**The novel desmin variant p.Leu115Ile is associated with a unique form of biventricular Arrhythmogenic Cardiomyopathy**

**Short title:** *DES* p.Leu115Ile as a cause of biventricular AC

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**Word count**: 5,045

**Brief Summary**

The *DES* p.Leu115Ile variant was identified in 2% of gene elusive Arrhythmogenic Cardiomyopathy index patients. Mutation carriers from all three families exhibited a malignant biventricular form of AC, characterized by LV dysfunction and a circumferential subepicardial distribution of myocardial fibrosis. Functional studies revealed that *DES* p.Leu115Ile leads to disruption of the desmin filament network although macroscopical Desmin aggregates were absent in myocardial histology.

**Abstract**

***Background***: Arrhythmogenic Cardiomyopathy (AC) is a heritable myocardial disorder and a major cause of sudden cardiac death. It is typically caused by mutations in desmosomal genes. Desmin gene (*DES*) variants have been previously reported in AC, but with insufficient evidence to support their pathogenicity.

***Methods***: We aimed to assess a large AC patient cohort for *DES* mutations and describe a unique phenotype associated with a recurring variant in three families. A cohort of 138 probands with a diagnosis of AC and no identifiable desmosomal gene mutation were prospectively screened by whole exome sequencing.

***Results***: A single *DES* variant (p.Leu115Ile, c.343C>A) was identified in three index patients (2%). We assessed the clinical phenotypes within their families and confirmed co-segregation. One carrier required heart transplantation, two died suddenly and one died of non-cardiac causes. All cases had right and left ventricular (LV) involvement. LV late gadolinium enhancement was present in all and circumferential sub-epicardial distribution was confirmed on histology. A significant burden of ventricular arrhythmias was noted. Desmin aggregates were not observed macroscopically but analysis of the desmin filament formation in transfected cardiomyocytes derived from induced pluripotent stem cells and SW13 cells revealed cytoplasmic aggregation of mutant desmin. Atomic force microscopy revealed that the mutant form accumulates into short proto-filaments and small fibrous aggregates.

***Conclusions***: *DES* p.Leu115Ile leads to disruption of the desmin filament network and causes a malignant biventricular form of AC, characterized by LV dysfunction and a circumferential subepicardial distribution of myocardial fibrosis.

**Introduction**

Arrhythmogenic Cardiomyopathy (AC) is a heritable heart muscle disorder and constitutes one of the major causes of sudden cardiac death (SCD) in the young. AC describes a phenotypic spectrum affecting the myocardium of the right, left or both ventricles: arrhythmogenic right ventricular cardiomyopathy (ARVC), left dominant AC and biventricular AC, respectively1. Advancements and increasing availability of genetic testing contributed to the identification of the broader concept of AC including all three phenotypes. In the classic ARVC form, more than half of probands carry pathogenic variants in one of the genes encoding desmosomal proteins, however an increasing number of non-desmosomal genes have been reported as causal, especially in the context of the broader AC spectrum2-4.

Desmin, encoded by the *DES* gene, is an intermediate filament protein expressed in skeletal, smooth muscle and cardiac myocytes. Desmin is known to be involved in mechanical integrity, cellular organization, and signal transduction within the cells5. *DES* variants have been reported in a number of heart muscle diseases, such as hypertrophic, restrictive, dilated, non-compaction cardiomyopathy and even ARVC6, 7.

Herein we report the incidence of *DES* variants in our AC cohort from a large tertiary centre and describe the phenotypes associated with a single variant, combined with functional analyses.

**Materials and methods**

The study cohort consisted of a total of 138 consecutive, desmosomal gene-negative probands presenting at the Inherited Cardiac Diseases clinics of the Heart Hospital (up to 2014) and St Bartholomew’s hospital (since 2015). All probands were enrolled on the basis of a definite diagnosis of ARVC based on the 2010 Revised Task-Force criteria (TFC)8. All individuals were clinically assessed with detailed medical and family history, 12-lead ECG, signal averaged ECG (SAECG), 24 h ambulatory ECG monitoring and standard 2D transthoracic echocardiogram. Cardiac magnetic resonance imaging (CMR) was performed in selected cases. Clinical phenotyping and genetic testing were offered to relatives based on pedigree analysis. This study conformed with the ethical guidelines of the Declaration of Helsinki and has received approval by the National Health Service (NHS) Ethics Committees (REC ID: 15/LO/0549, UK). Informed written consent for inclusion in the study was obtained from all participants or, in cases of minors or deceased individuals, from first-degree family members. For detailed methods regarding the clinical parameters, genetic analysis, histology, functional analyses and statistical analyses please refer to the supplement.

**Results**

*DES variants in an AC cohort*

A single *DES* gene missense variant, c.343C>A; p.Leu115Ile, was identified in three of the 138 probands (2%). The *DES*-p.Leu115Ile variant was the only candidate present in these unrelated families, all of Caucasian British descent (Families A-C, Figure 1A) and is not present in gnomAD (last accessed in 01/08/2019). Analysis of a putative damaging effect of this variant was assessed using the VarSome database entry for this variant at <https://varsome.com/variant/hg19/DES%20Leu115Ile> (accessed on 1st November 2020).9 A number of *in silico* prediction tools including MetaSMV10 which integrates nine prediction scores (SIFT, PolyPhen-2, GERP++, MutationTaster, Mutation Assessor, FATHMM, LRT, SiPhy and PhyloP) predicted that p.Leu115Ile would be damaging. This novel variant is located in the Coil 1 domain of the desmin protein which is highly conserved in different species (Figure 1B). In the absence of functional study data, based on the American College of Medical Genetics and Genomics (ACMG) guidelines, it would be classified as likely pathogenic 11, and our data supported a pathogenic role.

The first individual assessed from Family A (Figure 1A) was A-II-2, referred for screening due to a family history of heart transplantation (HTx) due to dilated cardiomyopathy (DCM) in her brother at the age of 53 years (A-II-1) and a diagnosis of DCM in her son (A-III-1), who had died suddenly at the age of 30 years. A-II-2 died after 17 years of follow-up due to non-cardiac causes. Further family screening revealed that all three daughters of the index patient fulfilled criteria for ARVC. In family B (Figure 1A), B-II-1 presented with SCD at the age of 33 years and further cardiac screening of his family revealed abnormal phenotypes in his mother (B-I-2) and brother (B-II-2). The initial diagnosis in B-II-2 was of DCM. In family C (Figure 1A), C-II-2 presented with syncopal episodes at the age of 40 years and was subsequently diagnosed with ARVC. Further screening was clinically positive in her brother (C-II-1) and father (C-I-1).

Genetic screening of the family members showed co-segregation of the variant with phenotype, and identified a total of seven relatives harbouring the p.Leu115Ile variant with a cardiac phenotype and two individuals who were non-carriers and did not have any signs of disease expression (Table 1). There were no variant-negative individuals with the phenotype.

Haplotype analysis of 29 polymorphic markers in individuals with WES data identified a shared haplotype in p.Leu115Ile carriers between rs2272017 and rs73991563 in a 290,168bp region on chromosome 2 which includes the entire desmin gene. We then genotyped three p.Leu115Ile carriers and two unaffected family members for specific single nucleotide variants spanning the entire 290Kb region but further refinement of the common haplotype was not possible (Supplementary Table S1).

*Clinical characteristics*

Detailed clinical information was available in a total of nine individuals who harboured the p.Leu115Ile variant (aged 45 ± 19 years). None of them had symptoms, signs or history of skeletal myopathy. Seven (88%) had a definite and two a borderline diagnosis, based on the 2010 TFC for ARVC. Eight cases (89%) had significant repolarization abnormalities either in the precordial or inferior leads. All cases (100%) had right ventricular (RV) wall motion abnormalities (WMA), six (75%) had dilated RV, six (75%) dilated LV and six (75%) LV dysfunction, which was mild in five and severe in one patient. LV late gadolinium enhancement (LGE) was present in all seven cases that had available CMR and all had a circumferential sub-epicardial distribution (Figure 2). Cases A-III-2 and A-IV-1 who were negative for *DES*-p.Leu115Ile did not have LGE or any wall motion abnormalities in both ventricles. The ventricular ectopic burden per 24 h ranged from 451 to 10583. Seven cases received an implantable cardioverter defibrillator (ICD). Non-sustained ventricular tachycardia (VT) was present in six (75%) and sustained VT/appropriate ICD therapy in three cases (33%). One patient received HTx and died later due to cancer (A-II-1), two suspected carriers died suddenly (A-III-1, B-II-1) and two died of non-cardiac causes (B-I-2).

*Histology and Immunohistochemistry*

Cardiac tissue was available from the explanted heart of A-II-1 following HTx and from the post-mortem of A-II-2. Histology revealed extensive left ventricular fibrosis in both cases with the co-presence of adipose tissue in case A-II-2 (Figure 3A and 3B). Immunofluorescence revealed plakoglobin, desmoplakin, SAP97 and GSK3β signal intensity and distribution similar to control, and reduced connexin 43 signal intensity in A-II-2. Desmin staining revealed a similar distribution between the two patient samples and the control (Figure 3C).

*Functional analyses*

To examine the functional impact of *DES*-p.Leu115Ile we transfected SW-13 cells, which do not express any cytoplasmic intermediate filament protein and cardiomyocytes derived from hiPSC. In both cell types, the recombinant mutant desmin mainly forms cytoplasmic aggregates, whereas the wild-type assembles into regular intermediate filaments (Figure 4). In addition, we purified recombinant mutant and wild-type desmin and analysed the filament assembly in vitro by atomic force microscopy at the single molecular level (Figure 4). *In vitro*, the wild-type desmin assembles into typical intermediate filaments whereas the mutant form accumulates into short proto-filaments and small fibrous aggregates. These data support a pathogenic role of *DES*-p.Leu115Ile.

**Discussion**

We have identified a novel *DES* variant previously considered of uncertain significance in three desmosomal gene-negative families. We have shown that although some clinical features consistent with typical desminopathy are absent, the p.Leu115Ile variant is responsible for aberrant desmin filament network formation and causes a biventricular AC phenotype with high penetrance.

The concept of AC illustrates the continuously evolving spectrum of myocardial disease definitions3. The classic description of ARVC has changed to a more complex understanding, which includes RV dominant, LV dominant and biventricular forms1. Current diagnostic criteria are focused mainly on the RV dominant forms8. In this report, although the inclusion criterion for the probands to undergo genetic screening was the fulfilment of the 2010 revised TFC, we identified three out of 138 desmosomal negative (2%) unrelated families presenting with a biventricular form of the disease and harbouring a likely pathogenic *DES* gene variant that had not been reported before and is not present in gnomAD.

Desmin variation has been previously reported in several diseases that involve skeletal, cardiac muscle or combined types. These include dilated, hypertrophic, restrictive, arrhythmogenic cardiomyopathies as well as myofibrillar myopathy, limb girdle muscular dystrophy and desminopathy related syndromes12.

In relation to AC, the *DES* gene has been implicated, initially in 2009, in five Dutch families carrying the p.Ser13Phe variant, with variant carriers presenting a spectrum of cardiomyopathies, including AC in 13%7. Notably, desmin aggregates and peripheral myopathy were observed in some cases. Other small series (1 to 5 cases) have also identified further missense *DES* gene variants implicated in cardiac disease consistent with ARVC with predominantly right ventricular disease (Table 2). The most recent, and larger, report (variant p.Glu401Asp) described a cardiac phenotype similar to the one presented in this report, including repolarization abnormalities, circumferential subepicardial fibrosis, ventricular arrhythmia and absence of desmin aggregates from myocardial tissue samples from heterozygous mutation carriers 13. This represents a phenotype more consistent with what is increasingly described as left dominant AC14.

In all our cases carrying the *DES*-p.Leu115Ile variant, structural abnormalities were demonstrated in both ventricles. A circumferential pattern of left ventricular subepicardial myocardial fibrosis was demonstrated in all cases with an available CMR, and therefore deemed the earliest finding of left ventricular involvement. A similar pattern of non-ischaemic scar is observed in patients with AC carrying mutations in the genes encoding for desmoplakin (*DSP*) or filamin C (*FLNC*)15. In addition, all cases had right ventricular disease with at least right ventricular wall motion abnormalities and in some cases chamber dilatation. Although the scar pattern was similar to the one reported in cases carrying *DES*-p.Glu401Asp variant, a more biventricular form of the disease was demonstrated in our cases, carrying the p.Leu115Ile variant.

Almost all carriers of the p.Leu115Ile variant had LV structural/functional abnormalities, such as LV dilatation, LV dysfunction or LV WMA and fulfilled clinical criteria for the diagnosis of DCM (Table 1). Case B-I-2 did not exhibit any of these features but significant LV LGE was present. In two of the families (A and B) the diagnosis at presentation of the probands was also DCM. These cases illustrate the increasingly recognised overlap between AC and DCM. A similar overlap has been observed with carriers of mutations in *FLNC* and *DSP16, 17*. Although evidence-based diagnostic criteria are yet to be established, clinicians should suspect AC in patients with DCM features that present with arrhythmia and family history of sudden cardiac death4. Appropriate diagnosis can be important in guiding genetic testing but also disease stratification18.

A prominent arrhythmic component was observed, with all mutation carriers manifesting increased arrhythmogenesis either as increased ventricular ectopy, presence of non-sustained ventricular tachycardia (VT) or sustained VT. In regard to SCD, there was only one family member (B-II-1) who suffered SCD without any previous cardiac manifestations. None of the family members were professional athletes or participated in competitive sports. In terms of conduction disease, first degree atrioventricular block and various types of bundle branch block were observed, but no individuals with higher degrees of AV block that might require pacing were identified, contrary to what has been described in other *DES* series19, 20.

Fibro-fatty replacement of the myocardium has been considered to be one of the key components of ARVC histopathology21. Although the subepicardial distribution of fibrous replacement lesions is present in the left dominant and biventricular forms, the identification of fat has not been consistently reported22, 23. In both cases (A-II-1 and A-II-2) where myocardial histology was available, fibrous replacement of the myocardium was predominantly observed, although fatty tissue was also present in case A-II-2.

Regarding the immunofluorescence study of the myocardial samples, neither a consistent pattern or a typical protein distribution (translocation of plakoglobin from the intercalated discs, GSK3b to the cell membrane and SAP97 signal is significantly decreased in both sarcomeric and junctional pools24) was observed. However, in left-dominant forms of the disease, such as in *FLNC* gene mutation carriers, a different protein distribution signature to classic ARVC has been demonstrated25. Interestingly, desmin staining was comparable to the control tissue. A similar finding was observed by Bermudez-Jimenez et al in samples from carriers of the p.Glu401Asp variant13. Connexin 43 intensity was reduced only in A-II-2, who was documented to have significant arrhythmias including an appropriate ICD intervention, a marker that has been previously associated with increased arrhythmogenesis26.

Multiple variants have been previously reported in the Coil 1 region of the *DES* gene, that are associated with cardiomyopathy27 (Figure 1C) but the observed phenotypes seem to be variable even in closely neighbouring regions. The p.Leu115Ile variant reported in this study is located in the α-helical segment 1A of the desmin protein. α-helical segments 1A are completely conserved not just among orthologues of desmin in different species but also in human vimentin, neurofilament L protein, cytokeratins 8 and 18, and nuclear lamins A and B128. Limited phenotypic similarities of p.Leu115Ile with another known *DES* mutation, p.Leu136Pro, can be noted, the latter being associated with biventricular disease leading to heart failure and transplantation at a young age (but importantly without an arrhythmic phenotype) but spared the peripheral muscles29. The p.Leu136Pro variant affects the d-position within the heptad repeat and is predicted to destabilize the desmin molecule by the loss of a stabilizing hydrogen bond within the backbone of the α-helix29. Indeed, cell transfection experiments indicated that mutant p.Leu136Pro desmin is associated with a severe filament assembly defect and aggregates in the cytoplasm29. Similarly, p.Leu115Ile also affects the d-position of the preceding heptad sequence and we show similar deleterious effects on protein function *in vitro*. The variant p.Glu114del has been reported to be associated with biventricular dysfunction and ventricular arrhythmias but were accompanied with restrictive function abnormalities and peripheral myopathy30. The variant p.Asn116Ser has also been associated with ARVC but with the presence of peripheral myopathy and functional studies revealed impairment of intermediate filament formation31. In contrast, variant p.Gln113\_Leu115del was associated with an entirely different phenotype with left ventricular hypertrabeculation, conduction disease and peripheral myopathy with desmin aggregates being present on cardiac pathology32. Similarly, the variant p.Leu115Phe presented predominantly with cardiac conduction disease33.

Interestingly, the clinical and histological phenotype associated with p.Leu115Ile had more in common with the distant p.Glu401Asp rather than any of the other Coil 1 variants, even the ones located at the same or immediately next to the 115 amino acid location. In both variants, disruption of desmin filament network has been observed in the absence of macroscopical myocardial desmin aggregates. This suggests that the mechanisms responsible for the development of AC-related manifestations from *DES* variants and the mechanisms causing the classic forms of desminopathy, are divergent.

*Study limitations*

This study is by definition limited by the inclusion criterion of probands fulfilling the 2010 TFC for ARVC, which have a low yield of identifying forms of the disease that do not affect predominantly the right ventricle and therefore it is likely that the yield of *DES* related variants as a cause of AC is underestimated. Cases with *DES* disease causing variants are likely to remain underdiagnosed or erroneously labelled as DCM in more severe forms.

**Conclusions**

In conclusion, in a study of 138 AC probands without desmosomal gene causal variants, a novel missense variant in the *DES* gene, p.Leu115Ile was found co-segregating with disease in three families of British, Caucasian descent. Functional data further supported its pathogenicity. Absence of desmin aggregates in myocardial staining is not sufficient to disprove the significance of *DES* variants, in cases with an AC phenotype. A phenotype consistent with biventricular AC and significant overlap with DCM was observed in all individuals harbouring this variant. Increased arrhythmogenesis, LV dysfunction and a circumferential subepicardial distribution of myocardial fibrosis were dominant features of the phenotype. Clinicians treating patients with cardiomyopathy and/or heart failure should be aware of this clinical phenotype and instruct family screening and disease stratification accordingly. Putative *DES* mutations, as genetic causes of AC should be considered, when no classical desmosomal gene mutations have been identified; especially if a biventricular form is present.

**Acknowledgements**

﻿The authors are grateful to the patients and families for their continuous contributions and support for research.

**Funding Sources**

Dr. Protonotarios is supported by a British Heart Foundation clinical research fellowship grant (FS/18/82/34024). Dr. Asimaki is supported by a British Heart Foundation project grant (PG/18/27/33616). Prof. Dr. Anselmetti and Prof. Dr. Milting were supported by a grant of the *Deutsche Forschungsgesellschaft* (DFG). Dr Lopes is funded by an Medical Research Council UK Clinical Academic Partnership Award. This research study is also supported by the Fondation Leducq Transatlantic Networks of Excellence Program grant (no. 14 CVD03).

**Disclosures**

The authors have no conflicts of interest to report.

**Figure Legends**

**Figure 1**

**A:** Pedigrees of Families A, B and C according to the carrier status of the p.Leu115Ile DES gene variant. Males and females are marked with squares and circles, respectively. Mutation carriers are marked with (+) and non-carriers with (-). Suspected mutation carriers are marked with (S). Phenotype positive individuals are marked in black. Deceased individuals are annotated by a slash. Probands are indicated with black arrows. **B:** DNA sequencing electropherogram of *DES* exon 1 showing the presence of the c.343C>A; p.Leu115Ile variant in individual C-I-1 (left). Multiple protein alignment of the Coil 1 domain in desmin orthologues shows complete evolutionary conservation in different species. C: Localization of reported *DES* variants including the p.Leu115Ile (asterisk) in Arrhythmogenic Cardiomyopathy within the *DES* gene.

**Figure 2**

In this figure the typical and consistent ECG and CMR abnormalities for Leu115Ile *DES* gene carriers are illustrated (Case A-III-3). The ECG (top panel) is characterized by low limb lead voltages and T-wave inversions present throughout the precordial and inferior leads. CMR demonstrates extensive circumferential subepicardial late gadolinium enhancement (lower panel).

**Figure 3**

Eosin/haematoxylin staining of myocardial samples from A-II-1 (**A**) and A-II-2 (**B**). Extensive subepicardial myocardial fibrosis was noted in both cases, whereas there was increased presence of adipose tissue in A-II-2. **C:** Immunofluorescence staining of myocardial samples from A-II-1, A-II-2 and normal control. N-Cadherin staining showed equivalent signal intensity between the 2 patients and the control sample.

**Figure 4**

**A:** Representative confocal microscopy images of SW-13 cells, transfected with pmRuby-N1-DES-WT and pmRuby-N1-DES-p.L115I. Scale bars = 10 nm. **B:** Quantitative analysis of aggregate formation. \* p<0.05, n=4. The nonparametric Mann–Whitney test was used for statistical analysis. **C:** Representative confocal microscopy images of hiPSC-CM transfected with pmRuby-N1-DES-WT and pmRuby-N1-DES-p.L115I. Scale bars = 25 nm. **D:** Representative AFM topography scans acquired under environmental conditions of *in vitro* assembled desmin. Wild-type desmin clearly exposes long fibrous constructs with a typical length from some 100 nm to several micrometers. In comparison to the wild-type control, DES-p.L115I filament assembly is seriously impaired. The mutant form exhibits only short proto-filaments with a length in the range of approx. 250nm that often appear in a circular configuration (inset, 50 nm scale bar). Furthermore, also small filamentous aggregates can be found.

**Table 1:** Clinical characteristics of studied individuals (p.Leu115Ile variant carriers and non-carriers)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Case No** | **Age (years)** | **Sex** | **Dilated RV** | **RV WMA** | **RV LGE** | **Dilated LV** | **LV WMA** | **LV systolic dysfunction** | **LV LGE** | **TAD** | **TWI** | **Conduction Disease** | **SAECG** | **VE /24h** | **ICD** | **Complex VAs** |
| A-II-2+ | 58 | Female | Yes | Yes | NA | Yes | Yes | Severe | NA | NA | V1-V3 | 1stAVB+ RBBB+LAFB | NA | NA | Yes | NSVT, appropriate ATP |
| A-III-2- | 29 | Male | No | No | No | No | No | None | No | 40 | No | No | Negative | 3 | No | No |
| A-III-3+ | 52 | Female | Yes | Yes | No | Yes | Yes | Mild | Yes | 50 | V1-V4, I, II, aVL | No | Positive | 505 | Yes | NSVT |
| A-III-4+ | 52 | Female | No | Yes | Yes | No | Yes | Mild | Yes | 50 | V1-V3 | No | Positive | 451 | Yes | No |
| A-III-5+ | 54 | Male | Yes | Yes | No | Yes | Yes | Mild | Yes | 50 | V1-V5 | No | Positive | 820 | Yes | NSVT |
| A-IV-1- | 20 | Female | No | No | No | No | No | None | No | 40 | V1 | No | Negative | 0 | No | No |
| B-I-2+ | 81 | Female | No | Yes | No | No | No | None | Yes | NA | V1-V2 | 1stAVB | Positive | NA | No | NSVT |
| B-II-2+ | 49 | Male | Yes | Yes | NA | Yes | Yes | Severe | NA | 60 | V4-V6, II, III | 1stAVB +LAFB | NA | 2821 | Yes | Sustained VT |
| C-I-1+ | 73 | Male | No | Yes | No | No | Yes | Severe | Yes | 65 | II, III, aVF | Incomplete RBBB | NA | 10103 | No | No |
| C-II-1+ | 42 | Male | Yes | Yes | Yes | Yes | Yes | Mild | Yes | 60 | V4-V6, III, aVF | No | Negative | 6183 | Yes | NSVT |
| C-II-2+ | 40 | Female | Yes | Yes | No | Yes | Yes | Mild | Yes | 40 | V2-V6, I, II, aVF | No | Positive | 10583 | Yes | Sustained VT, NSVT |

1stAVB=First-degree atrioventricular block; ATP=Anti-tachycardia pacing; ICD=Implantable cardioverter defibrillator implantation; LAFB=Left anterior fascicular block; LGE=Late gadolinium enhancement; LV=Left ventricle; NA=Not available/applicable; NSVT=Non-sustained ventricular tachycardia; RBBB=Right bundle branch block; RV=Right ventricle; SAECG=Signal averaged ECG; TAD=Terminal activation duration (maximum between leads V1, V2, V3); TWI=T-wave inversion; VA=Ventricular arrhythmia; VE=Ventricular ectopy; VT=Ventricular tachycardia; WMA=Wall-motion abnormalities. (+) and (-) mark the presence or absence of the p.Leu115Ile *DES* gene mutation, respectively.

**Table 2**. Literature review of the reported *DES* gene variants associated with Arrhythmogenic Cardiomyopathy and reported clinical and pathological abnormalities

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Publications** | **Variant** | **Cases (n)** | **ECG abnormalities** | **Ventricular involvement** | **Ventricular Arrhythmia** | **Heart failure** | **Histology and IHC** |
| ﻿van Tintelen et al.7 and van Spaendonck-Zwarts et al.34 | ﻿c.38C>T, p.Ser13Phe | 2 | Low-voltage, precordial TWI (n=1) | RV predominance (n=2) | None | Yes (n=2) | NA |
| Vernengo L et al.30 | c.340\_342del, p.Glu114del | 3 | RBBB, LAFB | RV or LV predominance | VE | Yes | Desmin aggregates in peripheral muscle biopsy |
| ﻿Klauke et al.31 | ﻿c.347A>G, p.Asn116Ser | 1 | Precordial TWI | RV predominance | None | Yes | Myocardial fibrofatty replacement |
| Lorenzon et al. | ﻿﻿c.721A>G, p.Lys241Glu\* | 1 | Precordial TWI | RV predominance | VT | None | NA |
| ﻿van Spaendonck-Zwarts et al.34 and Otten et al.20 | ﻿c.1024A>G, p.Asn342Asp | 3 | Precordial TWI (n=1), First degree AVB (n=2) | RV or LV predominance | VE and VT (n=1), SCD (n=1) | None | Desmin aggregates in peripheral muscle biopsy |
| Bermudez-Jimenez et al.13 | ﻿ ﻿c.1203G>C , p.Glu401Asp | 23 | Low voltage (n=12), Precordial TWI (n=14), Inferior TWI (n=11) | LV predominance (n=15) | VE (n=17), VT (n=6), SCD (n=4) | Yes (n=9) | Myocardial degeneration, adipose tissue infiltration. Reduced ID DSP and PG. No desmin aggregates. |
| Ripol-Vera et al.35 | ﻿p.R415E† | 1 | None | LV predominance | VT, SCD | None | NA |
| Oomen et al.19 and Otten et al.20 | c.1360C>T. p.Arg454Trp | 5 | Complete AVB (n=3) | LV predominance (n=3) | VE and VF (n=1) and VT (n=1) | Yes (n=3) | Myocardial fibrosis (n=2) and inflammation (n=1). Reduced ID DSP, PKP2, CX43 (n=2). Desmin aggregates (n=2). |

AVB=Atrio-ventricular block; CX43=Connexin 43; DSP=Desmoplakin; EW=Epsilon wave; ID=Intercalated disc; IHC=Immunohistochemistry; LAFB=Left anterior fascicular block; LV=Left ventricular; NA=Not available; PKP2=Plakophilin-2; PG=Plakoglobin; RBBB=Right bundle branch block; RV=Right ventricular; SCD=Sudden cardiac death; TWI=T-wave inversion; VE=ventricular ectopy; VF=Ventricular fibrillation; VT=Ventricular tachycardia. (\*) A plakophilin-2 gene variant was also present. (†) cDNA change not available and variant likely erroneously reported, as, assuming the presence of a single nucleotide substitution, Arginine cannot mutate to Glutamic acid based on the human genetic code.

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