ONLINE METHODS AND RESULTS

Human Protection

The Institutional Review Board at Beth Israel Deaconess Medical Center approved the study. The study was conformed to the declaration of Helsinki. Fully anonymized hearts and brain tissue and available clinical data were obtained from organ donors with family approval through the National Disease Research Interchange (NDRI) supported by the National Institute of Health. All donations were with family approval. Strict confidentiality was maintained to protect the privacy of all patients and donors.

Human subjects

A population of cases of AD was selected from the BIDMC clinical database (Clinical Query-2) and compared to age/gender/ethnicity matched control cases. Cases were selected using a query for the clinical diagnosis of AD (Online Figure 1 and Table 1). For one patient who deceased, the clinical diagnosis of AD was confirmed at pathology (indicated in Supplementary Table 1 with the # symbol). Three patients (indicated in Supplementary Table 1 with the § symbol) presented with a family history of AD. Patients with diagnosis of all other forms of dementia were excluded. Pathological conditions as primary or secondary forms of stages from 1 to 3 of hypertension, history of coronary artery disease, myocardial infarction, hypertrophic or dilated cardiomyopathy, primary or secondary amyloidosis, endocarditis, chemo or radiotherapy at the time of echocardiographic assessment were considered exclusion criteria. Controls were selected from the same query without a diagnosis of AD, from age/gender/ethnicity matched with a 1:2 ratio, when possible. Retrospective demographic, electrocardiographic and echocardiographic parameters were collected to evaluate myocardial function (Online Table 2 and 3). In some cases (indicated in the Online Table 1 with asterisk) echocardiographic recordings was performed before the diagnosis of AD. However in these cases, myocardial dysfunction would have been underestimated.

Human tissue

Whole heart was obtained from four cases with clinical diagnosis of AD. All cases were without known history of cardiac disease. Organ or tissue harvesting was performed either post-mortem or in patients under respiratory support for other organ explant for donation. Hearts were kept in cold oxygenated Wisconsin cardioplegic solution for transportation to the laboratory.

Brain tissue was obtained from the cortex. The entire brain from the 70 years-old case was sent to the department of neurology at the Johns Hopkins Hospital (JHH). The pathological diagnosis of AD was obtained from this case from the Investigators at the JHH.

Tissue processing

The hearts were dissected by regions (Anterior Wall - AW, Posterior Wall - PW, Lateral Wall -LW, Septal Wall –SW of the left ventricle -LV, and right ventricular wall -RV). Tissue from each region was frozen in liquid nitrogen and stored at -80°C for molecular biology tests. A sample from the anterior wall was collected in 4% paraformaldehyde-lysine-sodium metaperiodate in 0.1M NaPO4 (PLP) for immunohistochemistry. Approximately $1mm^3$ of the left ventricular AW was also collected in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer for electron microscopy. 1g of myocardial tissue removed from the LV-AW from the 58 years-old CF case was used to isolate cardiomyocytes by enzymatic digestion as previously described(1,2).

Transmission Electron Microscopy (TEM)

$1mm^3$ of LV-AW, fixed with gutaraldheyde was dehydrated, infiltrated with EPON-812 resin and embedded in capsules. The enclosed tissue was cut in super-thin sections on a Reichter Ultracut E ultramicrotome. The sections were collected on a formvar-coated slot grid, post stained with uranyl

acetate and lead citrate and viewed in a transmission electron microscope (JEM 1011 transmission electron microscope with digital acquisition of images) at 80 kV (Online Figure 2).

Immunoblotting

Dot blot was used to test the specificity and sensitivity of the antibodies against $A\beta_{40}$ and $A\beta_{42}$. Purified $A\beta_{40}$ and $A\beta_{42}$ were spotted on nitrocellulose membrane at increasing concentrations (5 to 200ng) for sensitivity. Anti $A\beta_{40}$ antibody was used to identify the $A\beta_{40}$ peptide and purified $A\beta_{42}$ was used as control for specificity. Anti $A\beta_{42}$ was used to identify $A\beta_{42}$ peptide and purified $A\beta_{40}$ was used as control (Online Figure 3).

For immunoblotting, frozen human tissue was lysated in Tris-HCl 50 mM, NaCl 150 mM and Halt™ Protease and Phosphatase Inhibitor cocktail (Thermo). The protein concentration of tissue lysate preparations was measured using the Bradford method(3). 16% Tricine gels were used for electroblotting and the membranes were incubated in Phosphate Buffer Saline (PBS) into a steamer to expose the peptides epitopes(4). For the immunoreactions, the blots were incubated separately with antibodies for anti-$A\beta_{40}$ (D8Q7I) (Cell Signaling, #12990) or anti-$A\beta_{42}$ (D3R10) (Cell Signaling, #12843) (Online Figure 4).

Enzyme-Linked-Immun-Sorbent Assay (ELISA)

Expression levels of $A\beta_{40}$ and $A\beta_{42}$ peptides were quantified using ELISA Novex kit KHB3481 and KHB3441 according to the manufacturer's instructions.

Structural staining and Immunohistochemistry

Frozen brain tissues were sectioned at 8µm slices. Sections were blocked with rabbit serum and incubated overnight at 4°C post with anti-oligo A11 antibody (Invitrogen AHB0052) at a concentration of 1:300. After extensive washing, Alexa green 568 secondary antibody of appropriate concentrations was

incubated for 1 hour at room temperature. Aggregates were stained using 0.01% Thyoflavin-S. Sections were imaged with a Zeiss confocal laser-scanning microscope (LSM550 or LSM700).

Isolated cardiomyocytes

Adult ventricular cardiomyocytes were isolated by enzymatic digestion from the heart of the 58 years old patient from 1g of myocardial tissue removed from the anterior wall of the left ventricle as previously described(2,5). Isolated cardiomyocytes were placed in a chamber on the stage of an inverted microscope, superfused with oxygenated Krebs-Henseleit solution, and electrically stimulated with a biphasic pulse (0.2 Hz, 50 % above threshold). Contraction amplitude and rates of contraction and relaxation were recorded online using a video-edge-detection system and data acquisition software (IonOptix). The fluorescent Ca^{2+} indicator Fura-2 (Molecular Probes) was used to measure intracellular Ca^{2+} through the use of a dual excitation spectrofluorometer, as previously described(6) (Online Figure 5).

Statistical analysis

Continuous variables (ultrasound, EKG, and laboratory parameters) are reported as means ± S.E.M. if normally distributed, or median and interquartile range for non-normally distributed. Groups were compared using Fisher's *t*-test for categorical and 2-sample Student *t*-test for continuous variables. Mann-Whitney test was employed for non-normally distributed variables and expressed as medians and IQR. Categorical variables (patients demographics, clinical features, and medications) are reported as percentage and analyzed by Fisher's exact test.

Linear regression was used to evaluate the association between group and cardiac parameters adjusted for age, and gender. The effect of age category by group on these parameters was evaluated by likelihood ratio test. Statistical significance was set at the 0.05 level. In case of non-normally distributed residuals, cardiac parameters were eventually transformed by log or square root. ANOVA test or Kruskal-Wallis H

test followed by Bonferroni or Dunn post-hoc analysis was conducted when appropriate for more than two groups comparisons.

Statistical analysis was performed with STATA14 (StataCorp LP, College Station, TX) for MacOS.

Online data abbreviations

AAG=Anti-aggregant

AARRH=Antiarrhythmic

ACE=Angiotensin Converting Enzyme

ACE-I=Angiotensin Converter Enzyme Inhibitor

AchE=Acetilcholinesterase

AD=Alzheimer Disease

AF=Atrial Fibrillation (paroxysmal and non-paroxysmal)

ARB=Angiotensin Receptor Blocker

ASA=Aspirin

AV=Aortic Valve

BB=Beta Blocker

CCB=Calcium Channel Blocker

CM=Caucasian Male

Crtl=Controls

COPD=Chronic Obstructive Pulmonary Disease

CVA=Cerebrovascular Accident

DM=Diabetes Mellitus

FS=Fractional Shortening

High BP=High Blood Pressure Readings.

LA=Left Atrium

LVDD=Left Ventricular End Diastolic Dimensions

LVDS= Left Ventricular End Systolic Dimensions

LVEF=Left Ventricular Ejection Fraction

LVILT=Left Ventricular Infero-Lateral Wall Thickness

LVSWT=Left Ventricular Septal Wall Thickness

MV=Mitral Valve

NSAID=Non Steroidal Anti-inflammatory Drugs

OAC=Oral Anticoagulant

RA=Right Atrium

RBC=Red Blood Cells

SSRI/SNRI=Selective Serotonin/Serotonin-Norepinephrine Reuptake inhibitor, T4=Levothyroxine

4C=Four Chamber view

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ONLINE FIGURES LEGEND

Online Figure 1: Flow chart of the clinical data query.

Online Figure 2: **Protein aggregates in AD hearts.** Transmission electron micrographs of all four cases of AD. In each case the image is presented with progressive magnification of significant regions.

Online Figure 3: **Aβ antibody specificity and sensitivity:** Dot blot for the validation of the sensitivity and specificity of the $A\beta_{1-40}$ and $A\beta_{1-42}$ fragments. Upper row: purified $A\beta_{40}$ is spotted at increasing concentrations from 0 to 200ng and detected with anti $A\beta_{40}$ antibody, but not the anti $A\beta_{42}$. The opposite is shown in the lower row (purified $A\beta_{42}$ at 0 to 200ng detected with the anti $A\beta_{42}$, but not the anti $A\beta_{40}$ antibody. As controls the highest concentration of purified $A\beta_{42}$ is spotted at the end of the series of purified $A\beta_{40}$ and vice-versa.

Online Figure 4: **SDS page of Aβ40 and 42**: Immunoblotting for $A\beta_{40}$ and $A\beta_{42}$ exposed simultaneously for comparison.

Online Figure 5: ***In-vitro*** **cardiomyocytes function in AD**: Single myocyte representative tracing of cell shortening and Ca^{2+} transient from a myocyte isolated from the 58yrs-old AD case (**A**) showing slow contractility and defect in Ca^{2+} handling compared to control (**B**). **C**) Schematic representation of the transients parameters measured using the Ion-Optix software. Abbreviations: TTP: Time to peak; R50-R90 time to 50% and 90% relaxation.

Online Figure 6: Proteolytic pathways of APP and Aβ biogenesis: Schematic representation of the APP processing by the secretase complex to produce $A\beta_{1-40}$ and $A\beta_{1-42}$ fragments. $A\beta_{40}$ and $A\beta_{42}$ originate from the last 28 aminoacids of the APP N-terminal ectodomain and the first 12 or 14 residues of the transmembrane domain following its cleavage by the alternative protease β-secretase (BACE1). This results in a c99 residue that is processed by the γ-secretase complex to release the Aβ fragments and the APP intracellular domain (AICD). APP processing by α- and γ-secretase is a normal event whereas the β-

and γ-secretase processing favors the production of the self-aggregating, amyloidogenic $A\beta_{1-42}$ variant over the shorter, less hydrophobic and amyloidogenic $A\beta_{1-40}$

Online Table 1:
Individual Patients (AD and controls) Demographics and Medications

Age	Ethnicity/ Gender	Group	Notable Associated Diseases	Therapy at the time of cardiac ultrasound
53	CM	AD *	Trisomy 21	T4, SSRI/SNRI
58	CM	AD *	Dyslipidemia, Benson Dementia	Donepezil, Memantine
62	CF	AD *	Dyslipidemia, Neoplasia	Statins, T4
63	CM	AD *#		SSRI
67	CM	AD	Dyslipidemia	ASA, Statins, Memantine, Vit E
73	CM	AD	AF	Rivastigmine
75	CF	AD §	Neoplasia	ASA, ACE-i, Donepezil, Memantine, SSRI/SNRI
75	CF	AD *		ASA, T4
76	CF	AD	Dyslipidemia	ASA, Statins, Donepezil, Vit E
78	CF	AD		
79	CM	AD *	Associated Vascular dementia	
79	CM	AD	Dyslipidemia, Neoplasia	ASA, Donepezil, Vit E, SSRI/SNRI
81	CM	AD	DM, Neoplasia	
81	CF	AD §	Dyslipidemia	ASA, NSAID, Statins, T4, Donepezil
82	CM	AD *§		CCB
83	CF	AD		ASA, T4
84	CM	AD	Dyslipidemia, Neoplasia, CVA Alcohol abuse	ASA, NSAID, Statins
85	CF	AD	Neoplasia, AF, CVA	Statins, AAG, T4, Galantamine, Memantine, SSRI/SNRI
86	CF	AD	AF	ASA, BB, T4
86	CF	AD		AAG
89	CM	AD	Neoplasia	ASA, Vit E
92	CF	AD	Dyslipidemia	ASA, Statins, SSRI/SNRI
53	CM	Ctrl	AF	OAC, CCB, Statins, AARRH,
53	CM	Ctrl		ASA, BB,
58	CM	Ctrl		ASA
58	CM	Ctrl		
62	CF	Ctrl		T4
62	CF	Ctrl		
63	CM	Ctrl	DM	Diuretics
63	CM	Ctrl		OAC, T4, SSRI
67	CM	Ctrl		OAC
67	CM	Ctrl		ASA, BB,
73	CM	Ctrl	Dyslipidemia, Neoplasia	Statins,
73	CM	Ctrl	Neoplasia, AF	ASA, OAC, BB, Statins, Digitalis, T4
75	CF	Ctrl	Dyslipidemia, AF	ASA, OAC, BB, Statins, Digitalis, T4
75	CF	Ctrl	High BP	ASA, BB, Diuretics, Statins, Vit E
75	CF	Ctrl		T4
76	CF	Ctrl	Dyslipidemia, COPD	BB, Diuretics, T4
76	CF	Ctrl		
78	CF	Ctrl	DM, COPD, High BP	ASA, ARBs
78	CF	Ctrl	COPD, Neoplasia	SSRI/SNRI
79	CM	Ctrl		BB, CCB
79	CM	Ctrl	Neoplasia, AF	OAC, BB, AARRH
81	CM	Ctrl	AF	OAC, BB, AARRH
81	CM	Ctrl	n/a	n/a
81	CF	Ctrl	Dyslipidemia, Neoplasia	ASA, OAC, BB, AARRH,
81	CF	Ctrl	Neoplasia	ASA, BB, T4
82	CM	Ctrl	High BP, CVA	ASA, Statins
83	CF	Ctrl		ASA, Diuretics, Statins, Digitalis
83	CF	Ctrl	AF	BB, AARRH, SSRI/SNRI
84	CM	Ctrl	Neoplasia,	ACE-i, Diuretics,

85	CF	Ctrl	COPD, Neoplasia	ASA,
86	CF	Ctrl		
86	CF	Ctrl	n/a	n/a
89	CM	Ctrl	Neoplasia, AF	
92	CF	Ctrl	Dyslipidemia, Neoplasia, High BP	OAC, BB, ACE-I, Diuretics, Statins, Digitalis
92	CF	Ctrl	n/a	n/a

Demographic and medications of the individual cases from the clinical cohort. Asterisk (*) indicates the patients with echocardiography performed before the diagnosis of AD; (#) indicates AD diagnosis at pathology; (§) indicates family history of AD. Abbreviations: AD=Alzheimer's Disease, CF=Caucasian Female, CM=Caucasian Male, Crtl=Controls.

Disease Abbreviations: AF=Atrial Fibrillation (paroxysmal and non-paroxysmal), COPD=Chronic Obstructive Pulmonary Disease, CVA=Cerebrovascular Accident, DM=Diabetes Mellitus, High BP=High Blood Pressure Readings.

Therapy Abbreviations AARRH=Antiarrhythmic, AAG=Anti-aggregant, ACE-I=Angiotensin Converter Enzyme Inhibitor, ARB=Angiotensin Receptor Blocker, ASA=Aspirin, BB=Beta Blocker, CCB=Calcium Channel Blocker, NSAID=Non Steroidal Anti-inflammatory Drugs, OAC=Oral Anticoagulant, SSRI/SNRI=Selective Serotonin/Serotonin-Norepinephrine Reuptake inhibitor, T4=Levothyroxine.

Online Table 2A
Cardiac Ultrasound Parameters of Control and Alzheimer's

Measurement	Control (n=35)	Alzheimer's (n=22)
LVEF > 55%	31/35 (88.6)	20/22 (90.9)
LVEF ≤ 55%	4/35 (11.4)	2/22 (9.1)
Left Atrium Long Axis (cm)	3.88±0.13	3.80±0.16
Left Atrium Four Chambers (cm)	5.20 (4.58-5.60)	4.85 (4.38-5.50)
Right Atrium Four Chambers (cm)	5.04±0.12	4.74±0.13
Left Ventricle Septal Wall Thickness (cm)	1.01±0.04	1.12±0.05
Left Ventricle Inferolateral Thickness (cm)	1.00±0.03	1.07±0.05
Left Ventricle Diastolic Dimension (cm)	4.62±0.09	4.34±0.19
Left Ventricle Systolic Dimension (cm)	2.79±0.09	2.69±0.12
Left Ventricle Fractional Shortening	0.40±0.01	0.40±0.01
Aortic Valve Peak Velocity (m/s)	1.40 (1.20-1.70)	1.70 (1.40-2.45)
Mitral Valve E Wave (m/s)	0.70 (0.60-1.00)	0.70 (0.60-0.88)
Mitral Valve A Wave (m/s)	0.80 (0.60-1.00)	0.80 (0.73-1.00)
Mitral Valve E/A Ratio	1.00 (0.71-1.29)	0.89 (0.75-1.00)
Mitral Valve E Wave Deceleration Time (ms)	240 (188-275)	245 (211-321)

Online Table 2B
Electrocardiographic Parameters of Control and Alzheimer's

Measurement	Control (n=16)	Alzheimer's (n=13)
Rate (bpm)	69 (63-77)	71 (58-75)
PR interval (ms)	179.50±12.04	155.20±9.56
QRS interval (ms)	93.08±4.55	93.69±5.75
QT interval (ms)	391.23±11.39	403.85±11.99
QTc interval (ms)	426.23±5.68	427.46±8.59

Online Table 2C
Laboratory Parameters of Control and Alzheimer's

Measurement	Control (n=25)	Alzheimer's (n=13)
RBCs (10^6/mL)	4.16±0.16	4.20±0.14
Hemoglobin (g/dL)	12.85±0.43	12.86±0.45
Hematocrit (%)	38.47±1.31	38.80±1.37
Glucose (mg/dL)	100.75±6.56	94.09±3.69
Blood Urea Nitrogen (mg/dL)	19 (15-28)	16 (14-19)
Creatinine (mg/dL)	1 (0.80-1.15)	0.8 (0.78-1)
Cholesterol (mg/dL)	197.75±15.71	197.75±19.12
HDL (mg/dL)	83.25±9.32	52.00±10.22
LDL (mg/dL)	95.75±11.95	123.33±10.27
Cholesterol/HDL ratio	2.43±0.19	4.03±0.41

Clinical parameters from the clinical cohort: A) Echocardiographic, **B)** Electrocardiographic and **C)** Laboratory data for control and AD patients. Data are expressed as mean±S.E.M. or median and IQR as appropriate. Electrocardiographic and laboratory parameters were similar in the two groups except for the total/HDL cholesterol ratio, as previously described(7-16).

Abbreviations: LVEF=Left Ventricular Ejection Fraction, RBC=Red Blood Cells.

Online Table 3A
Linear Regression of Cardiac Ultrasound Parameters

MV E/A ratio	β	95% CI	
Age	-0.0151163	-0.0235409	-0.0066918
Male	0.0823106	-0.0920983	0.2567195
AD	-0.0822727	-0.2491803	0.0846349
Constant	2.128903	1.45208	2.805725

MV E wave	β	95% CI	
Age	-0.0002008	-0.0074949	0.0070933
Male	-0.1212021	-0.2722075	0.0298033
AD	-0.0030187	-0.1475293	0.1414919
Constant	0.8653827	0.2793816	1.451384

MV E wave dec	β	95% CI	
Age	0.0967297	-2.098246	2.291705
Male	14.93527	-29.11576	58.9863
AD	28.95534	-15.16514	73.07582
Constant	226.8564	52.59449	401.1183

MV A wave	β	95% CI	
Age	0.0103221	0.0039144	0.0167298
Male	-0.2008249	-0.3334789	-0.0681709
AD	0.0621433	-0.0648053	0.1890918
Constant	0.1422067	-0.3725789	0.6569922

AV Peak V	β	95% CI	
Age	0.0167994	-0.0030387	0.0366375
Male	-0.1458605	-0.5834821	0.291761
AD	0.528849	0.0938788	0.9638193
Constant	0.2903946	-1.319209	1.899999

LA 4C	β	95% CI	
Age	0.0177469	-0.0063522	0.0418459
Male	-0.0597217	-0.5558531	0.4364096
AD	-0.1151634	-0.5918724	0.3615457
Constant	3.85819	1.932203	5.784176

RA 4C	β	95% CI	
Age	0.0130019	-0.0056556	0.0316593
Male	-0.0250765	-0.4091807	0.3590276
AD	-0.3341673	-0.7032348	0.0349002
Constant	4.081661	2.590565	5.572757

LVSWT	β	95% CI	
Age	0.0061874	0.0000449	0.0123299
Male	0.1509267	0.0227124	0.2791411
AD	0.0910206	-0.0331357	0.2151769
Constant	0.4766544	-0.0122128	0.9655216

LVILT	β	95% CI	
Age	0.0043108	-0.0007339	0.0093556
Male	0.1233948	0.0191218	0.2276679
AD	0.0518126	-0.0475914	0.1512167
Constant	0.6183428	0.2185107	1.018175

LVDD	β	95% CI	
Age	-0.0087981	-0.0271146	0.0095184
Male	0.1490012	-0.2371799	0.5351822
AD	-0.2765309	-0.648296	0.0952943
Constant	5.196918	3.734811	6.659025

LVSD	β	95% CI	
Age	-0.0063883	-0.0217811	0.0090044
Male	0.0330009	-0.2745182	0.34052
AD	-0.0951309	-0.3922099	0.201948
Constant	3.245579	2.033175	4.457983

FS	β	95% CI	
Age	0.0007699	-0.0012754	0.0028152
Male	0.0086295	-0.0326669	0.0499259
AD	-0.0062402	-0.0458714	0.0333909
Constant	0.3420622	0.1805283	0.5035961

Online Table 3B
Linear regression model (transformed) for Cardiac Ultrasound Parameters

logMV E/Aratio						
logMV E/A ratio	β	Std. Error	t	P	95% CI	
Age	-0.014979	0.0040098	-3.74	0.000	-0.0230215	-0.0069364
Male	0.0769907	0.0830116	0.93	0.358	-0.0895094	0.2434909
AD	-0.064544	0.0794412	-0.81	0.420	-0.2238829	0.094795
Constant	1.058021	0.32214	3.28	0.002	0.4118895	1.704152

Delta-method					Prob > χ^2 = 0.9603		
		Margin	Std. Err.	t	P>t	[95% CI]	
Tertile	Group						
1	Ctrl	0.2366164	0.1075695	2.20	0.032	0.0205566	0.4526761
1	AD	0.118562	0.1506242	0.79	0.435	-0.1839756	0.4210997
2	Ctrl	-0.0175869	0.0827459	-0.21	0.833	-0.1837869	0.148613
2	AD	-0.0814613	0.105437	-0.77	0.443	-0.2932379	0.1303152
3	Ctrl	-0.1916971	0.0804634	-2.38	0.021	-0.3533125	-0.0300817
3	AD	-0.2409354	0.0946027	-2.55	0.014	-0.4309506	-0.0509202

logMV E wave						
logMV E wave	β	Std. Error	t	P	95% CI	
Age	-0.000492	0.0042456	-0.12	0.908	-0.0090076	0.0080236
Male	-0.1393212	0.0878944	-1.59	0.119	-0.315615	0.0369725
AD	-0.007378	0.084114	-0.09	0.930	-0.1760893	0.1613334
Constant	-0.1765437	0.3410885	-0.52	0.607	-0.8606806	0.5075933

Delta-method					Prob > χ^2 = 0.8123		
		Margin	Std. Err.	t	P>t	[95% CI]	
Tertile	Group						
1	Ctrl	-0.2274815	.1130847	-2.01	0.050	-.4546189	-.0003441
1	AD	-0.3574009	0.1583469	-2.26	0.028	-0.6754499	-0.0393519
2	Ctrl	-0.2679996	0.0869883	-3.08	0.003	-0.4427208	-0.0932784
2	AD	-0.2215672	0.1108429	-2.00	0.051	-0.4442017	0.0010673
3	Ctrl	-0.3260913	0.0845888	-3.86	0.000	-0.4959929	-0.1561897

3	AD	-0.3178453	0.0994531	-3.20	0.002	-0.5176028	-0.1180879

sqrtMV E wave deceleration time						
sqrtMV Edecel	β	Std. Error	t	P	95% CI	
Age	0.0010724	0.0339143	0.03	0.975	-0.0670809	0.0692257
Male	0.4852337	0.6806268	0.71	0.479	-0.882537	1.853004
AD	0.8593871	0.6816999	1.26	0.213	-0.5105402	2.229314
Constant	15.07472	2.692498	5.60	0.000	9.663947	20.4855

Delta-method					Prob > χ^2 = 0.4609		
		Margin	Std. Err.	t	P>t	[95% CI]	
Tertile	Group						
1	Ctrl	14.59658	0.8558782	17.05	0.000	12.87378	16.31937
1	AD	16.96439	1.676133	10.12	0.000	13.59051	20.33827
2	Ctrl	15.66798	0.6577161	23.82	0.000	14.34407	16.99189
2	AD	16.77607	0.8380844	20.02	0.000	15.08909	18.46305
3	Ctrl	15.60497	0.6612462	23.60	0.000	14.27395	16.93599
3	AD	15.64007	0.7907345	19.78	0.000	14.0484	17.23174

logMV A wave						
logMV A wave	β	Std. Error	t	P	95% CI	
Age	0.0146267	0.0038278	3.82	0.000	0.0069491	0.0223044
Male	-0.2159069	0.0792446	-2.72	0.009	-0.3748515	-0.0569624
AD	0.0559627	0.0758363	0.74	0.464	-0.0961457	0.208071
Constant	-1.243943	0.3075217	-4.05	0.000	-1.860754	-0.6271331

Delta-method					Prob > χ^2 = 0.6853		
		Margin	Std. Err.	t	P>t	[95% CI]	
Tertile	Group						
1	Ctrl	-0.4635664	0.1051381	-4.41	0.000	-0.6747426	-0.2523903
1	AD	-0.4818107	0.1472196	-3.27	0.002	-0.77751	-0.1861114
2	Ctrl	-0.2494845	0.0808755	-3.08	0.003	-0.4119278	-0.0870412
2	AD	-0.1388562	0.1030538	-1.35	0.184	-0.3458459	0.0681335
3	Ctrl	-0.1322672	0.0786446	-1.68	0.099	-0.2902296	0.0256952
3	AD	-0.0748289	0.0924644	-0.81	0.422	-0.2605492	0.1108913

logAV Peak Velocity						
logAV PeakV	β	Std. Error	t	P	95% CI	
Age	0.008478	0.005027	1.69	0.100	-0.0017078	0.0186637
Male	-0.1027935	0.1108949	-0.93	0.360	-0.3274879	0.1219009
AD	0.2490862	0.1102231	2.26	0.030	0.0257531	0.4724193
Constant	-0.2334682	0.4078796	-0.57	0.571	-1.059911	0.5929743

Delta-method					Prob > χ^2 = 0.1705		
		Margin	Std. Err.	t	P>t	[95% CI]	
Tertile	Group						
1	Ctrl	0.2753672	0.1276608	2.16	0.038	0.0159292	0.5348052
1	AD	0.248852	0.1918487	1.30	0.203	-0.1410315	0.6387355
2	Ctrl	0.4697653	0.1248326	3.76	0.001	0.2160749	0.7234557
2	AD	0.5973677	0.1354982	4.41	0.000	0.3220023	0.8727332
3	Ctrl	0.3531786	0.0919523	3.84	0.001	0.1663091	0.540048
3	AD	0.8194304	0.1477948	5.54	0.000	0.5190752	1.119786

logLA 4C						
logLA 4C	β	Std. Error	t	P	95% CI	
Age	0.0034866	0.0022465	1.55	0.127	-0.0010256	0.0079988
Male	-0.004094	0.0462488	-0.09	0.930	-0.0969876	0.0887995
AD	-0.0280638	0.0444383	-0.63	0.531	-0.1173208	0.0611932
Constant	1.371344	0.1795385	7.64	0.000	1.01073	1.731957

Delta-method					Prob > χ^2 = 0.5153		
		Margin	Std. Err.	t	P>t	[95% CI]	
Tertile	Group						
1	Ctrl	1.592617	0.0564024	28.24	0.000	1.47915	1.706084
1	AD	1.460568	0.0903913	16.16	0.000	1.278724	1.642412
2	Ctrl	1.635592	0.043251	37.82	0.000	1.548582	1.722601
2	AD	1.576816	0.0590644	26.70	0.000	1.457994	1.695638
3	Ctrl	1.646784	0.0439277	37.49	0.000	1.558412	1.735155
3	AD	1.679975	0.0494364	33.98	0.000	1.580522	1.779428

Echocardiographic parameters from the clinical cohort adjusted for age and gender: A) Linear regression model of the cardiac ultrasound parameters adjusted for age, gender, and clinical status indicating how the echocardiographic parameters change are attributable to each variable. Age and gender are the major risk factors in AD with higher prevalence in women at all age ranges(17). In our analysis gender is also a predicting variable for functional changes, however here we focused on the contribution of age as described in our results section. **B)** Linear regression model and estimates for transformed data after visually inspecting the distribution of residuals. Data represent the χ^2 analysis of the ones in part illustrated in Figure 5B.

Abbreviations: LA=Left Atrium, RA=Right Atrium, 4C=Four Chamber view, LVSWT=Left Ventricular Septal Wall Thickness, LVILT=Left Ventricular Infero-Lateral Wall Thickness, LVDD=Left Ventricular End Diastolic Dimensions, LVDS= Left Ventricular End Systolic Dimensions, FS=Fractional Shortening, AV=Aortic Valve MV=Mitral Valve.

Online Table 4
Clinical Characteristics of Control and Alzheimer's with known drug management

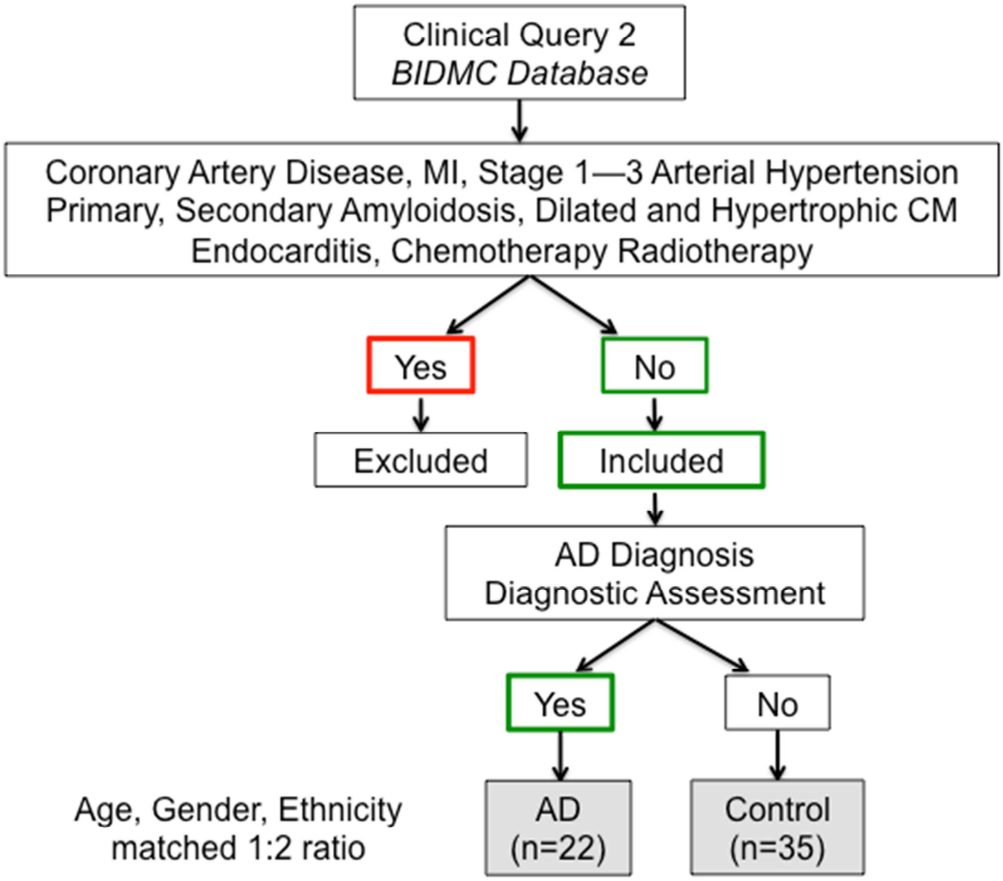
Feature	Control with β-blocker (n=13)	Control without β-blocker (n=19)	Alzheimer's (n=22)
Age – Median (IQR)	79 (75-81)	75 (62.5-82.5)	79 (73.5-83.8)
Male gender – no. (%)	6/13 (46.2)	10/19 (52.6)	11/22 (50)
Caucasian Ethnicity – no. (%)	13/13 (100)	19/19 (100)	22/22 (100)
Medical History – no. (%)			
Diabetes	0/13 (0)	2/19 (10.5)	1/22 (4.5)
Dyslipidemia	4/13 (30.8)	1/19 (5.3)	8/22 (36.4)
Neoplasia	5/13 (38.5)	5/19 (26.3)	7/22 (31.8)
High Blood Pressure Readings	2/13 (15.4)	2/19 (10.5)	0/22 (0)
Atrial Fibrillation	5/13 (38.5)	2/19 (10.5)	3/22 (13.6)
Cerebrovascular Accidents	0/13 (0)	1/19 (5.3)	2/22 (9.1)
Medications – no. (%)			
Aspirin	7/13 (53.9)	5/19 (26.3)	11/22 (50)
NSAIDs	0/13 (0)	0/19 (0)	2/22 (9.1)
Oral Anticoagulants	6/13 (46.2)	3/19 (15.8)	0/22 (0)
Antiaggregants	0/13 (0)	0/19 (0)	2/22 (9.1)
Statins	4/13 (30.8)	4/19 (21.1)	7/22 (31.8)
Antiarrhythmics	4/13 (30.8)	1/19 (5.3)	0/22 (0)
Digoxin	3/13 (23.1)	1/19 (5.3)	0/22 (0)
β-blockers	13/13 (100)	0/19 (0)	1/22 (4.5)
Calcium channel blockers	1/13 (7.7)	1/19 (5.3)	1/22 (4.5)
ACE inhibitors	1/13 (7.7)	1/19 (5.3)	1/22 (4.5)
ARBs	0/13 (0)	1/19 (5.3)	0/22 (0)
Diuretics	3/13 (23.1)	3/19 (15.8)	0/22 (0)
Levothyroxine	4/13 (30.8)	3/19 (15.8)	7/22 (31.8)
Any AchE inhibitors	0/13 (0)	0/19 (0)	7/22 (31.8)
Donepezil	0/13 (0)	0/19 (0)	5/22 (22.7)
Rivastigmine	0/13 (0)	0/19 (0)	1/22 (4.5)
Galantamine	0/13 (0)	0/19 (0)	1/22 (4.5)
Memantine	0/13 (0)	0/19 (0)	4/22 (18.2)
SSRI/SNRI	1/13 (7.7)	2/19 (10.5)	6/22 (27.3)
Vitamin E	1/13 (7.7)	0/19 (0)	4/22 (18.2)

Demographic features, clinical presentation and drug prescriptions recorded at the time of cardiac ultrasound dividing controls based on the presence of beta-blockers therapy and AD patients. Abbreviations in alphabetical order: ACE=Angiotensin Converting Enzyme, AchE=Acetilcholinesterase, ARB=Angiotensin Receptor Blocker, SSRI/SNRI=Selective Serotonin/Serotonin-Norepinephrine Reuptake inhibitors.

Online Table 5A Cardiac Ultrasound Measurements	**Subgroups**			**ANOVA or H test with post-hoc analysis**			
Feature	**Control with β-blocker (n=13)**	**Control without β-blocker (n=19)**	**Alzheimer's (n=22)**	**p**	**p (BB+ vs BB-)**	**p (AD vs BB+)**	**p (AD vs BB-)**
LA Long Axis (cm)	4.05±0.17	3.74±0.21	3.80±0.16	0.534	0.856	1.000	1.000
LA Four Chambers (cm)	5.40 (5.20-5.80)	5.10 (4.18-5.38)	4.85 (4.38-5.50)	0.049	0.009	0.022	0.339
RA Four Chambers (cm)	5.25±0.16	4.82±0.17	4.74±0.13	0.071	0.202	0.082	1.000
LV Septal Wall Thickness (cm)	1.05±0.05	0.98±0.04	1.12±0.05	0.175	1.000	1.000	0.190
LV Inferolateral Thickness (cm)	1.00±0.04	0.98±0.04	1.07±0.05	0.356	1.000	0.993	0.520
LV Diastolic Dimension (cm)	4.65±0.17	4.58±0.11	4.34±0.19	0.352	1.000	0.592	0.784
LV Systolic Dimension (cm)	2.85±0.18	2.79±0.10	2.69±0.12	0.695	1.000	1.000	1.000
LV Fractional Shortening	0.39±0.02	0.40±0.01	0.40±0.01	0.955	1.000	1.000	1.000
AV Peak Velocity (m/s)	1.60 (1.50-1.90)	1.20 (1.15-1.45)	1.70 (1.40-2.45)	0.024	0.021	0.422	0.006
MV E Wave (m/s)	0.70 (0.60-1.00)	0.70 (0.60-0.95)	0.70 (0.60-0.88)	0.915	0.359	0.348	0.492
MV A Wave (m/s)	0.80 (0.70-0.90)	0.80 (0.60-0.95)	0.80 (0.73-1.00)	0.425	0.337	0.235	0.098
MV E/A Ratio	1.00 (0.88-1.00)	1.00 (0.76-1.36)	0.89 (0.75-1.00)	0.439	0.487	0.160	0.125
MV E Wave Deceleration Time (ms)	235 (219-240)	240 (176-277)	245 (211-321)	0.437	0.444	0.132	0.143

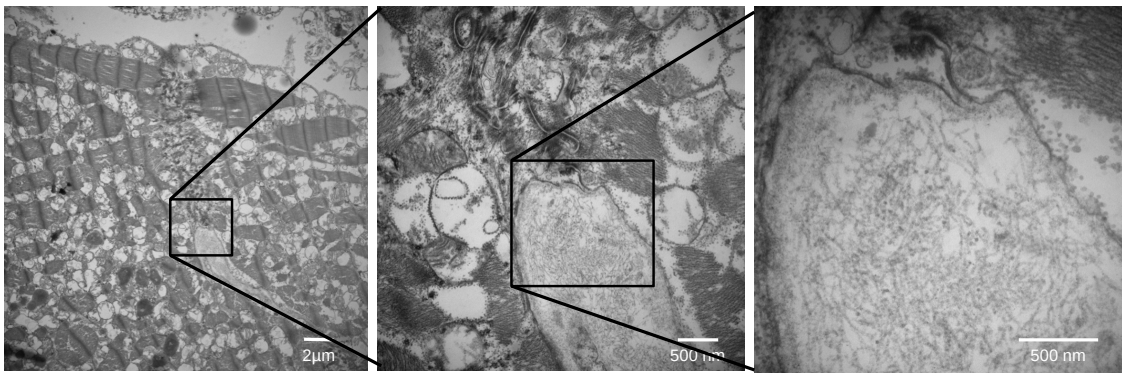
Online Table 5B Electrocardiographic Measurements	**Subgroups**			**ANOVA or H test with post-hoc analysis**			
Feature	**Control with β-blocker (n=8)**	**Control without β-blocker (n=8)**	**Alzheimer's (n=13)**	**p**	**p (BB+ vs BB-)**	**p (AD vs BB+)**	**p (AD vs BB-)**
Rate (bpm)	68 (63-71)	75 (65-84)	71 (58-75)	0.667	0.189	0.356	0.262
PR interval (ms)	185.33±11.72	158.8±13.53	155.20±9.56	0.159	0.477	0.198	1.000
QRS interval (ms)	93.75±3.57	93.25±6.81	93.69±5.75	0.998	1.000	1.000	1.000
QT interval (ms)	393±16.08	398.5±13.45	403.85±11.99	0.850	1.000	1.000	1.000
QTc interval (ms)	416.38±7.96	431.25±6.41	427.46±8.59	0.489	0.780	1.000	1.000

Clinical parameters from the clinical cohort dividing controls based on the presence of beta-blockers therapy and AD patients A) Echocardiographic, **B)** Electrocardiographic data are expressed as mean±S.E.M. or median and IQR as appropriate. Data indicate that diastolic function was not affected by the therapeutic regimen. Abbreviations: LA=Left Atrium, RA=Right Atrium, 4C=Four Chamber view, LVSWT=Left Ventricular Septal Wall Thickness, LVILT=Left Ventricular Infero-Lateral Wall Thickness, LVDD=Left Ventricular End Diastolic Dimensions, LVDS= Left Ventricular End Systolic Dimensions, FS=Fractional Shortening, AV=Aortic Valve MV=Mitral Valve.

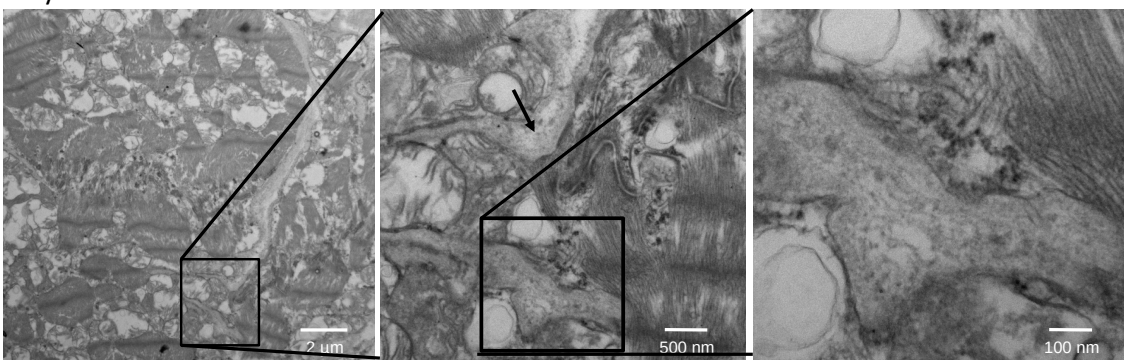


Online Figure 1

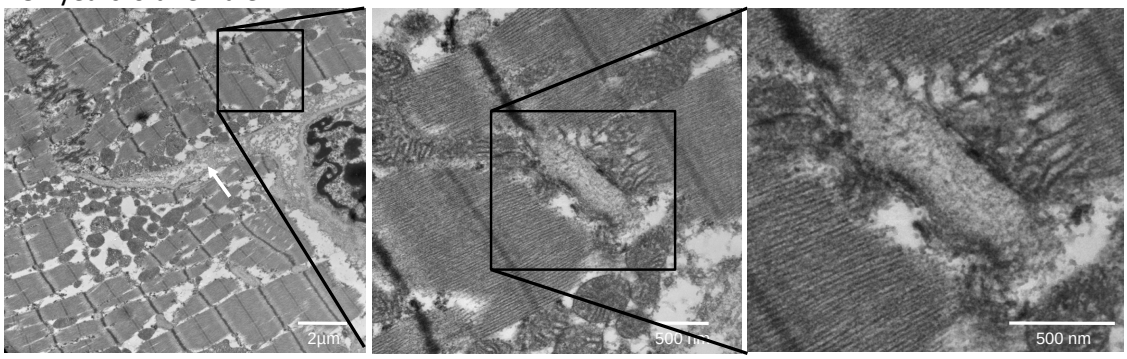
58 years-old female



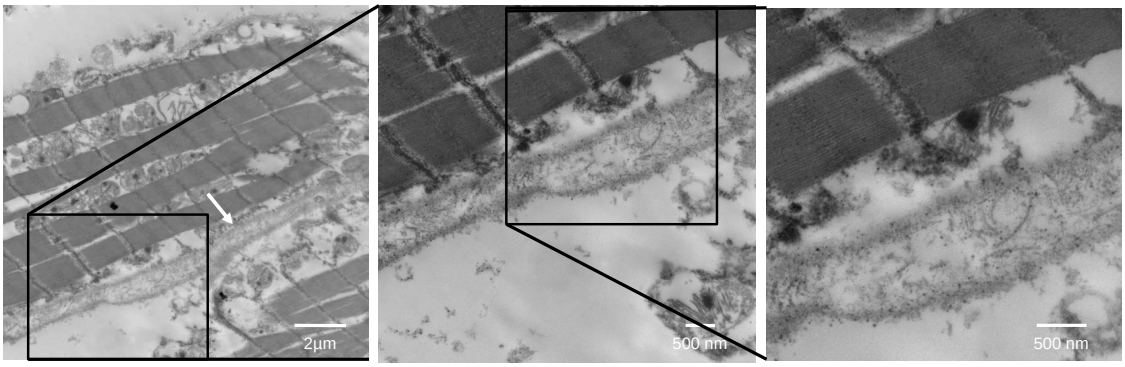
70 years-old female



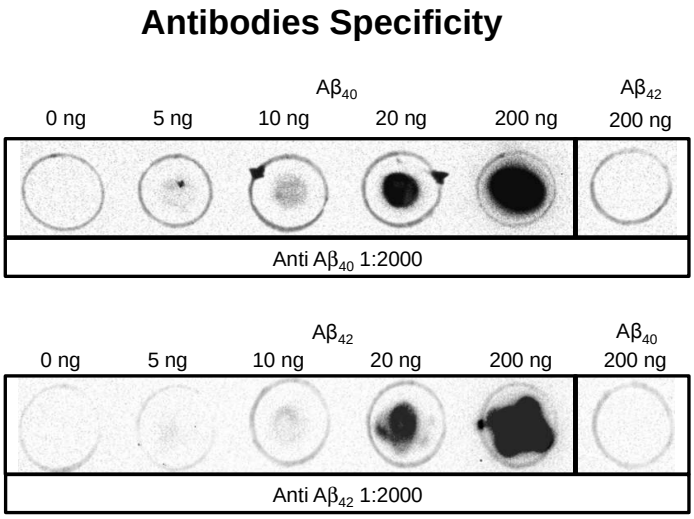
84 years-old female



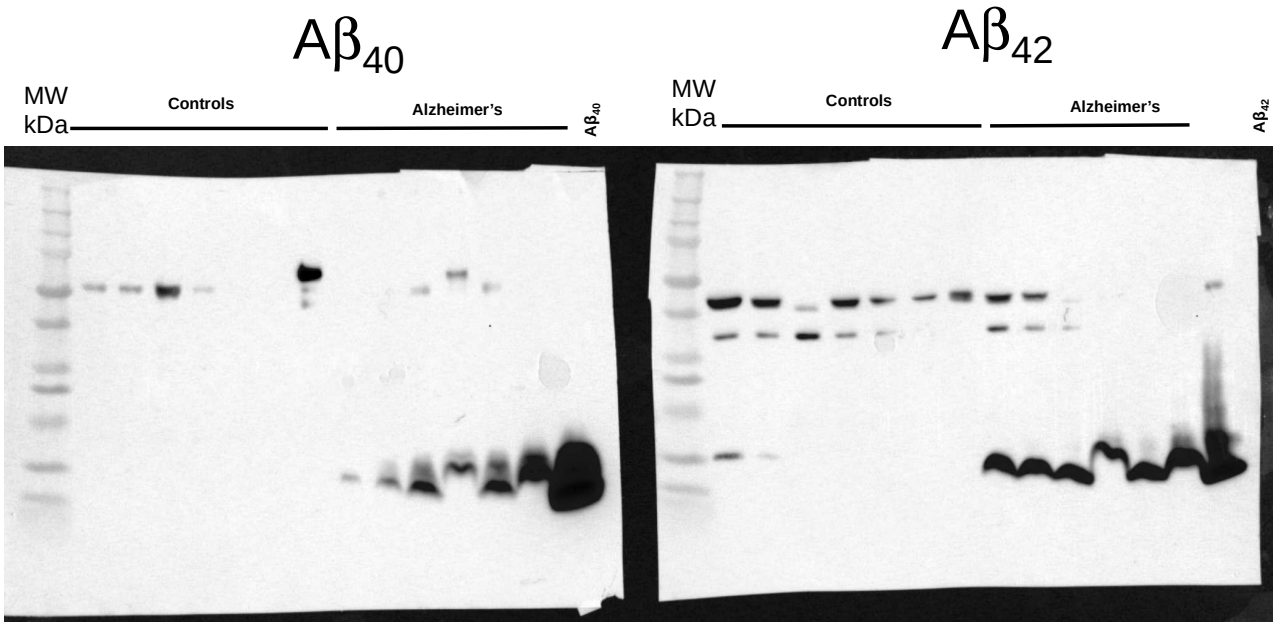
91 years-old male



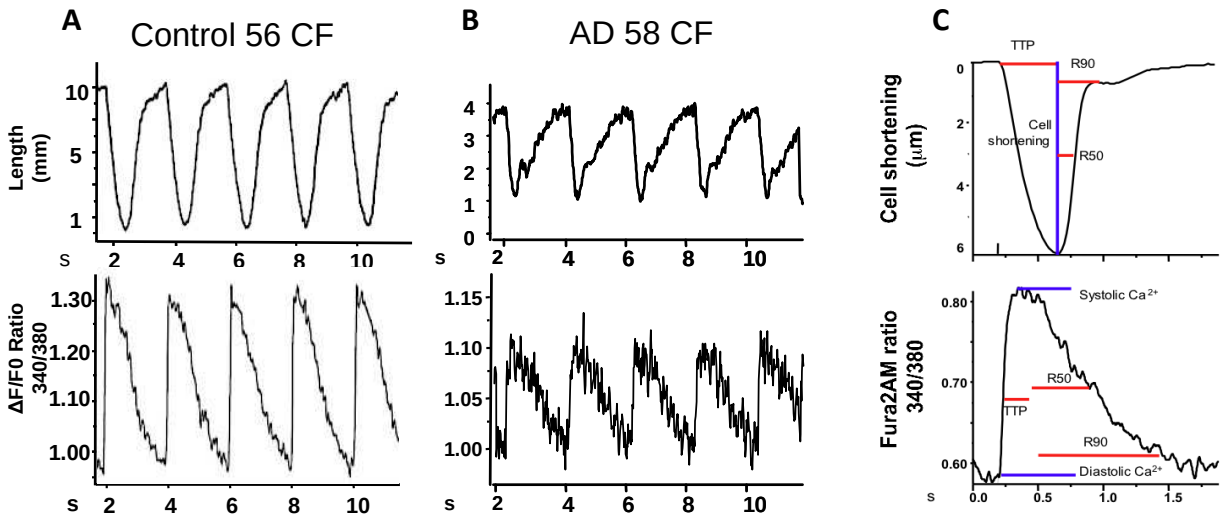
Online Figure 2



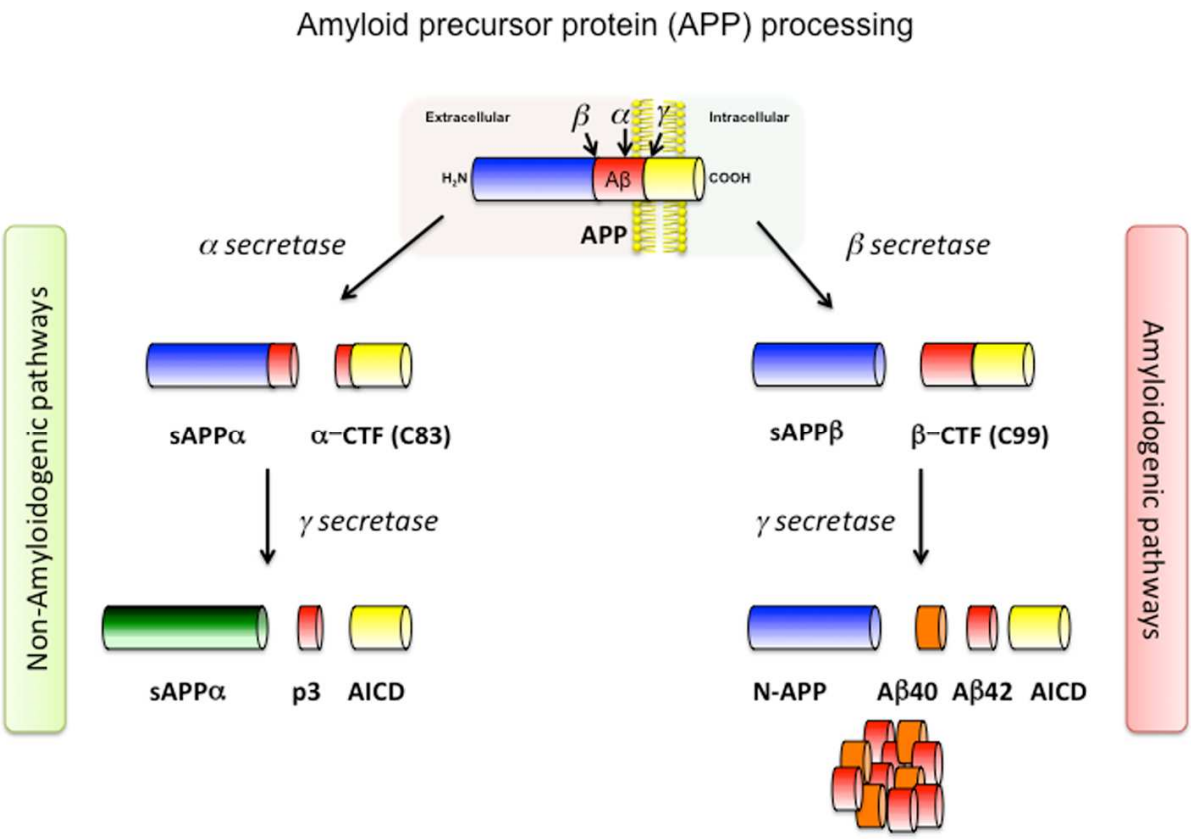
Online Figure 3



Online Figure 4



Online Figure 5



Online Figure 6