REPORT

Haplotype-Sharing Analysis Implicates Chromosome 7q36 Harboring *DPP6* in Familial Idiopathic Ventricular Fibrillation

Marielle Alders,[1,10] Tamara T. Koopmann,[2,10] Imke Christiaans,[1,3] Pieter G. Postema,[3] Leander Beekman,[2] Michael W.T. Tanck,[4] Katja Zeppenfeld,[5] Peter Loh,[6] Karel T. Koch,[3] Sophie Demolombe,[7,8,9] Marcel M.A.M. Mannens,[1] Connie R. Bezzina,[2] and Arthur A.M. Wilde[2,3],*

Idiopathic Ventricular Fibrillation (IVF) is defined as spontaneous VF without any known structural or electrical heart disease. A family history is present in up to 20% of probands with the disorder, suggesting that at least a subset of IVF is hereditary. A genome-wide haplotype-sharing analysis was performed for identification of the responsible gene in three distantly related families in which multiple individuals died suddenly or were successfully resuscitated at young age. We identified a haplotype, on chromosome 7q36, that was conserved in these three families and was also shared by 7 of 42 independent IVF patients. The shared chromosomal segment harbors part of the *DPP6* gene, which encodes a putative component of the transient outward current in the heart. We demonstrated a 20-fold increase in *DPP6* mRNA levels in the myocardium of carriers as compared to controls. Clinical evaluation of 84 risk-haplotype carriers and 71 noncarriers revealed no ECG or structural parameters indicative of cardiac disease. Penetrance of IVF was high; 50% of risk-haplotype carriers experienced (aborted) sudden cardiac death before the age of 58 years. We propose *DPP6* as a gene for IVF and increased *DPP6* expression as the likely pathogenetic mechanism.

Sudden cardiac death (SCD) is the major cause of mortality in developed countries, and the majority of SCD is caused by ventricular fibrillation (VF).[1–3] In the absence of identifiable structural heart disease or known repolarization abnormalities, it is referred to as idiopathic ventricular fibrillation (IVF [MIM 603829]). IVF accounts for as many as 10% of sudden deaths, mainly in the young.[2] The recurrence of VF in patients with IVF is about 30%, and the only effective therapy is implantation of an implantable cardioverter defibrillator (ICD).[2,4] In up to 20% of IVF cases, a family history of sudden cardiac death or IVF is present, suggesting that at least a subset of IVF is hereditary.[5–7] Because no cardiac abnormalities are observed in IVF patients, family members that may be at risk cannot be identified. Elucidation of underlying genetic defects will provide more insight into the pathogenesis of the disorder and, crucially, will allow presymptomatic identification of individuals at risk.

The identification of genes involved in IVF is very difficult. Classical linkage analysis is hampered for several reasons. Unlike that of other monogenic arrhythmia syndromes, the diagnosis of the disorder cannot be made on the basis of ECG abnormalities; it can be made only after the occurrence of (aborted) sudden cardiac death. Many affected patients die young, thus leaving only small numbers of patients and material available for analysis.

We set out to identify the culprit gene in three families (families A, B, and C; Figure 1) in which multiple individuals died suddenly or were successfully resuscitated from VF at young age. These families originate from the same area in The Netherlands and are genealogically linked through multiple lines. This unique situation of three distantly related IVF families enabled a gene-identification strategy consisting of searching for haplotypes shared in affected patients. This method identifies chromosomal segments that are identical by descent (IBD) and are likely to harbor the disease-causing gene.[8,9] Informed consent was obtained from all individuals studied.

The proband of family A (A-1) visited our cardiogenetics outpatient clinic because of the SCD of her younger brother and sister. Her brother died suddenly at age 31, in the early morning, and postmortem examination did not reveal a cause of death or cardiac abnormalities. Her sister died suddenly at age 31, at night. She underwent complete cardiac examination, which was unremarkable, a month before her death. Cardiac evaluation of the proband A-1 did not reveal any abnormalities. Six months after this examination, she suffered VF at age 44 and was successfully resuscitated. Implantation of an ICD followed, and it has never discharged during a follow-up period of 2.5 years. Just a few months later, her nephew (A-2) was also resuscitated from VF. He had an ICD implanted, and it discharged appropriately several times in the next two years after his first event.

The proband of family B (B-1) and his children contacted our cardiogenetics outpatient clinic because of the SCD of his two sons, who died at age 37 in rest and at age 32 during sleep, respectively. Postmortem examination revealed no cause of death, and cardiac abnormalities were absent in

[1]Department of Clinical Genetics, [2]Heart Failure Research Center, Department of Experimental Cardiology, [3]Department of Cardiology, [4]Department of Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, The Netherlands; [5]Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands; [6]Department of Cardiology, University Medical Center, Utrecht, The Netherlands; [7]INSERM, UMR915, L'Institut du Thorax, Nantes, France; [8]CNRS, ERL3147, Nantes, France; [9]Université de Nantes, Nantes, France
[10]These authors contributed equally to this work
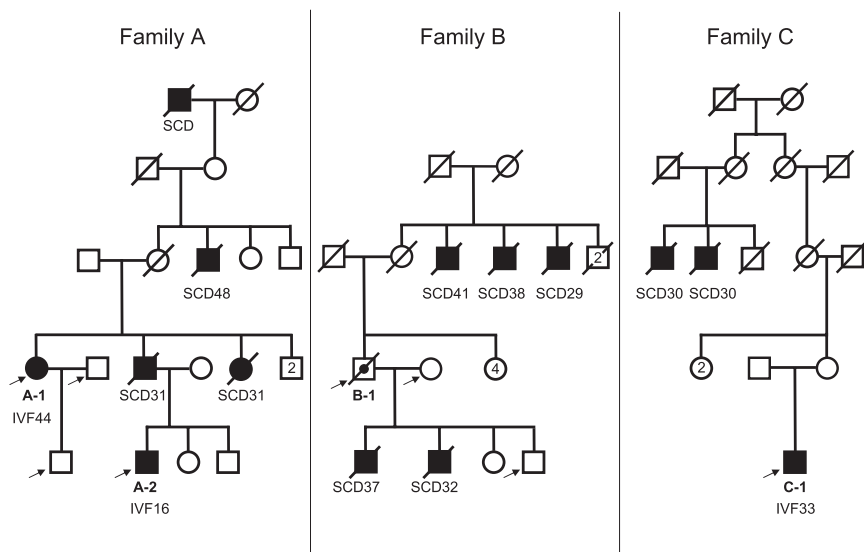*Correspondence: a.a.wilde@amc.uva.nl

Figure 1. Pedigrees of the Three IVF Families Used for the Haplotype-Sharing Analysis

Affected subjects (SCD or resuscitated after IVF) are shown as filled circles (females) or squares (males). A slash indicates that the subject is deceased, and a dot indicates obligate carrier. The numbers after SCD or IVF indicate the age of the subject at the time of the event. Individuals included in the analysis are indicated with an arrow. Numbers inside symbols indicate the number of individuals present of that sex and status; for example, individual B-1 has four unaffected sisters.

both. Although B-1 had no history of arrhythmias, he was believed to be an obligate carrier of the disease, because three siblings of his mother also died suddenly at young age. Cardiac examination ([exercise-]ECG, echocardiogram) of the proband and his children revealed no cardiac abnormalities.

The proband of family C (C-1) was admitted to our hospital after resuscitation from VF at age 33. His cardiac examination revealed no abnormalities, and an ICD was implanted. During a follow-up of four years, he had several appropriate ICD discharges. Two sons of his great grandmother had died suddenly at age 30.
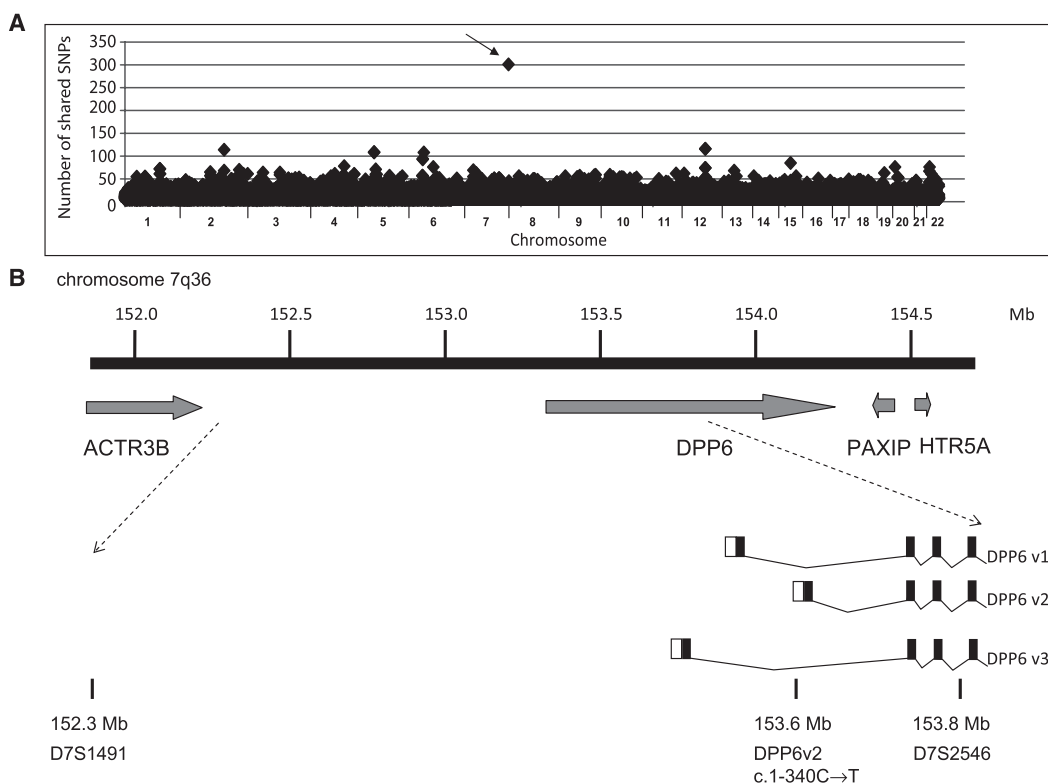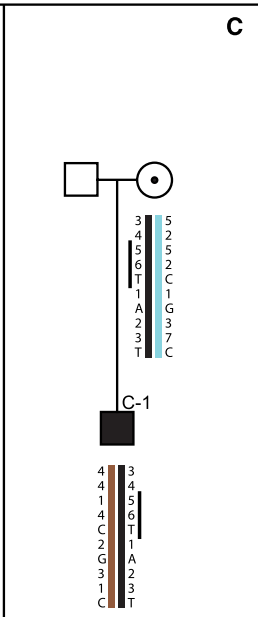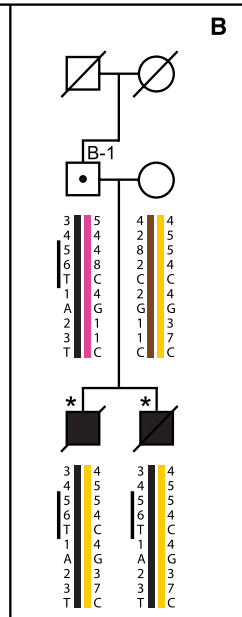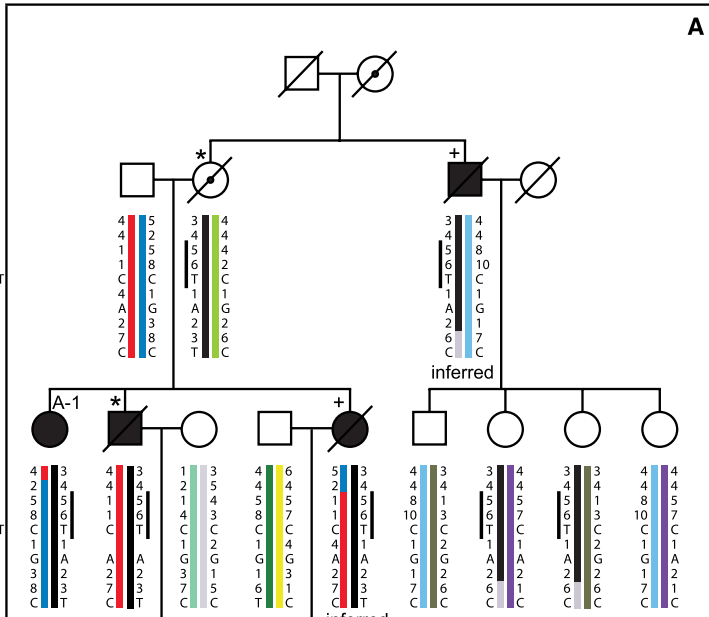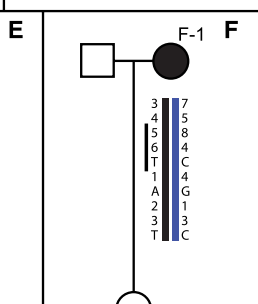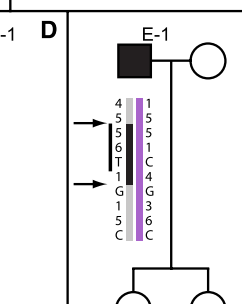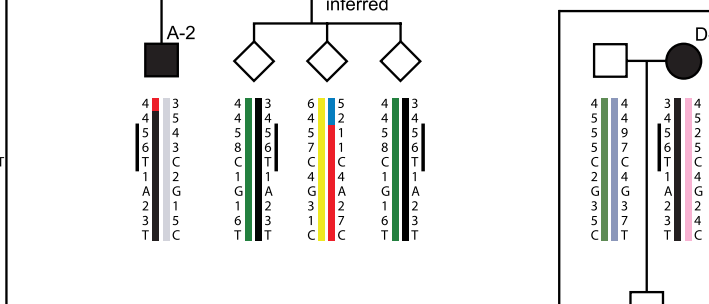

Figure 2. Mapping of the IVF Locus
(A) Result of the haplotype-sharing analysis. The length of the shared segment in number of SNPs is plotted, and the largest shared haplotype, of 301 contiguous SNPs on chromosome 7q36, is indicated by an arrow.
(B) Shared 7q36 chromosomal region, discovered after initial haplotype-sharing analysis and after extended haplotyping in additional families. The position of the markers and genes (in Mb) is shown, according to the human genome build 36.3. Isoforms 1, 2, and 3 of *DPP6* are also known as isoforms L, S, and K, respectively. Only validated genes are indicated.
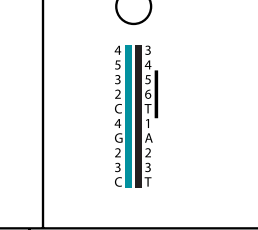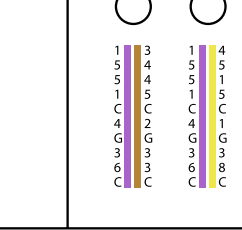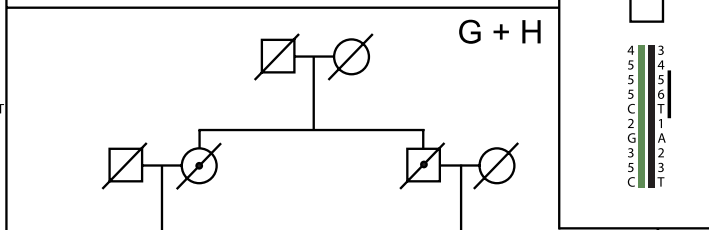
Eight individuals from these three families, indicated by arrows in Figure 1, were genotyped by genome-wide SNP analysis with the use of Illumina HumanHap 300 Bead-Chips. The obtained genotypes were analyzed in Microsoft Office Access. Haplotypes of individuals A-1 and B-1 were constructed by defining which allele was and which allele was not transmitted to the offspring. Each haplotype of A-1 was compared with each haplotype of B-1 and with the genotypes of patients A-2 and C-1. Shared segments of >5 contiguous SNPs were plotted (Figure 2A). One large haplotype of 301 contiguous SNPs on chromosome 7q36 was shared between the three families (Figure 2A), and this was confirmed by additional genotyping of microsatellite markers within this segment (D7S483, D7S798, D7S1491, D7S2462, D7S2546, D7S2447, D7S1823). Identity by descent of four smaller shared segments of >100 SNPs on chromosomes 2, 5, 6, and 12 was excluded by microsatellite analysis (chr12: D12S88, D12S365; chr2: D2S2196, D2S1334; chr5: D5S2086, D5S2024; chr6: D6S2986). Primer sequences of all markers were obtained from the UniSTS database (see Web Resources). Genomic DNA was amplified in the presence of Cy3 dCTP, and fragments were separated on an ABI310 genetic analyzer (Applied Biosystems). Results were processed with Gene-mapper software (Applied Biosystems).

The shared haplotype on chromosome 7q36 was actually 350 SNPs in length when patients B-1, A-1, and C-1 were compared. A recombination occurred when the haplotype was transmitted to A-2, decreasing the size of the shared haplotype to 301 SNPs. This haplotype was bordered by SNPs rs940261 and rs4960710. It was 2.5Mb in length and contained only four validated genes: HTR5A [MIM 601305], PAX-IP1 [MIM 608254], DPP6 [MIM 126141], and exons 10–12 of ACTR3B (Figure 2B). DPP6 encoding dipeptidyl-peptidase 6 was considered particularly interesting, because it encodes a putative subunit of potassium-channel complexes. It is predominantly expressed in brain, where it is associated with Kv4 potassium channels and Kv channel-interacting protein (KChIP) and underlies the transient subthreshold-activating, somatodendritic A-type potassium current (I_{SA}).[10] Studies by Radicke et al.[11] showed expression in heart and are suggestive for a role of DPP6 in cardiac transient outward current (I_{to}). Three human isoforms are known, and they differ at the N terminus (GenBank, NIH). Direct sequencing of all 28 coding exons in patients B-1, A-1, and C-1 revealed no mutations in the coding sequences of DPP6. However, a C→T transition 340 bases upstream of the ATG initiation codon of isoform 2 of the DPP6 gene (NM_001936) was identified in all three patients. It was not present in a control group of 350 Dutch individuals of European descent (700 alleles) and is specific for this particular haplotype.

For examination of the involvement of DPP6 in other IVF families, 42 probands of additional, independent families with one or more cases of IVF were screened for mutations in DPP6. These probands contacted our cardiogenetics outpatient clinic or were admitted to our hospital within the last 12 years (1996–2008). IVF was diagnosed if spontaneous VF occurred in the absence of electrolyte abnormalities, antiarrhythmic drugs, and any known cardiac disease as based on routine cardiac investigation, including ECG, exercise-ECG, Holter monitoring, echocardiography, coronary angiography, and MRI. In the case of unexplained sudden cardiac death, IVF was diagnosed in the absence of an identifiable cause of death upon autopsy and/or no cardiac abnormalities in relatives upon cardiac investigation.

No mutations were detected in the coding region of DPP6 in these 42 probands. However, the same variant c.1–340C→T in isoform 2 of DPP6 was identified in seven additional families. Additional haplotyping with microsatellite markers revealed that the extended haplotype was also shared between these families. However, in four of these families (families E, G, H, and I; Figure 3), the shared chromosomal region did not span the full length of the original haplotype. Recombinations must have occurred between D7S2546 and rs3807218 in families G and E, between the DPP6 variant c.1–340C→T and D7S2546 in intron 3 of DPP6 in family H, and between D7S1491 and D7S798 in families E and I (Figure 3, recombinations indicated by arrows), reducing the size of the risk haplotype to approximately 1.5 Mb and, importantly, excluding ACTR3B, PAXIP1, and HTR5A from the shared region (Figure 2B).

The identification of the variant in a substantial proportion of this independent patient group (7/42; 16.6%) and its absence in 350 controls strongly implies that this haplotype contains the disease-causing mutation ($p = 1.2 \times 10^{-7}$, Fisher's exact test). It also implies that along with families A, B, and C, the seven additional families are also descended from the same ancestor. Genealogical research identified links between most families >5 generations ago. The probands of families H and G, however, were linked more closely and were found to be third cousins. Multipoint parametric linkage analysis in the families with multiple affected individuals (families A, B, G+H, and I) was

Figure 3. Haplotypes in the Ten IVF Families
Haplotype analysis in the ten families (A to J) with the risk haplotype. Probands are labeled −1. Affected subjects (SCD or resuscitated after IVF) are shown as filled circles (females) or squares (males). A slash indicates that the subject is deceased, and a dot indicates an obligate carrier. Individuals included in the haplotype-sharing analysis are indicated (B-1, A-1, A-2, C-1), as well as individuals from whom a cardiac biopsy was used for expression studies (A-2, B-1, H-2, I-1, and J-1). The risk haplotype is colored black. Families E, G, H, and I carry recombinants, which are indicated with an arrow. The smallest shared segment, based on these recombinations, is indicated with a black bar next to the haplotype. From individuals labeled with an asterisk, DNA was extracted from paraffin-embedded tissues, and the haplotypes of individuals labeled with a "+" were inferred from their children.
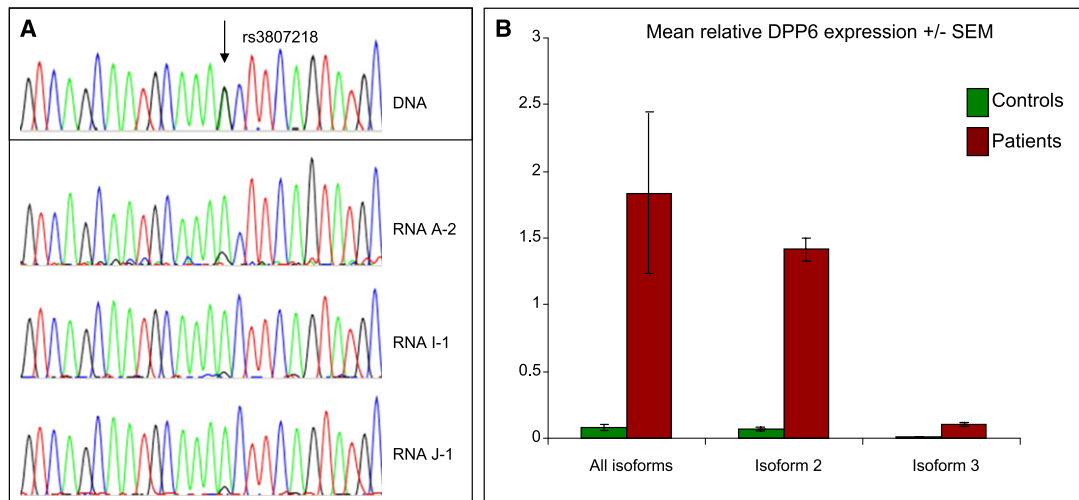
Figure 4. Expression Analysis of the *DPP6* Gene

(A) Reverse Transcriptase PCR on biopsies of three patients heterozygous for SNP rs3807218. The contribution of the A allele, which is on the risk haplotype, is increased relative to that of the G allele.

(B) *DPP6* mRNA expression in cardiac biopsies from affected individuals, relative to HPRT expression. The quantification of all *DPP6* isoforms with primers in exon 6 and 8 was performed on five patient samples, and isoform-specific analysis was performed on two patient samples. Bars represent standard errors.

performed with the use of the easyLinkage software package[12] running the GENEHUNTER v2.1 program,[13,14] with the assumption of an autosomal-dominant pattern of inheritance, a disease-allele frequency of 0.0001, and a disease penetrance of 0.70. This yielded a maximum additive LOD score of 5.9.

Despite the lack of mutations in the coding sequence of *DPP6*, it remains the only gene in the shared region. Because mutations outside coding sequences are also known to affect gene expression, we analyzed expression of *DPP6* in heart biopsies of five risk-haplotype carriers (A-2, C-1, H-2, I-1, and J-1; Figure 3). The cardiac biopsies from these patients were taken from the right interventricular septum (IVS) and were immediately frozen in liquid nitrogen. Control heart biopsies (n = 13) were taken from the IVS of hearts of controls (n = 3; Academic Medical Center Amsterdam) or from explanted nondiseased human hearts (n = 10, University of Szeged) that were technically unusable for transplantation and previously described by Gaborit et al.[15] All experimental protocols were approved by the Ethical Review Committees of the Academic Medical Center Amsterdam and the Medical Center of the University of Szeged (No. 51-57/1997 OEJ). Total RNA was extracted from pulverized heart tissue with RNA-Bee reagent (Tel-Test) or RNeasy Fibrous Tissue Mini Kit (QIAGEN) and reverse transcribed with Superscript II or Superscript III (Invitrogen) and random hexamers, in accordance with the manufacturer's protocol.

All five patients were heterozygous for SNP rs3807218 in exon 7 of *DPP6*. Amplification of *DPP6* cDNA, with the use of primers in exons 6 and 8 (present in all isoforms), and subsequent sequencing showed an imbalance in expression between the two alleles (Figure 4A). The A allele of rs3807218, representing the *DPP6* risk haplotype (see Figure 3), was overrepresented relative to the G allele, suggesting increased expression of *DPP6* from the risk-haplotype allele. To confirm upregulation of *DPP6* expression in these heart biopsies, we performed real-time quantitative PCR on a Roche LightCycler 480 Real-Time PCR System, using primers that recognize all *DPP6* transcripts and primers specific for transcript isoforms 2 and 3 (primer sequences available upon request). Isoform 1 was not tested, because no expression of this isoform could be detected in heart. Values were normalized to hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) expression levels, and measurements were done in triplicate. Data were analyzed with LinRegPCR software and were corrected for between-session variation as described previously.[16]

Average overall *DPP6* expression was increased 22-fold in patients compared to controls (p = 0.01, Mann-Whitney U test) (Figure 4B). Both isoforms 2 and 3 exhibited increased expression, although increased expression was more pronounced for isoform 2 (21-fold; p = 0.03, Mann-Whitney U test) than for isoform 3 (8-fold; p = 0.03, Mann-Whitney U test). Expression of the neighboring genes *ACTRB3* and *PAXIP1* was not elevated (data not shown), suggesting that the upregulation of *DPP6* is specific rather than due to a general increase of expression of genes in the region.

For evaluation of the clinical consequences of carrying the risk haplotype, a total of 155 relatives from the ten IVF families sharing the identical haplotype on chromosome 7q36 were genotyped and risk-haplotype carrier status was determined. Individuals with otherwise unexplained SCD at an age of <50 years were defined as risk-haplotype carriers when they had first-degree relatives carrying the risk haplotype. Haplotyping in these families also yielded obligate carriers and an obligate noncarrier of

Table 1. Demographic Data, Method of Identification, Events, and ECG Characteristics in 155 Individuals from Ten Families with IVF Linked to 7q36: Defined by Haplotype

	Risk-Haplotype Carrier (n = 84)	Risk-Haplotype Noncarrier (n = 71)	p Value
Demographics			
Mean age (years)	41 ± 20	32 ± 21	0.01
Age < 50 years of age	58 (69)	54 (76)	0.37
Male (%)	51 (61)	30 (24)	0.02
Method of Identification			
DNA haplotype	70 (83)	70 (99)	
Obligate*	6 (7)	1 (1)	
SCD <50 years of age and first-degree relative of carrier*	8 (10)	0 (0)	
Events			
Death	25 (30)	1 (1)	
SCD	19 (76)	0 (0)	
Other causes	6 (24)	1 (100)	
Resuscitated from VF	11 (13)	0 (0)	
Setting of SCD or VF			
Sleep	9 (30)	-	
Rest	17 (57)	-	
Activity	4 (13)	-	
ECG Characteristics			
PQ (msec)	161 ± 26	151 ± 23	0.07
QRS (msec)	91 ± 13	91 ± 12	0.85
QTc (msec)	395 ± 26	389 ± 29	0.32
Heart rate (bpm)	68 ± 14	71 ± 17	0.30

Data are mean ± SD or number of patients (%). SCD denotes sudden cardiac death, asterisk indicates haplotype inferred. ECG characteristics are obtained from 47 haplotype carriers and 43 noncarriers, all >15 years of age. Differences between high- and low-risk patients were analyzed with SOLAR.[23] p values < 0.05 were considered significant.

the haplotype. Available data on echocardiography, cardiac MRI, exercise, and baseline ECG were studied, as well as data on overall mortality and clinical events (resuscitated from VF or unexplained SCD).

As shown in Table 1, 84 (54%) individuals were identified as carriers of the risk haplotype. Echocardiographic and cardiac MRI data (including delayed gadolinium-enhanced imaging) in, respectively, 23 and 10 high-risk individuals did not reveal any significant or consistent abnormality. No arrhythmias or significant ECG changes were recorded during exercise testing or Holter monitoring. Baseline ECG characteristics of relatives >15 years of age were not significantly different between carriers and noncarriers of the risk haplotype (Table 1). ECG characteristics of early repolarization[17] were found in 3 of 47 risk-haplotype carriers (6%) and in none of the noncarriers (not significant). Ajmaline provocation tests in six high-risk individuals (three of which had had an event) did not reveal any sign of Brugada syndrome.

In three individuals who were resuscitated from VF, mode of spontaneous VF onset was recorded from their ICD (n = 3) or during an invasive electrophysiological study (EPS; n = 1). This showed relatively short-coupled isolated monomorphic extrasystoles, occasionally eliciting immediate VF (Figure 5). No specific electrocardiographic abnormalities (e.g., early repolarization, right precordial ST elevation, QT prolongation) preceded the VF episode. A part of spontaneous VF episodes was preceded by several single monomorphic extrasystoles (such as in Figure 5), whereas other episodes were initiated from regular sinus rhythm by one single extrasystole. During EPS, earliest activation of the extrasystoles was mapped to the right ventricular lower free anterior wall, compatible with the morphology on the 12-lead ECG (Figure 5).

Because no abnormalities can be demonstrated using cardiac diagnostic methods in IVF, the only phenotype of the disease that can be studied is (aborted) SCD. Median survival (50%) in risk-haplotype carriers was 58 years (95% confidence interval 46–69 years; Figure 6). For the noncarriers, median age of survival could not be calculated because of the lack of events. Only one noncarrier died (no SCD); his haplotype could be reconstructed by haplotyping of his children. All other noncarriers are alive; this corresponds with survival being necessary for the obtaining of a blood sample for DNA tests. Thirty risk-haplotype carriers experienced a clinical event: 19, unexplained SCD; 11, resuscitated VF. The youngest individual experiencing an event was 16 years of age, and the oldest was 77 years of age. The mean age at event was 36 ± 13 years. Males were overrepresented in this group: they had 23 events, whereas there were seven events in females.

Unlike other arrhythmia syndromes, such as Brugada syndrome or Long-QT syndrome, penetrance of IVF cannot be assessed on the basis of an electrocardiographic phenotype. In these syndromes, penetrance of ECG characteristics is variable but relatively high compared to the low penetrance of arrhythmic (lethal) events.[18] However, penetrance of lethal events linked to the chromosome 7q36 risk haplotype is very high; only 50% survival at 58 years. Therefore, any method that allows identification of the subset of the presymptomatic family members at such a high risk of arrhythmic events is potentially life-saving. Currently, we use haplotype analysis to identify relatives at risk and treat accordingly (i.e., ICD implant). Furthermore, noncarriers of the risk haplotype in these families can be reassured of the absence of an increased risk of SCD for themselves and their offspring. This strategy is, at this time, applicable only to the families carrying this specific haplotype, and a reliable classification is made only in cases in which the entire shared haplotype (between D7S1491 and D7S2546) is present or absent. Because the causal mutation might be present anywhere on the shared segment, the risk status of asymptomatic individuals carrying part of the risk haplotype (as a consequence of a recombination event) is considered uncertain.

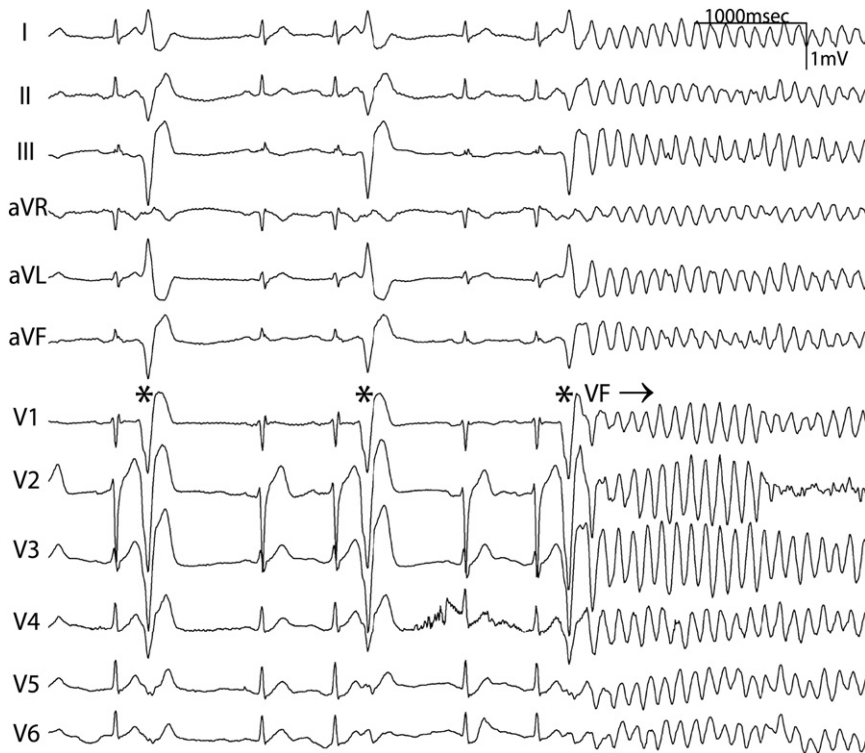Currently, we can speculate only on how overexpression of *DPP6* causes IVF. DPP6 is predominantly expressed in

brain, where it is a component of the neuronal K^+ current I_{SA}, but it is also considered an essential component of the native transient K^+ current (I_{TO}) channel complex in human heart.[11] Recent studies have shown that the characteristic kinetics of the native human cardiac I_{TO} are recapitulated only when Kv4.3 is coexpressed with DPP6 in addition to the β-subunit KChIP2.[11] In particular, DPP6 significantly alters the inactivation kinetics of both Kv4.2 and Kv4.3 and promotes expression of these alpha subunits in the cell membrane, as observed in different heterologous expression systems.[10,11,19]

Kv4 subunits appear to assemble in a 1:1 stoichiometry, meaning that such channels carry four subunits each of Kv4 and DPP6 subunits.[19] Studies using Kv4-DPP6 fusion proteins enforcing a 1:1 Kv4:DPP6 subunit ratio generated channel complexes with biophysical properties similar to those of naturally assembled channels. Of relevance to our finding of increased *DPP6* expression in the patients, coexpression of additional free DPP6 to these Kv4-DPP6 fusion channels did not affect channel kinetics, although it is well possible that translocation of Kv alpha subunits at the cell membrane is promoted, given that some increase in current amplitude was observed.[19]

The I_{TO} current, which mediates early (phase 1) cardiomyocyte repolarization, is distributed heterogeneously across the ventricular wall, with I_{TO} being more prominent in epicardium compared to endocardium. *DPP6* mRNA expression, however, appears similar between epicardium and endocardium,[11] and unless the risk haplotype disrupts this uniform expression, any increase in I_{TO} resulting from increased *DPP6* mRNA in the patients might not necessarily disturb the transmural I_{TO} gradient. In such a scenario, any increase in I_{TO} amplitude on the cardiac action-potential morphology would be expected to follow the background I_{TO} levels in the different myocardial layers. In epicardium, a deeper phase 1 may ensue with the appearance of a J-wave on the electrocardiogram,[20] a phenomenon that has recently been shown to precede ventricular arrhythmias in patients with idiopathic VF (the "early repolarization syndrome").[17] An additional increase in I_{TO} potentially leads to ST-segment elevation. However, no electrophysiological abnormalities were observed on the baseline ECG in carriers of the risk haplotype and no differences in prevalence of early repolarization were observed between haplotype carriers and noncarriers. Importantly, arrhythmic episodes appeared not to be preceded by any discernable electrocardiographic abnormality. Any morphological action-potential changes
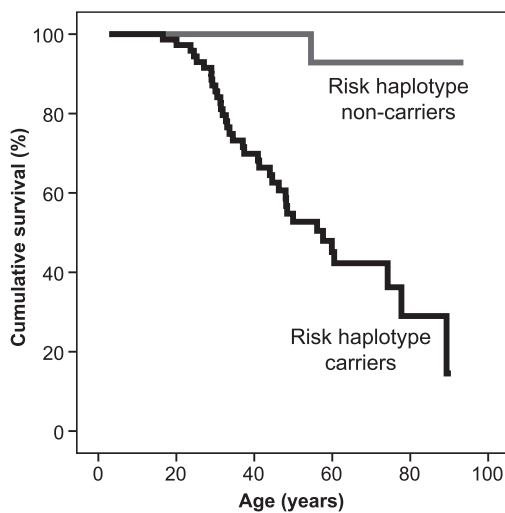
| No. of non-carriers | 71 | 46 | 20 | 10 | 1 | 0 |
| No. of carriers | 84 | 71 | 40 | 16 | 2 | 0 |

Figure 6. Survival
The survival curve of risk-haplotype carriers (black) and noncarriers (gray), demonstrating a median survival of 58 years for individuals carrying the high-risk haplotype.

that might be present are apparently not sufficient to inscribe on the ECG and, therefore, are as yet obscuring the nature of the arrhythmogenic substrate, which in fact might be very local, in the lower part of the right ventricular free wall.

The increased *DPP6* mRNA levels observed are expected to be the consequence of mutations in regulatory sequences of the gene. The c.1-340C→T variant on the risk haplotype could underlie this effect. It is in a stretch of bases conserved among dog, mouse, and rat, and it potentially creates a GATA-1-like binding site (TGATAC) on the reverse strand.[21]

However, it also remains possible that on the risk haplotype, an as yet unidentified sequence change, which could even be located tens or hundreds of kilobases away from the initiation codon, could still underlie the increased *DPP6* expression from the risk haplotype.[22]

In total, 10 of the 45 families studied carry the risk haplotype, suggesting that approximately 20% of cases in our clinic (which serves as a referral center for a large area in The Netherlands) are due to a founder mutation. Additional studies in cohorts of other ethnicities will have to establish to which extent *DPP6* underlies IVF in other populations.

In conclusion, we provide evidence for a familial component in IVF and identified a risk locus at chromosome 7q36. Our data support a role for *DPP6* as the causal gene, and we propose overexpression of this gene as the pathogenic mechanism. Identification of the genetic basis of IVF enables presymptomatic genetic diagnosis for a disorder of which the first and only symptom is potentially lethal arrhythmia.

Web Resources

The URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/

NCBI genome browser, http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

UniSTS database, http://www.ncbi.nlm.nih.gov/sites/entrez?db=unists

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