Genetics

Yield of Molecular and Clinical Testing for Arrhythmia Syndromes
Report of 15 Years’ Experience

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Background—Sudden cardiac death is often caused by inherited arrhythmia syndromes, particularly if it occurs at a young age. In 1996, we started a cardiogenetics clinic aimed at diagnosing such syndromes and providing timely (often presymptomatic) treatment to families in which such syndromes or sudden cardiac death existed. We studied the yield of DNA testing for these syndromes using a candidate-gene approach over our 15 years of experience.

Methods and Results—We analyzed the yield of DNA testing. In subanalyses, we studied differences in the yield of DNA testing over time, between probands with isolated or familial cases and between probands with or without clear disease-specific clinical characteristics. In cases of sudden unexplained death (antemortem or postmortem analysis of the deceased not performed or providing no diagnosis), we analyzed the yield of cardiological investigations. Among 7021 individuals who were counseled, 6944 from 2298 different families (aged 41±19 years; 49% male) were analyzed. In 702 families (31%), a possible disease-causing mutation was detected. Most mutations were found in families with long-QT syndrome (47%) or hypertrophic cardiomyopathy (46%). Cascade screening revealed 1539 mutation-positive subjects. The mutation detection rate decreased over time, in part because probands with a less severe phenotype were studied, and was significantly higher in familial than in isolated cases. We counseled 372 families after sudden unexplained death; in 29% of them (n=108), an inherited arrhythmia syndrome was diagnosed.

Conclusions—The proportion of disease-causing mutations found decreased over time, in part because probands with a less severe phenotype were studied. Systematic screening of families identified many (often presymptomatic) mutation-positive subjects. (Circulation. 2013;128:1513-1521.)

Key Words: arrhythmias, cardiac ▼ cardiomyopathies ▼ death, sudden, cardiac ▼ genetic counseling ▼ predictive genetic testing

Sudden cardiac death (SCD) at a young age is often caused by an inherited arrhythmia syndrome (primary electric disease or cardiomyopathy).1-4 Disease-carrying relatives of the SCD victim may also be at higher risk of untimely SCD. An increased awareness of this over the past 2 decades has led to the widespread belief that predictive screening for these diseases may prevent future SCD cases in affected families. Our ability to diagnose these diseases has been strongly aided by the discovery of disease-causing gene variants. This has led to prophylactic treatment in substantial numbers of asymptomatic mutation-positive subjects with such variants.5 Recently, the Heart Rhythm Society and European Heart Rhythm Association have issued a joint consensus statement on the role of predictive counseling and genetic testing in affected families.6 For many disorders, there is clear consensus that a proactive approach is warranted, because the yield of molecular-genetic (DNA) testing (finding the familial disease-causing gene variant) is relatively high, and potentially lifesaving therapies can be readily provided. The published yield of molecular testing differs between the various diseases, ranging from 20% (Brugada syndrome [BrS]) to 65% (long-QT syndrome [LQTS]) in primary electric diseases and from 20% to 52% in cardiomyopathies.6-13 Accordingly, there is widespread growing interest in investing in cardiogenetic care and an increasing need to establish the yield of cardiogenetic care.

Clinical Perspective on p 1521

In 1996, our group was one of the first worldwide to start a specialized cardiogenetic outpatient clinic where cardiologists, clinical genetic counselors, and molecular geneticists act synergistically.14 This design has allowed us to adopt a candidate-gene approach to find the familial disease-causing gene.

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variant, often driven by specific knowledge about inherited arrhythmia syndromes provided by the participating cardiologists. At the same time, specific molecular-genetic expertise has been deployed to determine whether gene variants thus found can be regarded as the disease-causing gene defects. Over the 15 ensuing years, we have counseled well over 7000 individuals (probands and family members) in this way. Yet it is expected that the development and imminent rapid implementation of novel high-throughput DNA analysis methods (next-generation sequencing [NGS]) will increase the yield of molecular genetics and may importantly change the ways in which cardiogenetic care will be provided in the near future.\(^{15}\)

In light of the increasing awareness of the relevance of cardiogenetic care, the widespread interest in setting up cardiogenetic care, and the expectation that DNA testing will soon change fundamentally, we here report our experience from one of the largest and longest-running cardiogenetic cohorts worldwide. We focused on the yield of DNA testing using a candidate-gene approach.

Methods

Study Patients

All counseled patients and family members were included in a research database of the Cardiogenetics Department of the Academic Medical Center (Amsterdam, Netherlands). In this retrospective analysis, we included all families (probands and relatives) who were counseled in the study period January 1, 1996, through January 1, 2011, because of suspected or confirmed primary electric disease, cardiomyopathy, or sudden unexplained death (SUD) in the family. SUD was defined as death in a person with no family history of known heart disease, which occurred suddenly (1 hour after complaints or within 12 hours of the victim being seen alive) and which was unexplained (because a relevant documented medical history [eg, syncope, seizures, palpitations] and antemortem cardiological tests were discussed with each counselee and informed consent was obtained, we performed DNA testing where appropriate. DNA testing usually started with the proband (the first person in the family with symptoms of an inherited disorder) and was conducted according to a candidate-gene approach. Thus, the choice of which gene to test was based on age, symptoms, triggers of symptoms, and results of cardiological investigation of the proband and medical reports from possibly affected relatives. As new genes were discovered during the period of the present analysis, families were revisited to test these new genes where appropriate.

To perform DNA testing, DNA was extracted from peripheral blood lymphocytes or from stored tissue specimens after autopsy. The polymerase chain reaction technique amplified the coding exons of the gene(s), and mutation detection was performed by either direct Sanger sequencing of the regions of interest, single-stranded conformational polymorphism analysis, or denaturing high-performance liquid chromatography, followed by sequencing of only those fragments with abnormal profiles. DNA variants thus identified were classified into 1 of 5 groups: Nonpathogenic variant; variant of unknown significance (VUS) type 1, 2, or 3; or pathogenic mutation.

We used a list of mutation-specific features based on in silico analysis with the mutation interpretation software Alamut (version 1.5; Interactive Biosoftware, Rouen, France). A score is given depending on the outcome of a prediction test for each feature (ie, Grantham distance, PolyPhen [polymorphism phenotyping], SIFT [sorting intolerant from tolerant amino acid substitutions], and evolutionary conservation). Depending on the total score and the availability of the variant in ≥300 ethnically matched control alleles (data obtained from the literature or the Internet, eg, 1000 Genomes, http://browser.1000genomes.org/index.html or Exome Variant Server, http://evs.gs.washington.edu/EVS, or from our own control alleles), we classified them as pathogenic, not pathogenic, or VUS (VUS1, unlikely to be pathogenic; VUS2, uncertain; or VUS3, likely to be pathogenic). Family information ( cosegregation) or functional analysis was needed to classify a variant as pathogenic. For this, we used strict criteria (Table I in the online-only Data Supplement, from van Spaendonck-Zwarts et al\(^{12}\)).

When a DNA variant was found in the proband that was considered pathogenic, subsequent cascade screening was performed. To this end, the probands were requested to distribute informative letters and application forms, written by the counselors, to their families.

In the case of arrhythmogenic right ventricular cardiomyopathy, such a classification is very uncertain at present. Often, multiple DNA variants in multiple arrhythmogenic right ventricular cardiomyopathy–linked genes are found in a single individual. Incomplete penetrance or nonpenetration is common in arrhythmogenic right ventricular cardiomyopathy; this makes it extremely difficult to establish whether these DNA variants are pathogenic\(^{16–19}\) and whether they cosegregate within families.\(^{20}\) Thus, we did not include arrhythmogenic right ventricular cardiomyopathy in our primary and secondary analyses.

### Table 1. Demographic Characteristics

<table>
<thead>
<tr>
<th>Primary Arhythmia Syndromes: LOTS, BrS, and CPVT</th>
<th>Cardiomyopathies: HCM, DCM, NCCMP, ARVC</th>
<th>Others*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probands, n</td>
<td>571</td>
<td>134</td>
<td>583</td>
</tr>
<tr>
<td>Relatives, n</td>
<td>1956</td>
<td>1868</td>
<td>832</td>
</tr>
<tr>
<td>Mean age, y (SD)</td>
<td>39 (20)</td>
<td>44 (18)</td>
<td>40 (17)</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>3416/3528</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LOTS, long-QT syndrome; and NCCMP, noncompaction cardiomyopathy.

*Others: progressive cardiac conduction disease, familial atrial fibrillation, sudden cardiac death, DPP6-related ventricular fibrillation, short-QT syndrome, Carney complex, mitochondrial disease, and premature arteriosclerosis.

Primary and Secondary Analyses

In our primary analysis, we studied the yield of DNA testing in each disease, defined by the numbers of pathogenic variants, VUS3 and VUS2, or, in the case of "idiopathic" ventricular fibrillation, a risk

![Figure 1. Number of counselees per year.](http://circ.ahajournals.org/)}
haplotype. In the case of familial SUD, we analyzed the yield of cardiological investigation (ECG, exercise test, provocation test, Holter monitoring, and cardiac imaging such as echocardiography and cardiac magnetic resonance imaging) using diagnostic strategies that we have reported previously.

In our secondary analyses, we divided the probands with a clear clinical diagnosis into isolated or familial cases and analyzed the yield of DNA testing in both groups. Cases were defined as familial if >1 person in the family was clearly affected or had symptoms typical for the disease or if SUD had occurred at ≤40 years of age (for primary arrhythmia syndromes) or ≤45 years (for cardiomyopathies) in a first-, second-, or third-degree relative. In another secondary analysis, we studied the yield of DNA testing over time by classifying the probands into one of three 5-year study periods (1996–2000, 2001–2005, and 2006–2010). Finally, we analyzed disease-specific clinical characteristics of the probands and compared them between the different study periods.

**Statistical Analysis**

Statistical analysis was performed with SPSS (IBM SPSS Statistics 19; IBM, Armonk, NY). Continuous variables were expressed as mean±SD. Group comparisons were made by Fisher exact test or with χ² test for trend. P<0.05 was considered statistically significant.

**Results**

During the study period, 7021 individuals (probands and family members) were counseled. After the exclusion of 77 individuals (those with isolated congenital heart disease or healthy partners of diseased probands), 6944 counselees (aged 41.5±18.9 years; 3416 male [49%]) from 2298 different families, of whom 946 were aged <18 years, were included in the study population (Table 1). The yearly number of counselees increased to reach ≈1000 in the last years of the present study (Figure 1).

Figure 2 shows the distribution of diseases among counselees (probands and family members). Overall, in 702 families (31%), a possible disease-causing DNA variant was detected (pathogenic mutation, VUS3 or VUS2; Figures 3 and 4). Most mutations/VUS were found in probands with LQTS (47%) and hypertrophic cardiomyopathy (HCM, 46%; Table 2). Subsequent cascade screening revealed 1539 additional mutation-positive subjects (relatives), which yielded an average of 3.2 mutation-positive subjects (proband included) per family. In relatives of families with VUS2 and VUS3, predictive DNA testing was combined with cardiological investigation. The number of relatives who tested negative for the familial mutation was 1941; they were reassured. Thus, the mean number of predictively tested relatives per proband was (1539+1941)/702=4.96±9.7 (median, 2).

We compared the yield of DNA testing among probands between isolated cases and familial cases of LQTS, BrS, catecholaminergic polymorphic ventricular tachycardia (CPVT), HCM, and dilated cardiomyopathy. In all diseases, the yield was significantly higher in familial cases than in isolated cases: LQTS, 82% (90/110) versus 29% (42/146, P<0.0001); BrS, 44% (33/75) versus 21% (29/138, P=0.0003); CPVT, 73% (11/15) versus 12% (3/25, P=0.0001); HCM, 65% (169/260) versus 40% (117/292, P=0.0010); and dilated cardiomyopathy, 39% (37/95) versus 10% (8/77, P<0.0001).

In general, the yield of DNA testing decreased over time, both in familial cases and in isolated cases. For instance, in LQTS, it decreased from 74% (28/38 families) in 1996–2000 to 55% (51/93) in 2001–2005 and 35% (53/150) in 2006–2010. Similar time-dependent changes were observed in HCM.
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and CPVT (Figure 5). This trend was explained in part by the fact that analysis of clinical characteristics in all probands (both familial and isolated cases) revealed a less severe phenotype over time: In the more recent years, LQTS probands had significantly shorter QTc intervals ($P=0.0058$), a lower proportion of BrS probands had a spontaneous (ie, without drug-provocation testing) type 1 BrS ECG ($P=0.0010$), and HCM probands had less septal hypertrophy ($P=0.0100$). In CPVT probands, however, the phenotype was not significantly different over time (Table 3).

Three hundred seventy-two families were counseled because of SUD in at least 1 close relative (aged ≤45 years). In 108 of these families (29%), an inherited disease was diagnosed (n=59 with a structural disease and n=49 with a primary electric disease). In 62% of these families (n=67), this was confirmed by the identification of a pathogenic mutation, VUS3 or VUS2. This includes a risk haplotype on chromosome 7 that contains the DPP6 gene, which we found to be associated with idiopathic ventricular fibrillation; this haplotype was detected in 15 families (Figure 6). Among all SUD families (including DPP6), 371 people tested positive for the familial mutation (including 24 probands for whom DNA was available and was tested postmortem), which yielded an average of 5.5 people per family who tested positive.

We found a substantial number of recurrent mutations (Table 4). Three MYBPC3 mutations, the risk haplotype on chromosome 7 associated with idiopathic ventricular fibrillation, several recurrent mutations in LQTS, and a PLN mutation demonstrated a founder effect. Thirty-three percent (747/2241) of all positively genotyped individuals carried 1 of these founder mutations. Testing for the risk haplotype on chromosome 7 had the largest impact, yielding an average of 7.9 mutation-positive subjects per family.

**Discussion**

In the present study cohort, the yield of DNA testing of probands with primary electric diseases was 47% in LQTS, 26% in BrS, and 37% in CPVT. Previous studies on disease-specific (LQTS, CPVT, or BrS) cohorts reported similar percentages: 30% to 64% in LQTS, 9,29,30 11% to 20% in BrS,10,31 and 35% to 47% in CPVT.9,32 In cardiomyopathies, the yield was

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**Figure 3.** Yield of molecular-genetic testing in primary electric diseases. Numbers indicate numbers of families. BrS indicates Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; LQT1, long-QT syndrome type 1; LQT2, long-QT syndrome type 2; LQT3, long-QT syndrome type 3; VUS 2, variant of unknown (uncertain) clinical significance; and VUS 3, variant of unknown (likely) clinical significance.

**Figure 4.** Yield of molecular-genetic testing in cardiomyopathies. A, Hypertrophic cardiomyopathy. B, Dilated cardiomyopathy. Numbers indicate numbers of families. LMNA indicates lamin A/C gene; MYBPC3, myosin binding protein C3-gene; MYH7, beta myosin heavy chain 7- gene; PLN, phospholamban gene; VUS 2, variant of unknown (uncertain) clinical significance; and VUS 3, variant of unknown (likely) clinical significance.
Table 2. Yield of Molecular-Genetic Testing, Expressed as Number of Different Mutations, VUS3, or VUS2 Found and Number of Mutation-Positive Subjects of a Mutation, VUS3, or VUS2

<table>
<thead>
<tr>
<th>Mutation, n (%)</th>
<th>Families, n</th>
<th>No. of Carriers</th>
<th>No. of Noncarriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS 132 (47)</td>
<td>281</td>
<td>552</td>
<td>516</td>
</tr>
<tr>
<td>CPVT 17 (37)</td>
<td>46</td>
<td>140</td>
<td>151</td>
</tr>
<tr>
<td>BrS 64 (26)</td>
<td>244</td>
<td>220</td>
<td>197</td>
</tr>
<tr>
<td>PCCD 17 (37)</td>
<td>46</td>
<td>77</td>
<td>73</td>
</tr>
<tr>
<td>HCM 300 (46)</td>
<td>648</td>
<td>789</td>
<td>556</td>
</tr>
<tr>
<td>DCM 48 (22)</td>
<td>219</td>
<td>97</td>
<td>104</td>
</tr>
<tr>
<td>ARVC 96 (39)</td>
<td>246</td>
<td>215</td>
<td>113</td>
</tr>
<tr>
<td>NCCMP 6 (29)</td>
<td>21</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>DPP6 15 (15)</td>
<td>15</td>
<td>119</td>
<td>188</td>
</tr>
<tr>
<td>Others 7 (2)</td>
<td>532</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>Total 702 (31)</td>
<td>2298</td>
<td>2241</td>
<td>1941</td>
</tr>
</tbody>
</table>

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; DPP6, DPP6-related ventricular fibrillation; HCM, hypertrophic cardiomyopathy; LQTS, long-QT syndrome; NCCMP, noncompaction cardiomyopathy; PCCD, progressive cardiac conduction disease; VUS2, variant of unknown significance type 2 (pathogenic status uncertain); and VUS3, variant of unknown significance type 3 (likely to be pathogenic).

The yield of DNA testing decreased over the observed 15-year period, both in familial cases and in isolated cases. Analysis of disease-specific clinical characteristics in probands revealed significant differences over time in probands with LQTS, BrS, and HCM, which probably explains, at least in part, this decreasing molecular yield. In the most recent study period, less severely affected probands were referred for genetic counseling and molecular diagnostics (Table 3). Moreover, families with a smaller number of clearly affected members were referred in later study periods (data not shown). With the growing number of referrals, such changes in referral pattern may be expected, that is, more referrals of patients with unclear diagnoses, often from relatively small families, in whom an inherited arrhythmia syndrome was considered, although not evident. Still, this is not necessarily undesirable; in patients with severe arrhythmias that are not fully understood, from whom an inherited arrhythmia syndrome was considered, although not evident. Still, this is not necessarily undesirable; in patients with severe arrhythmias that are not fully understood, cardiacogenic workup and DNA testing may yield a diagnosis. This may have therapeutic impact and improve prognosis; moreover, predictive testing, lifestyle advice, and timely treatment of relatives may prevent future SCD cases.

We also analyzed the yield in familial or isolated cases separately. The yield was higher in familial cases with primary electric diseases than in isolated cases and also tended to be higher in familial cases with cardiomyopathies. Still, the yield was also high in isolated cases, which indicates that DNA testing should not be limited to probands with a clear family history. Our findings support previous reports. In LQTS, Tester et al found that the proportion of genotype-positive individuals was 46% among probands with positive family history and 38% among probands without positive family history; these differences, however, were not statistically significant. In a small cohort of BrS patients, Schulze-Bahr et al reported that 6 of 16 probands with a positive family history but none of 27 probands without a positive family history were SCN5A positive. Similarly, Inglés et al reported that a family history in HCM probands is the key predictor for a positive genetic diagnosis.

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQT, long-QT syndrome.
analysis renders it likely that they may carry the mutation (e.g., if the patient’s ancestors lived in the geographic region where the founder mutation originated). Such a strategy has been implemented in the routine clinical care provided at our clinic.

### Sudden Unexplained Death

SUD at a young age (≤45 years) is often caused by an inherited arrhythmia syndrome, such as cardiomyopathy (HCM, dilated cardiomyopathy, arrhythmogenic right ventricular
diabetic cardiomyopathy; and LQTS, long-QT syndrome.

*Percentage of probands with corrected QT interval ≥480 ms or familial cases with corrected QT interval ≥460 ms.
†Percentage of probands with spontaneous type-1 Brugada ECG.
‡Percentage of probands with typical arrhythmias or typical history.
§Percentage of probands with interventricular septum ≥15 mm or familial cases with interventricular septum ≥13 mm.

Patients with restrictive and noncompaction cardiomyopathy were also included.
cardiomyopathy) or primary electric disease (LQTS, CPVT, BrS). To uncover the cause of SUD, we conducted systematic cardiological and molecular-genetic testing in relatives of the SUD victim, as we reported previously.2,5 We put great effort into obtaining the medical details of the SUD victim, the circumstances/triggers of SUD, and the results of autopsy. Given that protocols used in autopsy may vary, we found it useful to consider reexamination of the specimens by a pathologist with specialized knowledge of inherited heart disease. Moreover, we routinely retrieved tissue (preferably frozen) from the SUD victim for postmortem DNA analysis.37–39 With this strategy, we found familial disease in 29% of the entire study cohort. Similar to the yield of DNA testing in the study cohort as a whole, the yield of cardiological investigation of SUD families declined over the years.2,5 This probably reflects cohort as a whole, the yield of cardiological investigation of study cohort. Similar to the yield of DNA testing in the study cohort. The mean number of relatives per family who were referred went active cascade screening was 12.6.41 Various factors may account for this difference. Family screening for familial hypercholesterolemia is performed in a far more proactive fashion, whereby up to third- or fourth-degree relatives (as far as possible with the help of the relatives) are actively approached by a genetic service, the Foundation for Tracing Hereditary Hypercholesterolemia.42 Moreover, there may be insufficient recognition by referring physicians (and relatives of probands or SUD victims) that heart disease and SCD may be heritable and that appropriate diagnostic strategies can reveal such diseases in a large proportion of presymptomatic carriers, allowing for timely treatment. Education must thus be intensified to close this recognition gap. The institution of more cardiogenetics clinics (covering more potential referral areas) is expected to aid in these efforts.

Great technological advances in DNA testing have put us on the brink of a new era in cardiogenetics. With the advent of NGS techniques (eg, whole-exome sequencing), the number of DNA variants to be found, both disease-causing mutations and VUS, in both known and unknown genes is expected to increase drastically. This will likely increase the yield of DNA testing and cardiological testing in SUD. The present study, the largest to date (and possibly one of the last) on the yield of DNA testing in the pre-NGS era, may be a valuable reference point to quantify the advances that NGS may bring to this field. This is of particular relevance because it is likely that the increased number of DNA variants found with NGS will pose a new set of challenges. We are often faced with difficulties in interpretation of molecular-genetic data. For instance, it may be uncertain whether a found DNA variant is pathogenic (mutation or VUS?); also, there may be noncosegregation between a DNA variant and the disease phenotype.43,44 In our experience, such difficulties can only be resolved by the concerted efforts of cardiologists and (molecular) geneticists. We anticipate that with the enormous amount of molecular-genetic data that NGS will produce, intensive collaboration between cardiologists, genetic counselors, and (molecular) geneticists who combine research and patient care in specialized cardiogenetics centers and share their knowledge in international databases will become even more important.

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Disclosures
Dr Wilde is a member of the advisory board for Sorin. The remaining authors report no conflicts.

References

Table 4. Recurrent and Founder Mutations

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Mutation</th>
<th>Probands, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS</td>
<td>KCNQ1</td>
<td>p.Phe296Ser</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p.Tyr184Ser</td>
<td>7</td>
</tr>
<tr>
<td>HCM</td>
<td>MYBPC3</td>
<td>c.2373dup (p.Trp792fsX17)</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.2827C&gt;T (p.Arg943X)</td>
<td>21</td>
</tr>
<tr>
<td>DCM/ARVC</td>
<td>PLN</td>
<td>c.41_42del (p.Arg14del)</td>
<td>15</td>
</tr>
<tr>
<td>IVF</td>
<td>DPP6</td>
<td>Haplotype</td>
<td>15</td>
</tr>
<tr>
<td>PCCD</td>
<td>SCN5A</td>
<td>c.2582_2583delTT</td>
<td>12</td>
</tr>
</tbody>
</table>

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; IVF, idiopathic ventricular fibrillation; LQTS, long-QT syndrome; and PCCD, progressive cardiac conduction disease.


This study reports on 15 years of experience in genetic counseling, DNA testing, and cascade screening in cardiogenetics. In 1996, we started a cardiogenetics clinic in Amsterdam aimed at diagnosing inherited arrhythmia syndromes (primary electric diseases and cardiomyopathies), and providing timely (often presymptomatic) treatment to individuals from families who have such syndromes or have experienced a sudden cardiac death. We studied the yield of targeted genetic testing in almost 2300 probands. We found that the overall yield of DNA testing was as high as 47% (in long-QT syndrome and hypertrophic cardiomyopathy). This yield decreased over time, which was explained, at least in part, by the referral of probands with a less severe phenotype in more recent years. Moreover, the yield was significantly higher in familial cases than in isolated cases. These findings demonstrate that a clear phenotype is associated with a higher yield of DNA testing. Although these findings may be intuitive, this study is the first large study to provide solid evidence to verify this intuition. Moreover, with the rapid emergence of next-generation sequencing and exome-sequencing techniques, this study may be used as a reference to compare the yield of this targeted manner of DNA testing to newer high-throughput methods.
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Supplemental Figure 1

Classification of variations/mutations

To classify the sequence variants we have used two scoring lists. Scoring list 1 was used for missense and in-frame variants. Scoring list 2 was used for non-sense and frame-shift variants. For each variant we have analysed the outcome of many mutation specific features. If a feature could not be calculated, we selected “not possible”. This score was not taken into account for the final classification. With these scoring lists the variants were classified in 5 different classes: not pathogenic, variant of unknown clinical significance 1, 2, 3 (VUS1, unlikely to be pathogenic; VUS2, uncertain; VUS3, likely to be pathogenic), (putative) pathogenic.

Scoring list 1 (see below) consists of two parts. In the first part mutation specific features are calculated and scored based on in silico analysis using the mutation interpretation software AlaMut (version 1.5, parts 1-6 and 9) and protein alignments as offered by AlaMut and/or home made (part 6 and 7). All these features are basically based on conservation and the alteration in biophysical characteristics of the amino acid substitution. The score given to each feature is based on years of experience in interpreting sequence variants in autosomal dominant cardiac conditions in DNA diagnostics. Based on the presence and the frequency of the variant in a (preferably ethnically matched) control population a score is given (part 8 and table 1). For substitutions the exome variant server (http://evs.gs.washington.edu/EVS/) can be very useful. If a variant (i.e. insertions or deletions) was not available in the exome variant server, internet (databases, publications) or information from our own laboratory (number of index cases, control population) was used. A score is given based on the frequency and number of control alleles analysed see table 1 for the conversion. In part 9 a score is given based on splice site prediction obtained by several splice site prediction software’s available in AlaMut. Table 2 is used for the conversion. The scores obtained from the “in silico” part (1-9) are added and used to determine a sub-classification. Based on this information the highest score a variant can get is a VUS3. Family information (co-segregation), phenotypic features and/or functional analysis are needed to classify a variant as (putative) pathogenic (part 10 and 11).

Scoring list 2 (see below) consists also of two parts. The first part is based on splice predictions, general characteristics of the mutation type of variant and frequency in a control population (parts 1 till 3). Table 1 and 2 are used to determine the score with respect to frequency of the variant in controls and splice site prediction, respectively. The scores obtained from the first part (1-3) are added and used to determine a sub-classification. Based on this information the highest score a variant can get is a VUS3. Family information (co-segregation), phenotypic features and/or functional analysis are needed to classify a variant as (putative) pathogenic (part 4 and 5).
Scoring list 1

1. **PolyPhen:**
   a. HumDiv
   - Probably damaging => score 1
   - Possibly damaging => score 0.5
   - Benign => score 0
   not possible or Score: .......
   b. HumVar
   - Probably damaging => score 1
   - Possibly damaging => score 0.5
   - Benign => score 0
   not possible or Score: ..... 

2. **SIFT**
   - 0.00-0.05 : intolerant => score 1
   - >0.05 : tolerant => score 0
   not possible or Score: ..... 

3. **Grantham dist (0-215)**
   - Large distance (>140) => score 2
   - Moderate distance (≤140) => score 1
   - Benign (≤70) => score 0
   not possible or Score: ..... 

4. **Align-GVGD**
   - Class C65 most likely => score 1.25
   - Class C55 => score 1
   - Class C45 => score 0.75
   - Class C35 => score 0.5
   - Class C15/25 => score 0.25
   - Class C0 => score 0
   not possible or Score: ..... 

5. **Blosum 62**
   - ≥ -2 => score 1
   - -1 => score 0.5
   - ≥0 => score 0
   not possible or Score: ..... 

6. **Conservation between species using protein aligments**
   (use at least human, 3 other mammals and 3 lower animals like bird, frog, fly, fish)
   - All mammals and almost all lower animals => score 1
   - All mammals and a few lower animal => score 0.75
   - Almost all mammals and no lower => score 0.5
   - Other => score 0
   not possible or Score: .....
7. Conservation between isoforms (different genes) using protein alignments

- 75-100% conserved => score 0.5
- 35-74% conserved => score 0.25
- 0-34% conserved => score 0

**not possible or Score: .....**

8. Frequency in control population

a. Ethnical background of the patient matches the control population:
   - Yes
   - Unknown
   - No

b. Exome variant server (http://evs.gs.washington.edu/EVS/):

<table>
<thead>
<tr>
<th># variant alleles</th>
<th># total alleles</th>
<th>freq in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ........</td>
<td>........</td>
<td>EA (European American) alleles</td>
</tr>
<tr>
<td>2. ........</td>
<td>........</td>
<td>AA (African American) alleles</td>
</tr>
<tr>
<td>total: ........</td>
<td>........</td>
<td></td>
</tr>
</tbody>
</table>

c. Own laboratory:
   - freq. of the variant in index cases:
     \[100 \times (\text{variant in index} / \text{total number of index cases}) = \text{......\%} \]
   - freq. of the variant in controls:
     \[100 \times (\text{variant in controls} / \text{total number of controls}) = \text{......\%} \]

d. Other sources (databases, literature ...):

Determine the score based on table 1 **not possible or Score: .....**

9. In silico analysis of splicing

(Splice prediction using AlaMut, see table 2 for grouping)

- Very likely (group 4) => score 2
- Probably (group 3) => score 1
- Possibly (group 2) => score 0.5
- Not likely (group 1) => score 0

**not possible or Score: .....**

**Total score for 1 till 9:** 
**Maximum score possible**: 
("not possible” is excluded)

10. Family information/Phenotype?

The information can also come from other families or literature.
Summaries below all available information and evidence (e.g. literature references).

.. ..............................................................................................................
Very likely pathogenic => score 4
(de novo mutation or ≥6 affected family members with the mutation and no affected without the mutation*)

Probably co-segregation => score 3
(5 affected family members with the mutation and no affected without the mutation*)

Possible co-segregation => score 2
(3-4 affected family members with the mutation and no affected without the mutation*)

Co-segregation unclear => score 1
(2 affected family members with the mutation and no affected without the mutation*)

Only index => score 0

No co-segregation => score 0
(affected family member without mutation)

Score: .....

*This does not count when the phenotype is likely due to a non genetic cause like hypertension in cardiac hypertrophy or when it is likely that more than a single mutation explains the phenotype in a severely affected patient).

11. Functional analysis
Experimentally:
Is the variant functionally tested in vitro, in culture or in an animal model? If so judge based on the method used and the experimental data how convincing the conclusion is. This is important because functional assays are often not well validated.
Summaries below all available information and evidence (e.g. literature references):
…………………………………………………………………………………………
…………………………………………………………………………………………

Convincingly functionally aberrant => score 3
Possibly functionally aberrant => score 1
Unclear or not functionally aberrant => score 0

not possible or Score: .....


Sub-classification based on parts 1-9:

Calculate % score:
100* (total score for 1 till 9/ maximum score possible for 1 till 9) = …. %

% score ≥ 70%  ➔ VUS3
45% ≤ % score < 70%  ➔ VUS2
25% ≤ % score < 45%  ➔ VUS1
% score < 25%  ➔ not pathogenic*

*missense mutations which get a sub-classification “not pathogenic” but were less than two times observed in a large (>10000) population of control alleles will be upgraded to a VUS1

Final classification (including part 10 and 11):

Family information (co-segregation), phenotypic features and/or functional analysis are needed to classify a variant as (putative) pathogenic.

1. A combined score of 2 or 3 for part 10 and 11 will upgrade the score from the sub-classification one level.
2. A maximum score for part 11 (functional analysis) and a score 0 for part 10 (Family information) upgrades every sub-class to a VUS3 (a functional test on its own is not enough to give a variant the classification pathogenic).
3. A combined score of 4 for part 10 and 11 and none of the parts have a maximum score will upgrade the score from the sub-classification to a VUS3.
4. When in part 10 a maximum score is obtained the sub-classification is upgraded to a pathogenic mutation.
5. A combined score of 5 or 6 for part 10 and 11 and part 10 has not the highest score will upgrade the score from the sub-classification to a pathogenic mutation.

Score part 10 (family information): …….
Score part 11 (functional analysis): …….
Total score parts 10 & 11: …….

Conclusion:
1. Not pathogenic (neutral variant or weak modifier)
2. VUS1 (unlikely pathogenic)
3. VUS2 (unclear)
4. VUS3 (likely pathogenic)
5. Pathogenic (putative)

Comment:
If the scoring list is not in agreement with other information not included in this list indicate this below and correct the conclusion accordingly.

…………………………………………………………………………………………..
1. **Non-sense variant or predicted affect on splicing (table 2)**
   c.x-1 or -2 (acceptor) or c.y+1 or +2 (donor) and clear reduction of splice site
   Predicated values in AlaMut (group 5) => Score 4
   Stop or frameshift mutation* => Score 4

**New or altered splice site predicted (see table 2)**
   - Probably functional (group 4) => score 2
   - Possibly functional (group 3) => score 1
   - Unlikely (group 2) => score 0.5
   - Not very likely (group 1) => score 0

* When the stop or frameshift mutation is in the last 2 exons this may result in a stable protein and if the N-terminal part of the protein is not well conserved it remains uncertain whether the variant will be pathogenic.

2. **Does the mutation type fit with the disease?**
   (Think about gain or loss of function, dominant negative, haplo-insufficiency etc)
   Analysis of “real” control alleles are not necessary in case of stop, frame-shift or clear splice site mutations (c.x-1 or -2 or c.y+1 or +2) and this type of mutation fits with the disease.
   - Type of variant fits with the disease => score 5
   - Type of variant not described before in disease => score 1
   - Unlikely disease causing => score 0.5
   (Not) pathogenic because: ..............................................................
   .............................................................................................................

   **not possible or Score: …..**

3. **Frequency in control population**
   a. Ethnical background of the patient matches the control population:
      - Yes
      - Unknown
      - No

      
      | variant alleles | total alleles | freq in % |
      |-----------------|---------------|-----------|
      | 1.              |               | EA (European American) alleles  | ......% |
      | 2.              |               | AA (African American) alleles   | ......% |
      | total           |               |           | ......% |

   c. Own laboratory:
      freq. of the variant in index cases:
      100*(...... variant in index / ...... total number of index cases) = ......%  
      freq. of the variant in controls:
      100*(...... variant in controls / ...... total number of controls) = ......%  

   Other sources (databases, literature …): ..............................................................
   .............................................................................................................

   **Score: ……..**
Determine the score based on table 1 not possible or Score: ..... 

4. Family information/Phenotype?
The information can also come from other families or literature.
Summaries below all available information and evidence (e.g. literature references):

…………………………………………………………………………………………
……………………………………………………………………………………

Very likely pathogenic => score 4
(de novo mutation or ≥6 affected family members with the mutation and no affected without the mutation*)

Probably co-segregation => score 3
(5 affected family members with the mutation and no affected without the mutation*)

Possible co-segregation => score 2
(3-4 affected family members with the mutation and no affected without the mutation*)

Co-segregation unclear => score 1
(2 affected family members with the mutation and no affected without the mutation*)

Only index => score 0

No co-segregation => score 0
(Affected family member without mutation)

Score: ..... 

*This does not count when the phenotype is likely due to a non genetic cause like hypertension in hypertrophy or when it is likely that more than a single mutation explains the phenotype in a severely affected person).

5. Functional analysis

Experimentally:
Is the variant functionally tested in vitro, in culture or in an animal model? If so judge based on the method used and the experimental data how convincing the conclusion is. This is important because functional assays are often not well validated.
Summary of all available information and evidence (e.g. literature references):

…………………………………………………………………………………………
……………………………………………………………………………………

Convincingly functionally aberrant => score 3
Possibly functionally aberrant => score 1
Unclear or not functionally aberrant => score 0

not possible or Score: .....
Sub-classification based on parts 1-3:

Calculate % score:
100* (total score for 1 till 3/ Maximum score possible for 1 till 3) = …. %

% score ≥ 70% ➔ VUS3
45% ≤ % score < 70% ➔ VUS2
25% ≤ % score < 45% ➔ VUS1
% score < 25% ➔ not pathogenic*

Final classification (including part 4 and 5):

Family information (co-segregation), phenotypic features and/or functional analysis are needed to classify a variant as (putative) pathogenic.

1. A combined score of 2 or 3 for part 4 and 5 will upgrade the score from the sub-classification one level.
2. A maximum score for part 5 and a score 0 for part 4 upgrades every sub-class to a VUS3 (a functional test on its own is not enough to give a variant the classification pathogenic).
3. A combined score of 4 for part 4 and 5, and none of the parts have a maximum score, will upgrade the score from the sub-classification to a VUS3.
4. When in part 4 a maximum score is obtained the sub-classification is upgraded to a pathogenic mutation.
5. A combined score of 5 or 6 for parts 4 and 5, and part 4 has not the highest score, will upgrade the score from the sub-classification to a pathogenic mutation.

Score part 4 (family information): ……
Score part 5 (functional analysis): ……
Total score parts 4 & 5: ……

Conclusion:
1. Not pathogenic (neutral variant or weak modifier)
2. VUS1 (unlikely pathogenic)
3. VUS2 (unclear)
4. VUS3 (likely pathogenic)
5. Pathogenic (putative)

Comment:
If the scoring list is not in agreement with other information not included in this list indicate this below and correct the conclusion accordingly.

____________________________________________________________________________
____________________________________________________________________________
Table 1: Score table for the frequency of the variant in control alleles

### ≤1500 control alleles analysed:

<table>
<thead>
<tr>
<th># variant observed</th>
<th># alleles analysed</th>
<th>Action</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x in index</td>
<td>&lt; 200 index alleles</td>
<td>Analyse &gt; 300 “real” control alleles</td>
<td></td>
</tr>
<tr>
<td>1x in index</td>
<td>1x in &gt;200 index alleles and/or not in &gt;300 “real” control alleles (unknown or same ethnical background)</td>
<td>Analyse &gt;500 “real” control alleles</td>
<td>1.5</td>
</tr>
<tr>
<td>1x in index + SNP database (not genotyped)</td>
<td>1x in &lt;500 index alleles</td>
<td>Analyse &gt;500 “real” control alleles</td>
<td>1.0</td>
</tr>
<tr>
<td>1x in index + SNP database (not genotyped)</td>
<td>1x in &gt;500 index alleles or not in &gt;500 “real” control alleles</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1x in index + SNP database (genotyped)</td>
<td>≥ 2x in &gt;200 “real” control alleles (freq ≥ 1%)</td>
<td>Not pathogenic*</td>
<td></td>
</tr>
<tr>
<td>1x in index + SNP database (genotyped)</td>
<td>≤ 2x in &gt;500 “real” control alleles (freq &lt; 1%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥2x in index</td>
<td>Analyse &gt;500 “real” control alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2x in index</td>
<td>Not in &gt;500 “real” control alleles (unknown or same ethnical background)</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>≥2x in index</td>
<td>Not in &gt;500 “real” control alleles (different ethnical background)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥2x in index</td>
<td>≥1 in &gt;500 “real” control alleles</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>≥2x in index</td>
<td>1x SNP database (not genotyped) and not in &gt;500 “real” control alleles</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

### >1500 control alleles analysed:

<table>
<thead>
<tr>
<th>Variant allele frequency (%)</th>
<th>Match in ethnical background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not present</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>0&lt;freq&lt;0.02</td>
<td>Not important</td>
</tr>
<tr>
<td>0.02&lt;freq≤0.05</td>
<td>Not important</td>
</tr>
<tr>
<td>0.05&lt;freq≤0.1</td>
<td>Not important</td>
</tr>
<tr>
<td>&gt;0.1</td>
<td>Not important</td>
</tr>
<tr>
<td>In &gt; 40 control alleles</td>
<td>Not important</td>
</tr>
</tbody>
</table>
variant should not be known as a founder mutation (at least 200 index alleles should have been analysed)
Real control alleles indicate DNA from healthy people or patients that suffer from a non cardiac disease.
Table 2: Classification of potential splice site mutations using splice site predictions in AlaMut

<table>
<thead>
<tr>
<th>Classification</th>
<th># programs with (aberrant) values</th>
<th>Difference between the potential splice site and genuine splice site in %*</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aberrant values of the genuine splice donor or acceptor site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2 (2 or more)</td>
<td>All: 70≤%diff≤100%</td>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&lt;70%</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>≥2 (2 or more)</td>
<td>At least one program: 40≤%diff≤70%; Others 70≤%diff≤100%</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>≤5 (5 or less)</td>
<td>One program: ≤40%; Others 40≤%diff≤100%</td>
<td>Group 3</td>
<td></td>
</tr>
<tr>
<td>≥2 (2 or more)</td>
<td>At least two programs: ≤40%</td>
<td>Group 4</td>
<td></td>
</tr>
<tr>
<td>c.x-1 or -2 (acceptor) or c.y+1 or +2 (donor)</td>
<td></td>
<td></td>
<td>Group 5</td>
</tr>
<tr>
<td>New donor site or different value of predicted donor site that is not used in the wt gene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>downstream site</td>
<td>%diff &lt;20%</td>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>≤5 (5 or less)</td>
<td>At least one program: 80≤%diff≤100%</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>At least one program: %diff≥100%</td>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>upstream (in the exon)</td>
<td>%diff &lt;50%</td>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>≤5 (5 or less)</td>
<td>%diff &lt;90%</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>≥2 (2 or more)</td>
<td>At least one program: 50≤%diff≤60%; Others &lt;60%</td>
<td>Group 3</td>
<td></td>
</tr>
<tr>
<td>upstream (in the exon)</td>
<td>%diff ≥90%</td>
<td>Group 3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>%diff≥90%</td>
<td>Group 3</td>
<td></td>
</tr>
<tr>
<td>upstream (in the exon)</td>
<td>%diff &gt;90%; Others &gt;20%</td>
<td>Group 4</td>
<td></td>
</tr>
<tr>
<td>upstream (in the exon)</td>
<td>≥2 (2 or more)</td>
<td>At least one program: %diff&gt;90%; Others &gt;20%</td>
<td>Group 4</td>
</tr>
<tr>
<td>New acceptor site or different value of predicted acceptor site that is not used in the wt gene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downstream (in the exon)</td>
<td>%diff &lt;80%</td>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>≤5 (5 or less)</td>
<td>One program: 80≤%diff≤100%; Others: %diff &lt;80%</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>One program: &gt;100%</td>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others: %diff &lt;100%</td>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upstream site</td>
<td>%diff &lt;60%</td>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>≤5 (5 or less)</td>
<td>At least one program: 50≤%diff≤60%; Others &lt;60%</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>Upstream site</td>
<td>≥2 (2 or more)</td>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>Upstream site</td>
<td>1</td>
<td>%diff≥90%</td>
<td>Group 3</td>
</tr>
<tr>
<td>---------------</td>
<td>---</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Upstream site</td>
<td>≥2 (2 or more)</td>
<td>At least one program: 60%&lt;%diff≤90%; Others &lt;60%</td>
<td>Group 3</td>
</tr>
<tr>
<td>Upstream site</td>
<td>≥2 (2 or more)</td>
<td>At least one program: %diff&gt;90%; Others &gt;20%</td>
<td>Group 4</td>
</tr>
</tbody>
</table>

% chosen arbitrarily