Linkage and association analyses in an extended kindred with sodium channelopathy identifies a novel locus for cardiac conduction

In preparation

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ABSTRACT

BACKGROUND
The monogenic arrhythmia syndromes, caused primarily by mutations in genes encoding cardiac ion channel subunits, exhibit extensive clinical variability in ECG manifestations and occurrence of arrhythmias. Important modifiers such as age, gender and drugs are recognized. However, while evidence points to a role for genetic modifiers in phenotypic variability, these remain largely unknown. Through linkage and association analysis in a large family with sodium channelopathy we aimed to identify genetic variants influencing heart rate and ECG indices of conduction and repolarization.

METHODS AND RESULTS
This study was performed in a very large family carrying the SCN5A-1795insD mutation, manifesting as an overlap disorder of long-QT syndrome, Brugada syndrome and cardiac conduction disease. We investigated a total of 1329 tag-SNPs located in or around 18 candidate genes encoding ion channel subunits or interacting proteins for effects on ECG parameters by linkage and association analyses.

Linkage analysis identified significant linkage (LOD=3.7) for PQ-interval at the region of chromosome 21 harboring the KCNE1 and KCNE2 candidate genes. Suggestive linkage was detected on chromosome 3 in the region of the SCN5A, GPD1L and CAV3 genes for heart rate (LOD=2.0) and PQ-interval (LOD=3.1). SNP rs2834506, selected for analysis by virtue of its location upstream of the KCNE1 gene, encoding the I_{Ks} subunit MinK, was associated with PQ-interval with a p-value (p=9.8e-08) that exceeded our pre-specified Bonferroni-corrected threshold for significance (p=3.8e-06). A closer examination of the location of this SNP identified that it is actually located within intron 3 of the RCAN1 gene, located a few kilo base-pairs upstream of KCNE1. The RCAN1 gene encodes ‘Regulator of Calcineurin 1’ which is highly expressed in heart and regulates calcineurin, a calcium-activated phosphatase that promotes hypertrophic growth of the heart. Transgenic mice overexpressing constitutively active calcineurin display premature sudden death and profoundly prolonged PQ-intervals, and cells isolated from these mice before the development of hypertrophy display reduced I_{Na}.

CONCLUSIONS
We here identify a locus on chromosome 21q22.12 as a modulator of cardiac conduction, providing highly relevant candidate target genes for future studies aimed at deciphering the molecular basis of cardiac conduction.
INTRODUCTION

The genetic basis of the monogenic cardiac arrhythmia syndromes associated with sudden cardiac death (SCD) has been brought into focus over the last decade and a large spectrum of mutations, primarily in genes encoding components of cardiac ion channels, has been reported.1 This has had remarkable impact on the management of these disorders since it enabled genetic testing, allowing for presymptomatic identification and gene specific treatment of patients at risk of developing fatal arrhythmias. Moreover, the ability to diagnose patients independently from electrocardiographic (ECG) and arrhythmic manifestations led to the realization that these disorders are not spared from the genetic phenomena of reduced penetrance and variable expression typical of monogenic disorders.2,3 Thus, in the monogenic cardiac arrhythmia syndromes extensive variability in clinical manifestations may be observed among family members carrying an identical ion channel gene mutation. Some individuals carrying the mutation may exhibit overt ECG abnormalities and suffer fatal arrhythmias, whereas others might not have the ECG changes and may never develop arrhythmias. Ever since their inception in genetic research, these phenomena have been tightly linked to interactions between environmental and genetic modifiers with the particular pathogenic mutation. Some important modifiers such as age, gender, heart rate and drugs are already recognized.4-7 However, while evidence points to a role for genetic modifiers in phenotypic variability,3,8-12 these remain largely unknown.

In the present study we aimed to identify genetic variants influencing cardiac electrical behavior by linkage and association analysis at chromosomal loci harboring 18 candidate genes involved in cardiac electrical activity. This was performed in a very large family with cardiac sodium channelopathy as a consequence of the SCN5A-1795insD mutation, manifesting as an overlap disorder of long-QT syndrome (type 3), Brugada syndrome and progressive cardiac conduction disease.4,5,13-15 The pleiotropic effects of this particular mutation makes this family an ideal sensitized “model” to uncover genetic factors modulating both cardiac depolarization as well as repolarization at both the atrial and ventricular levels. Accordingly, we sought to identify loci modulating heart rate and ECG indices of atrial, atrio-ventricular and ventricular conduction and ventricular repolarization. Our data provides strong evidence for a role of chromosome 21q22 in the region of the KCNE1, KCNE2 and RCAN1 genes in cardiac conduction.

METHODS

Study population
The study population consisted of a large white Dutch kindred segregating the SCN5A-1795insD mutation associated with manifestations of long-QT syndrome (type 3), Brugada
syndrome and progressive conduction disease occurring either in isolation or in combinations thereof.\textsuperscript{4,5,13,14} Carrier status for SCN5A-1795insD was determined by direct sequencing as described previously.\textsuperscript{13} Only individuals with DNA and ECG available were included. All study participants provided their (written) informed consent and the study was approved by the institutional review boards.

**Phenotyping**

Heart rate and ECG indices of conduction and repolarization were measured from the last available resting ECG in the absence of anti-arrhythmic drugs. All ECGs were digitalized and analyzed using ImageJ (http://rsb.info.nih.gov/ij/). Only sinus rhythm complexes were analyzed. Measurement of all parameters (heart rate, PQ-interval, QRS-duration and QT-interval) were done manually on-screen, in lead II whenever possible.\textsuperscript{16} Parameters were averaged from up to 3 consecutive beats with similar preceding RR-intervals. For QT and heart rate corrected QT (QTc), the tangent method with Bazett’s correction was used.\textsuperscript{17} Additionally we determined right precordial ST-elevation as the maximal ST-elevation at the J-point in any lead among leads V1-V3.

**SNP selection and genotyping**

Eighteen candidate genes likely to modulate heart rate or ECG parameters of conduction and repolarization (Table 1) were selected on the basis of either being (1) disease causing genes of cardiac arrhythmia syndromes, (2) associated with QT-interval in genome-wide association studies in the general population, and (3) genes encoding key subunits of the ion channels encoded by the aforementioned genes.

Single nucleotide polymorphisms (SNPs)
for genotyping were selected from all HapMap SNPs (http://www.hapmap.org/) available for the CEU population (Utah residents with ancestry from northern and western Europe) within these genes and 50 kb upstream and downstream of these genes. Tag SNPs were selected using the Tagger program\textsuperscript{18} such that all SNPs with a minor allele frequency >5% were captured with \( r^2 > 0.8 \). SNPs with low Illumina quality design scores were replaced where possible by another SNP tagging the same haplotype block. A total of 1428 SNPs were derived in this way for genotype analysis in the family.

SNP genotyping was performed using a custom assay (GoldenGate) on an Illumina-BeadStation500GX (Illumina Inc., SanDiego, USA). SNPs and samples with call rates <95% were removed from further analyses. Data were checked for Mendelian inconsistencies using the mistyping method implemented in Mendel 8.0.\textsuperscript{19}

**Statistical analyses**

Except for ST-elevation, phenotypic data for mutation carriers and non-carriers were normally distributed (Shapiro-Wilk-test, \( W > 0.90 \)) and are reported as the mean±standard deviation. ST-elevation is reported as median and interquartile range and was rank-transformed for subsequent analyses. Differences in ECG characteristics of mutation carriers and non-carriers and effects of gender and age on these ECG-characteristics were analyzed using SOLAR 4.1.6. with a threshold for significance of 0.05.\textsuperscript{20} Correlations between ECG characteristics were expressed as Pearson correlation coefficients (\( r \)).

Genetic distance maps were calculated using the kosambi mapping function (Mendel). As this is a family-based study, very little recombination was observed resulting in very small distances between the genes (0.001-3.49cM). Estimation of (multipoint) identity by descent and linkage analyses were carried out using SOLAR. Two linkage models were used; 1) adjusted for age and gender, and 2) additionally adjusted for SCN5A-1795insD mutation carrier status. The latter model was used to identify effects of SNPs independent of the (large) effect of the SCN5A-1795insD mutation. All logarithm of the odds (LOD)-scores reported were corrected for inaccuracy caused by possible non-normal trait distribution using the empirical LOD-adjustment in SOLAR. We considered a LOD-score >1.9 as suggestive linkage and >3.3 as genome-wide significant linkage following proposed thresholds.\textsuperscript{21}

<table>
<thead>
<tr>
<th>Location</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p13.3-p11</td>
<td>CASQ2</td>
</tr>
<tr>
<td>1q23.3-q43</td>
<td>NOS1AP, RYR2</td>
</tr>
<tr>
<td>2p23.3</td>
<td>FKBP1B</td>
</tr>
<tr>
<td>3p21-p25</td>
<td>SCN5A, GPD1L, CAV3</td>
</tr>
<tr>
<td>4q25-q27</td>
<td>ANK2</td>
</tr>
<tr>
<td>7q21-q35</td>
<td>AKAP9, KCNH2</td>
</tr>
<tr>
<td>11p15.5</td>
<td>KCNQ1</td>
</tr>
<tr>
<td>11q23.3</td>
<td>SCN4B</td>
</tr>
<tr>
<td>12p13.3</td>
<td>CACNA1C</td>
</tr>
<tr>
<td>17q23-q25.3</td>
<td>SCN4A, KCNJ2</td>
</tr>
<tr>
<td>19q13.1</td>
<td>SCN1B</td>
</tr>
<tr>
<td>21q22.12</td>
<td>KCNE2, KCNE1</td>
</tr>
</tbody>
</table>
Family based association analyses were performed using the linear mixed effect model function (lmekin) in the Kinship package (1.1.0-22, Atkinson&Therneau, 2008) in R (http://www.r-project.org/). For each SNP-phenotype relationship, an additive genetic model was initially assumed. To test this assumption, a heterozygosity indicator variable was added to the additive model. In case of a significant (p<0.1) deviation from additivity, the SNP-phenotype relationship was modeled using either the dominant or recessive genetic model, depending on the direction of the deviation. Similar to the linkage analysis, two models were used; 1) adjusted for age and gender, and 2) additionally adjusted for carrier status.

A total of 1329 out of 1428 SNPs (93%) passed the quality control criteria and were included in the linkage and association analyses. The number of independent SNPs was 1308 (Nyholt’s method). The significance threshold for association was calculated by dividing α (0.05) by the number of independent tests (1308) x6 phenotypes and x2 models, resulting in a stringent (bonferroni corrected) significance threshold of p<3.8e-06.

RESULTS

Study population and ECG parameters
An extensive genealogical study allowed us to trace the family back to the eighteenth century (Figure 1). For this study, DNA and ECG was available for 217 cases (101 carriers) from the last 4 generations (Table 2). Gender, age and heart rate distributions were similar in carriers versus non-carriers. As expected, conduction and repolarization parameters were significantly prolonged in carriers versus non-carriers and carriers displayed more ST-elevation than non-carriers (2). There was a striking variability in all ECG-parameters, both among carriers and non-carriers (Figure 2). QRS-duration was correlated to PQ-interval in both carriers and non-carriers, but this was considerably more pronounced in carriers (carriers: r=0.50, p=1.1e-07; non-carriers r=0.24, p=0.01, Figure 2A). The heart rate dependency of

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Baseline and ECG characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carriers (n=101)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33±20</td>
</tr>
<tr>
<td>Male gender</td>
<td>48(48)</td>
</tr>
<tr>
<td>Hear rate (bpm)</td>
<td>75±19</td>
</tr>
<tr>
<td>PQ interval (ms)</td>
<td>181±28</td>
</tr>
<tr>
<td>QRS interval (ms)</td>
<td>102±17</td>
</tr>
<tr>
<td>QTc interval (ms)</td>
<td>453±32</td>
</tr>
<tr>
<td>Max. ST elevation V1-V3 (0.1 mV*)</td>
<td>0.5(0.25-1.00)</td>
</tr>
</tbody>
</table>

Data are mean±SD, n(%) or median (interquartile range). *, corresponding to 1mm on a standard calibrated ECG
ventricular repolarization can be appreciated in Figure 2B. The correlation between QTc and heart rate differs in the carriers (r=-0.23, p=1.9e-0.2) and non-carriers (r=0.42, p=3.2e-06). While in non-carriers QTc decreases with decreasing heart rate, we confirm earlier findings that in carriers QTc increases with decreasing heart rate.13

Influence of age and gender

Both age and gender affected the ECG parameters, particularly conduction indices (Table 3). Females had shorter PQ and QRS-intervals and lower ST-elevation. PQ- and QTc-intervals increased with increasing age, while heart rate decreased with increasing age. For QRS-duration, the increase with increasing age was significantly larger for mutation carriers compared to non-carriers, 0.47 (p=4.5e-09) vs. 0.12 (p=0.06), respectively (Figure 3). ST-elevation seemed to increase with age and specifically in mutation carriers. The proportion explained variance by the model including sex and age ranged from 2–12%. Carriers had significantly prolonged conduction (PQ, QRS) and repolarization (QTc) indices and inclusion of carrier status in the model increased the amount of explained variance considerably and up to 56% for QTc.

Linkage analyses

LOD scores obtained in linkage analysis using age and sex as covariates (model 1), exceeding the thresholds for significant and suggestive linkage are presented in Table 4. As expected due to the effects of the SCN5A-1795insD mutation, using this linkage model, high LOD-scores were observed for conduction (PQ, QRS) and repolarization (QTc) indices, at the region of SCN5A on chromosome 3 (LOD-scores of 12.9, 6.3 and 19.5, respectively).

Using the model with additional correction for SCN5A-1795insD mutation carriership, linkage emerged that was not detected in the previous analysis (Table 4).
Chapter 06

TABLE 3  EFFECTS OF AGE, GENDER AND CARRIER STATUS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Female vs. male</th>
<th>Age (per year)</th>
<th>Carriers vs. non-carriers</th>
<th>Age x carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β±SE</td>
<td>p-value</td>
<td>β±SE</td>
<td>p-value</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>2.7±2.1</td>
<td>0.204</td>
<td>0.27±0.06</td>
<td>2.6e-06</td>
</tr>
<tr>
<td>PQ (ms)</td>
<td>-8.4±2.9</td>
<td>0.004</td>
<td>0.72±0.07</td>
<td>3.3e-20</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>-8.2±1.7</td>
<td>2.8e-06</td>
<td>0.09±0.06</td>
<td>0.118</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>2.8±3.5</td>
<td>0.423</td>
<td>0.38±0.09</td>
<td>4.8e-05</td>
</tr>
<tr>
<td>ST (0.1mV) †</td>
<td>lower 4.1e-04</td>
<td>none</td>
<td>higher 3.4e-04</td>
<td>increase 0.016</td>
</tr>
</tbody>
</table>

* Model 1: gender and age; model 2: gender, age and carrier status (and interaction if p<0.01). SE, standard error; †, rank transformed

chromosome 21 locus in the region of KCNE1 and KCNE2 showed significant linkage to PQ-interval (LOD= 3.7), while the chromosome 3 region harboring the candidate genes SCN5A, GPD1L and CAV3 displayed suggestive linkage with PQ interval (LOD=3.1) and heart rate (LOD=2.0).

ASSOCIATION ANALYSIS

In association analysis with correction for age and gender, all phenotypes were found to be highly associated with SNPs in and around the SCN5A gene on chromosome 3 (data not shown). In association analysis with additional correction for carrier status of the mutation, association passing our stringent significance threshold was detected between SNP rs2834506 on chromosome 21 in the region of the KCNE1 and KCNE2 genes and PQ-interval (p=9.8e-08; Figure 4). A significant dominance deviation was found for this relationship and the best fitting model was a dominant genetic model. The G-allele was associated with increased PQ-intervals both in mutation carriers as well as in non-carriers (Table 5). Although the effect of the G-allele tended to be greater in mutation carriers as compared to non-carriers, an interaction model between SNP rs2834506 and carrier status showed only a weak trend for an interaction between the effect of this SNP and the mutation (p=0.27). Adding the SNP rs2834506 to the linkage model for
PQ-interval resulted in a complete disappearance of the linkage signal on chromosome 21, indicating that this SNP underlies the linkage peak in the linkage analysis.

Relaxing the p-value threshold to the arbitrary level of \( p<5.0e^{-05} \) identified two additional associations, rs7539281 upstream of the NOS1AP was associated with QTc-interval (\( p=4.0e^{-05} \)) and rs1842082 within the RYR2 gene was associated with QRS-duration (\( p=1.2e^{-05} \)).

**DISCUSSION**

In this study we investigated the role of candidate genes encoding ion channel subunits in modulation of heart rate, and ECG indices of cardiac conduction and repolarization in a large Dutch pedigree harboring the SCN5A-1795insD mutation. Through linkage and association analyses in this family, we identified for the first time a region on chromosome 21 (21q22.12), in the region of the KCNE1 and KCNE2 genes, associated with atrio-ventricular conduction. We also validate SNPs associated to ECG parameters in recent GWAS studies. Furthermore, the large number of SCN5A-1795insD mutation carriers in this family, allowed us to extend on previous observations that conduction disease due to SCN5A mutation is progressive and that the natural age-related slowing of conduction is exacerbated in SCN5A mutation carriers.

Recent genome-wide association studies in the general population have uncovered a number of SNPs in various genes associated with heart rate, and ECG indices of conduction (PQ, QRS and repolarization (QTc)).\(^{23-25}\) As for most biological traits the loci identified display small effect sizes and in aggregate explain only a small fraction of the total heritability for a given trait. Thus a large portion of heritability remains unexplained. A complementary approach to such population studies are family-based studies which although requiring appreciable effort

### Table 3 | Models

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>R²</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1*</td>
<td>Model 2*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>PQ (ms)</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td>ST (0.1mV) †</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

### Table 4 | LOD-scores and corresponding chromosomal position

<table>
<thead>
<tr>
<th></th>
<th>Heart rate</th>
<th>PQ</th>
<th>QRS</th>
<th>QTc</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>With adjustment for age and gender</td>
<td>-</td>
<td>12.9 (3p21-p25)</td>
<td>6.3 (3p21-p25)</td>
<td>19.5 (3p21-p25)</td>
<td>-</td>
</tr>
<tr>
<td>With adjustment for age, gender and SCN5A-mutation carriership</td>
<td>2.0 (3p21-p25)</td>
<td>3.7 (21q22.12)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Only loci with logarithm of the odds (LOD)-scores above the threshold for suggestive linkage (LOD>1.9) are displayed.
and resources for recruitment of family members, offer distinct advantages. They are robust against population admixture and stratification and, allow both linkage and association to be tested. The family studied here also offers an additional advantage in that it represents a sensitized model for the traits of interest since it segregates the SCN5A-1795insD mutation associated with prolonged conduction and repolarization.

We provide strong evidence in support for a role of genetic variation on chromosome 21q22.12 in modulation of PQ interval. Evidence obtained from linkage analyses is corroborated by the highly significant association with PQ-interval obtained for multiple SNPs in this chromosomal region. The most significant SNP (rs2834506) within this region displayed a p-value (p=9.8e-08) that not only passed our stringent pre-determined threshold for significance (p<3.8e-06) but was even borderline significant when one considers the commonly used genome-wide significance p-value cut-off of p<5.0e-8 corresponding to Bonferroni adjustment for 1 million independent tests. However, a role for this chromosomal region harboring KCNE1 and KCNE2 in regulation of atrio-ventricular conduction is rather unexpected. These genes encode β-subunits, respectively MinK and MiRP-1, of the major repolarization currents $I_{Ks}$ and $I_{Kr}$. Thus, genetic variation at these genes would be expected to affect cardiac repolarization rather than conduction. However, although it is commonly held that the MinK subunit modulates the function of the $I_{Ks}$ α-subunit encoded by KCNQ1, while MiRP-1 modulates the function of the $I_{Kr}$ α-subunit encoded by KCNH2, the exclusivity of these interactions has been a subject of debate and there is evidence from co-expression studies in heterologous cells that the interaction of these β-subunits might not be restricted to one particular α-subunit. For instance, MiRP-1 has been reported to alter the functional expression of Kv4.2, KCNQ1, and HCN channels, and mutations in KCNE2 seem to modulate $I_{to}$ generated by the Kv4.3 subunit. One could therefore speculate that MinK or MiRP1 could potentially also affect channel subunits involved in conduction. In support of the idea that auxiliary channel subunits modulate multiple ionic currents, post-transcriptional gene silencing of KChIP2 in neonatal rat cardiomyocytes suppressed both the repolarizing current $I_{to}$ as well as $I_{Na}$, consistent with a functional coupling of these channels.

Another intriguing possibility is that the linkage and association signal detected for PQ at chromosome 21q22.12 is not arising through effects from either of the KCNE1 and

<table>
<thead>
<tr>
<th>Table 5</th>
<th>PQ Interval per Genotype at rs2834506 in Carriers and Non-Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (n=94)</td>
</tr>
<tr>
<td>Carriers</td>
<td>170±27 (n=51)</td>
</tr>
<tr>
<td>Non-carriers</td>
<td>150±22 (n=43)</td>
</tr>
</tbody>
</table>

Data are mean ± SD.
Novel locus for cardiac conduction

KCNE2 genes but rather through another gene in the region. Inspection of the association signals within this region shows that the most highly associated SNP, rs2834506, which we selected for genotyping by virtue of the fact that it lies upstream of the KCNE1 gene, actually lies within intron 3 of the RCAN1 gene (previously called MCIP) encoding ‘Regulator of Calcineurin 1’ (Figure 4). Rcan1 is highly expressed in heart and regulates calcineurin, a calcium-activated phosphatase that promotes hypertrophic growth of the heart.34 Interestingly, transgenic mice overexpressing constitutively active calcineurin display premature sudden death and profoundly prolonged PQ-intervals.34-36 Action potential recordings in neonatal cells from these mouse hearts before the development of hypertrophy showed decreased action potential upstroke velocity (dV/dtmax) and decreased I_{Na}, suggesting that alternations in this current are directly and independently linked to the same calcineurin signaling pathway as myocardial hypertrophy.37 One study also showed decreased SCN5A mRNA and Nav1.5 protein in calcineurin-overexpressing mice.36 Taken together, these data support a role for calcineurin in regulation of atrio-ventricular conduction through I_{Na} and therefore provide evidence for a potential role of Rcan1, as an important regulator of calcineurin, in conduction. Moreover, studies of calcium homeostasis in myocytes isolated from knock-in mice harbouring the mouse homolog of the mutation in the family;15,38 demonstrated increased intracellular Ca^{2+} as a consequence of the mutation which could be expected to activate calcineurin (C.A. Remme, A. Baartscheer, unpublished data). If this is the case and if the RCAN1 SNP we identified affects the calcineurin pathway, then one would expect to find more pronounced effects of rs2834506 in mutation carriers versus non-carriers. Indeed, although formal testing for an interaction between rs2834506 and mutation carrihership only showed a trend for an interaction, the difference in PQ-interval as a function of genotype at this SNP was much more pronounced in carriers versus non-carriers. In addition, while there were four mutation
carriers who died suddenly but who did have a pacemaker, three of them were homozygous for this SNP.

In this study we also report suggestive linkage of chromosome 3 in the region of the CAV3, GPD1L and SCN5A genes in modulation of heart rate and PQ-interval. SCN5A encodes the pore-forming α-subunit of the cardiac sodium channel, while CAV3 and GPD1L encode respectively caveolin-3 and glycerol-3-phosphate dehydrogenase 1-like protein, both interacting with the sodium channel α-subunit. This effect was detected in the analysis correcting for carriership of the SCN5A mutation, suggesting that genetic variability within this region, separate from the causal mutation in this family, also impacts on heart rate and atrio-ventricular conduction.

In recently published GWAS meta-analysis studies for QT-interval in the general population, genetic variation within or upstream of the NOS1AP gene was consistently the most significant association with this trait. In both of these studies rs12143842 emerged as the most strongly associated SNP in this region. Although in our study, this SNP was not genotyped directly, it was captured by SNP rs16847584 with an $r^2$ of 0.82 (HapMap CEU) which was in turn significantly associated with QTc in our data ($p=4.0e-04$). The SNP most strongly associated with QTc in our data was however rs7539281 ($p=4.0e-05$).

In this study, as spontaneous type-1 Brugada ECGs were sparse within the family, we chose for the intermediate continuous phenotype of ST-elevation. Although carriers showed more ST-elevation than non-carriers, we did not detect linkage or association for the region of SCN5A with this parameter.

Cardiac conduction deteriorates with aging, presumably due to degenerative changes (fibrosis) in the conduction system. In Lenegre-Lev disease, a disorder associated with mutations in SCN5A, conduction disease as a consequence of the sodium channelopathy displays a progressive nature and a French study in a family with this disorder showed that above 40 years of age, worsening of conduction with increasing age was greater in individuals carrying the SCN5A mutation as compared to non-carriers. Our data, carried out in a much larger kindred confirms these findings. Although cardiac histological studies of carriers with SCN5A-1795insD are not available, the mouse model carrying this mutation indeed displays age-related development of fibrosis not present in wild-type, attesting to a role of the SCN5A mutation in this process.

**Limitations**

As this was a candidate gene approach we are currently not informed on the effect of other genes. Although intuitive, the relevance of the present findings outside this family needs to be confirmed in other monogenic arrhythmia syndromes and population based studies.
Conclusions

We here identify a locus on chromosome 21q22.12 as a modulator of cardiac conduction, providing highly relevant candidate target genes for future studies aimed at deciphering the molecular basis of cardiac conduction.

Acknowledgements

We are indebted to the family for their participation and their cooperation throughout the years. We thank Tineke van der Laan for her genealogical research in the family. We also thank Wilma van der Roest, Eline Nannenberg, Murat Kiliçarslan and Maik Grundeken for their help. We are grateful to all the previous participants in studies related to this family as well as to all referring physicians. Dr. J.J. Houwing (Leiden University Medical Center) and Dr. E.E.J. Creemers are thanked for helpful discussion.

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