Subtle killers and sudden death: Genetic variants modulating ventricular fibrillation in the setting of myocardial infarction

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Citation for published version (APA):

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Chapter 7

SNPs modulating inflammatory biomarkers as intermediate phenotype of susceptibility to ventricular fibrillation in the setting of myocardial infarction

Manuscript in preparation for submission

Abstract

**Background:** Sudden cardiac death from ventricular fibrillation (VF) during acute myocardial infarction (MI) is a leading cause of total and cardiovascular mortality. Genetic factors underlying risk for VF in the setting of acute MI remain largely unknown. Epidemiological and experimental data suggest that inflammation may be involved in susceptibility to VF. In this study we investigated the role of single nucleotide polymorphisms (SNPs) modulating inflammatory biomarkers on risk of VF in the setting of MI.

**Methods:** The study was performed in the context of the Arrhythmia Genetics in the NEtherlands Study (AGNES), an ongoing case-control study aimed at identification of genetic risk factors of VF. In total, 1433 AGNES patients with a first acute MI (672 of which had VF, ‘cases’, and 761 of which had no VF, ‘controls’) were analysed. We selected independent SNPs previously linked to inflammatory biomarkers by genome-wide association studies (GWAS) in the general population. We then tested these SNPs for modulatory effects on VF risk. We included SNPs associated with C-reactive Protein (CRP), interleukin 1, interleukin 6, interleukin 18, erythrocyte sedimentation rate (ESR), and monocyte chemotactic protein 1 (MCP-1).

**Results:** Two CRP-modulating SNPs (rs6901250 and rs4420638) were associated with VF at nominally significant $P$-value threshold ($P < 0.05$). Each additional A-allele at rs6901250 increased the risk of VF by 1.2 (95% CI: 1.03, 1.4; $P = 0.02$) while each additional A-allele at rs4420638 increased the risk of VF by 1.2 (95% CI: 1.01, 1.5; $P = 0.05$). Considering the 18 CRP SNPs in aggregate in the form of a weighted genetic risk score (wGRS), we demonstrated an association between the wGRS and VF. VF risk increased with 1.58 (95% CI: 1.01, 2.49; $P = 0.04$) per point wGRS increase.

**Conclusions:** Although this data awaits further verification, these initial findings may suggest that pro-inflammatory SNPs may play a role in arrhythmia susceptibility.
Introduction

Sudden Cardiac Death (SCD) is a major cause of cardiovascular mortality and is commonly caused by ventricular fibrillation (VF) in the setting of sequelae of coronary artery disease in adults\textsuperscript{1,2}. While a genetic component in susceptibility to VF in this setting is recognized\textsuperscript{3-6} the underlying genetic risk factors and molecular mechanisms involved in SCD are yet largely unknown.

Our current work focuses on the identification of genetic risk factors of VF in the setting of a first ST-segment elevation myocardial infarction (MI) in the Arrhythmia Genetics in the NEtherlandS (AGNES) study\textsuperscript{3}. To complement our previous agnostic approach entailing genome-wide association analysis (GWAS) for genetic risk variant discovery in AGNES\textsuperscript{7}, we here undertook a candidate approach testing the role of genetic variants previously associated with inflammation, a likely intermediate phenotype for VF. The underlying principal is that genetic variants modulating an intermediate phenotype for VF are likely to modulate VF susceptibility as well\textsuperscript{8}.

Inflammatory response increases dramatically during MI\textsuperscript{9,10}, and several lines of evidence support a role of inflammation in susceptibility to VF. A series of studies among patients with MI demonstrate an increased pro-inflammatory status among VF patients compared to those without VF\textsuperscript{11-13}. In a prospective study conducted in men from the Physician’s Health Study, a high baseline level of the inflammatory biomarker C-reactive protein (CRP) was significantly associated with SCD\textsuperscript{14}. Similar findings were reported for interleukin-6 (IL6) among participants from the Cardiovascular Health Study\textsuperscript{15}.

In patients treated with ICD after MI, an increased serum CRP was associated with a higher incidence of ventricular tachycardia\textsuperscript{16}. Furthermore, a common genetic variant in the promoter region of interleukin 18 gene (\textit{IL-18}) was found to be associated with SCD\textsuperscript{17,18}.

Studies conducted in vitro have shown that the pro-inflammatory cytokine interleukin-1\textbeta (IL-1\textbeta) produced by myofibroblasts downregulates the gap junctional protein Cx43, suggesting an effect of IL-1\textbeta on cardiac electrical function\textsuperscript{19}. A recent study conducted in mice provided evidence that myocardial inflammation contributes to adverse electrophysiological remodeling (prolonged action potential duration and slowed conduction) and increased post-MI arrhythmia risk\textsuperscript{20}.

Collectively the above studies support the general hypothesis that inflammatory biomarkers can be regarded as intermediate phenotypes for SCD and genetic variation influencing these inflammatory markers, are likely candidates for susceptibility to SCD. GWAS have in recent years identified single nucleotide polymorphisms (SNPs) modulating inflammatory biomarkers in the general population\textsuperscript{21-25}. In the current study, we hypothesized that SNPs modulating inflammatory biomarkers from these GWASes may impact on VF in the setting of a first ST-segment elevation MI. The aim of this study was to investigate this hypothesis in the AGNES population.
Methods

Study sample

The AGNES sample: Study individuals consisted of patients enrolled in the AGNES case-control study \textsuperscript{3,7}. The AGNES study is an ongoing study consisting of patients with a first acute ST-segment elevation MI. Patients who developed (ECG-documented) VF before percutaneous coronary intervention (\textit{i.e.} reperfusion) were defined as AGNES cases. Controls were defined as acute ST-segment elevation MI patients who did not develop VF in the course of their MI. All individuals included were Dutch and of self-declared European descent and were recruited at seven heart centers in the Netherlands from 2001–2011. Patients with a previous MI or major co-morbidities were excluded. Details on the AGNES study sampling have been described earlier \textsuperscript{3}.

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. The medical ethics committees of the hospitals participating in the study approved the study protocols, and all participants gave written informed consent.

Selection of inflammatory loci

We reviewed publications indexed in the National Center for Biotechnology Information (NCBI) PubMed database to identify genetic variants associated with inflammatory biomarkers at genome-wide statistical significance. We focused on full text publications, written in English, and studies performed among human subjects of European ancestry, published until 5\textsuperscript{th} October 2014. We used search queries that included: “single nucleotide polymorphism”, “genome-wide association”, “SNP”, “allele”, “C-reactive Protein”, “CRP”, “interleukin”, and “inflammation”. We excluded review articles and case reports. We additionally inspected the Catalog of Published Genome-Wide Association Studies (http://www.genome.gov/gwastudies/) to check for any additional SNPs modulating serum inflammatory biomarkers in populations of European ancestry and at genome-wide statistical significance. Independent SNPs were chosen for genotyping based on pairwise linkage disequilibrium (LD; $r^2 \leq 0.7$). We used LD information from 1000 Genomes pilot1 and HapMap projects compiled on the SNP Annotation and Proxy Search (SNAP) web page \textsuperscript{26}.

Genotyping, quality control and imputation

AGNES samples (n=1457) were genotyped using either the Illumina Human610-Quad (2001–2008) or the Illumina HumanOmni2.5 (2009–2011) arrays. Genotypes were called using the GenomeStudio V2011.1 software (Genotyping module version
1.9.0) and the GenCall score cutoff was 0.15 as recommended by Illumina. The average sample call rate was larger than 99%.

Multiple quality control measures were implemented before imputation. The estimated sex for each individual as determined by genotyping was compared with the phenotypic sex. Exclusion criteria were deviation from Hardy-Weinberg equilibrium at $P \leq 10^{-4}$ (estimated in controls), sample call rate < 0.95 and SNP call rate < 0.98. The minimal observed minor allele frequency (MAF) was 0.001 in the present sample and no selection based on MAF was performed.

To correct for possible genetic heterogeneity within the AGNES study sample, we performed principal component analysis using a multi-dimensional scaling technique applied on the Identity-By-State (IBS) matrix (R package GenABEL\textsuperscript{27}). Prim’s algorithm implemented in R package \texttt{nnclust}\textsuperscript{28} was used to identify clusters and outliers (threshold: 0.3). The outliers thus identified were excluded from the subsequent analyses. In total, 1433 AGNES cases and controls successfully passed all the quality control steps. The resulting outliers were excluded from the analysis.

Imputation of non-genotyped SNPs was done using the Markov-chain Monte Carlo method implemented in MACH1.0\textsuperscript{29,30} using the HAPMAP reference panel. After imputation, an $r^2$ threshold of 0.3 was implemented to identify and discard low-quality imputed SNPs.

### Statistical Analysis

Differences in continuous phenotypic variables between cases and controls were tested using an independent $t$ test where data was normally distributed or a Mann-Whitney $U$ test otherwise. Values are presented as means ± SD or median and interquartile range, respectively. Differences in categorical variables were compared using a Fisher exact test, and values are presented as number and percentages.

Logistic regression models assuming an additive genetic model of inheritance were used to test the association between pro-inflammatory SNPs and VF. We tested the additive assumption using the le Cessie - van Houwelingen - Copas - Hosmer unweighted sum of squares test for global goodness of fit using \texttt{rms} package in R\textsuperscript{31}. The analyses were adjusted for age, sex, and the first 2 principal components. In addition to main effects, possible interactions between a SNP and sex and/or age were also investigated.

Since at the CRP locus a total of 18 SNPs were investigated, we additionally tested the in-aggregate effect of these 18 SNPs on VF susceptibility by means of a weighted genetic risk score (wGRS) where the CRP increasing alleles were treated as coded allele. To calculate wGRS, the allele counts per SNP were multiplied by the size of the CRP increasing effect and were subsequently summed up. The weights used were based on the effect estimates per SNP from the original GWAS in the general popula-
tion. Nagelkerke’s R square\textsuperscript{32} was used as the estimate of percentage of variance in the risk of VF explained by individual SNPs or the SNPs in aggregate (combined in wGRS). A \( P \)-value of 0.05 with a Bonferroni correction for the number of independent SNPs was used as the threshold for statistical significance in the analysis involving individual SNP effects. A \( P \)-value of 0.05 was considered as the threshold for statistical significance in the genetic risk score analysis and as a nominal significance threshold for individual SNP analysis. All analyses were performed in R.

### Results

#### Patients

In total, 1433 AGNES patients with a first acute MI (672 patients with VF, ‘cases,’ and 761 without, ‘controls’) were included in the statistical analysis. Baseline characteristics of the study population are shown in Table 1. AGNES cases had a lower prevalence of diabetes mellitus and hypercholesterolemia, a lower average body mass index (BMI),

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N\textsuperscript{a}</th>
<th>Total (n = 1433)</th>
<th>Cases (n = 672)</th>
<th>Controls (n = 761)</th>
<th>( P )-value\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)\textsuperscript{c}</td>
<td>1433/672/761</td>
<td>1142 (80)</td>
<td>537 (80)</td>
<td>605 (79)</td>
<td>0.847</td>
</tr>
<tr>
<td>Age at MI (years)\textsuperscript{d}</td>
<td>1433/672/761</td>
<td>57.5 ± 10.85</td>
<td>56.7 ± 11.03</td>
<td>58.3 ± 10.63</td>
<td>0.004</td>
</tr>
<tr>
<td>ST segment deviation (mm)\textsuperscript{e}</td>
<td>491/251/240</td>
<td>15 (16)</td>
<td>18 (19)</td>
<td>14 (12)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MI location\textsuperscript{f}</td>
<td>1366/619/747</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior MI</td>
<td>726 (53)</td>
<td>359 (58)</td>
<td>367 (49)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Inferior MI</td>
<td>640 (47)</td>
<td>260 (42)</td>
<td>380 (51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB (μg/L)\textsuperscript{e}</td>
<td>1044/415/629</td>
<td>205 (277)</td>
<td>226 (356)</td>
<td>188 (244)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Family history of sudden death\textsuperscript{e}</td>
<td>1416/657/759</td>
<td>400 (28)</td>
<td>205 (31)</td>
<td>195 (26)</td>
<td>0.002</td>
</tr>
<tr>
<td>Beta blocker usage\textsuperscript{e}</td>
<td>1384/632/752</td>
<td>139 (10)</td>
<td>66 (10)</td>
<td>73 (10)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

**Cardiovascular risk factors**

| Current Smoking\textsuperscript{e} | 1374/627/747          | 809 (59)         | 396 (63)       | 413 (55)          | 0.003                         |
| Diabetes Mellitus\textsuperscript{e} | 1349/613/736          | 102 (8)          | 29 (5)         | 73 (10)           | 0.0004                        |
| Hypertension\textsuperscript{e}    | 1298/584/714          | 426 (33)         | 185 (32)       | 241 (34)          | 0.428                         |
| Hypercholesterolemia\textsuperscript{e} | 1250/563/687          | 406 (32)         | 164 (29)       | 242 (35)          | 0.022                         |
| BMI (kg/m\textsuperscript{2})\textsuperscript{d} | 1342/597/745          | 26.5 ± 3.86      | 26.1 ± 3.66    | 26.8 ± 3.99       | 0.002                         |

MI, myocardial infarction; CK-MB, creatine kinase-MB; \( ^{a} \)Sample sizes of the total, case and control sets for whom information is available are given. \( ^{b} \)\( P \)-value for comparison of cases and controls for each item; \( ^{c} \)Number (%); \( ^{d} \)Mean ± s.d.; \( ^{e} \)Median (interquartile range); \( ^{f} \)\( P \)-value for comparison of inferior and anterior MI between cases and controls.
and less frequency of smoking. AGNES cases more often had a family history of sudden cardiac death than the controls, were on average younger at the time of MI, and had higher creatine kinase-MB (CK-MB) levels than AGNES controls (Table 1).

**Inflammatory SNPs and VF**

By reviewing published GWAS, we selected 28 independent SNPs that modulate inflammatory biomarkers. These SNPs included 18 SNPs modulating serum CRP, 2 SNPs modulating serum erythrocyte sedimentation rate (ESR), 2 SNPs modulating serum monocyte chemotactic protein 1 (MCP-1), 4 SNPs modulating IL18, 1 SNP modulating serum interleukin 1 receptor antagonist (IL1ra), and 1 SNP modulating serum IL6.

The selected SNPs were independent ($r^2 \leq 0.7$) and were identified in the general population to be associated with inflammatory biomarkers in individuals of European descent at genome-wide significance level (see Table 2 where the coded allele is the biomarker increasing allele).

In the model corrected for age, sex and principal components, none of the 28 SNPs displayed an association with VF passing the Bonferroni-corrected significance threshold ($P \leq 0.002$; 0.05/28). Also, none of the SNPs showed interaction with age or sex at the Bonferroni-corrected threshold. Two CRP-modulating SNPs (rs6901250 and rs4420638) were associated with VF at nominally-significant $P$-value threshold ($P < 0.05$). Each additional A allele at rs6901250 increased the risk of VF by 1.2 (95% CI: 1.03, 1.4; $P = 0.02$) while each additional A allele at rs4420638 increased the risk of VF by 1.2 (95% CI: 1.01, 1.5; $P = 0.05$) (Table 2).

In a multivariable model restricted to the 18 CRP SNPs, rs6901250 and rs4420638 remained independently associated with VF ($P = 0.02$ for both). This model explained 2% of the variability in susceptibility to VF. Inclusion of the remaining 10 pro-inflammatory SNPs increased the explained variability to 3%. Addition of age, sex, and principal components increased the explained variance in susceptibility to VF to 6.6%.

**Genetic risk score analysis on CRP SNPs**

AGNES cases and controls carried at least 15 and at most 32 CRP-increasing alleles. For the wGRS (range 1.2 to 2.6; Figure 1) the risk of VF increased by 1.58 fold (95% CI: 1.01, 2.49; $P = 0.04$) per point wGRS increase, and the wGRS explained 0.3% of the VF risk. Frequency of VF increased with increasing wGRS quartile and was 34.9% in the lowest quartile and 44.2% in the highest quartile.
Table 2 | Association of inflammatory biomarker SNPs with VF in the AGNES sample

<table>
<thead>
<tr>
<th>SNP</th>
<th>CA(F)</th>
<th>Ref. GWAs</th>
<th>Chr Effect</th>
<th>Trait</th>
<th>Total AGNES (N = 1433)</th>
<th>P-value</th>
<th>P-int</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10745954</td>
<td>A(47)</td>
<td>21</td>
<td>0.039</td>
<td>12</td>
<td>CRP</td>
<td>0.94(0.81,1.1)</td>
<td>0.42</td>
<td>0.54/0.98 Near ASCL1</td>
</tr>
<tr>
<td>rs1183910d</td>
<td>G(70)</td>
<td>21,22</td>
<td>0.149</td>
<td>12</td>
<td>CRP</td>
<td>0.97(0.83,1.14)</td>
<td>0.72</td>
<td>0.30/0.21 TCF1</td>
</tr>
<tr>
<td>rs12037222</td>
<td>A(26)</td>
<td>21</td>
<td>0.045</td>
<td>1</td>
<td>CRP</td>
<td>1.06(0.89,1.27)</td>
<td>0.47</td>
<td>0.69/0.37 Near PABPC4</td>
</tr>
<tr>
<td>rs12239046</td>
<td>C(61)</td>
<td>21</td>
<td>0.047</td>
<td>1</td>
<td>CRP</td>
<td>1(0.85,1.17)</td>
<td>0.99</td>
<td>0.65/0.79 NLRP3</td>
</tr>
<tr>
<td>rs1260326</td>
<td>T(37)</td>
<td>21</td>
<td>0.072</td>
<td>2</td>
<td>CRP</td>
<td>1.06(0.91,1.24)</td>
<td>0.44</td>
<td>0.75/0.43 GCKR</td>
</tr>
<tr>
<td>rs13233571</td>
<td>C(87)</td>
<td>21</td>
<td>0.054</td>
<td>7</td>
<td>CRP</td>
<td>0.96(0.77,1.19)</td>
<td>0.74</td>
<td>0.22/0.03 BCL7B</td>
</tr>
<tr>
<td>rs1800961</td>
<td>C(97)</td>
<td>21</td>
<td>0.088</td>
<td>20</td>
<td>CRP</td>
<td>1.2(0.76,1.88)</td>
<td>0.44</td>
<td>0.35/0.29 HNF4A</td>
</tr>
<tr>
<td>rs2794520</td>
<td>C(68)</td>
<td>21</td>
<td>0.160</td>
<td>1</td>
<td>CRP</td>
<td>1.13(0.96,1.32)</td>
<td>0.15</td>
<td>0.78/0.04 Near CRP</td>
</tr>
<tr>
<td>rs2847281</td>
<td>A(59)</td>
<td>21</td>
<td>0.031</td>
<td>18</td>
<td>CRP</td>
<td>0.94(0.81,1.1)</td>
<td>0.40</td>
<td>0.40/0.08 PTPN2</td>
</tr>
<tr>
<td>rs340029</td>
<td>T(61)</td>
<td>21</td>
<td>0.032</td>
<td>15</td>
<td>CRP</td>
<td>1.11(0.94,1.29)</td>
<td>0.19</td>
<td>0.74/0.59 RORA</td>
</tr>
<tr>
<td>rs3845624</td>
<td>C(40)</td>
<td>21</td>
<td>0.10</td>
<td>1</td>
<td>CRP</td>
<td>0.98(0.84,1.15)</td>
<td>0.82</td>
<td>0.11/0.31 DARC</td>
</tr>
<tr>
<td>rs4129267</td>
<td>C(60)</td>
<td>21</td>
<td>0.079</td>
<td>1</td>
<td>CRP</td>
<td>0.95(0.81,1.11)</td>
<td>0.51</td>
<td>0.36/0.75 IL6R</td>
</tr>
<tr>
<td>rs4420065</td>
<td>C(60)</td>
<td>21</td>
<td>0.090</td>
<td>1</td>
<td>CRP</td>
<td>1.06(0.91,1.24)</td>
<td>0.46</td>
<td>0.95/0.11 Near LEPR</td>
</tr>
<tr>
<td>rs4420638d</td>
<td>A(82)</td>
<td>21,22</td>
<td>0.236</td>
<td>19</td>
<td>CRP</td>
<td>1.23(1.01,1.5)</td>
<td>0.05</td>
<td>0.68/0.85 Near APOC1</td>
</tr>
<tr>
<td>rs4705952</td>
<td>G(20)</td>
<td>21</td>
<td>0.042</td>
<td>5</td>
<td>CRP</td>
<td>0.92(0.77,1.1)</td>
<td>0.37</td>
<td>0.26/0.31 Near IRF1</td>
</tr>
<tr>
<td>rs6734238</td>
<td>G(40)</td>
<td>21</td>
<td>0.050</td>
<td>2</td>
<td>CRP</td>
<td>1.02(0.87,1.19)</td>
<td>0.82</td>
<td>0.12/0.28 Near IL1F10</td>
</tr>
<tr>
<td>rs6901250</td>
<td>A(31)</td>
<td>21</td>
<td>0.035</td>
<td>6</td>
<td>CRP</td>
<td>1.21(1.03,1.41)</td>
<td>0.02</td>
<td>0.91/0.11 GPRC6A</td>
</tr>
<tr>
<td>rs9987289</td>
<td>G(92)</td>
<td>21</td>
<td>0.069</td>
<td>8</td>
<td>CRP</td>
<td>1.12(0.83,1.5)</td>
<td>0.44</td>
<td>0.37/0.68 Near PPP1R3B</td>
</tr>
<tr>
<td>rs12034598</td>
<td>G(18)</td>
<td>25</td>
<td>0.14</td>
<td>1</td>
<td>ESR</td>
<td>1.07(0.88,1.3)</td>
<td>0.48</td>
<td>0.37/0.39 CR1</td>
</tr>
<tr>
<td>rs12075</td>
<td>A(55)</td>
<td>25</td>
<td>0.33</td>
<td>1</td>
<td>MCP-1</td>
<td>0.96(0.82,1.12)</td>
<td>0.57</td>
<td>0.99/0.46 DARC</td>
</tr>
<tr>
<td>rs1834481</td>
<td>G(26)</td>
<td>23</td>
<td>0.09</td>
<td>11</td>
<td>II18</td>
<td>1.03(0.86,1.23)</td>
<td>0.76</td>
<td>0.08/0.90 II18</td>
</tr>
<tr>
<td>rs2115763</td>
<td>T(31)</td>
<td>23</td>
<td>0.09</td>
<td>11</td>
<td>II18</td>
<td>0.93(0.81,0.99)</td>
<td>0.42</td>
<td>0.75/0.08 II18</td>
</tr>
<tr>
<td>rs2250417</td>
<td>T(45)</td>
<td>24</td>
<td>0.1</td>
<td>11</td>
<td>II18</td>
<td>1.01(0.86,1.18)</td>
<td>0.92</td>
<td>0.59/0.08 BCO2, IL18, TEX12</td>
</tr>
<tr>
<td>rs3026968</td>
<td>T(21)</td>
<td>25</td>
<td>0.22</td>
<td>1</td>
<td>MCP-1</td>
<td>0.9(0.76,1.08)</td>
<td>0.27</td>
<td>0.04/0.84 CADM3</td>
</tr>
<tr>
<td>rs4910742</td>
<td>G(5)</td>
<td>25</td>
<td>0.22</td>
<td>11</td>
<td>ESR</td>
<td>1.34(0.94,1.9)</td>
<td>0.12</td>
<td>0.29/0.76 HBB</td>
</tr>
<tr>
<td>rs643434</td>
<td>A(37)</td>
<td>25</td>
<td>0.22</td>
<td>9</td>
<td>IL-6</td>
<td>0.98(0.84,1.15)</td>
<td>0.83</td>
<td>0.11/0.70 ABO</td>
</tr>
<tr>
<td>rs6743376</td>
<td>A(63)</td>
<td>24</td>
<td>0.13</td>
<td>2</td>
<td>IL-1ra</td>
<td>1.13(0.96,1.32)</td>
<td>0.11</td>
<td>0.35/0.12 IL1F10</td>
</tr>
<tr>
<td>rs7577696</td>
<td>G(41)</td>
<td>24</td>
<td>0.08</td>
<td>2</td>
<td>IL18</td>
<td>1(0.85,1.17)</td>
<td>0.95</td>
<td>0.14/0.32 SRD5A2, DPY30, SPAST, SLC30A6, NLR4</td>
</tr>
</tbody>
</table>

CA(F), coded allele (frequency); Ref. reference; Chr, chromosome; CRP, c-reactive protein; OR, odds Ratio; P-int, P-value for interaction; “Allele refers to the allele that is associated with increased level of the corresponding inflammatory trait; “Beta coefficient represents 1-unit change in the natural log-transformed CRP (mg/L) per copy increment in the coded allele; “Effect estimate is given per copy of the CRP-increasing allele adjusted for age, and sex and PCs; “SNPs found in more than one study but could not be meta-analyzed due to incomparable unit differences. Coefficients from the larger sample size were then considered.
Pro-inflammatory SNPs and risk of VF in MI 139

Discussion

In the current study, we selected SNPs previously demonstrated modulating inflammatory biomarkers and subsequently tested their effect on susceptibility to VF in the setting of a first acute ST-elevation MI in the AGNES case-control set. We demonstrated that 2 SNPs, respectively at chromosome 6q22 (rs6901250) and chromosome 19q13 (rs4420638), displayed a suggestive association with the risk of VF. Considering CRP SNPs in aggregate in the form of a weighted genetic risk score (wGRS), we demonstrated an association between the wGRS and VF. Although this data awaits further verification, these initial findings may suggest that pro-inflammatory SNPs might play a role in arrhythmia susceptibility.

rs6901250

The data presented here may suggest that the CRP-increasing allele at rs6901250 may be associated with increased risk of VF in the setting of a first acute MI. The exact gene mediating the effect of rs6901250 on serum CRP is not known. rs6901250 is located in the GPRC6A gene that encodes the G protein-coupled receptor, family C, group 6, member A. GPRC6A which is expressed in multiple organs including heart, is a transmembrane calcium-sensitive receptor which is involved in numerous pathways including calcium signaling. It is known to be triggered by increased extracellular calcium as occurs for example during necrosis, to activate assembly of inflammatory cells.
rs4420638
The A-allele of rs4420638 which increases serum CRP in the general population was suggestively associated with VF in the current study. rs4420638, which is located immediately downstream of the apolipoprotein C-I (APOC1) gene has been linked to multiple phenotypes in GWAS including Lipoprotein-associated phospholipase A2 (Lp-PLA2) mass, coronary artery disease, and treatment response to lipid lowering function of statin and fenofibrates. As for rs6901250, the biological mechanism linking rs4420638 to variation in serum CRP are still unexplored. However, APOC1 is known to have immunosuppressive properties and to decrease secretion of pro-inflammatory cytokines from human macrophages. Furthermore APOC1 forms part of the statin pathway, which besides the major role in lipid metabolism has also been implicated in immunomodulation. Since rs4420638 is associated with multiple phenotypes in the general population, the effect of this SNP on CRP or VF may be possibly mediated through multiple factors and mechanisms.

Genetic risk score
Next to single SNP effects, we also constructed a weighted genetic risk score (wGRS) that considered the SNPs from the CRP loci in aggregate. This uncovered an association of the CRP wGRS with VF. This is the first time that SNPs impacting on phenotypes considered intermediate phenotypes for VF, were associated with VF when considered in aggregate. A similar approach has been employed by our group and others for SNPs related to ECG parameters. We previously tested the collective effect of ECG-modulating SNPs on risk of VF in the AGNES case-control set and did not demonstrate any effect for such score. Noseworthy et al. investigated the effect of SNPs modulating the QTc-interval on risk of SCD and did not demonstrate a linear relation between genetic risk score of QTc-interval SNPs on SCD risk.

Role of inflammation in VF susceptibility
The CRP genetic risk score in the current study explained a marginal part of the variance in risk of VF (0.3%). This is not surprising given the fact that the CRP-modulating SNPs explain only 5% of the variability in CRP in the general population which is rather small in relation to the 35-40% heritability estimated for CRP. Furthermore, VF in the setting of a first acute MI is most likely governed by numerous pathways of which inflammation may be one.

Mechanistic studies aimed at understanding the processes by which inflammation may affect cardiac electrophysiology and arrhythmia susceptibility are largely lacking. One recent study conducted in mice that investigated the role of post-MI inflammation on cardiac electrophysiology and arrhythmogenesis demonstrated prolonged repolarization, slowed conduction and exacerbation of arrhythmia as a consequence...
of inflammation. While this study supports a role for modulation of cardiac electrophysiology by inflammation, it looked at changes in hearts on the fifth date post-MI, which differs markedly from the timeline of arrhythmia in AGNES (where arrhythmias arise within 2 hours of commencement of complaints in almost 90% of patients). Furthermore, one should distinguish between the “pre-conditioning” effects of long-standing inflammation (existing prior to the MI, e.g. systemic) on cardiac electrical properties as opposed to the acute effects of inflammation on cardiac electrophysiology during the MI.

**Strengths and Limitations**

Considering the difficulty in ascertaining patients presenting with VF, the sample size of our study is rather large. Furthermore, we here study patients presenting with VF in the setting of a specific cardiac pathology entailing a first MI. This limits the variability in molecular mechanisms underlying VF which should enhance genetic variant discovery. However, we have here tested common genetic variants that have been associated with inflammatory markers in very large sets of the general population and which have been found to carry (very) small effect sizes (as expected of common variants). Thus although our current findings may be considered as encouraging evidence for an effect of pro-inflammatory SNPs (at least when considered in aggregate) in modulation of VF risk in acute MI, the current findings call for validation in independent patient sets and investigation in larger studies.

Our hypothesis that inflammation is involved in VF risk is entirely based on published epidemiological and experimental studies. We have as yet not studied the role of inflammation in predisposition to VF in the AGNES study where CRP measurements were available but only in a non-random subset of the total set. Further whenever CRP levels were determined, these measurements were done post MI and at various time-points after MI. For these reasons, we refrained from assessing the relation between serum CRP and VF risk in the AGNES study. This will require a dedicated study.

In our GWA studies on the AGNES sample (chapters 4 and 5 of this thesis), we identified a VF susceptibility locus at chromosome 21. Interestingly this locus harbors the Coxackie and Adenovirus Receptor (CAR) which besides playing a role in cardiac conduction has a long-recognized role as viral receptor in the pathogenesis of viral myocarditis. Of note, active coxsackie B virus infection has been reported at a high frequency in a group of individuals with MI who died suddenly. This may suggest a role for the genetic locus in mediating variability in susceptibility to myocarditis and ensuing inflammation that may in turn be pro-arrhythmic. While this remains purely speculative, these observations together with previously published observations emerged from epidemiological and experimental studies collectively argue for further investigation of the role of inflammation in risk of VF/SCD.
Conclusion

In the current study, two CRP SNPs located in the *GPRC6A* and *APOC1* gene were nominally associated with VF. Additionally, a genetic risk score constructed of CRP-increasing alleles appeared to increase the risk of VF in the AGNES study. The genetic underpinnings of inflammation system may play a role in arrhythmia susceptibility during MI and our findings here merit further validation and investigation in animal, clinical or population-based studies.

Acknowledgement

This study was supported by research grants from the Netherlands Heart Foundation (grants 2001D019, 2003T302 and 2007B202), the Leducq Foundation (grant 05-CVD) and the Interuniversity Cardiology Institute of the Netherlands.
References


