# 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).[1–6] 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.[7] 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.[8] 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 life-saving 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.[8–13] 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

Received November 22, 2012; accepted August 5, 2013.

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**The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.112.000091/-/DC1.**

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*Circulation* **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.112.000091**

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 [eg, ECG] were absent, and detailed postmortem macroscopic and microscopic examination of the heart and its vessels was either not performed [postmortem analysis is not mandatory in the Netherlands] or was performed but initially unable to provide an explanation).[2] We excluded partners of probands and patients with isolated congenital (structural) heart disease.

### Molecular-Genetic Testing

After the potential advantages and disadvantages of DNA testing 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 nonpenetrance 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.

### 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

**Table 1. Demographic Characteristics**

| | Primary Arrhythmia Syndromes: LQTS, BrS, and CPVT | Cardiomyopathies: HCM, DCM, NCCMP, ARVC | Others* | Total |
|---|---|---|---|---|
| Probands, n | 571 | 1134 | 583 | 2288 |
| Relatives, n | 1956 | 1868 | 832 | 4656 |
| Mean age, y (SD) | 39 (20) | 44 (18) | 40 (17) | 42 (19) |
| Male/female, n | | | | 3416/3528 |

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, 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.

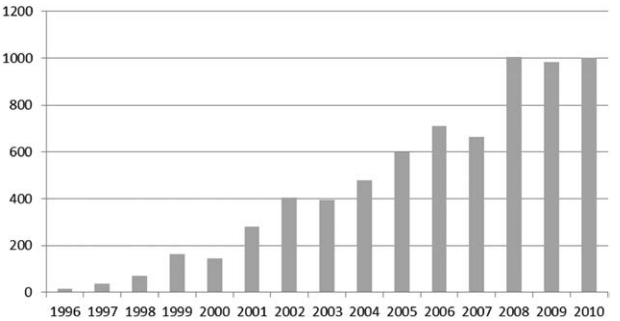



**Figure 1.** Number of counselees per year.

haplotype.[21] 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.[2]

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 $\chi^2$ 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

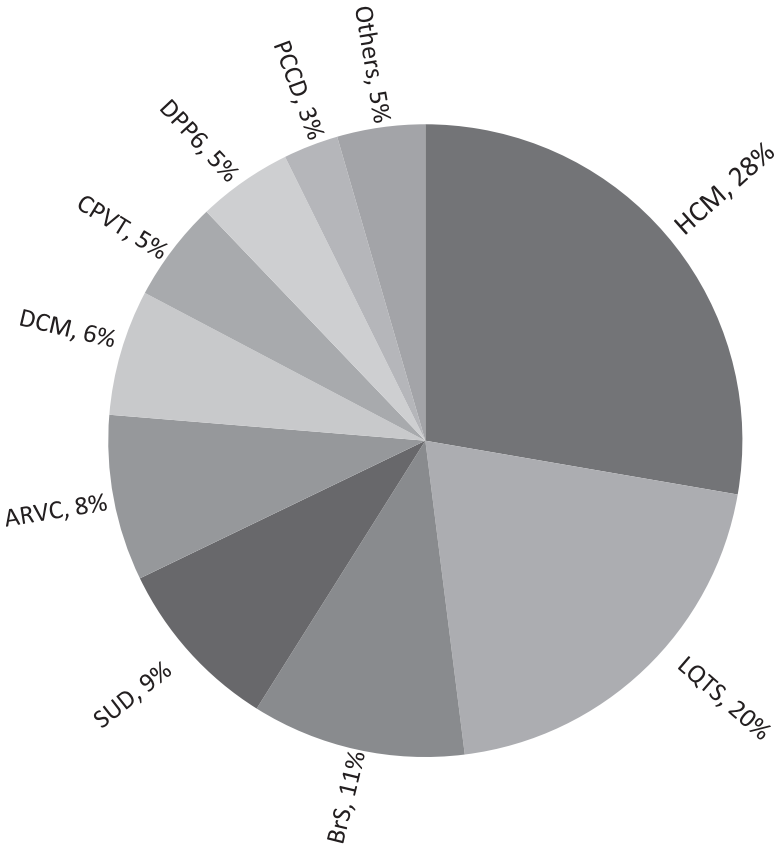



**Figure 2.** Distribution between diseases. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; DPP6, haplotype for ventricular fibrillation, including DPP6 gene; HCM, hypertrophic cardiomyopathy; LQTS, long-QT syndrome; PCCD, progressive cardiac conduction disease; and SUD, sudden unexplained death.

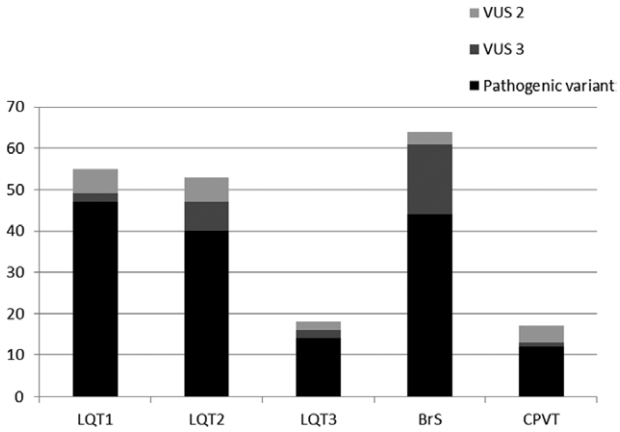



**Figure 3.** Yield of molecular-genetic testing in primary electric diseases. Numbers indicate numbers of families. BrS indicates Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; LQT1, long-QT syndrome type 1; LQT2, long-QT syndrome type 2; LQT3, long-QT syndrome type 3;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

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[21]; 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.[22–28] 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

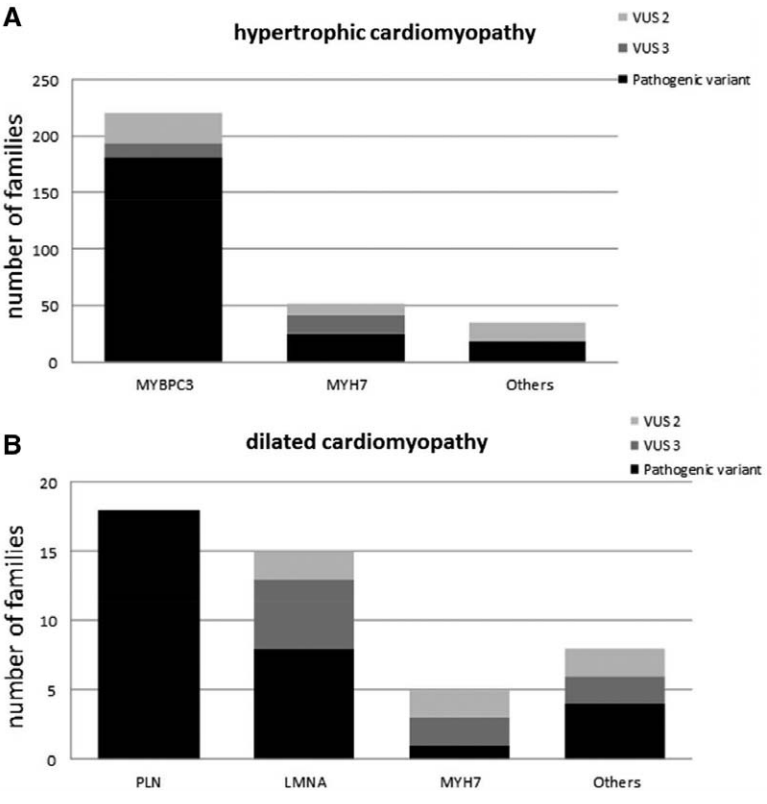



**Figure 4.** Yield of molecular-genetic testing in cardiomyopathies. **A**, Hypertrophic cardiomyopathy. **B**, Dilated cardiomyopathy. Numbers indicate numbers of families. LMNA indicates lamin A/C gene; MYBPC3, myosin binding protein C3-gene; MYH7, beta myosin heavy chain 7- gene; PLN, phospholamban 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**

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

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).

46% in HCM and 22% in dilated cardiomyopathy. These percentages compare equally well with published series: 38% to 52% in HCM[11,13] (smaller cohorts) and 20% in dilated cardiomyopathy (larger cohort that included patients from the present study).[12]

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[29] 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[31] 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[13] reported that a family history in HCM probands is the key predictor for a positive genetic diagnosis.

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, cardiogenetic 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.[8,33,34]

Through phenotype-directed DNA testing of patients with a (probable) inherited disease, we discovered a substantial number of recurrent or founder mutations, that is, mutations arising from a common ancestor, often many generations ago.[22–26,28] Phenotype-genotype studies in these large founder families have not only aided us in developing risk stratification and treatment strategies in the involved families[22] but have also allowed us to obtain novel insights into the role of the aberrant gene product and the molecular basis of SCD in general, sometimes through the construction of transgenic mouse models.[35] Large founder families are of particular interest because the relative homogeneity of the genetic substrate (identical primary mutation) is likely to increase the power of studies that aim to identify gene variants that modify the phenotype (genetic modifiers).[36] From a practical point of view, founder mutations are important because the yield of DNA testing may be increased and its cost reduced if known founder mutations are screened first in patients in whom phenotyping or pedigree

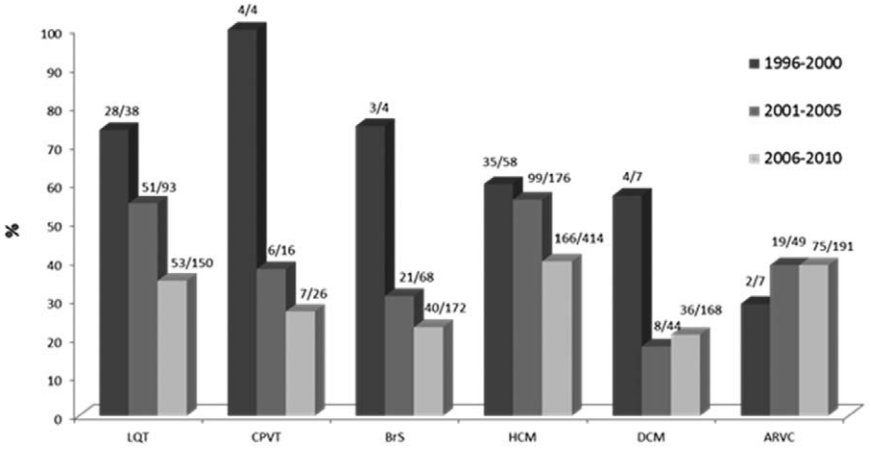



**Figure 5.** Yield of molecular-genetic testing in consecutive 5-year periods. Numbers above bars indicate cumulative number of mutations/number of families. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; and LQT, long-QT syndrome.

**Table 3. Phenotypic Characteristics Over Time**

| Disease Entity | 1996–2000 | 2001–2005 | 2006–2010 | *P* Value |
|---|---|---|---|---|
| LQTS | | | | |
| Total number of probands | 38 | 93 | 150 | |
| No. of probands with phenotype available | 35 | 89 | 131 | |
| % Phenotype present* | 51 | 48 | 31 | 0.0058 |
| CPVT | | | | |
| Total number of probands | 4 | 16 | 26 | |
| No. of probands with phenotype available | 4 | 15 | 20 | |
| % Phenotype present† | 100 | 47 | 65 | 0.6770 |
| BrS | | | | |
| Total number of probands | 4 | 68 | 172 | |
| No. of probands with phenotype available | 3 | 64 | 154 | |
| % Phenotype present‡ | 33 | 48 | 24 | 0.0010 |
| HCM | | | | |
| Total number of probands | 58 | 176 | 414 | |
| No. of probands with phenotype available | 43 | 123 | 294 | |
| % Phenotype present§ | 93 | 88 | 80 | 0.0100 |

BrS indicates Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; HCM, hypertrophic 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.

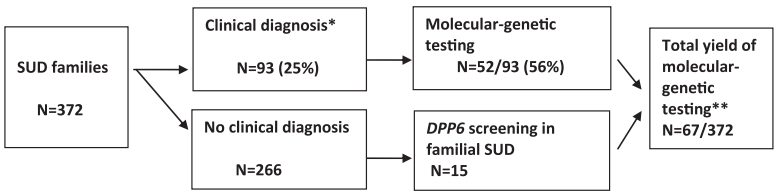
§Percentage of probands with interventricular septum ≥15 mm or familial cases with interventricular septum ≥13 mm. Patients with restrictive and noncompaction cardiomyopathy were also included.



analysis renders it likely that they may carry the mutation (eg, 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




| | * Clinical diagnosis, N | ** Molecular-genetic diagnosis, N |
|---|---|---|
| LQTS | 17 | 11 |
| CPVT | 6 | 5 |
| BrS | 9 | 2 |
| PCCD | 2 | 2 |
| HCM | 21 | 14 |
| DCM | 9 | 6 |
| ARVC | 27 | 12 |
| PAS | 2 | 0 |
| DPP6 | – | 15 |

**Figure 6.** Yield of clinical and molecular-genetic testing in sudden unexplained death (SUD). ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; DPP6, haplotype for ventricular fibrillation, including DPP6 gene; HCM, hypertrophic cardiomyopathy; LQTS, long-QT syndrome; PAS, premature arteriosclerosis; and PCCD, progressive cardiac conduction disease.

**Table 4. Recurrent and Founder Mutations**

| Disease | Gene | Mutation | Probands,* n |
|---|---|---|---|
| LQTS | *KCNQ1* | p.Phe296Ser | 4 |
| | | p.Tyr184Ser | 7 |
| | *SCN5A* | p.Ile1768Val | 9 |
| HCM | *MYBPC3* | c.2373dup (p.Trp792fsX17) | 105 |
| | | c.2827C>T (p.Arg943X) | 21 |
| | | c.2864_2865delCT (p.Pro955fsX95) | 3 |
| DCM/ARVC | *PLN* | c.41_42del (p.Arg14del) | 15 |
| IVF | *DPP6* | Haplotype | 15 |
| PCCD | *SCN5A* | c.2582_2583delTT | 12 |

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.

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 changes in referral pattern, that is, more referrals over time of patients in whom familial disease was less likely (older SUD victims and more isolated cases). Previous studies on the yield of cardiogenetic screening after SUD reported yields of 22% to 53%.[1–5] Comparison between the various previous studies and the present study is difficult because the study populations are not completely comparable in that the definitions, inclusion criteria, and workup differ considerably.

## Future Perspectives

The mean number of relatives per family who were referred for predictive testing was 4.96. This number was generally higher in families with primary electric diseases or SUD than in those with cardiomyopathies.[40] Clearly, it would be desirable to increase this number in the future, because this not only increases the likelihood of finding the diagnosis, for example, in SUD,[2] but also increases the number of relatives who benefit from timely treatment. Evidence from other fields in medicine indicates that it may be possible to increase this number and reveals how this may be achieved. For instance, in the Netherlands in 2010, the number of relatives per family of a proband with familial hypercholesterolemia who underwent 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.

## Acknowledgments

We thank Drs Yuka Mizusawa and Imke Christiaans for assistance with retrieving and analyzing additional data.

## Sources of Funding

Dr Tan was supported by the Netherlands Organization for Scientific Research (NWO, grant ZonMW Vici 918.86.616).

## Disclosures

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

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## CLINICAL PERSPECTIVE

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.