

## Functional Promoter Variant in *Desmocollin-2* Contributes to Arrhythmogenic Right Ventricular Cardiomyopathy

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### Clinical Case

A 31-year-old white male experienced ventricular fibrillation during exercise. He was successfully resuscitated without cerebral deficits and admitted to the Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Denmark. He had no medical history but had trained extensively the past year, including completing a marathon 6 months before the incident. Clinical workup showed a dilated right ventricle (Figure 1A) with segmental hypokinesia, but normal left ventricular size and function and no valvular abnormalities. ECG showed incomplete right bundle branch block with negative T-waves  $V_1$ – $V_2$ . A septal endomyocardial biopsy revealed fatty infiltrations and fibrosis (Figure 1B). On the basis of these findings, the patient was diagnosed with arrhythmogenic right ventricular cardiomyopathy (ARVC) according to Task Force criteria.<sup>1</sup> He was fitted with an implantable cardioverter–defibrillator. After 10 years of follow-up, the patient had received several appropriate implantable cardioverter–defibrillator therapies (Figure 1C). At the time of his diagnosis, no other family members were reported to have signs or symptoms of ARVC although none had been screened before presentation of the index patient. The patient was referred to genetic testing.

### Genetic Testing

The index patient was screened in the coding sequences and flanking introns of 65 known cardiomyopathy-associated

genes without identification of any disease-causing variants. Variants in desmosomal genes have been identified in approximately 40% of ARVC cases,<sup>2,3</sup> and the condition is widely regarded as a disease of the desmosome. We therefore chose also to screen a minimum of 1000 base pairs upstream of the transcriptional start site of the 5 desmosomal genes *DSC2*, *DSG2*, *DSP*, *JUP*, and *PKP2*. The upstream screening identified one rare heterozygous variant in the 5′-flanking region of desmocollin-2 (*DSC2*), –1445G>C (A in ATG=1). Variants in the coding sequence of *DSC2*, a desmosome component and member of the transmembrane cadherins, are found in ≤5% of patients, and both dominant and recessive modes of inheritance have been reported.<sup>4,5</sup> The identified –1445C variant was absent in 1300 ethnically matched Danish control chromosomes. The 1000 Genome project had no report of the –1445C allele in ethnically matched populations, but the allele has been found in individuals of African descent (rs75494355).

Family screening showed that only the index patient and his father carried the rare –1445C variant (Figure 1D), and an ECG revealed that the father had negative T-waves in  $V_1$ – $V_2$ . All other family members (brother, sister, and mother) did not carry the –1445C allele, were asymptomatic, and had a normal clinical workup.

The screening of the *DSC2* 5′-flanking region also revealed a common variant, –1304T>C (rs1790706), that was reported in an ethnically matched 1000 Genome group (minor allele frequency=allele T=18%).

### Investigation of the Noncoding *DSC2* Variant

Because the effect of regulatory *DSC2* promoter variants in ARVC is completely unknown, we performed molecular investigations of the detected –1445C variant. Multiple orthologous sequence alignment showed that *DSC2* position –1445 is evolutionary conserved (Figure 2A). In silico evaluation using web-based algorithms accessing the TRANSFAC database of transcription factors (TF) revealed an AT-rich promoter sequence around position –1445 and suggested altered binding of POU homeodomain TF OCT1 of the minor C allele (Figure 2B). The analysis also revealed a second conserved OCT1 binding site in close proximity to position –1445. In addition, a potential binding site for the myocyte-specific enhancer factor-2 (MEF2A) was detected at this AT-rich promoter sequence.

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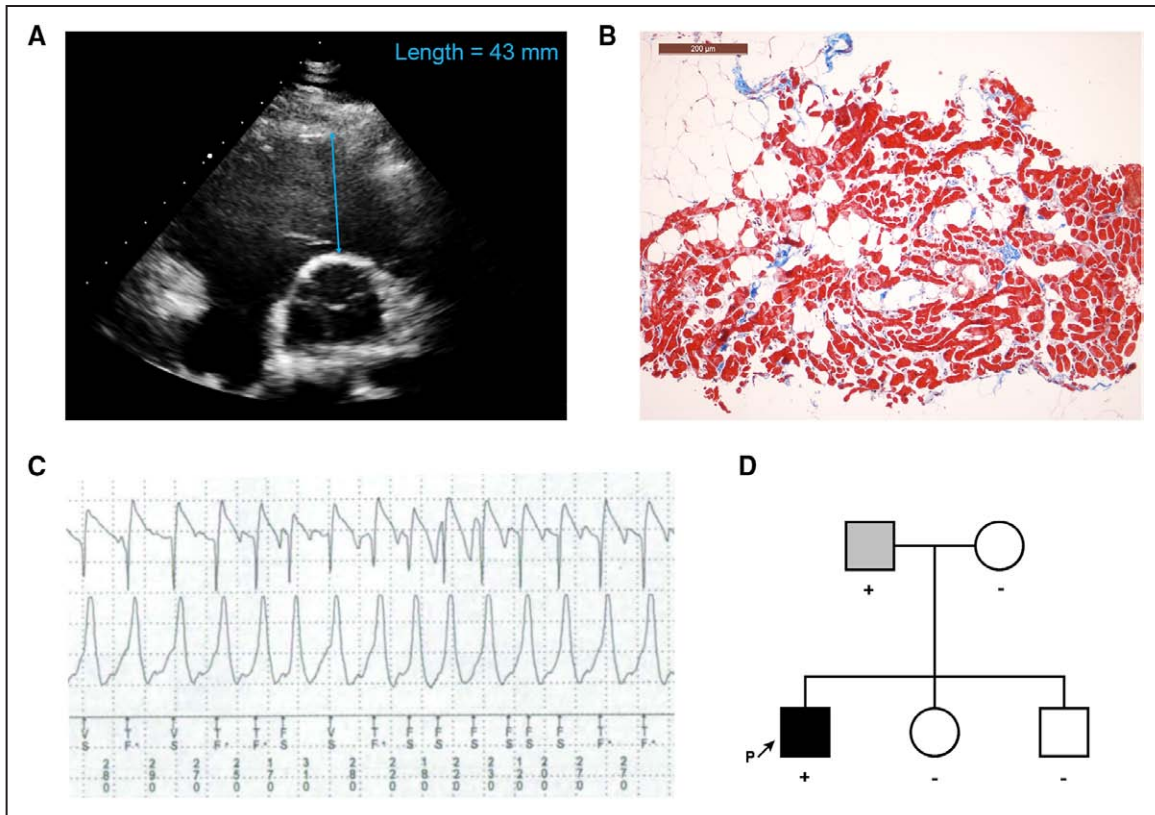
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**Figure 1.** Clinical characteristics of the index patient. **A**, Transthoracic echocardiography with short-axis parasternal view showing a dilated right ventricular outflow tract (measuring 43 mm). **B**, Septal myocardial biopsy from the index patient showing pronounced fatty infiltrations and fibrosis. **C**, Readout from the index patient's implantable cardioverter-defibrillator documenting fast ventricular tachycardia. **D**, Pedigree of the *DSC2* -1445G>C family. Women are illustrated by circles and men by squares. Filled circles/squares indicate ARVC (black), borderline ARVC (gray), or unaffected (white). The proband is marked with an arrow. Below the sex symbol, the molecular genetics finding is listed: *DSC2* -1445G>C carrier (+) or noncarrier (-).

Luciferase gene reporter assays were conducted to characterize the functional effect of the -1445C allele. The experiments revealed sufficient transcriptional activity of the *DSC2* wild-type -1445G construct under basic conditions (Figure 2C). Insertion of the mutant -1445C allele into the active *DSC2* construct resulted in a strong 5-fold reduction of *DSC2* promoter activity ( $P < 0.001$ ). Because the transforming growth factor (TGF)- $\beta$  pathway has been linked to ARVC,<sup>6</sup> *DSC2* expression is impaired in TGF- $\beta$ 1 receptor II knockout mice,<sup>7</sup> and loss of desmosome components affects TGF- $\beta$ 1/p38 mitogen-activated protein kinases signaling,<sup>8</sup> we applied TGF- $\beta$ 1 to cells transfected with the *DSC2* promoter fragments. Although we observed a significant  $\approx 1.5$ -fold activation ( $P < 0.001$ ) of the *DSC2* wild-type construct, TGF- $\beta$ 1 stimulation could not rescue the inhibitory effect of the mutant -1445C allele ( $P < 0.001$  for -1445G/TGF- $\beta$ 1 versus -1445C/TGF- $\beta$ 1).

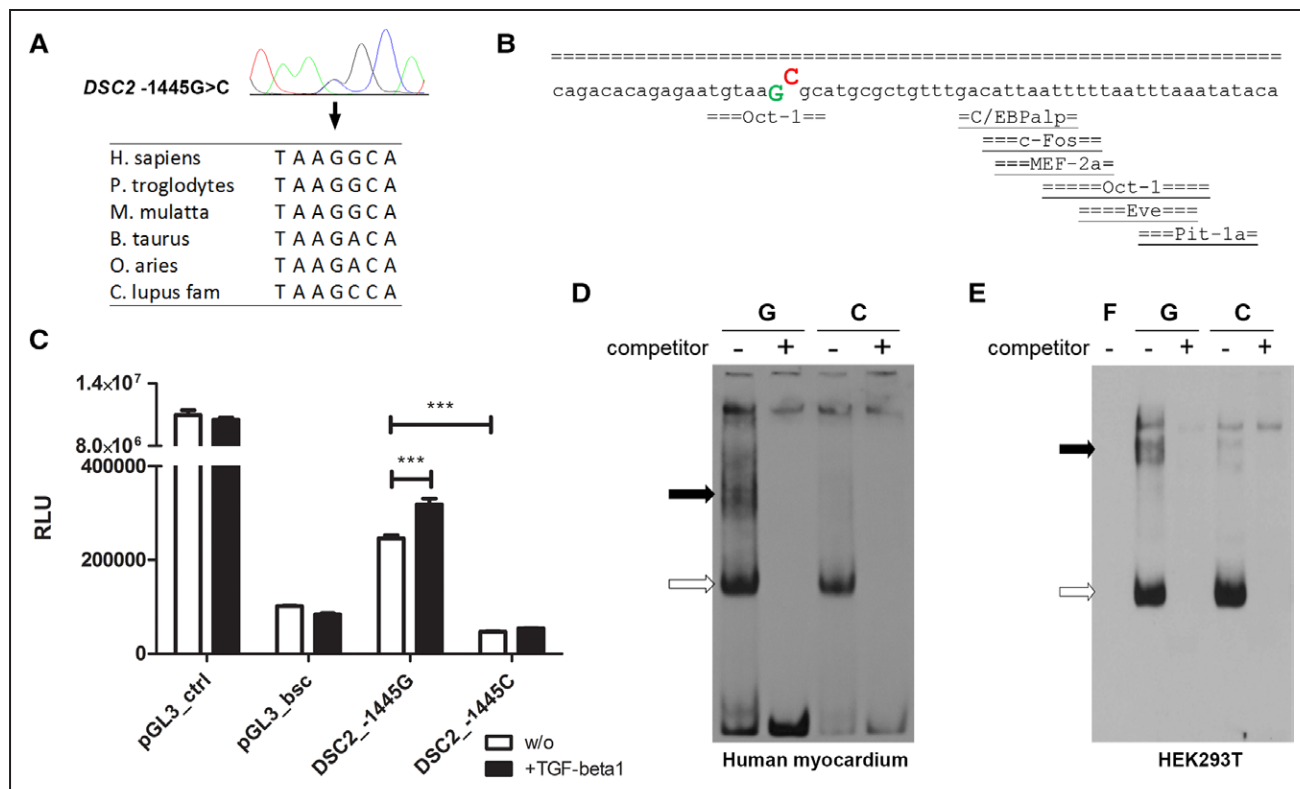
To evaluate the effect of *DSC2* -1445G>C on interaction of human myocardial TFs, electrophoretic mobility shift assays were performed. Using human nonfailing heart nuclear extracts and labeled oligonucleotides resembling the 5' sequence of *DSC2* harbouring position -1445, we detected altered TF binding in the presence of the mutant C allele (Figure 2D). Although 2 specific band shifts were detected for the wild-type G allele, 1 specific shift was lost in presence of the mutant C allele. The allele-specific difference in TF

binding was also observed using HEK293T nuclear extracts serving as an additional control (Figure 2E).

## Discussion and Conclusions

This clinical case highlights the complexity of genetic variants involved in ARVC. Reduced levels of myocardial desmocollin-2 expression have been mechanistically linked to ARVC, consistent with the generally accepted disease mechanism: desmosomal gene variants cause ARVC by lowering the amount of functional protein (loss-of-function or dominant-negative mutations) and thereby possibly altering Wnt/ $\beta$ -catenin signaling causing upregulation of fibrotic and adipogenic genes.<sup>9</sup> Furthermore, knockdown of the *DSC* orthologue in zebrafish caused a cardiomyopathy phenotype.<sup>4</sup> However, in our small family, we did not observe cosegregation with full expressivity. *DSC2* -1445C alone may not be sufficient to cause a complete ARVC phenotype, suggesting that additional genetic, epigenetic, or environmental factors are necessary for a full phenotype. Our findings are in line with reports on disease-modifying noncoding sequence variants for several other inherited cardiac diseases, including Brugada syndrome<sup>10</sup> and long-QT syndrome type 1.<sup>11</sup>

Interestingly, our *in silico* analyses suggest that -1445C interferes with OCT1 binding at this AT-rich promoter



**Figure 2.** Functional characterization of the *DSC2* -1445G>C variant. **A**, Chromatogram and orthologous sequence alignment with the -1445 position marked with an arrow. **B**, In silico evaluation of position -1445 suggested altered OCT1 binding at position -1445 as well as binding of OCT1 and MEF2A at the distal AT-rich promoter fragment. **C**, In vitro evaluation using luciferase assays transfected with *DSC2* wild-type (wt, -1445G) or mutant (-1445C) constructs. The wt construct exerted sufficient transcriptional activity in immortalized human kidney epithelial (IHKE) cells under basic conditions (white bars, w/o). Insertion of the mutant -1445C allele led to significantly decreased transcriptional activity. Stimulation with transforming growth factor (TGF- $\beta$ 1; 24 h, 10 ng/mL; black bars) activated the *DSC2* wt promoter. No significant activation was seen for the mutant -1445C allele. Transfection data are representative of 3 experiments. pGL3-control vector driven by a SV40 promoter and enhancer served as control for transfection efficiency. Promoter less pGL3-basic vector served as mock control. Transcriptional activity was assessed as relative light units (RLU). Data are given as mean $\pm$ SD. *P* values were calculated using ANOVA. \*\*\**P*<0.001, otherwise not significant. **D** and **E**, Immunoblot of gel shift experiments using a 3' biotinylated probe resembling position -1445 (wt -1445G or mutated -1445C allele) and human myocardial (**D**) or HEK293T (**E**) nuclear protein extracts. Specific shift are marked by arrows (filled arrow, G allele-specific shift). The unlabeled competitor (+) was applied at 200-fold excess. **F**, No nuclear extract. The free probe dissolved from the gel because of prolonged electrophoresis time.

fragment. We also detected a potential binding site of MEF2A in close proximity. It has been shown that OCT1 interacts with MEF2A in cardiomyocytes to regulate expression of genes such as cardiac troponin I (cTNI). In cTNI, MEF2A and OCT1 bind to a conserved AT-rich promoter sequence, and MEF2A transactivates the cTNI promoter.<sup>12</sup> Furthermore, MEF2-binding sites have also been described in the promoters of other cardiomyopathy-associated genes, including desmin and  $\alpha$ -myosin heavy chain.<sup>13,14</sup>

To our knowledge, this is the first report implicating regulatory desmosomal variants in the pathogenesis of ARVC. The presented data show a functional role of *DSC2* -1445G>C but does not allow classification of the variant as a definite monogenic cause of ARVC. Our case adds to the complexity of ARVC genetics, underlines the difficulty in defining causality of identified sequence variants even in known cardiomyopathy-associated genes, and the growing understanding that noncoding sequence variants also may contribute to the clinically observed phenotype.

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## Disclosures

None.

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