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ORIGINAL ARTICLE

# Acylation-stimulating protein and heart failure progression in arrhythmogenic right ventricular cardiomyopathy

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## Abstract

**Aims** Our previous studies suggested that the complement system was critical in the prognosis of arrhythmogenic right ventricular cardiomyopathy (ARVC). The acylation-stimulating protein (ASP), generated through the alternate complement pathway, was reported to regulate lipogenesis and triglyceride storage. This study aimed to investigate the role of ASP in predicting adverse cardiac events in an ARVC cohort.

**Methods and results** We enrolled 111 ARVC patients and 106 healthy volunteers, and measured their plasma ASP levels using enzyme-linked immunosorbent assays. Plasma ASP levels were significantly higher in the ARVC patients than in the healthy controls (2325.22 ± 20.08 vs. 2189.75 ± 15.55, $P < 0.001$), with a similar trend observed in the myocardial explant assay. Spearman correlation analysis indicated plasma ASP level associated with cardiac structural (right ventricular internal dimension, $P = 0.006$) and functional remodelling (left ventricular ejection fraction, $P = 0.002$) in ARVC patients. The ARVC patients were followed up for an average of 17.79 ± 1.09 months. Heart failure-associated events (HFAEs) were defined as heart transplantation, on a cardiac transplant list, or death due to end-stage heart failure. Plasma ASP levels in patients with HFAEs were significantly higher than in those without clinical events (2486.03 ± 26.70 vs. 2268.83 ± 23.51, $P < 0.001$) or those with malignant arrhythmic events (2486.03 ± 26.70 vs. 2297.80 ± 60.46, $P = 0.008$). LASSO (least absolute shrinkage and selection operator) and multivariable Cox regression analyses showed the ASP level (HR = 1.004, 95% CI [1.002,1.006], $P = 0.002$) was an independent predictor for adverse HFAEs in ARVC patients. The spline-fitting procedure was applied to illustrate the HFAE-free probabilities at different time points.

**Conclusions** Our results suggest that plasma ASP may be a useful biomarker in prediction of adverse HF-associated events in ARVC patients.

**Keywords** Arrhythmogenic right ventricular cardiomyopathy; ASP; Biomarker; Heart failure; Risk prediction

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## Introduction

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a genetic cardiomyopathy, with a prevalence of 0.02%–0.1% in the general population, and is one of the leading causes of sudden cardiac death (SCD) in people under the age of 35 years.[1,2] ARVC is characterized by malignant arrhythmias and right ventricular (RV) dysfunction, and sometimes left ventricular (LV) dysfunction.[3,4] The pathological hallmarks of ARCV include the progressive loss and replacement of cardiomyocytes with fibro-fatty tissue and infiltration of inflammatory cells.[5–7] Pathogenic mutations in genes encoding desmosomal proteins account for ~50% of ARVC cases. Other genes associated with ARVC include *TMEM43*, *PLN*, *LMNA*, *DES*, *CDH2*, and so on.[4,8] With the improved diagnosis of ARVC and subsequent prevention of malignant arrhythmias, heart

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failure (HF) prevalence in ARVC is increasing, drawing more attention.[9,10] Studies show that ARVC patients with HF are more likely to undergo heart transplantation or die during medical follow-up.[9]

Our previous deep-proteome study demonstrated that the complement system, an essential arm of the immune system, was highly activated in the hearts of ARVC patients.[11] The complement system plays a crucial role in immune regulation, phagocytosis, and cellular damage.[12,13] The uncontrolled activation of the complement system induces extensive inflammation and cardiac remodelling. Furthermore, complement activation was reported to promote disease progression in ARVC.[14,15] Recent studies suggested that the complement system was critical in pathomechanism of HF.[16–18] Elevated plasma levels of complement factors (such as C5a and sC5b9) were associated with adverse clinical events in HF patients.[19,20] Acylation-stimulating protein (ASP), also known as C3a des-Arg, is generated through the alternate complement pathway[21] and regulates lipogenesis and triglyceride storage.[22,23] Thus, it is important to assess changes in plasma ASP levels during HF progression in ARVC patients.

The current diagnosis and risk stratification of ARVC patients mainly focused on clinically detectable changes or biomarkers in arrhythmia burden.[24,25] Nevertheless, this study focused on the risk stratification and prognosis evaluation of the complement system in HF-associated events for ARVC patients. Through a 17.79 ± 1.09-month follow-up, we demonstrated that plasma complement factor, ASP/C3a des-Arg, significantly correlated with the severity of HF and could predict the HF-associated events for ARVC patients.

# Methods

## Study design

Our ARVC cohort included 118 unrelated probands from Fuwai hospital in Beijing, China from October 2015 to July 2018.[26,27] Adverse clinical events had already occurred before specimen collection in some patients. Of these, 111 ARVC patients (80 males, 38.46 ± 1.32 years) who had plasma samples were enrolled in this study, with seven patients not included in the analysis due to missing plasma samples. All the probands were Han Chinese. ARVC diagnosis was confirmed by two separate cardiologists (not privy to the assay results) using the 2010 Revised Task Force Criteria.[28] The healthy controls ($n$ = 106, 65 males, 42.11 ± 1.47 years) were confirmed to be free of any ARVC-related features using data from medical history, 12-lead electrocardiograph (ECG), and echocardiography. They were also confirmed to be free from pathogenic mutation using Sanger sequencing.

Baseline demographics and medical details of ARVC patients were retrospectively obtained from the chart review. The follow-up started with blood collection and ended with the observation of clinical endpoint events. Malignant arrhythmic cardiac events (MACE) were defined as SCD, survived SCD, ventricular fibrillation (VF), sustained ventricular tachycardia (VT), and appropriate implantable cardioverter-defibrillator (ICD) discharge. Heart failure-associated events (HFAE) were defined as heart transplantation, on a cardiac transplant list, or death due to end-stage heart failure. The diagnosis of ARVC was made at the time of blood sample collection. The study was approved by the Ethics Committee of Fuwai Hospital, China, and all participants provided hand-written informed consent.

## Measurement of circulating complement level

Peripheral venous blood was collected in pyrogen-free EDTA tubes and immediately centrifuged at 2000 g for 10 min at 4°C, and the plasma (supernatant) was stored at −80°C until assayed. Circulating levels of ASP/C3a des-Arg in plasma were assessed by an enzyme-linked immunosorbent assay (ELISA) (catalogue # ab133037, Abcam) according to the manufacturer's protocol.

## Statistical analysis

All continuous data were presented as mean ± standard error of the mean (SEM) and categorical variables as numbers (percentages). Categorical variables were analysed using $\chi^2$ and Fisher's exact tests, whereas continuous variables were analysed using the Mann–Whitney $U$ test for comparisons between two groups and the Kruskal–Wallis test for multiple groups (≥3 groups). The relationships between variables were tested using Spearman's rank correlation test. Event-free survival rates were estimated using Kaplan–Meier curves and compared by the log-rank test. LASSO (least absolute shrinkage and selection operator) regularization analysis was used to achieve shrinkage and variable selection - simultaneously using the glmnet package (http://cran.r-project.org/web/packages/glmnet/index.html) in R. Cox regression analyses were used for multivariate analysis. The spline-fitting procedure was applied to illustrate the HFAE-free probabilities at different time points in ARVC patients. A two-tailed $P < 0.05$ was considered significant. All statistical analyses were performed using SPSS Statistics, version 23.0 (IBM Corp, Armonk, NY, USA). A statistical chart was plotted using GraphPad Prism 7 (GraphPad Software Inc., CA, USA).

# Results

## Baseline characteristics and plasma ASP levels in ARVC patients

Clinical data and plasma levels of ASP/C3a des-Arg were presented in *Table 1*. The average age of ARVC-related symptom-onset was 28.17 ± 1.13 years, ranging from 2 to 60. At the time of blood sampling, 51 patients (46.79%) have suffered the baseline MACEs, whereas 40 patients (36.04%) had LV dysfunctions (defined as LVEF <50%). Similar to other cohort reports,[9] HF is highly prevalent among our ARVC patients.

## The ASP-C5L2 pathway is overexpressed in ARVC patients

As an intermediate product of the alternative complement pathway, ASP was mainly studied in adipocyte and metabolic homeostasis but not in myocardial tissue. Results showed that plasma ASP levels in the ARVC group were significantly higher than in the control group (2325.22 ± 20.08 vs. 2189.75 ± 15.55, $P < 0.001$) (*Figure 1A*). We further assessed myocardial ASP levels in an independent explanted cohort of 16 ARVC, 16 dilated cardiomyopathy (DCM), and 16 healthy heart explants (*Table* S1). Western blot analysis of the explant cohort showed a significant increase in ASP levels (fold change = 4.194, $P = 0.001$) in ARVC myocardium compared with DCM and healthy myocardium (*Figure 1B,C*). Meanwhile, C5L2, the ASP receptor, was significantly up-regulated in the ARVC myocardium, with an elevated mRNA expression level (fold change = 2.377, $P = 0.0013$) compared with the healthy donors (*Figure 1D*). Immunohistochemical staining revealed elevated levels of ASP was primarily expressed in the myocardial extracellular matrix of ARVC myocardium, especially around the fibrofatty infiltration area (*Figure 1E*). C5L2 was also overexpressed in residual cardiomyocytes surrounded by fibrofatty components (*Figure 1E*). Our previous study showed that plasma sC5b9 level significantly increased with progressive cardiac involvement in ARVC patients.[14] We found a significant correlation between plasma ASP and sC5b9 in this study (*Figure 1F*). Considering the ASP-C5L2 pathway regulates lipid storage, a hallmark pathology of ARVC, we further evaluated the correlation between ASP-C5L2 and adipogenesis in ARVC. Spearman correlation analysis revealed that the expression of *C5L2* was significantly correlated with *CEBPA* ($P < 0.001$), *PPARG* ($P < 0.001$), and *PLIN1* ($P = 0.006$) in ARVC myocardium (*Figure 1G*).

## Circulating ASP level is associated with cardiac dysfunction in ARVC patients

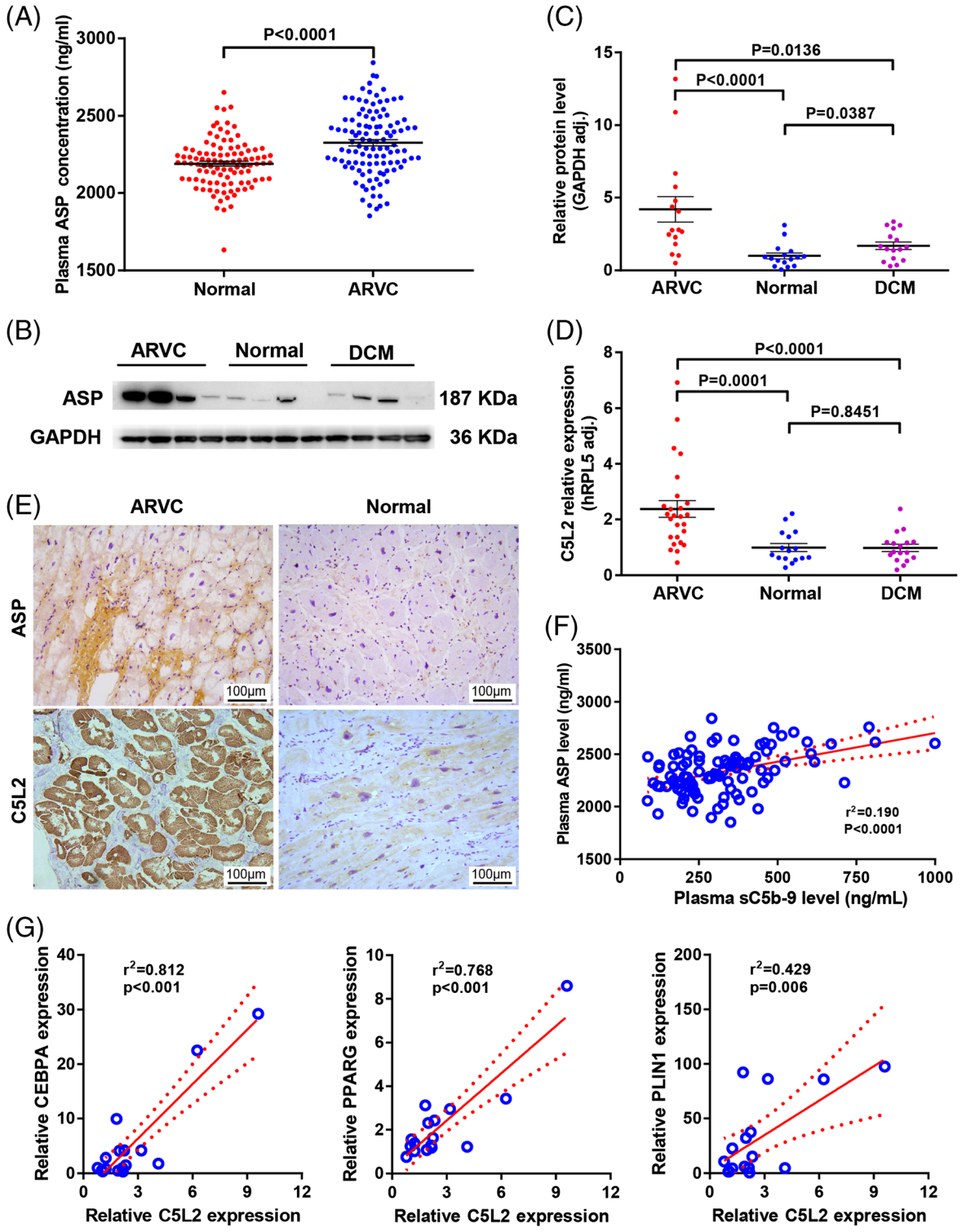
Results showed a significant correlation between plasma ASP levels and ARVC-associated clinical features, including left

**Table 1** Baseline characteristics and the complement level of patients with ARVC and controls ($N = 217$)

| Characteristics | ARVC ($N = 111$) | Control ($N = 106$) | $P$ |
|---|---|---|---|
| Male (%) | 80/111 | 65/106 | 0.113 |
| Age (year) | 38.46 ± 1.32 | 42.11 ± 1.47 | 0.089 |
| Weight (kg) | 67.28 ± 1.41 | 65.65 ± 1.36 | 0.644 |
| Height (cm) | 168.25 ± 1.36 | 166.62 ± 0.99 | 0.348 |
| Body mass index (kg/m$^2$) | 23.48 ± 0.50 | 23.53 ± 0.38 | 0.572 |
| IVS (mm) | 8.68 ± 0.16 | 8.27 ± 0.20 | 0.117 |
| RVID (mm) | 31.67 ± 1.12 | 22.42 ± 0.37 | <0.001 |
| LVEDD (mm) | 49.20 ± 0.97 | 45.90 ± 0.48 | 0.047 |
| LVEF (%) | 52.18 ± 1.67 | 63.81 ± 1.67 | <0.001 |
| Gene mutation (%) | 70/103 (67.96%) | | |
| Age of onset (year) | 28.17 ± 1.13 | | |
| Positive family history (%) | 25/108 (23.15%) | | |
| NYHA | | | |
| I (%) | 38/102 (37.25%) | | |
| II (%) | 25/102 (24.51%) | | |
| III (%) | 22/102 (21.57%) | | |
| IV (%) | 17/102 (16.67%) | | |
| Baseline MACE (%) | 51/109 (46.79%) | | |
| TWI (%) | 21/108 (19.44%) | | |
| ICD (%) | 33/107 (30.84%) | | |
| RFA (%) | 52/107 (48.60%) | | |
| NT-pro BNP (pg/mL) | 1405.48 ± 185.2 | | |
| ASP (ng/mL) | 2325.22 ± 20.08 | 2189.75 ± 15.55 | <0.001 |

*Note*: Data are expressed as either mean ± SEM or as number and percentage.
Abbreviations: ARVC, arrhythmogenic right ventricular cardiomyopathy; IVS, interventricular septal thickness; RVID, right ventricular internal dimension; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; MACE, malignant arrhythmic cardiac events; TWI, T-wave inversion; CRBBB, complete right bundle branch block; ICD, implantable cardioverter-defibrillator; RFA, radiofrequency ablation; ASP, acylation-stimulating protein.

**Figure 1** Elevated ASP-C5L2 pathway in patients with ARVC. (A) Plasma ASP levels in healthy and ARVC patients. (B,C) Representative western blot analysis and quantification of ASP in the myocardium of healthy controls, ARVC patients, and DCM patients ($n$ = 16/group). (D) of the difference in the transcription of C5L2 mRNA in cardiac tissue of ARVC patients and healthy controls or DCM ($n$ = 16 separately; hRPL5 was used as the reference gene). (E) Immunohistochemical staining of ASP and C5L2 in cardiac tissue sections obtained from healthy controls and ARVC patients. (F) Correlation between plasma ASP and plasma sC5b9 concentration in ARVC patients. The solid line represents the linear correlation, and the dotted lines represent 95% confidence intervals. (G) The association between the expression of *C5L2* gene and *CEBPA*, *PPARG*, and *PLIN1*. ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; ASP, acylation-stimulating protein.



ventricular ejection fraction (LVEF), New York Heart Association functional class (NYHA), and right ventricular internal dimension (RVID) (*Figure 2A–C*, *Table 2*). Similar results were obtained after applying age and sex correction to the correlation analysis (*Table S2*). Considering the significant variation in plasma ASP levels among ARVC patients, we divided the ARVC patients into non-dysfunction, isolated RV dysfunction, and bi-ventricular dysfunction groups. The clinical characteristics of the three groups were presented in *Table S3*. Plasma ASP levels were significantly higher in the bi-ventricular dysfunction group than in the other groups (*Figure 2D*). Meanwhile, plasma ASP levels were not associated with arrhythmias such as ventricular extrasystole (VE) ($P = 0.376$) and baseline MACEs ($P = 0.704$) (*Figure 2E*). In addition, there was no significant difference in plasma ASP levels between the males and females in the study cohorts (*Figure 2F*). Furthermore, plasma ASP levels were not different among ARVC patients with/without pathogenic mutations or family history (*Figure 3A,B*). A majority of patients (70/103) were genotype positive (*Figure 3C*), including 19 patients with plakophilin-2 (*PKP2*), 16 patients with desmoglein-2 (*DSG2*), 5 patients with desmoplakin (*DSP*), 4 patients with desmocollin-2 (*DSC2*) mutations, and 9 patients with more than one pathogenic variant (at least one desmosomal mutation each). The Kruskal–Wallis test showed that plasma ASP levels did not correlate with the underlying genetic mutations (*Figure 3D*).

## Plasma ASP levels predict adverse HF-associated events in ARVC patients

Given the SCD was effectively prevented by ICD in ARVC patients, HF was significantly associated with increased risk and poor prognosis in ARVC patients.[29] Our findings indicated that ARVC patients with cardiac dysfunction had higher plasma ASP levels than those without dysfunction. Thus, a follow-up study was conducted among 111 ARVC patients to evaluate the predicting value of plasma ASP on HF-associated events. During the 17.79 ± 1.09-month follow-up period, 2 patients were withdrawn, 34 patients experienced HFAEs, and 16 experienced MACEs. Plasma ASP levels were significantly higher in patients with HFAEs than in those without clinical events (2486.03 ± 26.70 vs. 2268.83 ± 23.51,

**Figure 2** The association between plasma ASP levels and the baseline characteristics of the ARVC patients. (A–C) There were significant differences in plasma ASP levels among groups with different NYHA, LVEF, and RV dilation in ARVC patients. (D) Plasma ASP levels were gradually up-regulated with the development of cardiac involvement, especially in ARVC patients with bi-ventricular dysfunction. (E, F) Plasma ASP levels had no association with the baseline MACE events and genders. ARVC, arrhythmogenic right ventricular cardiomyopathy; ASP, acylation-stimulating protein; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; MACE, malignant arrhythmic cardiac events.

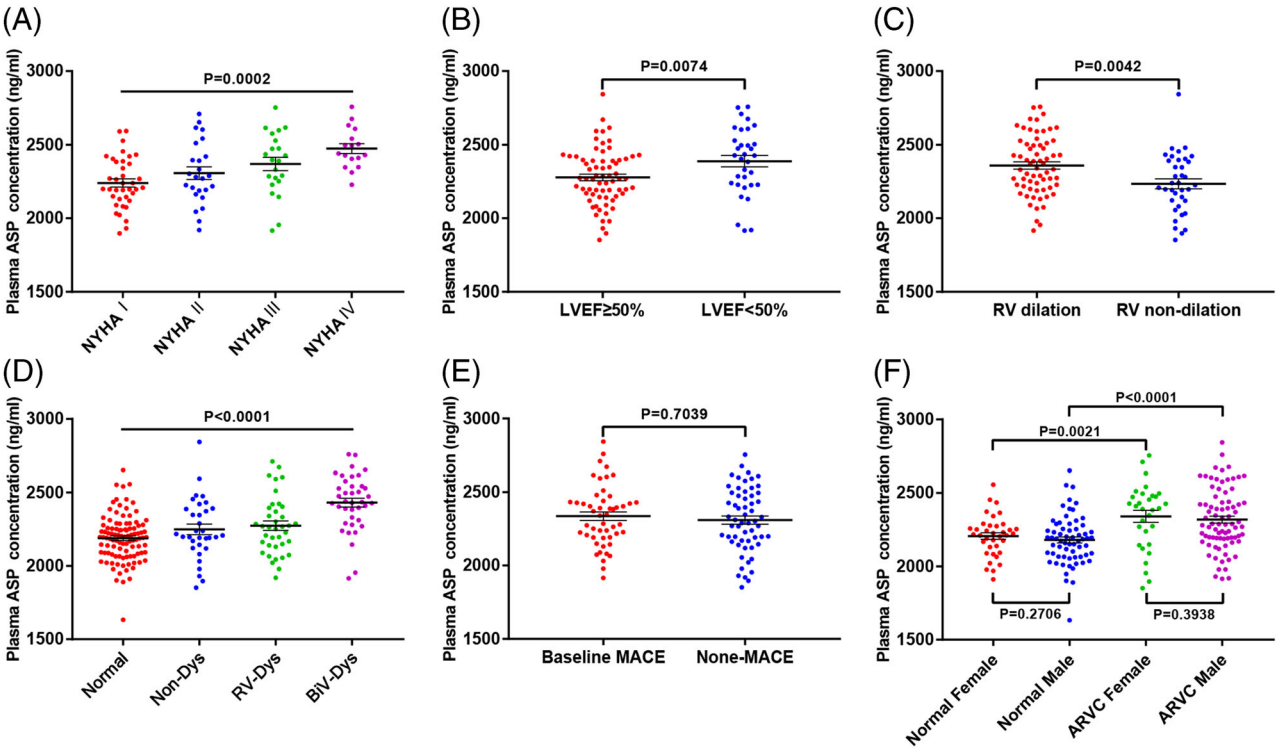


**Table 2** Plasma ASP correlation with clinical characteristics in ARVC patients

| Variables | Spearman's correlation (ASP) | *P* |
|---|---|---|
| Height (cm) | −0.137 | 0.192 |
| Weight (kg) | −0.192 | 0.066 |
| Body mass index (kg/m$^2$) | −0.120 | 0.230 |
| Age (year) | −0.155 | 0.112 |
| Age at onset (year) | −0.124 | 0.200 |
| Age at ICD (year) | 0.100 | 0.586 |
| Age at RFA (year) | −0.061 | 0.665 |
| VE (/24 h) | −0.209 | 0.376 |
| LVEDD (mm) | 0.112 | 0.261 |
| LAAP (mm) | −0.124 | 0.249 |
| IVS (mm) | −0.030 | 0.771 |
| RVID (mm) | 0.290 | **0.006** |
| LVEF (%) | −0.302 | **0.002** |
| NYHA | 0.397 | **<0.001** |
| Albumin (g/L) | −0.152 | 0.169 |
| AST (IU/L) | 0.125 | 0.212 |
| FFA (mmol/L) | −0.016 | 0.883 |
| BUN (mmol/L) | 0.102 | 0.314 |
| CK-MB (IU/L) | 0.115 | 0.260 |
| CHOL (mmol/L) | −0.246 | **0.016** |
| NT-proBNP (pg/mL) | 0.366 | **<0.001** |

*Note*: *P* values with significance were bolded.
Abbreviations: ARVC, arrhythmogenic right ventricular cardiomyopathy; ASP, acylation-stimulating protein; ICD, implantable cardioverter-defibrillator; RFA, radiofrequency ablation; VE, ventricular extrasystole; LVEDD, left ventricular diastolic end-stage diameter; LAAP, left atrial diameter; RVID, right ventricular internal dimension; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; AST, aspartate aminotransferase; CK-MB, creatine kinase-MB; CHOL, total cholesterol.

$P < 0.001$) or those with MACEs (2486.03 ± 26.70 vs. 2297.80 ± 60.46, $P = 0.008$) (*Figure 4A*). The relationship between plasma ASP level and ARVC progression was further assessed by Kaplan–Meier analysis, with the median value of ASP level (2321 ng/mL) serving as the group condition. The analysis showed a significant difference in HFAE-free rates between the groups (log rank $P < 0.001$) (*Figure 4B*). Four possible prognosis factors for HFAEs were screened out by the LASSO regression (*Figure 4C*). Multivariate Cox regression demonstrated plasma ASP level (HR = 1.004, 95% CI [1.002,1.006], $P = 0.002$) was identified as an independent prognostic factor for HF-associated events in ARVC patients (*Table 3*). The spline-fitting procedure was applied to illustrate the 3, 6, 12 and 24-month HFAE-free probabilities (*Figure 4D*). These results indicated that elevated levels of plasma ASP could predict HF progression in ARVC.

## Discussion

Previous studies on ASP-C5L2 mostly focused on adipocyte expansion, insulin resistance, and metabolic homeostasis. This is the first study to reveal that the ASP-C5L2 pathway is activated in the human heart and may contribute to lipid storage in ARVC. We also found that elevated plasma ASP levels were associated with cardiac dysfunction and could predict HF-associated events in ARVC patients.

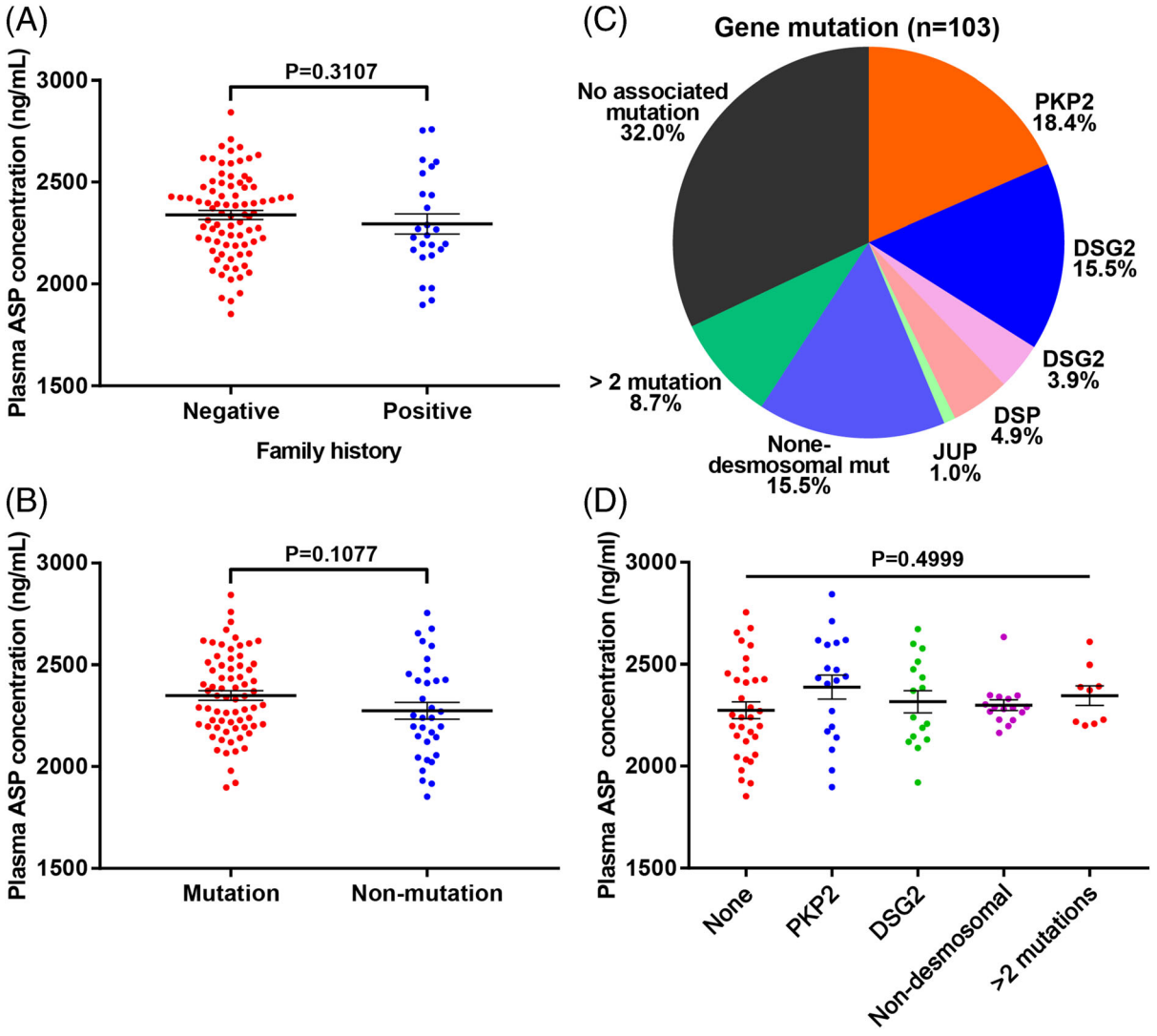
Based on our proteomic profiling and analysis of plasma complement factors, it can be inferred that the complement system is activated through the alternative pathway and influences the pathogenesis of ARVC.[11,14] ASP is a 76 amino-acid peptide derived from the activation of precursor protein complement 3 (C3).[21] C5L2 is the known receptor for ASP.[30] ASP-C5L2 signalling promotes fatty acid uptake, glucose transport, and triglyceride synthesis.[31] Because myocyte replacement with adipocytes is the primary pathogenesis of ARVC, we checked ASP accumulation in the myocardium and plasma, especially at the progressive/end stage of ARVC patients. We also found that *C5L2* was associated with adipogenesis-related genes, including *PPARG*, *CEBPA*, and *PLIN1*. Our previous study showed that *CEBPA* was involved in lipogenesis in ARVC. An iPSC-CM (induced pluripotent stem cells-cardiomyocyte) study revealed that *PPARG* was crucial in the pathogenesis of ARVC.[32] *PLIN1* is the marker gene for lipid droplets. These results indicated that activation of the ASP-C5L2 pathway might contribute to the progression of ARVC, although further gain-of-function study was needed to illuminate this pathogenesis.

Thus, it is speculated that the ASP-C5L2 pathway is activated secondary to alternative complement activation and further stimulates fat storage in the ARVC myocardium. However, as C5L2 is also the receptor of C5a and C3a, the inflammatory role of C3a/C5a-C5L2 activation remained controversial.[33] The interaction between C5L2 and C5aR in adipocytes and immune cells contributed to differential responses following ASP and C5a stimulation.[34] Therefore, it is necessary to further illuminate the role of the ASP-C5L2 axis in regulating inflammation and metabolic homeostasis using cell or animal models to provide new insights into the pathogenesis of ARVC.

Our current study focused on plasma complement system factors in ARVC patients. Through a follow-up of 17.79 ± 1.09 months, plasma ASP levels specifically increased in ARVC patients with bi-ventricle dysfunction and could predict HF-associated events. However, it is noteworthy that plasma ASP levels were normal in the isolated RV dysfunction stage, although patients showed severe RV failure phenotype. Patients with left ventricle dysfunction are more likely to undergo heart transplantation or die during follow-up.[9] The role of complement activation in LV remodelling remains undetermined. Future studies should explore the role of the ASP-C5L2 pathway in the pathogenesis of ARVC as a potential target for disease intervention.

Current studies mainly focused on the risk assessment and prognosis of malignant arrhythmias or SCD in ARVC. Although HF is also significantly associated with increased risk and poor prognosis in ARVC.[9,35] BNP/NT-proBNP, a common

**Figure 3** Plasma ASP levels in ARVC patients with different genetic mutations. (A) There is no significant difference in plasma ASP levels between ARVC patients with or without a family history. (B) Plasma levels of ASP did not differ significantly among ARVC patients with/without gene mutations. (C) Genetic mutation status of ARVC patients in this study ($n$ = 103). (D) Plasma ASP levels had no association with the underlying genetic mutation in ARVC patients.




biomarker in HF, could predict the risk of HF-associated events in ARVC patients.[36] Circulating troponin levels of ARVC patients negatively correlated with LVEF.[37] Novel biomarkers, such as tumorigenicity-2 (sST2), galectin-3 (Gal-3), and growth differentiation factor-15 (GDF-15), have been identified to be associated with adverse HF events in ARVC patients.[38] A cohort study based on 554 ARVC patients found that *DSP* gene mutation was the risk marker for LVEF.[39] The ECG indicators, first-degree atrioventricular block, epsilon waves, and lateral precordial T-wave inversions, were shown to be related to HF hospitalization in ARVC patients.[9,35] Our study also provided a novel biomarker and a possible mechanism for HF progression in ARVC patients.

## Limitations

There might be selection bias since it was an observational, retrospective study. Moreover, the small sample size of the study could influence the results. In this respect, additional multicenter studies involving large cohorts are needed. Because our hospital is the National Center for Cardiovascular Disease in China, we have a lot of referral patients from local hospitals, some of whom are in serious condition. This advanced stage of disease in our ARVC cohort may limit the extrapolation of the research results. Special considerations should also be made about the variable selection since there may be incomplete adjustments or unknown confounders not incorporated into the multivariable models. Directed

**Figure 4** The relationship between plasma ASP levels and adverse HF-associated events during the follow-up. (A) Plasma ASP levels are significantly higher in ARVC patients with HF-associated events, compared with other groups. (B) Kaplan–Meier survival curves of ARVC patients with the median value of ASP level (2321 ng/mL) serving as the group condition showed a significant difference in HFAE-free rates between the groups (log rank $P < 0.001$). (C) Variables screening process with the LASSO regression. (D) The spline-fitting procedure was applied to illustrate the HFAE-free probabilities at different time points. ASP, acylation-stimulating protein; HFAE, heart failure-associated events; MACE, malignant arrhythmic cardiac events; LASSO, least absolute shrinkage and selection operator.

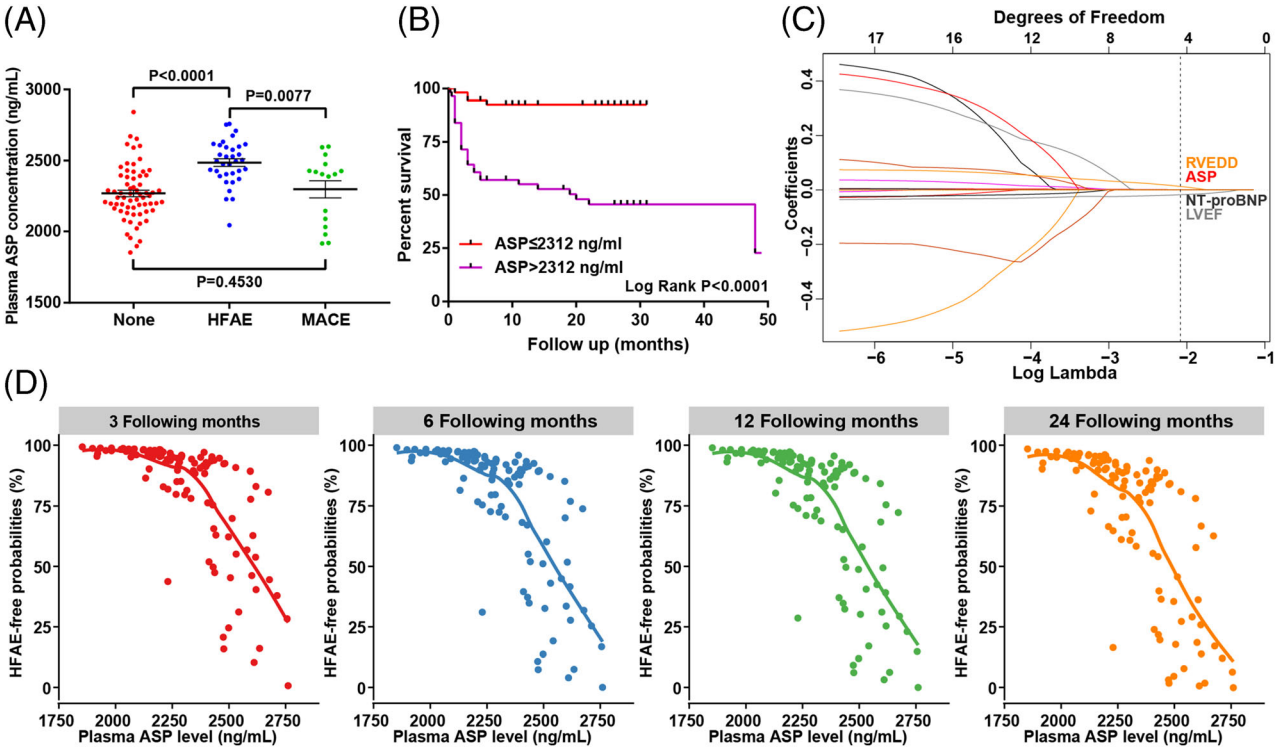



**Table 3** Increasing plasma ASP level is an independent predictor of heart failure-associated events in ARVC patients (Cox regression analysis)

| | Multivariate analysis | |
|---|---|---|
| Variables | HR (95% CI) | *P*-value |
| ASP (ng/mL) | 1.004 (1.002–1.006) | 0.002 |
| RVID (mm) | 1.042 (1.004–1.082) | 0.029 |
| NT-proBNP (pg/mL) | 1.000 (1.000–1.001) | 0.308 |
| LVEF (%) | 0.949 (0.914–0.985) | 0.005 |

Abbreviations: HR, hazard ratio, CI, confidence interval; RVID, right ventricular internal dimension; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide.

acyclic graphs (DAGs) may be useful in covariates adjustment or estimated residual confounding. Further work is also required for the comparison of the ASP and NT-proBNP.

## Conclusions

Plasma ASP levels in ARVC patients with bi-ventricular dysfunction are significantly higher than in those with non-dysfunction or isolated RV dysfunction. The high levels of plasma ASP are strongly linked to HF-associated events in ARVC patients.

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## Conflict of interest

None declared.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Clinical baseline of individuals involved in WB/qPCR analysis.

**Table S2.** Plasma ASP correlation with clinical characteristics after correction of age and sex.

**Table S3.** Baseline characteristics of ARVC patients in different sub-groups.

## References


1. Corrado D, Thiene G, Nava A, Rossi L, Pennelli N. Sudden death in young competitive athletes: clinicopathologic correlations in 22 cases. *Am J Med*. 1990; **89**: 588–596.
2. Azaouagh A, Churzidse S, Konorza T, Erbel R. Arrhythmogenic right ventricular cardiomyopathy/dysplasia: a review and update. *Clin Res Cardiol*. 2011; **100**: 383–394.
3. Corrado D, Link MS, Calkins H. Arrhythmogenic right ventricular cardiomyopathy. *N Engl J Med*. 2017; **376**: 61–72.
4. Corrado D, Basso C, Judge DP. Arrhythmogenic cardiomyopathy. *Circ Res*. 2017; **121**: 784–802.
5. Basso C, Ronco F, Marcus F, Abudureheman A, Rizzo S, Frigo AC, Bauce B, Maddalena F, Nava A, Corrado D, Grigoletto F, Thiene G. Quantitative assessment of endomyocardial biopsy in arrhythmogenic right ventricular cardiomyopathy/dysplasia: an in vitro validation of diagnostic criteria. *Eur Heart J*. 2008; **29**: 2760–2771.
6. Basso C, Thiene G, Corrado D, Angelini A, Nava A, Valente M. Arrhythmogenic right ventricular cardiomyopathy. Dysplasia, dystrophy, or myocarditis? *Circulation*. 1996; **94**: 983–991.
7. Corrado D, Basso C, Thiene G, McKenna WJ, Davies MJ, Fontaliran F, Nava A, Silvestri F, Blomstrom-Lundqvist C, Wlodarska EK, Fontaine G, Camerini F. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. *J Am Coll Cardiol*. 1997; **30**: 1512–1520.
8. Mayosi BM, Fish M, Shaboodien G, Mastantuono E, Kraus S, Wieland T, Kotta MC, Chin A, Laing N, Ntusi NB, Chong M, Horsfall C, Pimstone SN, Gentilini D, Parati G, Strom TM, Meitinger T, Pare G, Schwartz PJ, Crotti L. Identification of cadherin 2 (CDH2) mutations in Arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet*. 2017; **10**: e001605.
9. Gilotra NA, Bhonsale A, James CA, Te Riele ASJ, Murray B, Tichnell C, Sawant A, Ong CS, Judge DP, Russell SD, Calkins H, Tedford RJ. Heart failure is common and under-recognized in patients with Arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Circ Heart Fail*. 2017; **10**: e003819.
10. Mast TP, James CA, Calkins H, Teske AJ, Tichnell C, Murray B, Loh P, Russell SD, Velthuis BK, Judge DP, Dooijes D, Tedford RJ, van der Heijden JF, Tandri H, Hauer RN, Abraham TP, Doevendans PA, Te Riele AS, Cramer MJ. Evaluation of structural progression in Arrhythmogenic right ventricular dysplasia/cardiomyopathy. *JAMA Cardiol*. 2017; **2**: 293–302.
11. Chen L, Yang F, Chen X, Rao M, Zhang NN, Chen K, Deng H, Song JP, Hu SS. Comprehensive myocardial Proteogenomics profiling reveals C/EBPalpha as the key factor in the lipid storage of ARVC. *J Proteome Res*. 2017; **16**: 2863–2876.
12. Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: therapeutic interventions. *J Immunol*. 2013; **190**: 3839–3847.
13. Holers VM. Complement and its receptors: new insights into human disease. *Annu Rev Immunol*. 2014; **32**: 433–459.
14. Ren J, Tsilafakis K, Chen L, Lekkos K, Kostavasili I, Varela A, Cokkinos DV, Davos CH, Sun X, Song J, Mavroidis M. Crosstalk between coagulation and complement activation promotes cardiac dysfunction in arrhythmogenic right ventricular cardiomyopathy. *Theranostics*. 2021; **11**: 5939–5954.
15. Mavroidis M, Davos CH, Psarras S, Varela A, Athanasiadis CN, Katsimpoulas M, Kostavasili I, Maasch C, Vater A, van Tintelen JP, Capetanaki Y. Complement system modulation as a target for treatment of arrhythmogenic cardiomyopathy. *Basic Res Cardiol*. 2015; **110**: 27.
16. Zwaka TP, Manolov D, Ozdemir C, Marx N, Kaya Z, Kochs M, Hoher M, Hombach V, Torzewski J. Complement and dilated cardiomyopathy: a role of sublytic terminal complement complex-induced tumor necrosis factor-alpha synthesis in cardiac myocytes. *Am J Pathol*. 2002; **161**: 449–457.
17. Nijmeijer R, Krijnen PA, Assink J, Klaarenbeek MA, Lagrand WK, Veerhuis R, Visser CA, Meijer CJ, Niessen HW, Hack CE. C-reactive protein and complement depositions in human infarcted myocardium are more extensive in patients with reinfarction or upon treatment with reperfusion. *Eur J Clin Invest*. 2004; **34**: 803–810.
18. Lappegard KT, Bjornstad H. Anti-inflammatory effect of cardiac resynchronization therapy. *Pacing Clin Electrophysiol*. 2006; **29**: 753–758.
19. Palikhe A, Sinisalo J, Seppanen M, Haario H, Meri S, Valtonen V, Nieminen MS, Lokki ML. Serum complement C3/C4 ratio, a novel marker for recurrent cardiovascular events. *Am J Cardiol*. 2007; **99**: 890–895.
20. Speidl WS, Exner M, Amighi J, Kastl SP, Zorn G, Maurer G, Wagner O, Huber K, Minar E, Wojta J, Schillinger M. Complement component C5a predicts future cardiovascular events in patients with advanced atherosclerosis. *Eur Heart J*. 2005; **26**: 2294–2299.
21. Cianflone K, Xia Z, Chen LY. Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim Biophys Acta*. 2003; **1609**: 127–143.
22. Maslowska M, Wang HW, Cianflone K. Novel roles for acylation stimulating protein/C3adesArg: a review of recent in vitro and in vivo evidence. *Vitam Horm*. 2005; **70**: 309–332.
23. Saleh J, Al-Wardy N, Farhan H, Al-Khanbashi M, Cianflone K. Acylation stimulating protein: a female lipogenic factor? *Obes Rev*. 2011; **12**: 440–448.
24. te Riele AS, Bhonsale A, James CA, Rastegar N, Murray B, Burt JR, Tichnell C, Madhavan S, Judge DP, Bluemke DA, Zimmerman SL, Kamel IR, Calkins H, Tandri H. Incremental value of cardiac magnetic resonance imaging in arrhythmic risk stratification of arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated desmosomal mutation carriers. *J Am Coll Cardiol*. 2013; **62**: 1761–1769.
25. Akdis D, Saguner AM, Shah K, Wei C, Medeiros-Domingo A, von Eckardstein A, Luscher TF, Brunckhorst C, Chen HSV, Duru F. Sex hormones affect outcome in arrhythmogenic right ventricular cardiomyopathy/dysplasia: from a stem cell derived cardiomyocyte-based model to clinical biomarkers of disease

outcome. *Eur Heart J*. 2017; **38**: 1498–1508.
26. Ren J, Chen L, Zhang N, Chen X, Zhao Q, Chen K, Li X, Ruschitzka F, Duru F, Song J. Plasma testosterone and arrhythmic events in male patients with arrhythmogenic right ventricular cardiomyopathy. *ESC Heart Fail*. 2020; **7**: 1547–1559.
27. Chen L, Song J, Chen X, Chen K, Ren J, Zhang N, Rao M, Hu Z, Zhang Y, Gu M, Zhao H, Tang H, Yang Z, Hu S. A novel genotype-based clinicopathology classification of arrhythmogenic cardiomyopathy provides novel insights into disease progression. *Eur Heart J*. 2019; **40**: 1690–1703.
28. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, Corrado D, Cox MG, Daubert JP, Fontaine G, Gear K, Hauer R, Nava A, Picard MH, Protonotarios N, Saffitz JE, Sanborn DM, Steinberg JS, Tandri H, Thiene G, Towbin JA, Tsatsopoulou A, Wichter T, Zareba W. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Eur Heart J*. 2010; **31**: 806–814.
29. Tedford RJ, James C, Judge DP, Tichnell C, Murray B, Bhonsale A, Philips B, Abraham T, Dalal D, Halushka MK, Tandri H, Calkins H, Russell SD. Cardiac transplantation in arrhythmogenic right ventricular dysplasia/cardiomyopathy. *J Am Coll Cardiol*. 2012; **59**: 289–290.
30. Kalant D, MacLaren R, Cui W, Samanta R, Monk PN, Laporte SA, Cianflone K. C5L2 is a functional receptor for acylation-stimulating protein. *J Biol Chem*. 2005; **280**: 23936–23944.
31. Cui W, Lapointe M, Gauvreau D, Kalant D, Cianflone K. Recombinant C3adesArg/acylation stimulating protein (ASP) is highly bioactive: a critical evaluation of C5L2 binding and 3T3-L1 adipocyte activation. *Mol Immunol*. 2009; **46**: 3207–3217.
32. Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen HS. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature*. 2013; **494**: 105–110.
33. Li R, Coulthard LG, Wu MC, Taylor SM, Woodruff TM. C5L2: a controversial receptor of complement anaphylatoxin, C5a. *FASEB J*. 2013; **27**: 855–864.
34. Poursharifi P, Lapointe M, Petrin D, Devost D, Gauvreau D, Hebert TE, Cianflone K. C5L2 and C5aR interaction in adipocytes and macrophages: insights into adipoimmunology. *Cell Signal*. 2013; **25**: 910–918.
35. Kimura Y, Noda T, Matsuyama TA, Otsuka Y, Kamakura T, Wada M, Ishibashi K, Inoue Y, Miyamoto K, Okamura H, Nagase S, Aiba T, Kamakura S, Noguchi T, Anzai T, Satomi K, Wada Y, Ohno S, Horie M, Shimizu W, Yasuda S, Shimokawa H, Kusano K. Heart failure in patients with arrhythmogenic right ventricular cardiomyopathy: what are the risk factors? *Int J Cardiol*. 2017; **241**: 288–294.
36. Cheng H, Lu M, Hou C, Chen X, Wang J, Yin G, Chu J, Zhang S, Prasad SK, Pu J, Zhao S. Relation between N-terminal pro-brain natriuretic peptide and cardiac remodeling and function assessed by cardiovascular magnetic resonance imaging in patients with arrhythmogenic right ventricular cardiomyopathy. *Am J Cardiol*. 2015; **115**: 341–347.
37. Akdis D, Saguner AM, Burri H, Medeiros-Domingo A, Matter CM, Ruschitzka F, Tanner FC, Brunckhorst C, Duru F. Clinical predictors of left ventricular involvement in arrhythmogenic right ventricular cardiomyopathy. *Am Heart J*. 2020; **223**: 34–43.
38. Akdis D, Chen L, Saguner AM, Zhang N, Gawinecka J, Saleh L, von Eckardstein A, Ren J, Matter CM, Hu Z, Chen X, Tanner FC, Manka R, Chen K, Brunckhorst C, Song J, Duru F. Novel plasma biomarkers predicting biventricular involvement in arrhythmogenic right ventricular cardiomyopathy. *Am Heart J*. 2022; **244**: 66–76.
39. Protonotarios A, Bariani R, Cappelletto C, Pavlou M, Garcia-Garcia A, Cipriani A, Protonotarios I, Rivas A, Wittenberg R, Graziosi M, Xylouri Z, Larranaga-Moreira JM, de Luca A, Celeghin R, Pilichou K, Bakalakos A, Lopes LR, Savvatis K, Stolfo D, Dal Ferro M, Merlo M, Basso C, Freire JL, Rodriguez-Palomares JF, Kubo T, Ripoll-Vera T, Barriales-Villa R, Antoniades L, Mogensen J, Garcia-Pavia P, Wahbi K, Biagini E, Anastasakis A, Tsatsopoulou A, Zorio E, Gimeno JR, Garcia-Pinilla JM, Syrris P, Sinagra G, Bauce B, Elliott PM. Importance of genotype for risk stratification in arrhythmogenic right ventricular cardiomyopathy using the 2019 ARVC risk calculator. *Eur Heart J*. 2022; **43**: 3053–3067.