Non-atrial fibrillation cardiac phenotypes associated with common atrial fibrillation genotypic risk

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Abstract

Background: Atrial fibrillation (AF) genetics studies have focused on a linear genotype- phenotype relationship, i.e. genetic predisposition to the arrhythmia. Genome wide association studies have implicated numerous upstream mechanisms responsible for AF. Objective: We hypothesized that the genetic predisposing factors for AF might be associated with non-AF clinical phenotypes and sought to characterize electrophysiology parameters as a function of AF genetic risk. Methods:. Biosamples were obtained from 405 subjects for classification of carrier status at 12 single nucleotide polymorphisms with a known association to AF allowing calculation of a validated AF genetic risk score. We then analyzed subgroups within the total population; in order to understand the effect on (a) sinus node function and cardiac conduction (b) primary atrial flutter (c) left atrial appendage morphology. Results: We evaluated 405 patients consisting of a range of genetic risk scores from -1.016 to +2.178. Within this, we identified 86 patients without prescribed chronotropic pharmacotherapy with a 24-hour Holter recording to investigate sinus node function; 181 patients with invasive H-V measurement at the time of electrophysiologic study to investigate cardiac conduction; 78 undergoing cavotricuspid isthmus ablation for typical atrial flutter without prior diagnosis of AF; and 284 patients with cardiac imaging of the left atrial appendage. Conclusions: A common AF genetic risk score is associated with a number of non-AF electrophysiologic relevant phenotypes. Sinus node function, AV node physiology, post flutter ablation AF risk, atrial appendage morphology all appear to be associated with the common genetic AF risk.

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Short Title: AF Genotype/Non-AF Phenotype Relationships

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Keywords

Atrial fibrillation

Polygenic risk score

Atrial flutter

Cardiac electrophysiology

Left atrial appendage

Abbreviations List

AF atrial fibrillation

AFL atrial flutter

SNP single nucleotide polymorphism

SNV single nucleotide variant

GRS genetic risk score

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Introduction

Atrial fibrillation (AF) is the most common clinical arrhythmia. While some rare monogenic or Mendelian forms of AF have identified non-AF manifestations[1], the common forms of AF do not. Recent insights into the genetics of common AF suggest that the disease, while manifesting in the atrial myocardium, may also have a somatic "non-AF" phenotype[2]. Patients with AF often manifest with sinus node dysfunction, heart block, atrial flutter, cardiac thrombi, coronary disease and other atrial arrhythmias. The relationship between these discrete phenotypes is thought to be secondary to overlapping upstream biological risk factors. We sought to investigate the potential presence of a non-AF phenotype of AF through associations with polygenic AF risk.

Since the first identified single nucleotide variant (SNV) predisposing to AF was identified[3], a cascade of other variants have been implicated in the heritable contribution to AF. This has allowed the creation of polygenic

risk scores which aggregate risk across multiple loci to approximate the risk to an individual. While the clinical utility of such scores remains uncertain, they do offer opportunity to define whether discrete clinical features are associated with specific genetic risk. We sought to determine if more granular clinical features might be associated with a defined polygenic risk to AF.

Methods

Patient Selection

This study was conducted in accordance with the Partners Healthcare institutional review board approval. Consecutive patients presenting to the Brigham and Women's Hospital Cardiovascular clinic and Electrophysiology Lab for evaluation were approached for study entry and informed consent obtained. A retrospective chart review was completed to identify clinical features.

Genotyping Protocol

Venipuncture was performed and DNA was extracted using the Illustra Nucleon BACC3 Genomic DNA Extraction kit (GE Healthcare, Chicago, IL, USA). Samples were genotyped for the AF-associated variants using TaqMan (Applied Biosystems, Foster City, CA), a plate-based, 1-step reaction that identifies the SNP allele during polymerase chain reaction amplification. An AF genetic risk score was calculated using the literature risk estimates as weights for the minor allele of each of the 12 SNPs as previously reported[4]. All 12 SNPs were genotyped in all subjects without genotype imputation. In the atrial appendage cohort subgroup, we classified patients based on genetic risk score as well as the status of the RS2200733 SNV (a component of the polygenic risk score). This SNP was chosen as a stratifier for morphologic evaluation analysis given the known role of PITx2 in Left-Right signaling. Patients were categorized as SNV carriers if they were heterozygous (CT) or homozygous (TT) for the RS2200733 variant. Patients with the CC genotype were classified as non-carriers. Investigators were fully blinded to the genotype status of each subject until data analysis was complete.

Modeling of genetic risk score

The polygenic risk score utilized here includes 12 SNPs (Table 1), and is the most commonly studied such score as originally published by Tada et al and subsequently validated[3, 5-9]. The linkage disequilibrium between these SNPs is captured in the implementation of the score components[4]. The AF- genetic risk score (GRS) for each individual in the current study was calculated: for each SNP the natural log transformed risk estimate for the minor allele was multiplied by the number of minor alleles carried by that individual; these 12 products were then summed. The risk estimate for the minor allele at each SNP was obtained from the largest available data set.

Sinus Node Function

In order to ascertain baseline sinus function, we identified 86 patients who had a 24-hour Holter monitor performed when they were not using any chronotropic medications (beta blockers, calcium channel blockers, Class I-IV antiarrhythmics). Average, minimum and peak heart rate recorded as well as metrics of heart rate variability including SDNN (standard deviation of NN intervals in ms) and RMSDD (Root mean square of successive RR interval differences in ms) were documented[10]. We used SDNN as the index of heart rate variability over a 24 h period and RMSSD as the primary time-domain measure for estimation of the vagally mediated changes reflected in HRV[11].

Cardiac Conduction

In order to objectively define cardiac conduction in a cohort, we identified 181 patients who had invasive electrophysiologic measure of the His-Purkinje system (i.e. H-V interval measurement in ms) as well as a surface EKG measuring QRS duration (in ms). Repolarization via the surface EKG was also measured (maximum QT interval in ms). HV was measured via standard electrophysiologic lab practices[12]. During electrophysiologic study, patients were classified as having dual atrioventricular (AV) node physiology or

not having dual AV node physiology from the presence or absence of*discontinuous antegrade AV nodal conduction* in response to atrial programmed electrical premature stimulation using an abrupt increase in AV nodal conduction time of [?]50 ms in response to a decrement of 10 ms as the definition of dual AV nodal pathway physiology.

Post Atrial Flutter Ablation AF

We identified 78 patients in our cohort who presented for cavotricuspid ablation (CTI) ablation of typical atrial flutter between 2015-2019 and had no documented prior AF. Prospective enrollment of this population was specifically targeted among the total 405 person cohort. Patients were followed post successful CTI ablation with home telemetry monitoring at 1-2 months as well as at 6-12 months in addition to as-needed monitoring based on symptoms. The clinical records were extracted for diagnosis of AFL as well as subsequent AF.

Atrial Appendage Morphology

284 patients in the cohort had a cardiac CT or cardiac MRI performed for clinical indications. Three-dimensional structures of the left atrial appendge (LAA) were constructed using the volume-rendered post-processing technique. Standard measurements of LAA volume, os circumference, and diameters were obtained. The morphology of the LAA was also evaluated using multiplanar reconstruction. LAA morphologies were classified by the reading radiologist and confirmed by the primary investigator (SK). The LAA os was pre-determined to be the proximal border of the LAA and all visualized lobes were included in the volumes. LAA was reported as chicken wing (CW), cauliflower (CF), cactus (CS), and windsock (WS) as previously described[13]. Given the numbers and known stroke associations, we prespecified patients as CW (n=136) or non-CW (148) for analysis. In addition to stratifying by GRS, patients were stratified by the 4q25 variant of greatest significance to AF (r2200733 (NC_000004.12:g.110789013C>T) given the known association with left-right asymmetry. Patients were categorized at this SNV as carriers (C; n=109) if they were heterozygous (CT) or homozygous (TT). Patients with the CC genotype were classified as non-carriers (NC; n=175).

Statistical analysis

Continuous variables are expressed as mean ± SD and categorical variables. Analysis was performed using Prism (version 6.0d, GraphPad Software Inc, La Jolla, CA). Continuous variables were expressed as mean ± SD. The Student t test and Mann-Whitney U test were used for the comparison of continuous variables between groups. The paired t test and Wilcoxon signed-rank test were used for the comparison of continuous variables between echocardiographic follow-ups. The χ^2 test and Fisher exact test were used for noncontinuous variables. Bonferroni correction was used for multiple correction where applicable. A 2-sided P value <0.05 was considered statistically significant.

Results

The overall population of 405 subjects was evaluated for clinical features as stratified by quartiles of GRS. Dividing the population into quartiles via genetic risk required cutoffs of Low (L): GRS[?]0.2150; Low Intermediate (LI) GRS>0.2150 and [?]0.4445; High Intermediate (HI) GRS >0.4445 and [?]0.7479; High (H) GRS>0. 7479. In comparing baseline demographics of the four divided groups, the only difference identified was, as expected, presence of atrial fibrillation (L: 39%; LI: 50%; HI 48%; H: 60%; p=0.02).

Sinus Node Function

Figure 1A shows Holter observed heart rate metrics including minimum, average and peak heart rate (in beats per minutes / bpm) as stratified by AF GRS quartiles (n = L: 22; LI: 21; HI 23; H: 20). Baseline demographics were similar between the four quartiles labeled: age (L: 51 ± 8 years; LI: 52 ± 9 years; HI: 51 ± 9 years; H: 50 ± 9 years), male gender percent (L: 50%; LI: 53%; HI 50%; H: 50%); Body mass index (kg/m2) (L: 24.9 ± 4.4 ; LI: 24.1 ± 5.0; HI: 24.1 ± 4.6; H: 25.0 ± 6.1); ejection fraction (L: 54 ± 6 %; LI: 53 ± 8; HI: 54 ± 7; H: 56 ± 7). There is an observed association of increased minimum and average heart rate with increased GRS (minimum L: 55 ± 5 bpm; LI: 56 ± 5 bpm; HI: 58 ± 6 bpm; H: 62 ± 5 bpm; p

= 0.02; average L: 75 ± 6 bpm; LI: 74 ± 7 bpm; HI: 79 ± 8 bpm; H: 81 ± 8 bpm; p = 0.01). There is no association between peak heart rate and GRS (maximum L: 125 ± 9 bpm; LI: 123 ± 9 bpm; HI: 128 ± 9 bpm; H: 125 ± 8 bpm; p = 0.30). Figure 1B shows Holter observed heart rate variability metrics including SDNN and RMSDD as stratified by GRS quartiles. There is an observed trend of decreased HR variability with increased AF GRS (SDNN L: 121 ± 13 ms; LI: 120 ± 12 ms; HI: 114 ± 13 ms; H: 108 ± 14 ms; p = 0.01; RMSDD L: 25 ± 4 ms; LI: 24 ± 5 ms; HI: 22 ± 6 s; H: 21 ± 5 ms; p = 0.04).

Cardiac Conduction

Figure 2A shows cardiac conduction parameters observed as measured during an electrophysiologic study as stratified by AF GRS quartiles (n = L: 44; LI: 47; HI 45; H: 45). Baseline demographics were similar between the four quartiles labeled: age (L: 56 ± 10 years; LI: 55 ± 11years; HI: 56 ± 10 years; H: 57± 11 years), male gender percent (L: 50%; LI: 51%; HI 52%; H: 52%); Body mass index (kg/m2) (L: 26.9 ± 5.6 ; LI: 26.5 ± 6.6; HI: 26.4 ± 4.0; H: 27.0 ± 6.1); ejection fraction (L: 51 ± 0 %; LI: 52 ± 9; HI: 51 ± 8; H: 53 ± 9). There is no observed trend of HV (His-Ventricle) conduction time, QRS duration or QTc duration with increased AF GRS (QRS L: 102 ± 11 ms; LI: 103 ± 13 ms; HI: 99 ± 11 s; H: 105 ± 12 s; p = 0.11; HV L: 38 ± 9 ms; LI: 41 ± 8 ms; HI: 39 ± 9 s; H: 42 ± 11 s; p = 0.16; QTc L: 401 ± 24 ms; LI: 399 ± 27 ms; HI: 408 ± 30 s; H: 396 ± 26 s; p = 0.19). Figure 2B shows percent of total in each quartile of genetic risk with document dual AVN pathway. (L: 18%; LI: 23%; HI 42%; H: 67%; p< 0.01).

Post Atrial Flutter Ablation AF

The 78 subjects who underwent CTI without prior AF diagnosis consisted of patients in each of the GRS quartile [L, n=20, LI, n=19, HI, n=19, and H, n=20]. Baseline demographics were similar between the four quartiles labeled: age (L: 59 ± 6 years; LI: 55 ± 8 years; HI: 61 ± 5 years; H: 60 ± 7 years), male gender percent (L: 55%; LI: 53%; HI 58%; H: 60%); Body mass index (kg/m2) (L: 25.9 ± 4.7 ; LI: 28.1 ± 5.1; HI: 27.1 ± 4.4; H: 26.5 ± 6.5); hypertension (L: 15%; LI: 11%; HI 16%; H: 20%); diabetes (L: 15%; LI: 16%; HI 16%; H: 15%); coronary disease (L: 20%; LI: 16%; HI 11%; H: 20%); prior stroke (L: 10%; LI: 5%; HI 5%; H: 5%); ejection fraction (L: 55 ± 7 %; LI: 59 ± 8; HI: 61 ± 7; H: 62 ± 9) . Over an average of 2.4 years of follow up, 47 patients had documented AF. Figure 3 documents variable AF free survival post CTI ablation as stratified by AF genetic risk score (Log Rank Mantel-Cox test, p = 0.0004). At the end of follow up, 18 of the 20 high risk patients had documented AF while only 5 of the low risk.

Atrial Appendage Morphology

The 284 subjects with appendage imaging consisted of patients in each of the GRS quartile [L, n=71, LI, n=73, HI, n=69, and H, n= 71]. Baseline demographics were similar in age, gender, race (data not shown). Figure 4A shows no differences in left atrial morphology (L: 46%; LI: 46%; HI 45%; H: 54%), os circumference (L: 2.40 ± 0.60; LI: 2.60 ± 0.61; HI 2.51 ± 0.62; H: 2.57 ± 0.58; p =0.21), or indexed volume (L: 3.75 ± 0.81; LI: 3.80 ± 0.80; HI 3.70 ± 0.81; H: 3.82 ± 0.82; p =0.31) as stratified by GRS quartile. Figure 4B Baseline demographics showed no difference between carriers and non-carriers in age (C 61±8 vs NC 60±9 years), gender (C 27% vs NC 37% female), race (C 84% vs NC 81% white), hypertension(C 34% vs NC 29%), diabetes(C 5% vs NC 8%), coronary/vascular disease presence (C 12% vs NC 14%), heart failure presence(C 8% vs NC 7%), body-mass index (C 28.8 ± 4.5 vs NC 29.7 ± 4.6 kg/m2), serum creatinine (C 0.91± 0.11 vs NC 0.89 ± 0.12 mg/dl), percent paroxysmal AF (C 54% vs NC 52%) or calculated CHADVASC score (cardioembolic score that does not include stroke; C 2.20 ± 0.50 vs 2.1 ± 0.6). Of the 109 carrier patients, 89 (82%) had a non-CW morphology and 20 (18%) had a CW morphology. In the non-carrier 175 patient population, 47 (27%) had non-CW whereas 128 (73%) were CW. Carriers also had smaller LAA orifice (2.20 ± 0.05 cm vs 2.79 ± 0.07 cm ; $P < .01$) however no change in LA diameter (C 4.2 ± 0.50 cm vs NC 4.79 ± 0.6 cm), LA indexed volume (C 34.7 ± 10.2 vs NC 36.3 ± 11.2 ml/m2), or LVEF (C 55 ± 5 vs NC 58 ± 7 %) were noted. Carriers also had a larger volume of the LAA 4.10 ± 0.14 ml/m2 versus non-carriers 3.59 ± 0.18 ml/m2; $P < 0.01$.

Discussion

We observe a series of persistent phenotypes associated with an estimate of the underlying risk of AF. These findings highlight that multiple phenotypic features are represented in the common heritable contribution to risk of AF, and also suggest that more granular phenotypic resolution of the AF syndrome may enable the detection of larger effect size alleles underlying particular subsets.

Cardiac Electrophysiology

The known relationship between common allele risk scores and AF suggests a fundamental change in electrophysiologic substrate. This study finds an association between sinus node function and AF risk ,though the mechanism is unclear. Large longitudinal studies have noted that those with a higher resting heart have increased risk of incident AF[14, 15], consistent with our findings here. Changes in resting heart rate as well as heart rate variability suggest the mechanism may lie within the autonomic nervous system. The associations between common genetic risk for AF and both SDNN and RMSDD infer a consistent relationship possibly mediated through higher vagal tone. Similarly, large longitudinal studies suggest extremes of RMSDD and lower SDNN are independently associated with incident AF[14 16]. In addition, studies have shown that SDNN typically decreases immediately prior to a paroxysm of AF[17]. These findings suggest that the genetic loci associated with AF mediate at least part of their effects through sinus node and cardiac autonomic function.

While infrahisian conduction (HV and QRS) does not appear to be associated with common genetic risk for AF there does appear to be an association between dual AVN physiology and the risk score we have deployed. An association of AF genetic risk with dual AV node physiology suggests a potential link through AV node reentrant tachycardia (AVNRT), but it is unclear if this represents a functional or structural change in the AV node. Little is known of the genetics of AVNRT but a fundamental relationship between the substrate for AVNRT and that for AF is supported by an unexpectedly high incidence of new-onset AF in AVNRT patients following ablation [18]. Mechanisms of atrioventricular development have previously been implicated in the predisposition to AF with Wolff-Parkinson-White (WPW) syndrome patients also prone to paroxysmal AF at a young age[19]. Anatomic distribution of Cx43 in defined AV nodal structures has supported an anatomic basis for dual AV nodal physiology and aberrant Cx43 expression has been directly implicated in rare subsets of AF, as well as in the ongoing remodeling of atrial substrate after onset of the arrhythmia[20, 21].

Cardiac Structure

GWAS studies have also associated 4q25 with not only AF but an increased risk of stroke[22]. The mechanism of this association is thought to be AF itself, though associations of AF with stroke are notably present for both thrombotic and hemorrhagic forms. The LAA is a complex structure with important roles in both cardioembolism and AF arrhythmogenesis. Variations in LAA morphology are associated with discrete stroke risk[13, 23]. These studies have shown a non-chicken wing LAA morphology, a small LAA os and a larger LAA volume to be associated with an increased risk of stroke. Given that the 4q25 variants are strongly associated with both AF and stroke, and likewise LAA morphology is important in both AF and stroke pathogenesis, we sought to determine if the most common 4q25 risk variant is associated with LAA morphology. We observed that the RS2200733 4q25 variant is associated with LAA morphology with risk allele carriers 3.0 times more likely to have a non-CW morphology than non-carriers. Conversely, noncarriers were 4.0 times more likely to have a CW morphology compared to carriers. This finding does support cardiac development as a fundamental mediator of the PITX2 risk[24]. The complex relationship between appendage morphology, stroke risk, and atrial arrhythmogenesis will require experimental dissection, but this may be challenging outside large animal models.

Response to Therapy

After ablation (cavo-tricuspid isthmus or CTI ablation) for AFL, ˜60% of patients will exhibit AF over the subsequent 5 years[25]. Stratifying those patients presenting with AFL who are likely to develop AF has management implications with regards to ongoing arrhythmia surveillance, long-term anticoagulation and index ablation strategy. It has been suggested in some patients who present with AFL alone, a combined AFL/prophylactic AF ablation strategy is warranted[26]. To date, the clinical utility of polygenic risk scores in AF management has been limited[27]. Our data suggest a potential use case for an AF GRS in clinical

practice. AF polygenic risk appears to identify those patients presenting with AFL where future AF is likely.

Constellation of Risk Variants

The polygenic risk score did not predict appendage morphology but the PITX2 risk allele with the largest effect size was associated with non-CW morphology. Similarly, prior studies have shown the RS2200733 4q25 variant alone does not stratify CTI patients who will go on to develop AF[28], yet in this study a polygenic risk score was able to discriminate this group. These findings highlights the need for much broader clinical genotyping and much more granular phenotyping to fully understand the relationships between genetics and disease. Large-scale GWAS have now identified more than 100 loci associated with AF[29-31]. Despite the number, these loci explain only a modest proportion of the heritability[32], suggesting that more AF-related genetic loci remain to be identified. As the number of association loci increases, so effect sizes diminish and competing functions are more likely to obscure some orthogonal non AF phenotypic associations. For example, some variants might mediate arrhythmogenesis via a similar pathway to PITX2, yet others might act through unrelated but synergistic pathways[6]. As with much of medicine, the balance between personalization of care and grouping of data must be carefully considered.

Limitations

The statistical power of the present study may be limited given the small sample size of patients within each clinical subgroup / quartile. These findings may be limited since data of associations of SNP-exposure and SNP-outcome were derived from a single population. Larger scale studies with similarly detailed phenotyping will be necessary.

Conclusion

SNV that predispose to AF are also associated with discrete non-AF phenotypes. Our observations underscore the biological complexity of the AF genetics and its control of various aspects of cardiac development and the autonomic nervous system. These findings highlight a potential use for genetics at the intersection of atrial flutter and AF management. Whether such traits represent true endophenotypes in AF is not yet established, but they do support the general hypothesis that more granular phenotyping will be necessary to realize the full potential of human genetics in common disorders.

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Figure Title and Legends

Figure 1: Heart Rate (A) and Heart Rate Variability (B as Stratified by Atrial Fibrillation Genetic Risk Quartiles. BPM = beats per minutes; HR = heart rate; SDNN= standard deviation of NN intervals; RMSDD =Root Mean Square of the Successive Differences. * = P <0.05; n = 86.

Figure 2: Cardiac Conduction (A) and Atrioventricular Physiology (B) as Stratified by Atrial Fibrillation Genetic Risk Quartiles. HV = His-Ventricle Conduction time; * = P <0.05; n = 181.

Figure 3: AF Free Survival After CTI Ablation Stratified by Genetic Risk Score. Probability of atrial fibrillation over time (days) in follow up as stratified by genetic risk score in the post CTI population. Genetic risk score is stratified into quartiles ranging from Low to High genetic risk.

Figure 4: Left Atrial Appendage Morphology and AF Genetic Risk. (A) Appendage morphology (percent chicken wing), appendage os (circumference in cm) and indexed appendage volume stratified by polygenic risk of AF quartiles. (B) Appendage morphology (percent chicken wing), appendage os (circumference in cm) and indexed appendage volume stratified by RS2200733 single nucleotide variant carrier status. * = P <0.05

Table 1. Single Nucleotide Variants Comprising an AF Polygenic Risk Score

Locus	**Gene**	**SNP**	**Modeled allele**	**Other allele**	**Minor/Modeled Allele Frequency**	**Literatu**
1q21	KCNN3	RS13376333	T	C	0.34	1.13 (0.12
1q24	PRRX1	RS3903239	G	A	0.47	1.14 (0.13
4q25	PITX2	RS10033464	T	G	0.09	1.39 (0.33
4q25	PITX2	RS2200733	T	C	0.10	1.72 (0.54
4q25	PITX2	RS17570669	T	A	0.08	0.73 (-0.3
4q25	PITX2	RS3853445	C	T	0.27	0.86 (-0.1
7q31	CAV1	RS3807989	A	G	0.41	0.9 (-0.11
9q22	C9orf3	RS10821415	A	C	0.41	1.11 (0.10
10q22	SYNPO2L	RS10824026	G	A	0.16	0.87 (-0.1
14q23	SYNE2	RS1152591	A	G	0.49	1.13 (0.12
15q24	HCN4	RS7164883	G	A	0.17	1.19 (0.17
16q22	ZFHX3	RS2106261	T	C	0.18	1.24 (0.22

Table 2. Baseline Characteristics of 405 Cardiovascular Patients Stratified by Polygenic Risk Quartiles

	Low (n= 102)	Low-Intermediate (n=101)	High-Intermediate
Genetic risk score	< 0.2150	0.2150-0.4445	0.4445-0.7479
Clinical AF (%)	39	50	48

	Low (n= 102)	Low-Intermediate (n=101)	High-Intermediate
Male (%)	50	51	53
Congestive Heart Failure (%)	12	13	11
Left Ventricular Ejection Fraction (%)	52± 10	50± 9	50± 10
LA dimension (cm)	4.1± 0.5	4.0± 0.6	3.9± 0.5
Hypertension (%)	22	22	23
Age (years)	63±8	66±7	65±7
Diabetes Mellitus (%)	12	11	13
Stroke/Transient Ischemic Attack (%)	5	6	5
Coronary Artery Disease/Peripheral Arterial Disease (%)	16	19	17
Obstructive Sleep Apnea (%)	18	17	16

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