied Biosystems linkage analysis mapping set version 2.5 with a total of 400 polymorphic markers) and additional markers selected from the Marshfield genetic map. The average spacing of the markers was about 10 cM apart. The genome scan was performed in family A.

Analysis of Known Disease Genes

Genes previously reported as associated with isolated AS (*SCN5A* and *Cx40/GJA5*) and with AS in dilated cardiomyopathy (*LMNA*, *EMD*) were analyzed by direct bidirectional automated sequencing (ABI 3130*Xl* Genetic Analyzer, Applied Biosystems, Foster City, CA) in the affected members of the largest family A.

Linkage Analysis

Two-point and multipoint parametric linkage analyses were performed using version 5.08 of the EASYLINKAGE program.[27] From

the pedigree analysis, we assumed an autosomal recessive model of inheritance and a 0.001 disease allele frequency. Penetrance was set at 0% in <20 years of age, 20% in 21 to 40 years of age, 80% in 41 to 55 years of age, and 100% in >55 years of age based on the observed frequency of affected individuals in at-risk sibships. Nonparametric multipoint linkage analysis was then performed to validate inheritance hypothesis.

Association Analysis

An association analysis including 77 healthy living members of the 6 families and the 192 healthy controls from the same geographic area was performed to compare the genetic profile of healthy adult residents with that of the 8 living patients. The direct sequencing of the candidate gene identified by linkage analysis showed 10 single-nucleotide polymorphisms (SNPs), 9 known and 1 novel. For each SNP, a χ^2 test was done to assess whether the observed genotype frequencies were in Hardy–Weinberg equilibrium among controls. A recessive genetic model was tested. To take into account relatedness among patients, the association between disease and genotypes was evaluated using the Cochran Mantel–Haenzel test adjusted for clustered binary response.[28] In this analysis, each family was considered as a different cluster. $P<0.005$ was considered significant to adjust for multiple testing across the 10 SNPs. Analysis has been performed using gPLINK 2.050.

Biomarkers

Mid-regional proatrial natriuretic peptide (BRAHMS AG, Henningdorf), N-terminal probrain natriuretic peptide, C-reactive protein, and serum creatine phosphokinase were measured in the 8 living patients and 33 relatives. Mid-regional proatrial natriuretic peptide differences among homozygous, heterozygous, and wild-type groups were assessed by the Kruskal–Wallis test, followed by post hoc Wilcoxon–Mann–Whitney test for pairwise comparisons because values in the wild-type group were not normally distributed (Shapiro–Wilk test). Statistical analysis was performed with Origin 8.1 Pro (OriginLab Corporation, Northampton, MA).

Conservation Index and In Silico Analyses

The evaluation of the pathogenicity of the mutation was based on the following: (1) involvement of residues that are highly conserved throughout evolution (Conservation Index at http://evs.gs.washington.edu/EVS);
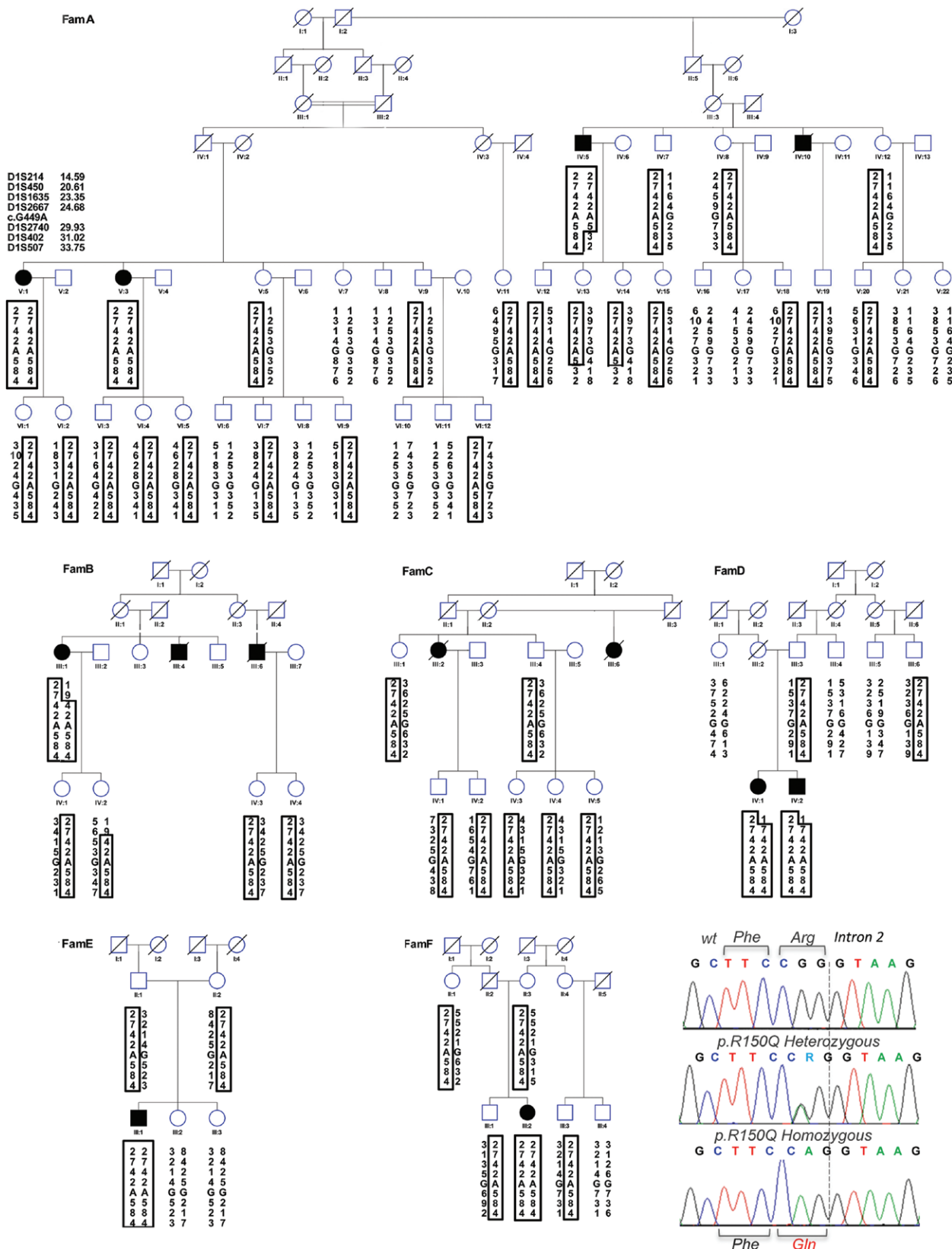
Figure 2. Pedigrees. The pedigrees of 6 families (**A–F**) showing the 13 affected subjects. Squares indicate males, circles females. Black-filled symbols indicate affected subjects. Slashes through the symbols indicate deceased subjects. Fine mapping markers that delimited the chromosome region 1p36.31-1p36.21 with maximum LOD score at marker D1S2740 that identified the locus region containing the *Natriuretic Peptide Precursor A* gene. Right-sided electropherograms show homozygous mutated, homozygous wild-type, and hetero-zygous cG449A mutation (p.Arg150Gln) in the *Natriuretic Peptide Precursor A* gene; the positively charged Arginine is substituted by the neutral Glutamine at position 150.

Table 2. Results of Genetic Testing in 477 Individuals

	c.G449A (p.Arg150Gln)			
	Homozygous	Heterozygous	Wild Type	Total
Living patients with complete phenotype	8	0	0	8*
Family members without phenotype	0	40	37	77
First control group: unrelated individuals from the same area	0	16	176	192
Second control group: individuals from the national ground	0	0	200	200
Total	8	56	413	477

*Five of the 13 patients with complete phenotype died before receiving genetic testing.

(2) in silico prediction of pathogenicity (Polyphen); (3) segregation of the mutation with the phenotype; and (4) absence of the homozygous mutation in 384 ethnically and geographically matched control chromosomes and in 400 ethnically matched control chromosomes. The National Heart, Lung, and Blood Institute (NHLBI) ESP Exome Variant Server (NHLBI Exome Sequencing Project [ESP], Seattle, WA) (http://evs.gs.washington.edu/EVS/) was last accessed in November 2012. The exomes included in the ESP5400 were selected from the populations listed on the ESP website (https://esp.gs.washington.edu/drupal/). The NCBI|dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and 1000 Genomes Project (http://www.1000genomes.org/) databases were last accessed in November 2012.

Results

Phenotype and Natural History

The 13 patients (6 males, 7 females) were 31 to 58 years of age at the time of diagnosis (Table 1). The diagnosis at onset was idiopathic AD (n=13) associated with AS in 7 and Brady–Tachy syndrome (BTS) in 6. These latter developed AS during follow-up. After standard pharmacological therapy first and pacing later, the hemodynamic status improved, and all patients were in New York Heart Association class I to II at last observation (4–37 years of follow-up). Eleven patients

received a single- or dual-chamber pacemaker in 0 to 15 years after first cardiac evaluation because of slow junction rhythm or BTS. Two patients had mildly increased blood pressure. To date, none of the patients required surgical atrial remodeling and tricuspid and mitral valvular annuloplasty.[15] Two patients developed Hodgkin lymphoma (E:III:1) and endometrial sarcoma (F:III:2) before the onset of the disease, at 28 and 35 years of age, respectively, and were successfully treated. At present, we have no data to correlate these diseases to *NPPA* mutation, although the atrial natriuretic peptide (ANP) exerts an anticancer effect in prostatic and pancreatic cancers.[29] Of the 8 patients diagnosed before 1983, 5 died 8 to 25 years after the first diagnosis because of stroke, after months of posttraumatic tetraplegia (n=1), and out-of-hospital sudden death (n=4). We have no data to establish the arrhythmic or thromboembolic cause of the sudden deaths (autopsy was not performed). None of them had shown sustained ventricular arrhythmias during the follow-up. Three patients diagnosed before 1983 and 5 diagnosed after 1983 are alive. Cerebral (n=7) and peripheral (n=1) embolic episodes occurred in 7 of 13 patients, at disease onset (n=1), after diagnosis before anticoagulation (n=5), and while taking anticoagulation (n=2, including the patient with fatal stroke; Table 1). Serial neurological evaluation in all patients and electromyography in 7 of the 8 living patients excluded clinically silent muscular dystrophy (Table 3). The ultrastructural study of abdominal fine needle fat biopsies excluded amyloid deposits. Right ventricular biopsy, performed in 2 patients after 35 and 2 years of follow-up, respectively, excluded myocarditis and cardiac amyloidosis.

Imaging Data

At first echocardiographic examination, AD was severe in 6 patients and moderate in 7 patients. The follow-up documented progressive biatrial enlargement, especially of the right atrium (Figures 1B and 3). At the last evaluation, atria were giant in the 4 patients with the longest follow-up (27–37 years) and severely enlarged in the remaining 9 patients with 4 to 25 years follow-up (Table 4). Atrial volumes measured by multislice tomography or Carto mapping were higher than those measured by echocardiography (Table 3), also because

Table 3. Laboratory and Instrumental Data of the 8 Living Patients

Family	Pedigree No.	MR-proANP	NT-proBNP	Am. in Abd. Fat biopsy	RV-EBM	EMG and NCV	CT LA Vol, mL/m²	CT RA Vol, mL/m²	Carto RA Vol, mL/m²	Carto RA Scar
A	IV:5	11	136	Absent	Yes	Normal	…	…	…	…
A	V:1	5	182	Absent	…	Normal	253	443	385	Diffused*
A	V:3	3	75	…	…	Normal	105	195	…	…
B	III:1	5	74	Absent	…	Normal	…	…	…	…
D	IV:1	39	142	Absent	…	Normal	72	98	82	Localized†
D	IV:2	45	166	Absent	…	…	…	…	106	Localized†
E	III:1	18	53	Absent	Yes	Normal	…	…	139	Localized†
F	III:2	42	181	…	…	Normal	…	…	66	Localized†

Am indicates amyloidosis in abdominal fat biopsy; CT, cardiac tomography; EMG, electromyography; MR-proANP, mid-regional proatrial natriuretic peptide, normal value 18–120 pmol/L; NCV, nerve conduction velocity; and NT-proBNP, N-terminal probrain natriuretic peptide, normal value <125 pg/mL, heart failure exclusion <300.
*Carto Mapping performed during complete atrial standstill.
†Carto Mapping performed during BTS.
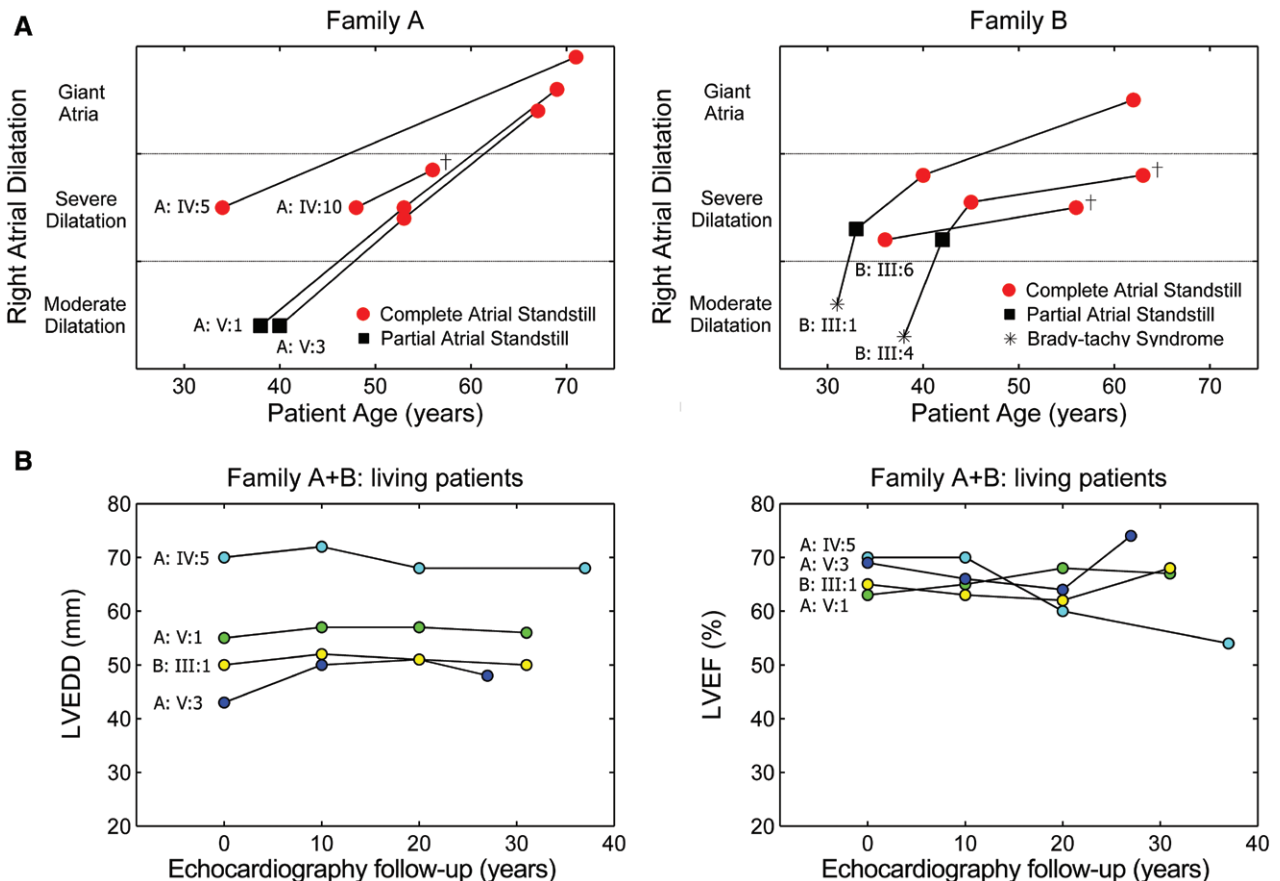
Figure 3. Natural history. (**A**) Disease evolution (degree of right atrial dilatation and type of atrial arrhythmia) of the patients with the longest follow-up (families A and B). Within the same family, affected members share age at diagnosis and disease evolution. Three patients died (†) during the follow-up. At echocardiographic examination, right atrial dilatation was graded as moderate (right atrial volume between 34 and 39 mL/m²), severe (≥40 mL/m²),[23] and giant (>80 mL/m²). (**B**) Echocardiographic follow-up of 4 living patients with the longest follow-up. Note the stability along the follow-up of the non-normalized left ventricle end-diastolic diameter (LVEDD) (**left panel**) and of the left ventricle ejection fraction (LVEF) (**right panel**).

of technical limits in echocardiographic reconstruction of atrial chambers with complex geometry changes (online-only Data Supplement Video).

Left ventricular (LV) ejection fraction, diastolic function, and morphology were normal in 8 living patients (Table 4 and Figure 3). Mild biventricular dilatation occurred in 4 deceased and in 2 living patients who developed atrioventricular valve regurgitation because of annular dilatation that worsened with progressive atrial enlargement after 37 and 31 years of follow-up, respectively. LV mass was increased in 8 of the 10 cases in which the data were available. Coronary arteries were normal.

Electrophysiological Studies

The electric disease was characterized by progressive lowering of atrial ECG voltages and BTS evolving to AS. Five patients, diagnosed in advanced phases of the disease, showed severe AD, complete AS with junctional bradycardia, and narrow QRS interval (Table 1, Figure 1A, and online-only Data Supplement Figure I); 1 of these 5 patients is living with giant atria and unmodified ECG pattern after 37 years of follow-up. In the remaining 8 patients, we documented the evolution of the atrial arrhythmia, from partial to complete AS (n=2), from BTS to partial (n=3), or complete AS (n=3). Overall, at last observation, all patients showed AS, complete in 10 and

partial in 3. Key markers of disease progression were similar in all patients (age, ECG findings, atrial enlargement; Figure 3). Complete AS matched only with severe AD or giant atria. In 4 patients who were studied when they had a BTS, the Carto mapping of the right atrium showed a localized scar area (atrial activity <0.05 mV at bipolar mapping) in the lateral wall, whereas in 1 with complete AS, the scar involved the whole right atrium (Figure 1C, Table 3).

Linkage and Mutational Analysis

Analysis of genome scan in family A showed a unique region of interest on chromosome 1p36.32-1p36.13 (D1S468, D1S2660, D1S2667, D1S2644, D1S199). Two-point linkage analysis yielded a maximum logarithm odds (LOD) score of 2.23 at the marker D1S2667 at recombination fraction 0 in family A and 2.52 adding families B and C. In families A, B, and C, multipoint linkage analysis yielded a maximum LOD score of 2.75 (*P*<0.001). For the fine mapping, additional markers were selected from the Marshfield genetic map, both proximal (D1S214, D1S450, D1S1635) and distal (D1S2740, D1S402, D1S2507) to marker D1S2667. Of the overall markers (the 5 genome scan markers, the additional 3 proximal, and 3 distal markers), 2 (D1S1635 and D1S2740) were further informative for linkage. The maximum 2-point

Table 4. Echocardiographic Measurement of the 13 Affected Patients at Last Observation

Family	Pedigree No.	LA APD, mm	LA Vol, mL/m²	RA Vol, mL/m²	LVEDD, mm/m²	LV mass, g/m²	RV	LVEF, %
A	IV:5	71	222	295	37	202	Dilated	54
A	IV:10	58	+++	+++	35	NA	Dilated	NA
A	V:1	63	174	255	35	98	Dilated	67
A	V:3	52	89	136	30	125	Normal	74
B	III:1	55	62	85	31	100	Normal	68
B	III:4	51	+++	+++	34	154	Dilated	57
B	III:6	57	+++	+++	30	142	Normal	63
C	III:2	53	+++	+++	42	NA	Dilated	NA
C	III:6	56	+++	+++	33	NA	Dilated	55
D	IV:1	44	47	41	27	137	Normal	63
D	IV:2	48	46	55	22	91	Normal	56
E	III:1	48	57	73	26	117	Normal	56
F	III:2	37	41	34	26	71	Normal	69

+++ indicates severe dilatation; APD, antero-posterior diameter (mm); LA, left atrium; LV mass, left ventricle mass (g)/body surface (m²); LVEDD/m², left ventricle end-diastolic diameter (mm)/body surface (m²); LVEF, left ventricle ejection fraction (%); NA, not assessed; RA, right atrium; RV, right ventricle; and Vol, volume (mL)/body surface (m²).

LOD scores were at the markers D1S2740 (LOD score=2.58) and D1S2667 (LOD score=2.52) and increased to 3.37 (θ=0) and 3.17, respectively, when the affected living members of the 3 additional families (families D–F) and unaffected relatives were added. Multipoint linkage analysis of the 6 families gave the maximum LOD score at marker D1S2740 (LOD score=3.38; *P*=0.002). Multipoint nonparametric analysis gave an LOD score of 3.05 at the same marker, confirming the autosomal recessive model of inheritance. Chromosomal recombination in individuals IV:5 of family A, III:1 of family B, and in the 2 affected sibs (IV:1 and IV:2) of family D contributed to the definition of proximal and distal boundaries of the locus (Figure 2). Markers D1S468 and D1S199, located 41.11 cM apart (Marshfield Map), can be identified as the distal and proximal flanking markers of our candidate locus region on 1p36 (online-only Data Supplement Figure II).

The LOD score for the most closely linked markers did not drop <3 when penetrance was varied between 60% and 100%. The disease region contains several annotated genes including the *5,10-Methylenetetrahydrofolate reductase, Chloride Channel 6, Natriuretic Peptide Precursor B, and NPPA genes.* We sequenced *5,10-Methylenetetrahydrofolate reductase, Chloride Channel 6, NPPA,* and *Natriuretic Peptide Precursor B* and found a homozygous transition (c.G449A) in exon 2 of *NPPA* that predicts the substitution of the positively charged Arginine with the neutral Glutamine at position 150 (p.Arg150Gln) of the prepro-ANP. The heterozygous mutation has been found in 2 of 13 006 alleles (*NPPA* NM_006172.3, T=2; C=13004; GLN, ARG, 150/152; minor allele frequency=0.0154%; all genotypes: TT=0; TC=2; CC=6501 at the Exome Variant Server, NHLBI ESP, Seattle, WA) (November 2012). Minor allele frequency was not reported in the 1000 Genome Project and dbSNP. The mutated residue was highly evolutionarily conserved in our evaluation as well as at the http://evs.gs.washington.edu/EVS where the Conservation Index (range, 0–1) among 17 vertebrate species is equal to 1. The Genomic Evolutionary Rate Profiling score was 5.65 (Genomic Evolutionary Rate Profiling range,

12.3–6.17; http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html). The Grantham Score, which categorizes codon replacements into classes of increasing chemical dissimilarity, was 43. The in silico evaluation predicted the mutation as probably damaging.

The homozygous mutation was absent in 269 healthy individuals from the same geographic area (including the 192 local controls and the 77 healthy living members of the 6 families) and in 200 healthy controls from the national ground. The heterozygous mutation was found in 40 relatives of the 13 patients, in 16 of 192 healthy controls from the same geographic area, and in none of the 200 controls from the national ground (Table 2).

Sequencing of the *NPPA* gene in 277 individuals, including the 8 living patients from the 6 families, their family members, as well as the controls from the local population with a nonmissing phenotype, identified 10 intragenic 10 SNPs. Genotype frequencies of all the SNPs analyzed resulted in Hardy–Weinberg equilibrium among controls. Genotype frequencies of the 10 SNPs and the c.G449A were compared between patients and controls (online-only Data Supplement Table I). The p.Arg150Gln was the only variant significantly associated with the disease risk under the recessive genetic model. *P* value was significant (*P*<0.0001) when the analysis was adjusted for relatedness among individuals. None of the other SNPs was significantly differently distributed between cases and controls.

Mid-regional proatrial natriuretic peptide levels were significantly lower in homozygous patients (median, 25%–75%, values of 14.5 [5–40.5] pmol/L versus 66.2 [60.4–80.9] pmol/L in heterozygous and 77.2 [55.6–83.6] pmol/L in wild-type subjects; *P*<0.001; Figure 4). Levels of N-terminal probrain natriuretic peptide, C-reactive protein, and serum creatine phosphokinase were within normal ranges in all patients.

Heterozygous Mutation Carriers
At the end of the study, we identified 40 heterozygous family members. Their ECG was normal, and none showed the
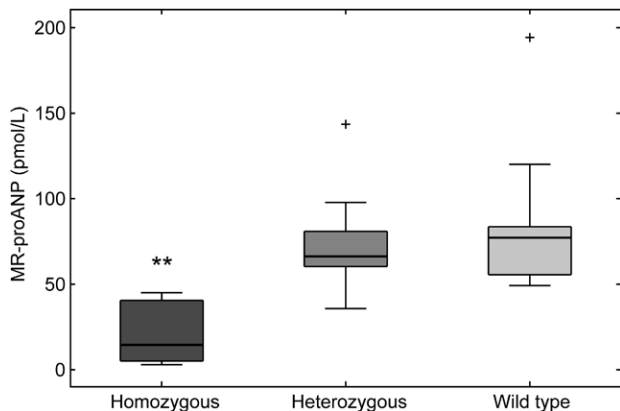
Figure 4. Mid-regional proatrial natriuretic peptide (MR-proANP) levels. MR-proANP levels in 8 patients with homozygous mutation, 11 family members with heterozygous mutation, and 21 family members with wild-type gene. MR-proANP levels were significantly lower in homozygous versus heterozygous and wild-type subjects (**$P<0.001$). For each distribution, values are given as median and interquartile ranges (IQRs, box), lower and superior adjacent values at 1.5×IQR (whiskers), and outliers (plus sign markers).

arrhythmias observed in the early and late phases of the disease in homozygous patients; only 1 of 40 had paroxysmal lone atrial fibrillation (AF). None had significant structural heart disease with the exclusion of older individuals. The follow-up of heterozygous family members ranged from 1 to 9 (mean 5) years, without evidence of an evolving phenotype. In the 192 unrelated individuals of the same geographic area, 16 were healthy carriers of the heterozygous mutation, whereas the 200 individuals from the national ground all had wild-type alleles.

Discussion

We describe a rare nosologically orphan autosomal recessive AD cardiomyopathy (ADCM) associated with homozygous mutation in the *NPPA* gene and clinically characterized by clinical onset in adulthood, biatrial dilatation up to giant size, early supraventricular arrhythmias with progressive loss of atrial electric activity to AS, stable normal LV function, long-term stable functional class, secondary thromboembolic complications, and severely decreased levels of ANP. The primary structural abnormalities of the atrial walls, leading to both AD and electric disease, is proven by the prolonged follow-up and progressive lowering of atrial voltages up to AS associated with scarring of the whole atrial wall as proven by the Carto study. ADCM involves primarily atrial walls; the LV shows regular morphology with normal LV ejection fraction and stable New York Heart Association functional class in the long-term follow-up (up to 37 years); brain natriuretic peptide values remain normal. As far as atrial enlargement progresses, atrioventricular valve annuli dilate and valve regurgitation worsens. Patients require pacemaker or cardioverter defibrillator implantation and chronic anticoagulation because of the high prevalence of thromboembolic complications. The full expression of the disease is age dependent.

The genetic data indicate that *NPPA* is the gene candidate for this rare autosomal recessive ADCM. The segregation of the homozygous p.R150Q mutation of the *NPPA* gene with the phenotype in the 6 families, the in silico prediction of

damaging mutation, the high conservation of the mutated residues, the absence of the homozygous mutation in 192 normal controls from the same geographic area and from 200 controls from the national ground, the allele frequency of 0.0154% in 6503 individuals from the NHLBI GO Exome Sequencing Project, the severely decreased levels of ANP in carriers of the homozygous mutation (similarly to the few reported cases of sporadic AS),[30] as well as the identical onset and progression of the disease by age in the affected family members support the causative role of the mutation. Because the sample size for cases is small and controls are oversampled, the test result may be biased. With this sampling scheme, the Cochran Mantel–Haenzel test distribution may not follow the χ^2 distribution. However, the strong significance of the association between the homozygous mutation of the *NPPA* gene and the phenotype (P=5.31; 10–8 for Cochran Mantel–Haenzel test) supports the validity of our results.

Experimental Models

While heterozygous *Nppa*+/− mice show normal phenotype, identical to that observed in *Nppa*+/+ mice,[31] transgenic mice with homozygous disruption of either *Nppa* or *Natriuretic Peptide Receptor A* genes show significantly increased weight of each cardiac chamber, particularly the atrial chambers.[31–33] The *Nppa*−/− and *Natriuretic Peptide Receptor A*−/− mice show hypertension, pressure-independent LV hypertrophy, and dilatation but normal ventricular performance.[32] With respect to the atria, both *Nppa*−/− and *Natriuretic Peptide Receptor A*−/− mice show increased atrial mass, but there are no data on AD and atrial arrhythmias. The cardiac walls demonstrate prominent interstitial fibrosis,[32] increased expression of extracellular matrix proteins,[33] and activation of proinflammatory cytokines.[34]

With respect to the ventricular phenotype, similar to knock-out mice, 6 of our 13 patients had upper limit of normal or increased LV end-diastolic diameter with normal LV function, and most of them showed an increase of the echocardiographic LV mass. Finally, only 2 patients had arterial hypertension; because all patients were treated with renin–angiotensin system inhibitors for the AD, the possibility exists that pressure levels were controlled by chronic treatment with renin–angiotensin system inhibitors. Overall, although in our patients the atrial phenotype is prominent, the ventricular phenotype is similar to that of knock-out mice and supports the case for a reduction of ANP having a pathogenic role.

Role of ANP in the Heart

ANP regulates intravascular blood volume and vascular tone through natriuresis, diuresis, and vasodilatation; modulates ion channel function; and prevents atrial electric remodeling.[35,36] In humans, ANP increases the intra-atrial conduction velocity and shortens the right atrial effective refractory period.[35] Either increase or decrease of ANP may perturbate these mechanisms.

Increased ANP levels were found in a family with autosomal dominant AF and absence of severe AD (in all but 1 patient) associated with a heterozygous frameshift mutation of the *NPPA* gene.[37] The high levels of ANP were explained as due to the resistance of the mutant peptide to proteolytic

degradation.[38] In the isolated whole-heart model, mutant ANP caused significant shortening of action potential duration[37] favoring AF. A second heterozygous missense mutation of the *NPPA* (p.S64R) causing augmented potassium current and shortening action potential duration was identified in a family with lone AF.[39] We found p.S64R in 5 individuals of our local control population, 4 healthy and 1 with lone AF, all with normal ANP levels. Their follow-up is ongoing.

The circulating levels of ANP were severely decreased in our homozygous carriers of the *NPPA* mutation, as in *Nppa* knock-out mice.[31] The possibility exists that long-term exposure to low levels of ANP, which seem to cause the extensive atrial fibrosis and myocyte damage in knock-out mice,[31–33] explains the enormous AD and the loss of electric activity in our patients with atrial cardiomyopathy, as confirmed by Carto mapping.

Epidemiology of ADCM
ADCM is a rare disease; the region that refers to the S. Chiara Hospital of Trento is constituted of ≈400 000 inhabitants, and although we have not screened the entire population, patients with cardiovascular diseases are primarily referred to this tertiary cardiology, making it unlikely that other undiagnosed, phenotypically overt cases exist. The identification of the c.G449A in the local population suggests an ancestral origin of this mutation. Individuals who carry the heterozygous mutation are now aware of their genetic background and of the possible implications in case of mating between healthy carriers. In the literature, there are genetically orphan, sporadic, and familial cases that look phenotypically similar to our cases. The Indian case described by Sajeev et al[10] was adult-onset and showed same phonotypical traits observed in our patients as the 2 Australian patients reported by Sanders et al,[11] whereas 3 Japanese siblings with isolated atrial amyloidosis described by Maeda et al[16] suggest a recessive inheritance and the possible contribution of atrial amyloid deposits to the pathological substrates of the AD and AS. The possibility exists that in cases with isolated atrial amyloidosis, amyloid fibrils display either atrial or probrain natriuretic peptide immunoreactivity, as we have shown in atria of human failing hearts,[40] thus suggesting amyloid to be secondary to a primary disease that causes AD and AS. Overall, 13 patients are reported phenotypically similar to our cases.[1,4,10,11,14,16]

Limitations
The limitation of the current work is the lack of atrial specimens for tissue studies. The 5 deceased patients did not undergo autopsy. Tissue studies could have been confirmatory of the extensive structural abnormalities of the atrial walls. However, data from Carto mapping support the extensive fibrosis and myocyte loss of the atrial wall.

Conclusions
Autosomal recessive ADCM associated with *NPPA* defects could represent the atrial counterpart of dilated ventricular cardiomyopathies. The characterization of this rare phenotype opens the field of investigation for atrial diseases not only on their arrhythmic phenotype but also on their genetic, structural, and functional background.

Disclosures
None.

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CLINICAL PERSPECTIVE

Primary atrial cardiomyopathy without significant ventricular involvement is a rare, nosologically and genetically orphan disease. We describe the phenotype of idiopathic atrial dilated cardiomyopathy in 13 affected members of 6 families, all from the same geographic area. In these families, atrial dilated cardiomyopathy was inherited as an autosomal recessive trait and was characterized by clinical onset in adulthood, significant biatrial dilatation, early supraventricular arrhythmias with progressive loss of atrial electric activity to atrial standstill, stable normal left ventricular function, long-term stable functional class, and secondary thromboembolic complications. By linkage analysis, we mapped a locus at 1p36.22 containing the *Natriuretic Peptide Precursor A* gene. By sequencing *Natriuretic Peptide Precursor A*, we identified a homozygous missense mutation (p.Arg150Gln) in all living affected individuals of the 6 families. All patients showed low serum levels of atrial natriuretic peptide. Heterozygous mutation carriers were healthy and demonstrated normal levels of atrial natriuretic peptide. The translational impact of this study includes: (1) early and correct diagnosis in carriers of the homozygous mutation, as the diagnosis allows timely medical treatment for negative atrial remodeling and prevention of thromboembolic complications; (2) identification of heterozygous mutation carriers that can be informed of the procreative risk in the case of mating between healthy carriers; and (3) the possibility of screening *Natriuretic Peptide Precursor A* or searching for novel disease genes in phenotypically similar, genetically orphan familial cases. Atrial dilated cardiomyopathy can be considered the atrial counterpart of dilated cardiomyopathy that typically affects the ventricles. Clinical and genetic investigation of atrial dilated cardiomyopathy may expand the field of research on primary atrial diseases.

SUPPLEMENTAL MATERIAL

Autosomal recessive atrial dilated cardiomyopathy with standstill evolution associated with mutation of *Natriuretic Peptide Precursor A*

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FIGURE 1 SUPPL.

Partial and complete atrial standstill ECG presentations

At the top the ECGs of patient A:V:1 in 1984 was present a partial atrial standstill (AS) with an irregular junctional rhythm (mean heart rate 65 bpm) without atrial activity and narrow QRS, while in 1995 a complete AS with bradycardic junctional rhythm (mean heart rate 38 bpm) was present, at the time of pace-maker implantation; the patient is living with a follow-up of 31 years. At the middle a complete AS with a bradycardic junctional rhythm (mean heart rate 33 bpm) was present in patient A:IV:5 in 1980, at the time of pace-maker implantation; the patient is living with a follow-up of 37 years. At the bottom a complete AS (mean heart rate 40 bpm) was present in patient B:III:4 in 1983, at the time of pace-maker implantation; the patient died suddenly after a follow-up of 25 years.
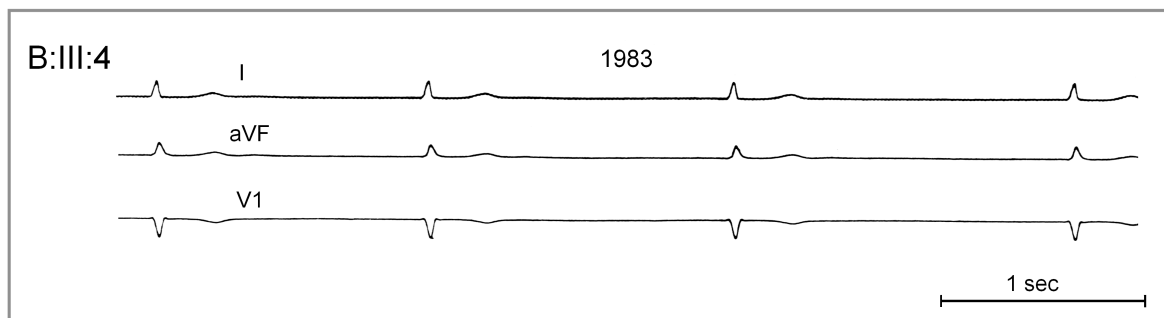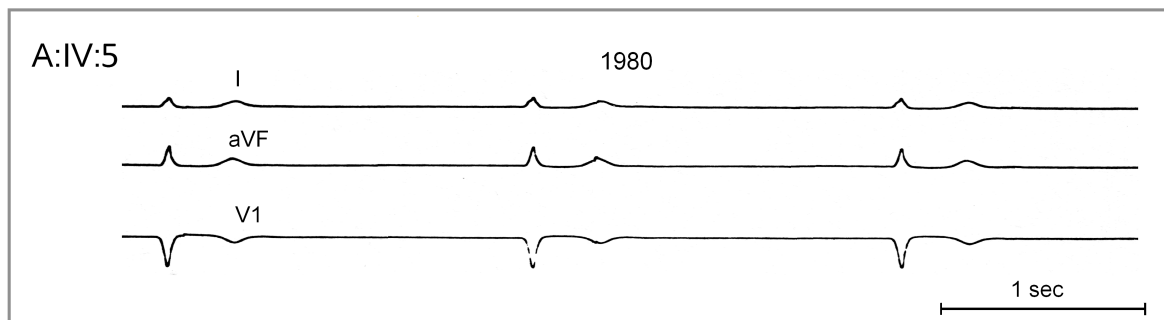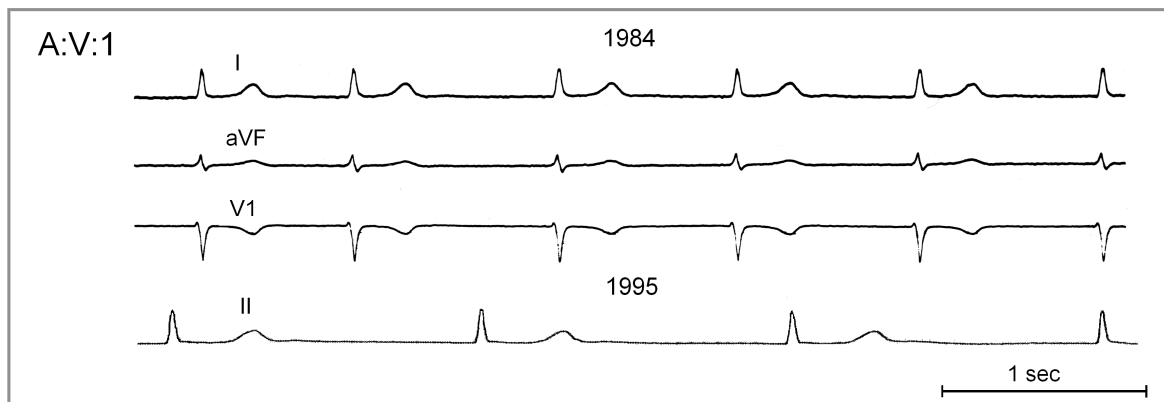
FIGURE 2 SUPPL.

Schematic showing the list of markers in the region and the disease interval marked.
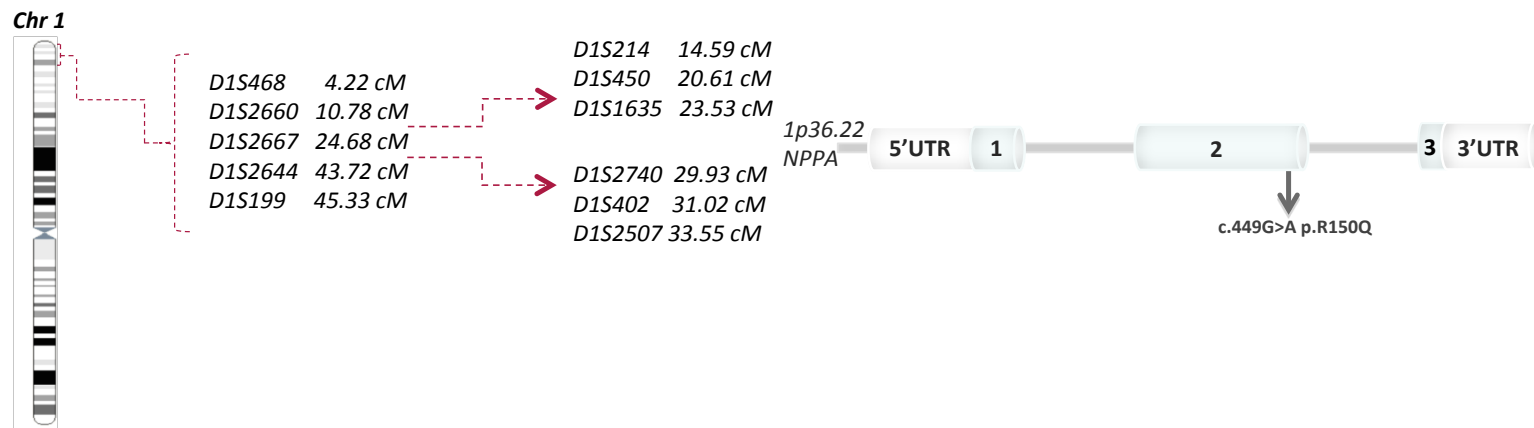
VIDEO LEGEND

The 3DCT reconstruction of the cardiac chambers (23) of patient A:V:1 shows the rotating view the cardiac chambers from all directions, thus getting an appreciation of the giant atria compared to the ventricles.

TABLE 1. Genotype distribution in 8 living patients with the phenotype and in 269 controls from the same geographic area.

SNP	Variant	Affected	Controls	p-value*
rs5063	AA	8	251	1
	AG	0	18	
	GG	0	0	
rs5064	AA	8	246	1
	AG	0	23	
	GG	0	0	
c.123+25T>C	TT	8	268	1
	TC	0	1	
	CC	0	0	
rs61757261	GG	8	264	1
	GT	0	5	
	TT	0	0	
c.G449A	**GG**	**0**	**213**	**<0.0001**
	GA	**0**	**56**	
	AA	**8**	**0**	
rs5065	AA	8	213	1
	AG	0	54	
	GG	0	2	
rs5066	GG	8	235	1
	GT	0	33	
	TT	0	1	
rs5067	AA	8	213	1

	AG	0	54	
	GG	0	2	
rs5068	CC	8	244	1
	CT	0	23	
	TT	0	2	
rs61764044	TT	8	244	1
	TC	0	23	
	CC	0	2	
rs63749086	WT/WT	8	234	1
	WT/ c.*250_*260delTGAAAGTGGTT	0	34	
	c.*250_*260delTGAAAGTGGTT/ *250_*260delTGAAAGTGGTT	0	1	

 * recessive model

Only c.G449A (p.Arg150Gln) variant (Ensemble Chromosome Position 11907171) was significantly associated to the disease risk under a recessive genetic model. P-value was significant (P<0.0001) when analysis was adjusted for relatedness among individuals. None of the other considered SNPs were differently distributed between cases and controls. Variant c.123+25T>C (Ensemble Chromosome Position 11907594) was reported here for the first time.

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