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

Running title: Disertoro et al.; Atrial cardiomyopathy by NPPA mutation

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Abstract:

**Background** - Atrial dilatation and atrial standstill are etiologically heterogeneous phenotypes with poorly defined nosology. In 1983 we described 8-years follow-up of idiopathic 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 a same geographic area in the North-East 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) bi-atrial dilatation up to giant size; 3) early supraventricular arrhythmias with progressive loss of atrial electrical activity to atrial standstill; 4) thromboembolic complications; 5) stable, normal left ventricular function and NYHA functional class during the long-term course of the disease. By linkage analysis we mapped a locus at 1p36.22 containing the *natriuretic precursor A* (*NPPA*) gene. By sequencing *NPPA* 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 (ANP). Heterozygous mutation carriers were healthy and demonstrated normal levels of ANP.

**Conclusions** - Autosomal recessive Atrial Dilated Cardiomyopathy is a rare disease associated with homozygous mutation of the *NPPA* gene and characterized by extreme atrial dilatation with standstill evolution, thromboembolic risk, preserved left ventricular function and severely decreased levels of ANP.

**Key words:** atrial natriuretic factor; genetics; NPPA gene; atrial cardiomyopathy
Introduction

Idiopathic atrial dilatation (AD) with disproportionately enlarged atria in the absence of other cardiac or hemodynamic abnormalities, and atrial standstill (AS) with loss of electrical and mechanical activity can occur as independent entities (1-8) or combined together (4,9-11). AS can also be associated with Ebstein’s anomaly (12), dilated cardiomyopathy (DCM) (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 DCM with catecholaminergic polymorphic ventricular tachycardia (20) and in DCM with Charcot-Marie-Tooth type 2 axonal neuropathy (21). The diagnosis may be incidental or coincide with the occurrence of atrial arrhythmias (4).

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, while idiopathic AS has been associated with combined heterozygous mutations of SCN5A and Connexin40 genes in two unrelated families in which only one of 5 members with AS also showed AD (6,7). AS has been also 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 the North-East of Italy (9) and we later identified 2 sibs and 2 unrelated apparently sporadic patients with identical phenotypes in 3 additional families. During a very 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 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 up to 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 semi-quantitatively evaluated. Three patients underwent multi-slice tomography with three-dimensional reconstruction of the cardiac chambers (24). All patients underwent at least one endocavitary electrophysiological (EP) study, at the time of diagnosis or at pacemaker implantation. Complete AS was defined as absence of atrial electrical activity on surface electrocardiogram (ECG), with junctional bradycardia, absence of atrial activity in endocavitary recordings, no response to stimulation during EP study, and an absence of A wave by echocardiography (9,14). Partial AS was defined as absence of atrial electrical 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-Webster, Diamond Bar, CA, USA): the areas displaying electric atrial activity <0.05 mV at bipolar mapping were considered as 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 neurologic 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 sampling for genetic studies. The Figure 2 shows the pedigrees of the 6 families including 64 of the overall 85 family members; 21 healthy relatives (all members of the Vth and VIth generations of family A) are not reported in the figure. Based on family pedigrees and clinical screening of the family members we hypothesized an autosomal recessive inheritance of the disease.

Since the origin of the six 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 to the population study call launched by the local authorities.

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 two 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 USA) quantified by spectrophotometric readings (optical density = 260”), diluted to 40 ng/μL and used for PCR amplification. For genome scan we used fluorescently labeled short tandem repeats (STR) 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 DCM (LMNA, EMD) were analyzed by direct bidirectional automated sequencing (ABI 3130xl Genetic Analyzer, Applied Biosystems) 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% under 20 years, 20% from age 21 to 40 years, 80% from age 41 to 55 years, and 100% over age 55 years, 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 vs. 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 Chi-square test was done to assess whether the observed genotype frequencies were in Hardy Weinberg Equilibrium (HWE) 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. A p-value < 0.005 was considered significant to adjust for multiple testing across the ten
SNPs. Analysis has been performed using gPLINK 2.050.

**Biomarkers**

Mid regional pro-atrial natriuretic peptide (MR-proANP) (BRAHMS AG, Henningdorf,
Germany), N-terminal pro-brain natriuretic peptide (NT-proBNP), C-reactive protein and serum
creatine phosphokinase were measured in the 8 living patients and in 33 relatives. MR-proANP
differences among homozygous, heterozygous and wild type groups were assessed by the
Kruskall-Wallis test, followed by post-hoc Wilcoxon-Mann-Whitney test for pairwise
comparisons, since 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, Massachusetts).

**Conservation index and in silico analyses**

The evaluation of the pathogenicity of the mutation was based on the followings: (i) involvement
of residues that are highly conserved throughout evolution (Conservation Index at
http://evs.gs.washington.edu/EVS); (ii) *in silico* prediction of pathogenicity (Polyphen); (iii)
segregation of the mutation with the phenotype; (iv) absence of the homozygous mutation in 384
ethnically and geographically matched control chromosomes and in 400 ethnically matched
control chromosomes. The NHLBI ESP Exome Variant Server [NHLBI Exome Sequencing
Project (ESP)] Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) was lastly accessed on
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 lastly accessed on November, 2012.

Results

Phenotype and natural history (Figure 1)

The 13 patients (6 males, 7 females) were 31-58 year-old 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 pharmacologic therapy first and pacing later, the hemodynamic status improved and all patients were in New York Heart Association (NYHA) class I-II at last observation (4-37 years of follow-up). Eleven patients received a single or dual-chamber pacemaker in 0-15 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’s lymphoma (E:III:1) and endometrial sarcoma (F:III:2) before the onset of the disease, at the age of 28 and 35 years, respectively, and were successfully treated. At present we have no data to correlate these diseases to NPPA mutation, although the 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 post-traumatic 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 the 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 neurologic
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 two 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. The follow-up documented progressive bi-atrial enlargement, especially of the right atrium (Figure 1B and Figure 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 4-25 years follow-up (Table 4). Atrial volumes measured by multi-slice tomography or by Carto mapping were higher than those measured by echocardiography (Table 3), also due to technical limits in echocardiographic reconstruction of atrial chambers with complex geometry changes (Online video Supplement).

Left ventricular ejection fraction (LVEF), 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 atrio-ventricular valve regurgitation due to annular dilatation that worsened with progressive atrial enlargement after 37 and 31 years of follow-up respectively. Left ventricular 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
Figure 1 Supplement); one 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), and 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 that 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 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], two [D1S1635 and D1S2740] were further informative for linkage. The maximum two-point LOD scores were at the markers D1S2740 (LOD score= 2.58) and D1S2667 (LOD score=2.52) and increased to 3.37 (Theta =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 non-
parametric analysis gave a LOD score=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 two 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 (Figure 2 suppl.).

The LOD score for the most closely linked markers did not drop below 3 when penetrance was varied between 60% and 100%. The disease region contains several annotated genes including the 5,10-Methylenetetrahydrofolate reductase (MTHFR), Chloride Channel 6 (CLCN6), Natriuretic Peptide Precursor B (NPPB), Natriuretic Peptide Precursor A (NPPA) genes. We sequenced MTHFR, CLCN6, NPPA and NPPB 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 13006 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 Exome Sequencing Project (ESP), Seattle, WA [November 2012] but was absent 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 =1. The Genomic Evolutionary Rate Profiling (GERP) score was 5.65 (GERP range:-12.3-6.17) (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html). The Grantham Score, that 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 non-missing phenotype, identified 10 intragenic Single Nucleotide Polymorphisms (10 SNPs). Genotype frequencies of all the SNPs analyzed resulted in HWE among controls. Genotype frequencies of the 10 SNPs and the c.G449A were compared between patients and controls (Table 1 Supplement). 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 were significantly differently distributed between cases and controls.

MR-proANP levels were significantly lower in homozygous patients (median, 25th-75th percentiles) 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 NT-proBNP, C-reactive protein and serum creatine phosphokinase were within normal ranges in all patients.

Homozygous mutation carriers

At the end of the study, we identified 40 heterozygous family members. Their ECG was normal and none showed the arrhythmias observed in the early and late phases of the disease in homozygous patients; only 1/40 had paroxysmal lone 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 had all wild type alleles.

Discussion

We describe a rare nosologically orphan autosomal recessive atrial dilated cardiomyopathy (ADCM) associated with homozygous mutation in the NPPA gene, and clinically characterized by clinical onset in adulthood, bi-atrial dilatation up to giant size, early supraventricular arrhythmias with progressive loss of atrial electrical activity to AS, stable normal left ventricular function, long-term stable functional class, secondary thromboembolic complications and severely decreased levels of ANP. The primary structural abnormalities of the atrial walls, leading both to AD and electrical disease, is proven by the prolonged follow-up and the progressive lowering of atrial voltages up to AS associated with scarring of whole atrial wall as proven by Carto study. ADCM involves primarily atrial walls; the LV shows regular morphology with normal LVEF and stable NYHA functional class in the long-term follow-up (up to 37 years); BNP values remain normal. As far as atrial enlargement progresses, atrio-ventricular valve annuli dilate and valve regurgitation worsens. Patients require pacemaker or cardioverter defibrillator implantation and chronic anticoagulation due to 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 six 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 as well
as 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. Since the sample size for cases is very small and controls are over sampled, the test result may be biased. With this sampling scheme the Cochran Mantel-Haenzel test distribution may not follow the Chi-squared 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 (Npra) genes show significantly increased weight of each cardiac chamber, particularly the atrial chambers (31-33). The Nppa−/− and Npra−/− mice show hypertension, pressure-independent LV hypertrophy and dilatation but normal ventricular performance (32). With respect to the atria, both Nppa−/− and Npra−/− mice show increased atrial mass but are no data on atrial dilatation and atrial arrhythmias. The cardiac walls demonstrate prominent interstitial fibrosis (32), increased expression of extracellular matrix proteins (33) and activation of pro-inflammatory cytokines (34).

With respect to the ventricular phenotype, similarly to KO mice, 6 of our 13 patients had upper limit of normal or increased LVEDD with normal LV function and most them showed increase of the echocardiographic LV mass. Finally, only two patients had arterial hypertension; since all patients were treated with renin-angiotensin-system inhibitors (RAS-I) for the atrial
dilatation, the possibility exists that pressure levels were controlled by chronic treatment with RAS-I. Overall, although in our patients the atrial phenotype is prominent, the ventricular phenotype is similar to that of KO mice and supports the case for a reduction of ANP having a pathogenic role.

**Role of atrial natriuretic peptide in the heart**

ANP regulates intravascular blood volume and vascular tone through natriuresis, diuresis and vasodilatation, modulates ion channel function and prevents atrial electrical 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 atrial dilatation (in all but one 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 significantly shortening of action potential duration (APD) (37) favoring AF. A second heterozygous missense mutation of the NPPA (p.S64R) causing augmented potassium current and shortening APD, 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 KO mice (31). The possibility exists that long-term exposure to low levels of ANP, that seem to cause extensive the atrial fibrosis and myocyte damage in KO mice (31-33), explains the enormous atrial dilatation and the loss of electric activity in our patients.
with atrial cardiomyopathy, as confirmed by Carto mapping.

**Epidemiology of atrial dilated cardiomyopathy**

ADCM is a rare disease; the region that refers to the S. Chiara Hospital of Trento is constituted of about 400,000 inhabitants and although we have not screened the entire population, patients with cardiovascular diseases are primarily referred to this tertiary cardiology, making 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 phenotypical traits observed in our patients as the 2 Australian patients reported by Sanders et al (11), while 3 Japanese sibs with “isolated atrial amyloidosis” described by Maeda et al. (16) suggest a recessive inheritance and the possible contribution of atrial amyloid deposits to the pathologic substrates of the atrial dilatation and standstill. The possibility exists that in cases with isolated atrial amyloidosis, amyloid fibrils display either atrial or brain 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 atrial dilatation and standstill. Overall, 13 patients are reported phenotypically similar to our cases (1,4,10,11,14,16).

**Limits**

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.

Acknowledgments: The authors thank for the collection of data during the years: Serena Belli MD, Maurizio Centonze MD, Danilo Dallafior, MD, Giovanni D’Onghia MD, Roberta Spadaro MD, Emanuela Toffalori MD, Prisca Zeni MD, Santa Chiara Hospital, Trento; Alessandro Cristoforetti, PhD, Department of Physics, University of Trento, Povo-Trento, Paolo Lazzaro MD, Baselga di Pinè, Trento; all in Italy. The authors are indebted with patients and families as well as with the population of Northeast of Italy for their participation and support to the present research. The authors would also like to thank the NHLBI GO Exome Sequencing Project and its ongoing studies which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the Heart GO Sequencing Project (HL-103010).

Funding Sources: This study was supported by: Grant 2010 Fondazione Cassa di Risparmio Trento e Rovereto, Italy; Grants European Union INHERITANCE project 241924, Health-2009-2.4.2-3. RC Heritable Cardiomyopathies, Italian Ministry of Health to the IRCCS Policlinico San Matteo; Cassa di Risparmio Province Lombarde Foundation and Fondo Investimenti Ricerca di Base n.BIP06PMF2, 2007, Italy.

Conflict of Interest Disclosures: None.

References:


**Table 1.** Clinical data of the 13 affected patients

<table>
<thead>
<tr>
<th>Family</th>
<th>Pedigree No.</th>
<th>Sex</th>
<th>1st Diagnosis yrs of age</th>
<th>Duration follow-up, yrs</th>
<th>1st Diagnosis symptoms</th>
<th>NYHA at 1st/last observation</th>
<th>Heart rhythm at 1st/last observation</th>
<th>PM yrs of age</th>
<th>Emb. yrs of age</th>
<th>Hypert.</th>
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<tbody>
<tr>
<td>A</td>
<td>IV:5</td>
<td>M</td>
<td>34</td>
<td>37</td>
<td>dyspnoea</td>
<td>II / II</td>
<td>Complete Atrial Standstill</td>
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<td>No</td>
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<td>M</td>
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<td>8 (†56 yrs)</td>
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<td>Complete Atrial Standstill</td>
<td>48</td>
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<td>palpitations</td>
<td>I / II</td>
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<td>31</td>
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<td>I / II</td>
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<td>I / II</td>
<td>BTS → Complete Atrial Standstill</td>
<td>47</td>
<td>46 - 63</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>III:6</td>
<td>M</td>
<td>36</td>
<td>20 (†56 yrs)</td>
<td>stroke</td>
<td>II / II</td>
<td>Complete Atrial Standstill</td>
<td>36</td>
<td>36</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>III:2</td>
<td>F</td>
<td>58</td>
<td>9 (†67 yrs)</td>
<td>dyspnoea</td>
<td>III / II</td>
<td>Complete Atrial Standstill</td>
<td>59</td>
<td>67</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>III:6</td>
<td>F</td>
<td>51</td>
<td>13 (†64 yrs)</td>
<td>dyspnoea</td>
<td>III / II</td>
<td>Complete Atrial Standstill</td>
<td>51</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>D</td>
<td>IV:1</td>
<td>F</td>
<td>41</td>
<td>9</td>
<td>no symptoms</td>
<td>I / I</td>
<td>BTS → Complete Atrial Standstill</td>
<td>43</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>D</td>
<td>IV:2</td>
<td>M</td>
<td>42</td>
<td>4</td>
<td>no symptoms</td>
<td>I / I</td>
<td>BTS → Complete Atrial Standstill</td>
<td>43</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>E</td>
<td>III:1</td>
<td>M</td>
<td>34</td>
<td>4</td>
<td>palpitations</td>
<td>I / I</td>
<td>BTS → Partial Atrial Standstill</td>
<td>34</td>
<td>37</td>
<td>No</td>
</tr>
<tr>
<td>F</td>
<td>III:2</td>
<td>F</td>
<td>36</td>
<td>5</td>
<td>palpitations</td>
<td>I / I</td>
<td>BTS → Partial Atrial Standstill</td>
<td>34</td>
<td>37</td>
<td>No</td>
</tr>
</tbody>
</table>

yrs = years; NYHA = New York Heart Association Class; † = Died (years of age); BTS = Brady-tachy Syndrome; PM = Pace-maker; Emb. = Embolism; Hypert. = Hypertension.

**Table 2.** Results of genetic testing in a total of 477 individuals.

<table>
<thead>
<tr>
<th></th>
<th>c.G449A (p.Arg150Gln)</th>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Wild type</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Living patients with complete phenotype</td>
<td></td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8*</td>
</tr>
<tr>
<td>Family members without phenotype</td>
<td></td>
<td>0</td>
<td>40</td>
<td>37</td>
<td>77</td>
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<tr>
<td>1st Control group: unrelated individuals from the same area</td>
<td></td>
<td>0</td>
<td>16</td>
<td>176</td>
<td>192</td>
</tr>
<tr>
<td>2nd Control group: individuals from the national ground</td>
<td></td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>8</td>
<td>56</td>
<td>413</td>
<td>477</td>
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</tbody>
</table>

* 5 of the 13 patients with complete phenotype died before receiving genetic testing.
Table 3. Laboratory and instrumental data of the 8 living patients.

<table>
<thead>
<tr>
<th>Family</th>
<th>Pedigree No.</th>
<th>MR-proANP</th>
<th>NT-proBNP</th>
<th>Am. in abd. fat biopsy</th>
<th>RV-EBM</th>
<th>EMG and NCV</th>
<th>CT LA Vol ml/m²</th>
<th>CT RA Vol ml/m²</th>
<th>Carto RA Vol ml/m²</th>
<th>Carto RA Scar</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>IV:5</td>
<td>11</td>
<td>136</td>
<td>absent</td>
<td>yes</td>
<td>normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>V:1</td>
<td>5</td>
<td>182</td>
<td>absent</td>
<td>-</td>
<td>normal</td>
<td>253</td>
<td>443</td>
<td>385</td>
<td>diffused†</td>
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<tr>
<td>A</td>
<td>V:3</td>
<td>3</td>
<td>75</td>
<td>-</td>
<td>-</td>
<td>normal</td>
<td>105</td>
<td>195</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>III:1</td>
<td>5</td>
<td>74</td>
<td>absent</td>
<td>-</td>
<td>normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>IV:1</td>
<td>39</td>
<td>142</td>
<td>absent</td>
<td>-</td>
<td>normal</td>
<td>72</td>
<td>98</td>
<td>82</td>
<td>localized†</td>
</tr>
<tr>
<td>D</td>
<td>IV:2</td>
<td>45</td>
<td>166</td>
<td>absent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>106</td>
<td>localized†</td>
</tr>
<tr>
<td>E</td>
<td>III:1</td>
<td>18</td>
<td>53</td>
<td>absent</td>
<td>yes</td>
<td>normal</td>
<td>-</td>
<td>-</td>
<td>139</td>
<td>localized†</td>
</tr>
<tr>
<td>F</td>
<td>III:2</td>
<td>42</td>
<td>181</td>
<td>-</td>
<td>-</td>
<td>normal</td>
<td>-</td>
<td>-</td>
<td>66</td>
<td>localized†</td>
</tr>
</tbody>
</table>

MR-proANP = Mid Regional pro Atrial Natriuretic Peptide, normal value 18-120 pmol/L; NT-proBNP = N-Terminal pro Brain Natriuretic Peptide, normal value <125 pg/mL, heart failure exclusion <300; Am. = amyloidosis in abdominal fat biopsy; EMG = electromyography; NCV = nerve conduction velocity; CT = cardiac tomography; *Carto Mapping performed during complete AS; † Carto Mapping performed during BTS

Table 4. Echocardiographic measurement of the 13 affected patients at last observation

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

LA = Left Atrium; APD = Antero-Posterior Diameter (mm); Vol = Volume (ml)/body surface (m²); +++ = severe dilatation; RA = Right Atrium; LVEDD/m² = Left Ventricle End-Diastolic Diameter (mm)/ body surface (m²); LV mass = LV mass (g)/ body surface (m²); RV = Right Ventricle; LVEF = Left Ventricle Ejection Fraction (%); NA = Not Assessed
Figure Legends:

**Figure 1.** Phenotype. **Panel A:** surface ECG with complete AS: bradycardic (39 bpm) junctional rhythm without atrial activity and narrow QRS (patient A:V:1). **Panels B:** giant atria are shown by ultrasound examination and by three-dimensional cardiac tomography (3DCT) imaging. On the left the apical four chambers 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 (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 video). **Panels C:** scars in the RA are shown by 3D voltage mapping (right anterior projection) in the patients D:IV:2 (at the left) and A:V:1 (at the right) with BTS and complete AS respectively. In the former the scar is localized at lateral wall while in the latter the scar is diffused (red color indicates voltages < 0.05 mV).

**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 *NPPA* gene. Right sided electrophorograms show homozygous mutated, homozygous wild type and heterozygous cG449A mutation (p.Arg150Gln) in the *NPPA* gene; the positively charged Arginine is substituted by the neutral Glutamine at position 150.
**Figure 3.** Natural history. **Panels 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 34-39 ml/m²) (23), severe (≥40 ml/m²) (23) and giant (>80 ml/m²). **Panels 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).

**Figure 4.** 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 (IQR, box), lower and superior adjacent values at 1.5*IQR (whiskers) and outliers (plus sign markers).
The box plot shows the distribution of MR-proANP (pmol/L) across different genotypes:

- **Homozygous**
- **Heterozygous**
- **Wild type**

The plot indicates a significant difference between the homozygous and heterozygous groups, marked by asterisks **.
Autosomal Recessive Atrial Dilated Cardiomyopathy with Standstill Evolution Associated with Mutation of Natriuretic Peptide Precursor A

Marcello Disertori, Silvia Quintarelli, Maurizia Grasso, Andrea Pilotto, Nupoor Narula, Valentina Favalli, Camilla Canclini, Marta Diegoli, Silvia Mazzola, Massimiliano Marini, Maurizio Del Greco, Roberto Bonmassari, Michela Masè, Flavia Ravelli, Claudia Specchia and Eloisa Arbustini

Circ Cardiovasc Genet. published online December 29, 2012;
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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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.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Variant</th>
<th>Affected</th>
<th>Controls</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs5063</td>
<td>AA</td>
<td>8</td>
<td>251</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>rs5064</td>
<td>AA</td>
<td>8</td>
<td>246</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>c.123+25T&gt;C</td>
<td>TT</td>
<td>8</td>
<td>268</td>
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<td>54</td>
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<td>Allele 2</td>
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<td>Count (Controls)</td>
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<td>----------</td>
<td>----------</td>
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<td>------------------</td>
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<td>1</td>
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</tbody>
</table>

* recessive model

Only c.G494A (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.