SNPs Identified as Modulators of ECG Traits in the General Population Do Not Markedly Affect ECG Traits during Acute Myocardial Infarction nor Ventricular Fibrillation Risk in This Condition

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Abstract

Background: Ventricular fibrillation (VF) in the setting of acute ST elevation myocardial infarction (STEMI) is a leading cause of mortality. Although the risk of VF has a genetic component, the underlying genetic factors are largely unknown. Since heart rate and ECG intervals of conduction and repolarization during acute STEMI differ between patients who do and patients who do not develop VF, we investigated whether SNPs known to modulate these ECG indices in the general population also impact on the respective ECG indices during STEMI and on the risk of VF.

Methods and Results: The study population consisted of participants of the Arrhythmia Genetics in the NEtherlandS (AGNES) study, which enrolls patients with a first STEMI that develop VF (cases) and patients that do not develop VF (controls). SNPs known to impact on RR interval, PR interval, QRS duration or QTc interval in the general population were tested for effects on the respective STEMI ECG indices (stage 1). Only those showing a (suggestive) significant association were tested for association with VF (stage 2). On average, VF cases had a shorter RR and a longer QTc interval compared to non-VF controls. Eight SNPs showed a trend for association with the respective STEMI ECG indices. Of these, three were also suggestively associated with VF.

Conclusions: RR interval and ECG indices of conduction and repolarization during acute STEMI differ between patients who develop VF and patients who do not. Although the effects of the SNPs on ECG indices during an acute STEMI seem to be similar in magnitude and direction as those found in the general population, the effects, at least in isolation, are too small to explain the differences in ECGs between cases and controls and to determine risk of VF.

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Introduction

Sudden cardiac death (SCD) is a leading cause of death in adults in the Western world [1]. The overwhelming majority (~80%) of SCDs in adults are caused by the sequelae of coronary artery disease, namely myocardial ischemia or acute myocardial infarction (MI) [2]. While the risk of VF is known to have a genetic component, the underlying genetic factors are yet largely unknown [3–5].

Electrocardiographic indices of conduction and repolarization are considered important quantifiable intermediate phenotypes for arrhythmia risk [6]. In support of this concept, ECG indices measured during the acute phase of ST elevation MI (STEMI) were associated with risk of ensuing VF [7]. A number of studies have demonstrated that heart rate, PR interval, QRS duration and QTc interval are heritable traits [8–10] and over the last years genome-wide association studies (GWAS) in large samples of the general population have uncovered several common single nucleotide polymorphisms (SNPs) affecting these ECG traits [11–21].

The objective of this study was to assess whether SNPs, previously associated with heart rate and ECG indices of conduction and repolarization in GWAS in the general population, modulate ECG indices and risk of VF during acute STEMI. Specifically, we hypothesized that ECG-related SNPs modulate...
Studies of SNPs and Genotyping

Ethics statement
The study protocol was approved by the Institutional Review Board of the Academic Medical Center, University of Amsterdam, and was conducted according to the principles of the Declaration of Helsinki. All participants gave written informed consent.

Study samples
The study population consisted of participants of the Arrhythmia Genetics in the Netherlands (AGNES) case-control study which has been described in detail previously [3,4]. In brief, the AGNES study enrols patients with a first acute STEMI. Cases are defined as patients who were successfully resuscitated after documented VF that occurred between the onset of symptoms and coronary intervention. Controls are defined as acute STEMI patients who did not develop VF. Patients with a previous MI or major co-morbidity were excluded. All individuals studied were of self-declared European ancestry.

Measurement of ECG indices
The first recorded ECG that was acquired during STEMI and before reperfusion treatment was used for analysis. For cases, both ECGs acquired pre-VF as well as post-VF were included. ECGs of insufficient quality (due to e.g. baseline drift or missing leads) and ECGs with rhythms other than sinus rhythm or atrial fibrillation (AF) were excluded. Patients with AF (n = 23) were excluded for the analyses of PR interval, but included for the other ECG indices. All ECGs were digitized at 400 dpi (giving a spatial resolution 0.064 mm or 1.6 ms / pixel on an ECG traced at 25 mm / sec). Calibrated measurements were performed on-screen after 4 times enlargement of the digitized ECGs in ImageJ (National Institutes of Health, Bethesda, Maryland, http://rsb.info.nih.gov/ij/). RR-interval (heart rate) was measured from the onset of ventricular depolarization. QRS duration was measured from the onset of ventricular depolarization to the J point. QTc interval was measured using the tangent method [22]. Lead II was used when the T wave was of sufficient amplitude to warrant QT measurement, otherwise leads V5 or V2 were used. QT was corrected for heart rate (QTc) using Fridericia’s formula (QTc = QT / (cube root (RR))). ST deviation was calculated as the sum of all ST deviation from baseline at 60 ms after the J point in all 12 leads. ST deviation was not reported if individual leads were disconnected or in patients with left bundle branch block. The mean of three consecutive beats wherever possible was measured for all parameters and used in subsequent analyses. Patients with AV block, PR interval ≥ 200 ms or QRS duration ≥ 120 ms were excluded from the ECG analyses involving the continuous endpoints PR interval, QRS duration and QTc interval. For PR interval and QRS duration, additional dichotomous endpoints were assessed i.e. PR interval ≥ 200 ms and QRS duration ≥ 120 ms. Patients with AV block were included in the dichotomized PR interval endpoint as PR > 200 ms.

Selection of SNPs and Genotyping
We inspected the published GWAS concerning heart rate and ECG indices of conduction and repolarization and identified SNPs reported to be associated with these parameters at genome-wide significant P-values (P<5×10^-8) [23]. In case of high linkage disequilibrium (r²≥0.75) between identified SNPs, only a single SNP, capturing the maximum amount of variation present in the correlated SNPs, was selected for our analysis. This was the case for SNPs at the SCN1A, SCN5A, KCNH2, KCNQ1, CNOT7, ARHGAP24, NOS1AP, CDKN1A, GJA1 and MYH6 loci. A total of 65 SNPs were identified in this way. Genotypes for these SNPs were either obtained by direct genotyping (Illumina610 Quad genotyping array) or were estimated by imputation using HapMap build 36 as the reference panel. Details on genotyping and imputation in the AGNES sample have been described previously [4].

Statistical Analysis
We tested differences between cases and controls in continuous variables with an independent sample t-test, or the Mann-Whitney U test when the data was not normally distributed. We compared differences in the percentages of categorical variables with the Pearson χ² test. We used linear regression modelling in association analyses of continuous endpoints and logistic regression modelling for association analyses of dichotomous endpoints (VF, PR≥200 ms and QRS≥120 ms). In all models, we assumed an additive genetic model and corrected for age, sex and the culprit artery (harbouring the occluding lesion) [7] as well as the possible interaction between culprit artery and the SNPs. The occurrence of the latter was first tested using a Wald test.

Our analysis plan had a two-stage design. In the first stage, we tested for association of the selected SNPs with the corresponding ECG parameter during STEMI. In the second stage, we selected those SNPs with a (suggestive) significant effect on the ECG parameter and analyzed their effect on the occurrence of VF.

The Bonferroni thresholds for statistical significance were P≤0.0002 for the first stage (corrected for two tests per SNP and six outcomes: HR, QTc, PQ, PQ, QRS≥200 ms, QRS and QRS≥120, resulting in a total of 210 tests) and P≤(0.05/number of SNPs carried over from stage 1) for the second stage. For both stages, P-values between the Bonferroni threshold and 0.05 were considered as a suggestive trend.

Power and detectable effects
Given the observed standard deviations in our study population for heart rate and the ECG indices, the reported effects of the identified SNPs would result in effect sizes ≤ 0.2 SD. With the present range in sample sizes (417 – 515), the power to detect an effect size of 0.2 of a SNP with a minor allele frequency (MAF) ranging from 0.05 to 0.5 would range from 1 to 31% for a two-sided p-value of 0.0002 (Bonferroni threshold) and from 24 – 90% for a two-sided p-value of 0.05 (suggestive trend). The present study, therefore, lacks the power to significantly detect effect sizes as found in the general population (from the GWAs). However, given the fact that our heart rate and ECG indices were measured in patients experiencing a STEMI, we hypothesized that the SNP effects might be markedly increased in this sensitized population.

Given the present range in sample sizes, the study had 80% power to detect effect sizes of 0.7 to 0.3 (± 5% explained variance) for the quantitative traits at MAFs ranging from 0.05 to 0.5 assuming an additive genetic model and a two-sided significance threshold of 0.0002 (Stage 1). For PR interval with an overall standard deviation of 20 ms, for example, this translates into detectable allele effects (beta) ranging from 14 to 6 ms. For the dichotomized endpoints, we were able to detect odds ratio’s ranging from 2.3 to 1.6 (Stage 1, α = 0.0002).
Results

VF and ischemic ECG indices

Baseline characteristics of the study population are shown in Table 1. As reported previously [3,4], AGNES cases had a lower prevalence of diabetes mellitus and hypercholesterolemia, and lower mean body mass index (BMI) than AGNES controls. AGNES cases more often had a family history of sudden cardiac death as compared to controls.

STEMI ECGs of sufficient quality were available for 599 patients. Of these, 79 (13%) patients had QRS\(\geq120\) ms, and 85 (14%) patients had a PR\(\geq200\) ms or higher degree AV block; several patients had a combination of these ECG abnormalities. Because of these exclusions and missing values, the number of available observations varied between 417 and 515. VF cases showed, on average, a shorter RR interval, a longer QTc interval, and a greater ST segment deviation and more often prolongation of the QRS interval\(\geq120\) ms as compared to controls (Table 1). Location of the culprit coronary lesion modified the effect of QRS duration and QTc interval on risk of VF (Table 2). For instance, QRS duration was more prolonged in cases compared to controls only among patients with a left circumflex artery (LCX) occlusion (\(P_{\text{interaction}}=0.002\)). QTc-interval tended to be more prolonged among patients with left anterior descending artery (LAD) or LCX occlusion (\(P_{\text{interaction}}=0.026\)).

ECG candidate SNPs and STEMI ECG indices (Stage 1)

None of the 65 SNPs tested displayed an association with the corresponding ECG trait that passed the Bonferroni-corrected significance threshold of \(P\leq0.0002\), nor showed an interaction with culprit artery. Eight SNPs displayed a trend (\(0.0002<\ P<0.05\)) in association with STEMI ECG indices (Table 3). Regarding the continuous ECG outcomes, SNPs rs223116 and rs291860 showed a trend for association with RR interval (\(P=0.004\) and 0.114, respectively) and SNP rs6795970 showed a trend for association with PR interval (\(P=0.004\)). SNPs rs186512 and rs883079 showed a trend for association with QRS duration (\(P=0.010\) and 0.007, respectively). SNPs rs17779747 and rs8049607 showed a trend for association with QTc interval (\(P=0.004\) and 0.036, respectively). Regarding the dichotomized ECG endpoints, the C-allele of SNP rs11708996 showed a trend for association with PR\(\geq200\) ms with an odds ratio of 2.39 (95% CI: 1.33 – 4.30; \(P=0.004\)). None of the QRS SNPs showed any significant association with QRS\(\geq120\) ms.

STEMI ECG SNPs and risk of VF (Stage 2)

We next tested the eight SNPs from Stage 1 showing a trend for association with the ECG parameters for association with VF. Of these eight, none passed the Bonferroni-corrected significance threshold (\(P\leq0.0063\)) for association with risk of VF during STEMI. However, two SNPs, namely rs6795970 and rs17779747, were nominally associated with VF (\(P=0.009\) and 0.026,

### Table 1. Baseline characteristics of the AGNES case-control set.

| Characteristic                  | N\(^{*}\) | Total (n = 969\(^{1}\)) | Cases (n = 516) | Controls (n = 453) |
|--------------------------------|-----------|-------------------------|-----------------|-------------------|
| Sex (male)                     |           | 783 (80.6)              | 412 (80.0)      | 371 (81.2)        |
| Mean age at myocardial infarction |           | 56.4 ±11               | 55.9 ±11.2      | 56.9 ± 10.8       | 0.174 |
| Family history of sudden death |           | 909, 459, 450          | 291 (32.0)      | 174 (37.9)        | 117 (26.0) |

### CVD risk factors

| Current smoking | 914, 473, 441 | 565 (61.8) | 303 (64.1) | 262 (59.4) | 0.1531 |
| Diabets mellitus | 893, 462, 431 | 62 (6.9) | 21 (4.6) | 41 (9.5) | 0.004 |
| Hypertension | 294 (30.2) | 149 (28.9) | 145 (31.7) | 0.3633 |
| Hypercholesterolemia | 297 (30.6) | 127 (24.7) | 170 (37.2) | < 0.0001 |
| Body mass index (BMI, kg/m\(^{2}\)) | 879, 439, 440 | 26.5 ±3.9 | 26.1 ±3.8 | 26.9 ± 4.0 | 0.0042 |
| Peak CK-MB | 735, 337, 398 | 227 [294] | 241 [354] | 215 [247] | 0.006 |
| Ischemic ECG |           |           |           |           |
| RR interval (ms) | 453, 214, 239 | 807 ±204 | 743 ±188 | 864 ±203 | < 0.0001 |
| ST segment deviation (mm) | 474, 188, 286 | 15 [17] | 20 [20] | 13 [12] | < 0.0001 |
| PR interval (ms) | 417, 195, 222 | 157 ±20 | 158 ±19 | 157 ±20 | 0.358 |
| QRS duration (ms) | 452, 214, 238 | 93 ±13 | 93 ±14 | 93 ± 13 | 0.860 |
| QTc interval (ms) | 424, 200, 224 | 410 ±35 | 415 ±37 | 405 ±31 | 0.003 |
| PR\(>200\) ms | 515, 249, 266 | 57 (11.0) | 32 (12.7) | 25 (9.5) | 0.153 |
| QRS\(>120\) ms | 509, 252, 257 | 57 (11.2) | 38 (15.1) | 19 (7.4) | 0.007 |
| Culprit artery | 811, 430, 381 |           |           |           |
| RCA | 220 (27.1) | 109 (25.3) | 111 (29.1) | 0.304 |
| LAD | 475 (58.6) | 260 (60.5) | 215 (56.4) | 0.202 |
| LCX | 116 (14.3) | 61 (14.2) | 55 (14.4) | 0.685 |

CK-MB, creatine kinase-MB; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery. *In case of missing values, the sample sizes of the total, case and control sets (total, case, control) for which information was available are given. ^Normally distributed continuous variables are presented as mean ± SD or as Median [interquartile range] otherwise. Categorical variables data are presented as number (%). † \(^{P}\) value for comparison of cases and controls using independent t-test, Mann-Whitney test, or chi-square test where appropriate.

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ECG candidate SNPs, STEMI ECG indices and VF

Discussion

In the current study, we confirm and extend previous observations that heart rate and ECG indices of conduction and repolarization differ between acute STEMI patients who develop VF and acute STEMI patients who do not develop VF. Although our analysis only detected nominal association with the STEMI ECG traits for only a minority of SNPs, the effect size and direction for the association of these SNPs was comparable to that found for the corresponding trait in the general population. Furthermore, of the eight SNPs displaying such association, three also displayed a trend for association with VF.

Ischemic ECG indices and VF

Ischemic ECGs of patients with VF (cases) showed, on average, shorter RR intervals, longer QTc intervals and more ST segment deviation than patients without VF (controls). When the culprit artery harbouring the occluding lesion was taken into account, cases with an LCX occlusion had a longer QRS duration. This finding is similar to our previous findings in a smaller sample of STEMI patients with and without VF [7]. However, the observation in this previous study of longer QRS interval in cases with an RCA occlusion was not observed in the present study. With respect to QTc interval, cases with either an LAD or LCX occlusion had a longer QTc-interval compared to controls. This extends our earlier findings, where similar (though non-significant) differences in QTc interval were found for the same culprit arteries. The observed longer QRS duration and QTc interval in cases with VF reflect cardiac conduction delay and prolonged repolarization time, respectively, consistent with a more pro-arrhythmic substrate in cases [24].

ECG candidate SNPs, STEMI ECG indices and VF

Recent GWAS have identified multiple common genetic variants affecting heart rate, conduction and repolarization in individuals from the general population [11–21]. However, whether these variants also influence ECG indices in the setting of STEMI has not yet been investigated. In our study, none of the association P-values exceeded our stringent pre-set threshold for statistical significance. Eight SNPs displayed a suggestive association to the corresponding STEMI ECG parameter, and of note all eight SNPs displayed an effect that was similar in direction and magnitude to that observed in the general population [11–21]. However our findings also demonstrate that SNPs which are known to exert only small effects on ECG parameters in the general population, do not have a markedly increased effect on ECG indices in the setting of acute STEMI.

Table 2. ECG characteristics of AGNES cases and controls according to the artery harbouring the stenotic lesion.

| Culprit artery | Cases (N) | Mean±SD | Controls (N) | Mean±SD | P value* | Interaction P value |
|----------------|----------|---------|--------------|---------|----------|-------------------|
| RR interval    |          |         |              |         |          |                   |
| LAD            | 130      | 728±170 | 124          | 822±180 | <0.0001  |                   |
| LCX            | 25       | 751±185 | 35           | 866±213 | 0.047    | 0.974             |
| RCA            | 43       | 791±230 | 70           | 944±214 | 0.001    |                   |
| PR interval    |          |         |              |         |          |                   |
| LAD            | 117      | 158±19  | 112          | 159±18  | 0.879    |                   |
| LCX            | 24       | 150±17  | 33           | 151±19  | 0.647    | 0.423             |
| RCA            | 39       | 163±20  | 67           | 157±22  | 0.168    |                   |
| QRS duration   |          |         |              |         |          |                   |
| LAD            | 130      | 93±13   | 123          | 91±13   | 0.288    |                   |
| LCX            | 25       | 98±11   | 35           | 88±9    | 0.002    | 0.002             |
| RCA            | 43       | 94±16   | 70           | 98±12   | 0.134    |                   |
| QTc interval   |          |         |              |         |          |                   |
| LAD            | 120      | 422±37  | 113          | 413±28  | 0.036    |                   |
| LCX            | 24       | 415±28  | 33           | 391±30  | 0.020    | 0.026             |
| RCA            | 40       | 399±38  | 68           | 399±35  | 0.939    |                   |

LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery

*P value of comparison between cases and controls using a logistic regression model adjusted for age and sex. (All patients with AV block or PR=200 ms or QRS≤120 ms & AF are excluded)

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respective) independent of the culprit artery. Another SNP, rs225116 displayed a nominal association with VF in patients with an occlusion in the LCX (P=0.039) or RCA (P=0.037) only (Table 4).
Table 3. Association analysis of SNPs with ECG indices of conduction and repolarization during myocardial ischemia.

| SNP | Coded/Non-Coded Allele | GWAS Minor Allele (Frequency) | Beta (SE) | P value | P value Interaction | Gene |
|-----|------------------------|-------------------------------|-----------|---------|---------------------|------|
| RR SNPs | | | | | | |
| rs11154022 | A/G | Inc | A (0.33) | 6.5 (14.9) | 0.664 | 0.975 | 8 kb from GJA1 |
| rs12666989 | C/G | Dec | C (0.20) | −10.7 (17.6) | 0.543 | 0.175 | LFSF1 |
| rs12731740 | T/C | Inc | T (0.12) | 10.3 (22.3) | 0.646 | 0.277 | CD3-MIR29C-MIR29B2 |
| rs17287293 | G/A | Inc | G (0.14) | 22.6 (19.7) | 0.273 | 0.128 | SOX5-BCAT1 |
| rs174547 | C/T | Dec | C (0.31) | −11.7 (15.0) | 0.438 | 0.343 | FADS1 |
| rs223116 | A/G | Dec | A (0.24) | −45.6 (15.9) | 0.004 | 0.461 | THTPA-NGDN-ZFHX2-MYH7 |
| rs2745967 | G/A | Inc | G (0.38) | −1.2 (14.1) | 0.932 | 0.168 | CD34-PLXNA2 |
| rs281868 | G/A | Dec | A (0.49) | −33.8 (13.8) | 0.014 | 0.061 | SLC35A1 |
| rs314370 | C/T | Dec | C (0.20) | −5.5 (17.4) | 0.751 | 0.197 | SLC12A9 |
| rs452036 | A/G | Dec | A (0.39) | 10.4 (14.5) | 0.472 | 0.823 | MYH6 |
| rs853899 | G/A | Dec | G (0.38) | 22.6 (19.7) | 0.273 | 0.128 | SOX5-BCAT1 |
| rs9398652 | A/C | Dec | A (0.12) | −9.2 (21.9) | 0.674 | 0.292 | GJA1-HSF2 |
| PR SNPs | | | | | | |
| rs11047543 | A/G | Dec | A (0.14) | −1.97 (1.94) | 0.312 | 0.696 | SOX5 |
| rs11708996 | C/G | Inc | C (0.16) | −0.64 (2.11) | 0.763 | 0.722 | SCN5A |
| rs11897119 | C/T | Inc | C (0.38) | 0.41 (1.42) | 0.775 | 0.407 | MEIS1 |
| rs1896312 | C/T | Inc | C (0.29) | 0.37 (1.49) | 0.807 | 0.399 | TBX5-TBX3 |
| rs251253 | C/T | Dec | C (0.41) | −1.59 (1.37) | 0.248 | 0.744 | N1K2-S |
| rs3807989 | A/G | Inc | A (0.42) | 0.17 (1.42) | 0.904 | 0.571 | CAV1-CAV2 |
| rs3825214 | G/A | Dec | G (0.20) | −0.16 (1.74) | 0.925 | 0.766 | TBX5 |
| rs4940092 | G/A | Dec | G (0.33) | −0.74 (1.48) | 0.616 | 0.462 | WNT11 |
| rs6795970 | A/G | Inc | A (0.39) | 4.06 (1.42) | 0.004 | 0.031 | SCN10A |
| rs7660702 | T/C | Inc | C (0.30) | −0.04 (1.42) | 0.979 | 0.462 | ARHGAP24 |
| QRS SNPs | | | | | | |
| rs10850409 | A/G | Dec | A (0.25) | −0.39 (0.98) | 0.696 | 0.380 | TBX3 |
| rs10865879 | T/C | Inc | C (0.25) | −0.45 (1.07) | 0.671 | 0.792 | SCN5A-EXOG |
| rs11153730 | C/T | Inc | T (0.49) | 0.72 (0.87) | 0.008 | 0.383 | C6orf204-SLC35F1-PLN-BRD7P3 |
| rs11708996 | C/G | Inc | C (0.16) | 0.94 (1.37) | 0.493 | 0.232 | SCN5A |
| rs11710077 | T/A | Dec | T (0.18) | 0.59 (1.12) | 0.596 | 0.517 | SCN5A |
| rs11848785 | G/A | Dec | G (0.27) | −0.34 (1.05) | 0.743 | 0.756 | S palabras |
| rs13165478 | A/G | Dec | A (0.37) | −1.45 (0.91) | 0.115 | 0.559 | HAND1-SAP30L |
| rs1321311 | A/C | Inc | A (0.24) | −0.43 (1.08) | 0.691 | 0.858 | CDKN1A |
| rs1362212 | A/G | Inc | A (0.16) | 0.65 (1.29) | 0.616 | 0.834 | TBX20 |
| rs17020136 | C/T | Inc | C (0.21) | 0.24 (1.08) | 0.827 | 0.251 | HEATR5B-STRN |
| rs1733724 | A/G | Inc | A (0.27) | −0.51 (1.03) | 0.621 | 0.274 | DKK1 |
| rs7784776 | C/T | Inc | C (0.13) | 1.31 (1.31) | 0.320 | 0.453 | GOSR2 |
| rs1886512 | A/T | Dec | A (0.37) | −2.39 (0.92) | 0.010 | 0.880 | KLF12 |
| rs2051211 | G/A | Dec | G (0.26) | −0.35 (1.03) | 0.734 | 0.271 | EXOG |
| rs2242285 | A/G | Inc | A (0.43) | −0.24 (0.87) | 0.784 | 0.888 | URI1G-SLC25A26 |
| rs4074536 | C/T | Inc | C (0.32) | 1.19 (0.99) | 0.232 | 0.749 | CASQ2 |
| rs4687718 | A/G | Dec | A (0.12) | 1.70 (1.34) | 0.207 | 0.487 | TTK-PKPCD-CACNA1D |
| rs6795970 | A/G | Dec | A (0.39) | 0.52 (0.91) | 0.569 | 0.326 | SCN10A |
| rs7342028 | T/G | Inc | T (0.25) | 1.89 (1.03) | 0.068 | 0.051 | VTI1A |
| rs7562790 | G/T | Inc | G (0.43) | −0.22 (0.91) | 0.812 | 0.529 | CRIM1 |
| rs7784776 | G/A | Inc | G (0.41) | −0.82 (0.93) | 0.383 | 0.265 | IGFBP3 |
| rs883079 | C/T | Inc | C (0.26) | 2.77 (1.02) | 0.007 | 0.147 | TBX5 |
| rs9436640 | G/T | Dec | G (0.48) | −0.68 (0.89) | 0.445 | 0.278 | NFIA |
small percentage of the variation in these indices. For instance, in a meta-analysis for identification of QTc-associating SNPs by Pfeufer and co-workers [15], SNPs at 10 different loci in aggregate explained only around 3% of the variance in this trait. The effects of these common genetic variants on the STEMI ECG are small and insufficient to explain the differences found in STEMI ECGs of cases and controls. This underscores the need for further studies aimed at uncovering additional genetic variants, such as rare variants associated with larger effects, which would lead to a more-

Table 3. Cont.

| SNP       | GWAS End point | Coded/Non Coded Allele | GWAS Effect | Minor Allele (Frequency) | Beta (SE) | P value | P value Interaction | Gene       |
|-----------|----------------|------------------------|-------------|--------------------------|-----------|---------|---------------------|------------|
| QRS SNPs  |                |                        |             |                          |           |         |                     |            |
| rs9851724 | QRS            | C/T                    | Dec         | C (0.35)                 | −1.25 (0.92) | 0.177   | 0.527               | SCN10A     |
| rs991014  | QRS            | T/C                    | Inc         | T (0.43)                 | −0.40 (0.91) | 0.665   | 0.427               | SETBP1     |
| rs9912468 | QRS            | G/C                    | Inc         | G (0.40)                 | 0.28 (0.96)  | 0.770   | 0.511               | PRKCA      |
| QTc SNPs  |                |                        |             |                          |           |         |                     |            |
| rs1091907 | QTc            | A/G                    | Inc         | G (0.11)                 | −0.01 (2.09) | 0.997   | 0.774               | ATP1B1     |
| rs11970286| QTc            | T/C                    | Inc         | T (0.47)                 | 0.13 (2.31)  | 0.954   | 0.996               | PLN        |
| rs1209454 | QTc            | A/G                    | Inc         | A (0.14)                 | 4.74 (3.20)  | 0.139   | 0.439               | NOS1AP     |
| rs12053903| QTc            | C/T                    | Dec         | C (0.33)                 | −4.13 (2.55) | 0.106   | 0.911               | SCN5A      |
| rs12143842| QTc            | T/C                    | Inc         | T (0.24)                 | 3.47 (2.69)  | 0.197   | 0.874               | NOS1AP     |
| rs12210810| QTc            | C/G                    | Dec         | C (0.04)                 | 1.34 (3.33)  | 0.801   | 0.590               | PLN        |

Table 4. Association analysis of SNPs with VF in AGNES cases versus AGNES controls.

| SNP       | GWAS End point | Coded/Non Coded Allele | GWAS Effect | Minor Allele (Frequency) | Odds ratio [95% CI]* | P value | P value Interaction | Gene       |
|-----------|----------------|------------------------|-------------|--------------------------|----------------------|---------|---------------------|------------|
| rs223116  | RR             | A/G                    | Dec         | A (0.24)                 | 0.86 [0.64 – 1.17]   | 0.340   | 0.0163              | THTPA-NGDN-|
| LAD       |                |                        |             |                          |                      |         |                     |            |
| LCX       |                |                        |             |                          | 2.08 [1.04 – 4.17]   | 0.039   |                     |            |
| RCA       |                |                        |             |                          | 1.61 [1.03 – 2.51]   | 0.037   |                     |            |
| rs281868  | RR             | G/A                    | Dec         | A (0.49)                 | 0.99 [0.81 – 1.21]   | 0.929   | 0.237               | SLC35F1    |
| rs6795970 | PR             | A/G                    | Inc         | A (0.39)                 | 0.77 [0.63 – 0.94]   | 0.009   | 0.897               | SCN10A     |
| rs11708996| PR=200 ms      | C/G                    | Inc         | C (0.16)                 | 1.24 [0.93 – 1.65]   | 0.144   | 0.235               | SCN5A      |
| rs1886512 | QRS            | A/T                    | Dec         | A (0.37)                 | 0.98 [0.80 – 1.20]   | 0.824   | 0.549               | KLF12      |
| rs883079  | QRS            | C/T                    | Inc         | C (0.26)                 | 0.90 [0.72 – 1.13]   | 0.359   | 0.819               | Tbx5       |
| rs17779747| QTc            | T/G                    | Dec         | T (0.34)                 | 1.27 [1.03 – 1.57]   | 0.026   | 0.921               | KCNJ2      |
| rs8049607 | QTc            | T/C                    | Inc         | C (0.49)                 | 1.05 [0.87 – 1.29]   | 0.597   | 0.942               | LITAF      |

*effect estimate is given per copy of the coded allele adjusted for age, sex and culprit artery. † P values for interaction between SNPs and culprit artery on risk of VF

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complete representation of the allelic architecture of these differences in STEMI ECGs.

Strengths and Limitations
In this study, we had the unique opportunity to study the effect of genetic variants, known to impact on ECG indices in the general population, on VF in a well-defined case-control set of patients with a first acute STEMI. Our study is rather unique in that the availability of STEMI ECGs and DNA from patients in whom STEMI is complicated by VF is very scarce due to the high mortality in these patients. On the other hand, as a consequence, our sample size is limited, which can result in insufficient statistical power. Furthermore, the STEMI ECGs used in this study were retrieved retrospectively and, therefore, the time between the onset of complaints and acquisition of the STEMI ECG varied among the study participants. Also since the ECGs were retrieved retrospectively, some were of insufficient quality for analysis, limiting the size of the sample available for analysis. The first recorded ECG that was acquired during STEMI and before reperfusion treatment was used for this analysis. Due to the nature of this complex and specific phenotype under investigation a resting ECG without STEMI prior to the index event is missing from these patients could, therefore, not be tested. Our analysis did not account for effects which may arise from differences in infarct size, intake of amiodarone or cardioversion. However, with respect to infarct size, additional adjustment for peak CKMB as a marker of infarct size, resulted in similar effect estimates but with larger confidence intervals, mainly related to the reduced sample size as peak CKMB was not available in all patients (Table S1).

Conclusion
In conclusion, ECG indices of conduction and repolarization differ between STEMI patients with VF and STEMI patients who do not develop VF. Although the effects of some SNPs on ECG parameters during an acute STEMI were similar in magnitude and direction as those found in the general population, the effects were too small to explain the differences in conduction and repolarization indices and to exert any marked impact on risk of VF. Nevertheless, rs6795970, located within the SCN10A gene, associated with longer PR interval in the general population and with longer PR interval and risk of VF during STEMI in our study, merits investigation in future larger studies.

Supporting Information
Table S1 Association analysis of SNPs with VF in AGNES cases versus AGNES controls with additional correction for peak CKMB levels.

Author Contributions
Conceived and designed the experiments: AAMW CRB LRCD MWTT. Performed the experiments: RFM NB. Analyzed the data: RP JSSGdJ MWTT. Wrote the paper: RP JSSGdJ RFM AAMW CRB MWTT.

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