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Common variants at 9p21 are associated with sudden and arrhythmic cardiac death

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Summary

Background—While a heritable basis for sudden cardiac death (SCD) is suggested by the impact of family history on SCD risk, genetic determinants have been difficult to identify. We hypothesized that a common variant at chromosome 9p21 related to myocardial infarction would influence SCD risk.

Methods and Results—Prospective, nested case-control analysis among individuals of Caucasian ancestry enrolled in six prospective cohort studies. Study subjects were followed for development of SCD, and genotypes for rs10757274 were determined for 492 sudden and/or arrhythmic deaths and 1460 controls matched on age, sex, cohort, history of cardiovascular disease (CVD) and follow-up time. Conditional logistic regression with fixed effects meta-analysis assuming an additive model was used to test for associations. When individual study results were combined in meta-analysis, each increasing copy of the G-allele at rs10757274 conferred a significantly elevated age-adjusted odds ratio for SCD equal to 1.21 (95% CI, 1.04–1.40; P=0.01). Control for cardiovascular and lifestyle risk factors strengthened these relationships (OR=1.29/G-allele copy, 95%CI: 1.09–1.53, p=0.003). These results were not materially altered in sensitivity analyses limited to definite SCDs or models that further controlled for the development of CVD or when a highly correlated variant rs2383207 was tested.

Conclusions—The major allele of a SNP previously associated with increased risk of coronary artery disease events is associated with increased risk of SCD in individuals of European ancestry. Study of the mechanism underlying this association may improve our understanding of lethal CVD.

Keywords

death, sudden; genetics; arrhythmia; risk factors; coronary disease

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Introduction

Sudden cardiac death (SCD) results in 250,000 to 400,000 deaths in the United States annually.[1–3] Coronary heart disease (CHD), with or without myocardial infarction (MI), is the most common substrate underlying SCD in the western world, being responsible for approximately 75% of SCDs[4–7]. However, the majority of individuals who die suddenly are not in recognized high-risk subsets[8], and over half have no clinically recognized heart disease prior to death[9]. Therefore, improved methods that more accurately identify individuals at risk for SCD within the general population are needed to significantly reduce the overall incidence of SCD. Because there is a heritable component to SCD risk[10–14], genetic factors may help us to better identify those at risk.

To date, rare Mendelian syndromes such as congenital Long QT Syndrome or hypertrophic cardiomyopathy have been found to contribute to a minority of SCD cases in adults[15] but are typically the result of rare variants, private to individual families[16]. Although the majority of SCD cases occur in the general population rather than among high-risk subsets[8], the overall annual incidence in middle-aged to older adults is low (0.1 to 0.4%)[17–19]. Thus, few prospective studies have adequate power to examine the role of genetic variants in the primary prediction of SCD, and at present, the search for common variants relevant at the population-level has met limited success.[20,21]

Common variants at a locus on chromosome 9p21 near the *DKN2A* and *CDKN2B* genes have recently been associated with CHD and MI,[22–26] as well as abdominal aortic and intracranial aneurysms.[27,28] We hypothesized that alleles of these common variants, which have been associated with multiple manifestations of vascular disease, might also be associated with SCD within the general population. In order to increase the number of SCD cases without decreasing our specificity for arrhythmic death, we chose to pool cases from six NIH-funded prospective cohorts within the Brigham and Women's Hospital and the Harvard School of Public Health to test for an association between a common polymorphism at the 9p21 locus and SCD among individuals of European ancestry.

Methods

Study Populations

The study design is a case-control investigation sampled from prospective cohorts and clinical trials, taking advantage of the time-to-event data by matching cases and controls on follow-up time. The prospective cohorts included in the present investigation include the Physicians' Health Study (PHS I and II), the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), the Women's Health Study (WHS), and the Women's Antioxidant Cardiovascular Study (WACS). Together, these cohorts include a total of 38,975 men and 67,093 women with stored blood samples. The details of the cohorts along with the blood sample collection are outlined in the supplement (Supplementary Table 1). In brief, the NHS and HPFS are prospective observational cohort investigations, the PHS I, WHS, and WACS studies were initially randomized trials of aspirin and/or vitamin supplements in which treatment has ended. Prospective follow-up is ongoing in PHS I and WHS. The PHS II is an ongoing randomized trial of vitamin supplementation. Information about medical history, lifestyle choices, and incident disease is assessed either annually or biennially by self-administered questionnaires.

Endpoint Confirmation

The study end points included incident cases of sudden and/or arrhythmic cardiac death that occurred after return of the blood sample and before April 1, 2007. All cohorts employed similar

methods to document the timing and mechanism of cardiovascular deaths[29]. First, next-of-kin or postal authorities report most deaths, and at the completion of each mailing cycle, the National Death Index is searched for names of non-respondents to the questionnaire. Death certificates are obtained from state vital statistics departments to confirm reported deaths; and for death certificates indicating possible cardiovascular disease, permission to obtain further information from medical records is requested from family members. For deaths that occurred outside of the hospital, descriptions regarding the circumstances surrounding these deaths were obtained from the next of kin. Medical records (hospital, emergency room, autopsy, and emergency medical services reports) and accounts of the death from next-of-kin for all possible cardiovascular deaths (excluding strokes) were then reviewed by two cardiologists, and deaths were classified according to timing (the length of symptoms preceding the terminal event) and according to mechanism (arrhythmic versus non-arrhythmic). Information from the death certificate was not used in the classification.

A cardiac death was considered a definite SCD if the death or cardiac arrest that precipitated death occurred within one hour of symptom onset as documented by medical records or next-of-kin reports (n=389, 72.6%) or had an autopsy consistent with SCD (i.e. acute coronary thrombosis or severe coronary artery disease without myocardial necrosis or other pathologic findings to explain death; n=23, 5.4%). Unwitnessed deaths or deaths that occurred during sleep where the participant was documented to be symptom free when last observed within the preceding 24 hours, and circumstances suggested that the death could have been sudden were considered probable SCDs (n= 93, 17.4%).[4,17,30]

Deaths were also classified as arrhythmic or non-arrhythmic based on the definition of Hinkle and Thaler[31]. An arrhythmic death was defined as an abrupt spontaneous collapse of the circulation (pulse disappeared) without evidence of prior circulatory impairment (shock, congestive heart failure) or neurologic dysfunction (change in mental status, loss of consciousness, or seizure). Deaths before which the pulse gradually disappeared and/or those preceded by circulatory or neurologic impairment were considered non-arrhythmic deaths, and these deaths were excluded from the SCD endpoint even if the death occurred within one hour of symptom onset. Deaths which fulfilled the criteria for arrhythmic death, but were preceded by greater than one hour of symptoms (n= 31, 5.8%) were also included in the combined endpoint of sudden and/or arrhythmic cardiac death.

A total of 540 sudden and/or arrhythmic deaths occurred among participants who donated blood samples at baseline (Supplementary table 1), and 536 of these cases had DNA samples that passed our quality control standards. Since very few SCD cases occurred in these predominantly Caucasian cohorts among other ethnicities (n= 20) and to reduce the risk of population stratification, analyses were limited to SCD cases among Caucasians (n=516).

Selection of Controls

Using risk-set sampling[32], for each case we randomly selected up to three controls from the same cohort. These controls were additionally matched on sex, age (1 year), ethnicity, smoking status (current, never, past), time and date of blood sampling, fasting status, and presence or absence of CVD (MI, angina, CABG, or stroke) at the time of blood draw. For the 69 cases that developed CVD after the blood draw but prior to their SCD, we selected a second set of three controls who developed CVD after the blood draw to explore how much of the overall association with SCD might be explained by the development of non-fatal CVD prior to SCD.

Genotyping and Quality Control

Genomic DNA was extracted from the buffy coat fraction of centrifuged blood using Qiagen Autopure kits (Valencia, CA) in NHS, HPFS, and WACS and from whole blood in PHS I. In

WHS and PHS I, DNA was extracted using the MagNA Pure LC instrument with the MagNA Pure LC DNA isolation kit (Roche Applied Science, Penzberg, Germany). All assays were conducted without knowledge of case status, and samples were labeled by study code only. Matched case-control pairs were handled identically, shipped in the same batch, and assayed in the same analytical run to avoid batch effects. A common variant at the chromosome 9p21 locus that has been associated with vascular disease (rs10757274)[23] was genotyped on the Sequenom platform (San Diego, CA) which resolves allele-specific single-base extension products using mass spectrometry (MALDI-TOF)[33] As a backup, a second highly correlated variant (rs2383207) was also genotyped using the same platform. Samples with successful genotyping on fewer than 60% of quality control SNPs selected from HapMap (typically performing well on this platform) were excluded from analysis. Genotypes for both SNPs passed our quality control thresholds (call rate ≥90%, Hardy-Weinberg equilibrium p>0.01 in controls). Blinded replicate quality control samples were included and genotyped with 100% concordance.

Statistical analysis

Means or proportions for baseline cardiac risk factors were calculated for cases and controls. The significance of risk factor associations was tested with the Chi-square statistic for categorical variables and with the Student's t-test for continuous variables. For each cohort, we investigated deviation from Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test. We analyzed the association between the SNP and the risk of sudden and/or arrhythmic cardiac death using conditional logistic regression analysis. With risk-set analysis, the odds ratio derived from the logistic regression directly estimates the hazard ratio, and thus, the rate ratio or relative risk[32]. Based upon the genetic model underlying associations with CHD in prior studies[22,23], odds ratios were estimated for each cohort separately under an additive model of inheritance. The primary test of association was a fixed effect meta-analysis conducted based on the summary conditional logistic regression results for each cohort34; PROC MIXED of SAS was used to pool effect estimates over study cohorts using inverse variance weights. Tests for heterogeneity of the genetic effect across sites were conducted using the Q-statistic[35].

In order to allow secondary analyses we examined pooled conditional logistic regression models that directly combined the individual cohort data. Results were similar, so secondary analyses combined the individual data for simplicity. In these pooled analyses, we examined as a secondary analysis a two-degree of freedom test of the three genotypes that did not assume an additive mode of inheritance. We tested for deviation from additivity by placing an additive term as well as a term for the heterozygotes in the pooled model. In secondary analyses, we explored the relation between genotype and SCD across categories of age, sex, smoking, or the prevalence of known CVD at the time of the blood draw both through pooled stratified models and by adding cross-product terms between genotype and the exposure of interest into the full pooled multivariable model. These secondary analyses were exploratory, and therefore, must be considered in light of multiple testing and should be considered hypothesis generating.

For all of the combined analyses (meta-analyses and pooled), conditional logistic regression models adjusting for increasing levels of CHD risk factors were performed. Of the matching variables, age and smoking were not perfectly matched, and therefore these variables were also entered into the conditional logistic regression models to avoid any potential for residual confounding. The first primary model adjusted for age only. The second further adjusted for standard cardiac risk factors including body-mass index; history of diabetes, hypertension, hyperlipidemia, and smoking (current, past, never). The third additionally adjusted for alcohol intake (<weekly, weekly, daily, 2 or more per day); physical activity (at least once per week) and aspirin use (> or = 22days/month). The fourth further controlled for a family history of

myocardial infarction. Statistical analysis was performed using SAS statistical software (SAS Institute Inc, Cary, NC), Version 9.1.

Results

Study sample characteristics

A total of 516 cases (188 women and 328 men) of sudden and/or arrhythmic cardiac death occurred among Caucasians in the six cohorts over an average follow-up of 13.0 years. The clinical characteristics recorded at the time of the blood draw for the 516 cases and the 1540 age-, sex-, smoking- and CVD- matched controls are displayed by study cohort in Table 1. The mean age of the cases was 64.2 and 132 (25.6%) reported a history of cardiovascular disease (CVD) at the time of the blood draw. In pooled analyses, cases were more likely to report a history of diabetes, hypertension, a higher body mass index ($p<0.001$ for all comparisons), and a family history of MI ($p=0.05$). Cases did not significantly differ with respect to a history of hypercholesterolemia and/or aspirin use from the controls. Among the controls, cardiac risk factors at baseline were not associated with genotypes of the rs10757274 variant at the chromosome 9p21 locus, with the exception of a weak association toward lower rates of diabetes with increasing copy of the G allele ($p=0.02$, Supplemental table 2).

Genotyping

The genotyping call rates were 99.4% for rs10757274 and 99.7% for the backup SNP rs2383207, respectively. There was no difference in the call rate between cases (99.2%) and controls (99.4%). Of the total study group, 492 cases and 1460 matched controls were successfully genotyped for rs10757274. No deviation from Hardy Weinberg equilibrium was detected in controls in any of the cohorts. As had been demonstrated in prior studies, rs10757274 and rs2383207 are in strong linkage disequilibrium ($r^2=0.87$).

Meta-Analyses

Table 2 displays the individual age-adjusted cohort-specific associations for rs10757274 and sudden/arrhythmic death from conditional logistic regression models under an additive mode of inheritance. Allele frequencies in the control groups were relatively homogeneous (P= 0.29) and the pooled frequency was similar to that previously reported in other population-based cohorts of European ancestry such as the Atherosclerosis Risk in Communities and the Copenhagen City Heart Study[22]. The association between rs10757274 and sudden/arrhythmic death was relatively consistent across the six studies, although within-study P-values for the most part did not reach significance likely due to the smaller sample size. In all of the cohorts, except for PHS-II, increasing copies of the G allele was associated with a higher odds ratio for sudden/arrhythmic death and the test for heterogeneity of the odds ratios was non-significant ($p=0.34$). When these odds ratios were combined in meta-analysis, rs10757274 was significantly associated with sudden/arrhythmic death (OR=1.21/G-allele copy, 95%CI: 1.04–1.40, $p=0.01$ (Table 2). When we repeated the age-adjusted analysis for the correlated SNP rs2383207 the results were consistent (OR=1.23/G-allele copy, 95%CI: 1.06–1.43, $p=0.005$, Supplemental Table 3).

Further control for other cardiovascular and lifestyle risk factors (Table 3) resulted in consistent associations for rs10757274, with slightly higher point estimates but overlapping confidence intervals. In the full multivariable model, the OR was 1.29 per G-allele copy (95% CI; 1.09 to 1.53, P=0.003). Results were not materially altered in sensitivity analyses limited to the definite SCDs (n= 384). Results were also unchanged after further adjustment for the interim development of non-fatal CVD after the blood draw (which occurred in 69 cases) by repeating the primary analysis with all cases but substituting a second set of controls matched for the development of CVD for these cases (Table 3).

Pooled Analyses

When the individual data from the cohort studies were pooled, and analyses were repeated, the results were similar to those from the primary meta-analysis. Each increasing copyof the G allele of rs10757274 was associated with a significantly elevated OR for sudden/arrhythmic death of 1.21 (95% CI, 1.05 to 1.41) in the age-adjusted analysis. Further control for other cardiovascular and lifestyle risk factors also had a similar impact on the risk estimate for rs10757274 (OR=1.27; 95% CI, 1.09–1.50; P=0.003). When examined in a two degree of freedom model, the results were consistent with an underlying additive model, and the test for deviation from additivity was nonsignificant (P=0.38). Compared to minor allele homozygotes, the OR for the heterozygotes in the full multivariable model was 1.12 (95% CI; 0.85–1.50) for rs10757274, and the major allele homozygotes had the highest risk (OR=1.60; 95% CI 1.16–2.20), consistent with an additive genetic model.

Stratified Analyses

In exploratory secondary analyses, we then repeated the full pooled multivariable analysis after individually stratifying on each of the matching variables of age, sex, smoking, and history of prior CVD individually (Table 4). The odds ratios associated with the risk allele were somewhat higher among those above the mean age of 64.2 years and among those with a history of prior CVD at the time of the blood draw, but the confidence intervals for the risk estimates widely overlapped and the P-values for interaction in the full multivariable model were not significant. However, our power to detect such interactions is limited. Secondary analyses of the correlated SNP rs2383207 were very similar (data not shown).

Discussion

In this combined nested case-control analysis from six prospective cohorts, a common variant at the chromosome 9p21 locus, previously associated with MI and CHD, was significantly associated with sudden and/or arrhythmic death in individuals of European ancestry even after matching for prior CVD and controlling for cardiovascular risk factors. The risk allele is common with approximately 50% of the population carrying one copy and having an estimated 29% increased risk of sudden/arrhythmic death after controlling for other CHD risk factors as compared to non-carriers. For the approximate 25% of the population who are homozygous for the risk allele, the odds ratio of sudden/arrhythmic death was elevated by an estimated 60% as compared to those homozygous for the minor allele.

These data have important pathophysiologic implications. The risk estimate observed here for SCD is remarkably similar to those previously reported for MI and CAD[22,23,25], and likely represents an association between this variant and a common underlying pathology (coronary atherosclerosis). However, the variant was associated with SCD even after matching for and further controlling for prior CVD. Therefore, the observed association is likely not entirely explained by the detection of underlying atherosclerosis. The present data along with prior associations documented between this chromosomal region and aneurysmal diseases (abdominal aortic and intracranial aneurysms)[27,28] and progression of atherosclerosis[36] suggests that the genetic locus may be involved in abnormal vascular remodeling and/or repair, which could increase the propensity toward more aggressive atherosclerosis and more unstable, ruptureprone atherosclerotic plaques, which could result in SCD.

Currently the causally responsible variant and mechanism underlying the association with vascular disease are not known. The SNP is located in a region on chromosome 9p21 of high linkage disequilibrium[24,26], which is devoid of known genes. The region is adjacent (~115kb) to the coding sequences of two cyclin dependent kinase inhibitor genes, *CDKN2A* and *CDKN2B*, which are known to have critical roles in cell proliferation, aging, senescence, and

apoptosis[37] and which could play a role in atherosclerosis through their role in TGF-beta induced growth inhibition.[38,39] However, it is also possible that a previously unrecognized gene or regulatory element within or near the region could be the causal variant. Deep resequencing of this region will be required to obtain the full spectrum of variation and to identify the causal variants.

These data may eventually have clinical implications as well. The modest risk elevation conferred by this variant is unlikely to have immediate implications for risk stratification in isolation[40]. However, given the 2-fold elevations associated with a family history of SCD and/or ventricular fibrillation, the 9p21 variant likely explains only a small fraction of the familial clustering of SCD. Therefore, it is likely that other susceptibility variants for SCD will be found as well, which in combination with other established and novel risk factors, may eventually allow the identification of a population at higher risk. Further investigation into the underlying mechanism may also lead to important insights regarding the underlying biology and point to novel causal pathways that can be targeted for intervention.

The present study has several strengths and limitations that warrant consideration. Strengths include the nested prospective case-control design, the large well-characterized cohorts, and the combined large number of rigorously confirmed sudden and/or arrhythmic cardiac deaths, a difficult phenotype to classify in population studies. Ascertaining cases and controls prospectively from a defined cohort reduces selection bias, and the ability to match on time at risk reduces the risk of survival bias. Potential limitations of this population-based study include the possibility of some degree of misclassification, particularly among the probable SCDs. However, results were unchanged when these probable SCDs were excluded from the analysis. Also, not all participants provided a blood sample (Supplementary table 1), and therefore, these cases and controls could have differed in some respects from their respective source populations. Given the low frequency at which SCD occurs in the general population, we needed to combine cases from independent cohorts to achieve adequate power without decreasing our specificity for arrhythmic death, and the small numbers within each cohort did not allow us to independently replicate the association. However, the association has previously been repeatedly replicated with respect to other CHD endpoints. Moreover, these cohorts were all composed of health professionals and utilized similar methodology for endpoint documentation and risk factor ascertainment.

Population stratification (differences in ancestry between cases and controls) is a concern in any genetic association study, and if present, can lead to false inference of association for variants that have differing frequencies in different ancestral groups. However, the two variants tested, rs10757274 and rs2383207, are only highly correlated in European ancestry samples, and the concordance of the association results for the two SNPs in our samples of self-described Caucasian individuals provide strong evidence against the possibility of false-positive association due to population stratification. Finally, the results observed in these cohorts composed entirely of health professionals may not apply to other socioeconomic strata who may be at higher risk for CVD or non-Caucasian populations, particularly African-Americans where the association between these variants and CHD has not to date been replicated[22].

In summary, in this combined population of Caucasian men and women, a common variant at the chromosome 9p21 locus was associated with significantly increased risks of sudden and/or arrhythmic cardiac death, and this relationship was independent of established risk factors for CHD and/or SCD. These findings emphasize the important role atherosclerosis plays in SCD even among relatively healthy free-living populations and may carry implications for further efforts to screen and prevent SCD in the general population. Since the majority of SCDs do not occur in high-risk individuals[8,9,29], improved identification of those at risk is crucial to reducing SCD mortality.

Clinical Summary

Sudden cardiac death (SCD) results in 250,000 to 400,000 deaths in the United States annually. While coronary heart disease is recognized to be a potent risk factor and acute mechanism for SCD, risk in individuals is poorly estimated and improved risk prediction is needed. Given the aggregation of SCD within families, we sought to identify genetic factors associated with SCD risk in cases and controls of European ancestry drawn from six NIH-funded prospective cohorts. We tested for association between SCD and a common polymorphism, rs10757274, at the 9p21 locus which has been robustly associated with coronary artery disease. We found that the G allele associated in prior studies with coronary artery disease was associated with 29% increased risk of SCD per allele copy after adjustment for cardiovascular and lifestyle risk factors (p=0.003). This finding suggests that genetic factors that contribute to coronary artery disease also contribute to SCD risk. It also raises the possibility that development of a genetic risk profile comprised of multiple SCD variants could ultimately improve risk prediction for a lethal and poorly predicted disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Cohort Specific and Pooled Prevalence Of Cardiac Risk Factors at the Time of the Blood Draw according to Case and Control Status

Cohort	Case/Control	n	Age (SD) years	Body mass index (SD) kg/m2	History of prior CVD N (%)	Current smoking N (%)	Diabetes N (%)	Hypertension N (%)	High cholesterol N (%)	Family History of MI N (%)	Aspirin Use N (%)
HPFS	case	120	68.0 (7.7)	26.5 (3.8)	46 (38.3%)	10 (8.9%)	18 (15.0%)	64 (53.3%)	62 (51.7%)	18 (15.0%)	44 (37.3%)
HPFS	control	366	67.7 (7.5)	25.6 (3.4)	138 (37.7%)	36 (10.1%)	24 (6.6%)	128 (35.0%)	162 (44.3%)	48 (13.1%)	151 (42.3%)
PHS I	case	133	59.8 (8.9)	25.4 (3.0)	4 (3.0%)	16 (12.0%)	14 (10.5%)	63 (47.7%)	14 (11.4%)	20 (15.0%)	65 (48.9%)
PHS I	control	395	59.7 (8.9)	24.4 (2.8)	9 (2.3%)	47 (11.9%)	14 (3.5%)	91 (23.3%)	52 (14.4%)	34 (8.6%)	183 (46.3%)
PHS II	case	75	73.1 (9.0)	26.0 (4.1)	22 (29.3%)	3 (4.0%)	13 (17.3%)	46 (61.3%)	30 (40.5%)	13 (17.3%)	36 (50.7%)
PHS II	control	224	72.9 (8.8)	25.3 (2.9)	65 (29.0%)	9 (4.2%)	12 (5.4%)	109 (48.7%)	96 (42.9%)	25 (11.2%)	119 (54.1%)
NHS	case	113	60.8 (6.1)	27.2 (5.4)	32 (28.3%)	30 (26.6%)	25 (22.1%)	69 (61.1%)	66 (58.4%)	30 (26.6%)	38 (33.6%)
NHS	control	338	60.8 (6.0)	26.4 (5.0)	96 (28.4%)	69 (20.4%)	27 (8.0%)	148 (43.8%)	187 (55.3%)	63 (18.6%)	74 (21.9%)
WACS	case	37	66.4 (7.1)	29.5 (7.1)	27 (73.0%)	9 (24.3%)	11 (29.7%)	30 (81.1%)	24 (64.9%)	10 (27.0%)	19 (51.4%)
WACS	control	109	66.1 (6.9)	29.0 (6.0)	80 (73.4%)	27 (24.8%)	20 (18.4%)	81 (74.3%)	84 (77.1%)	40 (36.7%)	55 (50.5%)
WHS	case	38	58.8 (8.5)	26.7 (4.6)	1 (2.6%)	10 (26.3%)	4 (10.5%)	20 (52.6%)	12 (31.6%)	3 (7.9%)	17 (44.7%)
WHS	control	108	58.4 (8.3)	26.3 (5.1)	1 (0.9%)	30 (27.8%)	5 (4.6%)	36 (33.3%)	44 (40.7%)	15 (13.9%)	63 (58.3%)
Total	case	516	64.2 (9.4)	26.5 (4.5)	132 (25.6%)	78 (15.3%)	85 (16.5 %)	292 (56.7 %)	208 (41.2 %)	94 (18.2 %)	219 (42.9 %)
Total	control	1540	64.1 (9.2)	25.7 (4.1)	389 (25.3%)	218 (14.2%)	102 (6.6 %)	593 (38.6 %)	625 (41.5%)	225 (14.6%)	645 (42.2%)

Table 2

Association of sudden cardiac death with two 9p21 variants

Shown are the individual cohort specific and combined metaanalysis age-adjusted odds ratios (95% CI) for increasing copy of the G allele of rs10757274 from conditional logistic regression models under an additive model of inheritance.

Cohort	Case/ control	rs10757274			
		n	Frequency of G Allele	OR per G Allele (95% CI)	P-value
HPFS	case	110	0.586	1.52 (1.12-2.07)	0.008
HPFS	control	330	0.496		
PHS I	case	129	0.543	1.13 (0.84- 1.52)	0.41
PHS I	control	387	0.510		
PHS II	case	74	0.480	0.86 (0.58- 1.27)	0.45
PHS II	control	218	0.514		
NHS	case	107	0.542	1.20 (0.87- 1.67)	0.26
NHS	control	317	0.505		
WACS	case	35	0.500	1.13 (0.66- 1.93)	0.66
WACS	control	104	0.466		
WHS	case	37	0.514	1.61 (0.89-2.89)	0.11
WHS	control	104	0.428		
Meta-analysis	case	492	0.538	1.21 (1.04 - 1.40)	0.01
Meta-analysis	control	1460	0.497		

Table 3

Multivariable and Sensitivity Analyses: Association of Sudden Cardiac Death with 9p21 variant. Shown are the Meta-analysis odds ratios (95% CI) with increasing levels of risk factor adjustment for increasing copy of the G allele of rs10757274

	OR (95% CI) for All SCD (Primary Analysis)	P-value	OR (95% CI) for Definite SCD [*] (N= 386)	P-value	OR (95% CI) Alternate Controls matched for Interim CVD [†]	P-value
Multivariable Model 1[‡]	1.24 (1.06- 1.46)	0.009	1.30 (1.08 - 1.57)	0.006	1.22 (1.04 - 1.43)	0.02
Multivariable Model 2[§]	1.29 (1.09- 1.53)	0.003	1.30 (1.08 - 1.60)	0.008	1.26 (1.06 - 1.49)	0.008
Multivariate Model 3 [//]	1.29 (1.09 - 1.53)	0.003	1.31 (1.08 - 1.60)	0.007	1.26 (1.07 - 1.50)	0.007

[*] **Sensitivity Analysis:** Utilizing only cases of definite sudden cardiac death defined as death within one-hour of the onset of symptoms or autopsy consistent with SCD ((i.e. acute coronary thrombosis or severe coronary artery disease withoutmyocardial necrosis or other pathologic findings to explain death

[†] **Secondary Analysis:** Analysis repeated for the 492 cases utilizing an alternative set of controls who developed CVD after the blood draw for the 69 cases who developed CVD after the blood draw.

[‡] **Multivariable Model 1:** Controlled simultaneously for age, smoking status (current, past, never), BMI (continuous), history of diabetes, hypertension, and high Cholesterol

[§] **Multivariable Model 2:** Controlled for variables listed above in Model 1 and alcohol intake (<weekly, weekly, daily, 2 or more per day); physical activity (at least once per week) and aspirin (> or = 22days/month).

[//] **Multivariable Model 3:** Controlled for variables listed above in Model 2 and family history of myocardial infarction

Table 4

Secondary Stratified Analyses

Shown are the multivariable* odds ratios (95%CI) for sudden cardiac death per G allele copy for rs10757274 from pooled conditional regression models with stratification on age, sex, history of CVD or smoking status.

Cardiac Risk Factor	Cases (n)	Controls (n)	OR (95%CI)*	P, for Sub-group	P, for Interaction
Age					
<Mean	236	667	1.14 (0.90 – 1.44)	0.29	0.18
≥Mean	254	719	1.40 (1.12 – 1.75)	0.003	
Sex					
Male	312	886	1.25 (1.02 – 1.53)	0.03	0.65
Female	178	500	1.36 (1.04– 1.80)	0.03	
History of CVD					
Yes	124	342	1.54 (1.11 – 2.13)	0.009	0.09
No	366	1044	1.18 (0.98 – 1.42)	0.09	
Smoking†					
Never	181	508	1.20 (0.91 – 1.58)	0.19	
Past	228	644	1.28 (1.01 – 1.61)	0.04	0.69
Current	70	198	1.31 (0.85 – 2.03)	0.23	0.60

*Multivariable controlled simultaneously for age, smoking status (current, past, never), BMI (continuous), history of diabetes, hypertension, and high Cholesterol, family history of myocardial infarction, alcohol intake (<weekly, weekly, daily, 2 or more per day); physical activity (at least once per week) and aspirin (> or = 22d/month).

†The smoking stratified analyses did not control for smoking status.