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Apolipoprotein A-IV concentrations and clinical outcomes in haemodialysis patients with type 2 diabetes mellitus – a *post hoc* analysis of the 4D Study

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Abstract. Kollerits B, Krane V, Drechsler C, Lamina C, März W, Ritz E, Wanner C, Kronenberg F & for the German Diabetes and Dialysis Study Investigators (Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria; Division of Nephrology, Department of Medicine, University of Würzburg, Würzburg; Department of Public Health, Social and Preventive Medicine, Mannheim Medical Faculty, University of Heidelberg, Mannheim; and Department of Nephrology, University of Heidelberg, Heidelberg, Germany). Apolipoprotein A-IV concentrations and clinical outcomes in haemodialysis patients with type 2 diabetes mellitus – a *post hoc* analysis of the 4D Study. *J Intern Med* 2012; **272**: 592–600.

Background. Apolipoprotein A-IV (apoA-IV) is an anti-atherogenic and anti-oxidative plasma glycoprotein involved in reverse cholesterol transport. The aim of this study was to examine the association between apoA-IV and all-cause mortality, cardiovascular endpoints and parameters of protein–energy wasting and nutrition in haemodialysis patients.

Methods. This *post hoc* analysis was performed in the German Diabetes Dialysis Study (4D Study) evaluating atorvastatin in 1255 haemodialysis patients with type 2 diabetes mellitus, followed for a median of 4 years. The association between apoA-IV and relevant outcomes was analysed using Cox proportional hazards regression analyses. Body mass index (BMI) was used as a marker of protein–energy wasting. In addition, a definition of extended wasting was applied, combining median values of BMI, serum albumin, creatinine and sensitive C-reactive protein, to classify patients.

Results. Mean (±SD) apoA-IV concentration was 49.8 ± 14.2 mg dL^{-1}. Age- and gender-adjusted apoA-IV concentrations were strongly associated with the presence of congestive heart failure at baseline [odds ratio = 0.81, 95% confidence interval (CI) 0.74–0.88 per 10 mg dL^{-1} increase; $P < 0.001$). During the prospective follow-up, the strongest association was found for all-cause mortality [hazard ratio (HR) = 0.89, 95% CI 0.85–0.95, $P = 0.001$), which was mainly because of patients with BMI > 23 kg m^{-2} (HR = 0.87, 95% CI 0.82–0.94, $P < 0.001$) and those in the nonwasting group according to the extended definition (HR = 0.89, 95% CI 0.84–0.96, $P = 0.001$). This association remained significant after additionally adjusting for parameters associated with apoA-IV at baseline. Further associations were observed for sudden cardiac death. ApoA-IV was less strongly associated with atherogenic events such as myocardial infarction.

Conclusions. Low apoA-IV levels seem to be a risk predictor of all-cause mortality and sudden cardiac death. This association might be modified by nutritional status.

Keywords: all-cause mortality, apolipoprotein A-IV, haemodialysis, nutrition, protein–energy wasting.

Introduction

Apolipoprotein A-IV (apoA-IV) is a 46 kDa glycoprotein [1] that is almost exclusively produced in intestinal enterocytes and secreted as one of the structural proteins of chylomicrons, very low-density lipoprotein, high-density lipoprotein (HDL) or unassociated with lipoproteins [2, 3]. *In vitro* studies

support a role for apoA-IV in reverse cholesterol transport [4, 5] discharging atherogenic LDL cholesterol from peripheral cells. It has been linked to fat absorption processes [6], hepatic lipid metabolism [7] and the physiological control of food intake and body weight [8]. Various experiments in rats have demonstrated that apoA-IV is a satiety factor that reduces nutritional intake [8]. Whether these findings can be extrapolated to humans remains to be determined. Moreover, apoA-IV has anti-oxidative and anti-atherogenic properties in both mice and humans, implying that low apoA-IV concentrations increase the risk of coronary heart disease [3, 9–15]. To date, a limited number of studies have analysed the impact of apoA-IV in patients with chronic kidney disease (CKD) and found a 2- to 3-fold elevation in apoA-IV in these patients [16–21]. Despite this elevation, CKD patients with atherosclerotic complications still have lower apoA-IV concentrations than those without atherosclerotic complications and a similar degree of kidney impairment [14, 17]. However, large prospective studies have not been conducted to investigate the role of apoA-IV in CKD.

Patients with CKD have a dramatically increased risk of all-cause and cardiovascular mortality [22, 23]. Protein–energy wasting is a well-known and common problem among dialysis patients and is characterized by poor food intake, muscle loss, inflammation and development of comorbidities [24, 25]. It has been associated with a high risk of mortality in chronic dialysis patients [26]. As apoA-IV levels have been associated with nutritional intake and cardiovascular outcomes in cross-sectional studies, we hypothesized that interaction between apoA-IV and various indicators of protein–energy wasting might have a direct effect on all-cause mortality and other outcomes in dialysis patients. Besides a low body mass index (BMI) and hypoalbuminaemia, which are indicators of protein–energy wasting [24], we additionally extended our analyses to other potential indicators, such as serum creatinine and sensitive C-reactive protein (sCRP) as discussed recently [24]. The aim of this study was to examine the associations between apoA-IV concentrations and all-cause mortality, cardiovascular endpoints and parameters of protein–energy wasting and nutrition in a *post hoc* analysis of a prospective study in diabetic haemodialysis patients.

Methods

Study design and participants

The methodology of the 4D Study has been described in detail previously [27]. Briefly, this study was a prospective, randomized controlled trial to evaluate the efficacy and safety of atorvastatin in 1255 patients with type 2 diabetes mellitus (T2DM). Patients were aged 18–80 years and had been on maintenance haemodialysis <2 years at baseline; the median follow-up was 4 years. Patients were recruited between March 1998 and October 2002 in 178 dialysis centres in Germany taking part in this study, and were randomly assigned in a double-blind manner to either atorvastatin ($n = 619$) or placebo ($n = 636$). Patients were regularly followed until death, censoring or to the end of the study in February 2004.

Endpoints

The endpoints considered in the study included all-cause mortality, death from cardiac causes, combined cardiac events, combined cerebrovascular events and combined cardiovascular events. Death from cardiac causes comprised fatal myocardial infarction, sudden cardiac death, death because of congestive heart failure and death because of coronary heart disease, including during or within 28 days after an intervention. Combined cardiac events were defined as death from cardiac causes (as above) and additionally nonfatal myocardial infarction and nonfatal percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG) interventions. Combined cerebrovascular events included fatal and nonfatal strokes (ischaemic, haemorrhagic and unclear types) and transient ischaemic attack/prolonged reversible ischaemic neurological deficit. Combined cardiovascular events included death from cardiac causes (as above), nonfatal myocardial infarction and fatal and nonfatal stroke. These endpoints were determined by three members of the endpoint committee consisting of one cardiologist and two nephrologists and blinded to study treatment, according to previously defined criteria [28]. The originally defined endpoints were used for all analyses in the present study.

Data collection

A detailed description of baseline data collection has been previously reported [29]. Data on comorbidities at baseline, such as coronary heart disease and congestive heart failure, and information on the duration of diabetes and dialysis treatment were provided by the patient's nephrologist. Plasma apoA-IV quantification was performed with a

double-antibody enzyme-linked immunosorbent assay using an affinity-purified polyclonal rabbit anti-human apoA-IV antibody for coating and the same antibody coupled to horseradish peroxidase for detection. Plasma containing a known concentration of apoA-IV served as the calibration standard. Each sample was analysed in duplicate, and intra- and inter-assay coefficients of variation were 2.7% and 6.0%, respectively [30]. ApoA-IV was measured in baseline samples which were used for data analysis. To investigate whether atorvastatin treatment has a differential effect on apoA-IV concentration, we measured the levels at baseline as well as at the 6-month follow-up visit in most patients in both the atorvastatin and placebo groups. Measurements of apoA-IV concentrations were performed at the Innsbruck Medical University. All other laboratory measurements in the 4D Study were carried out centrally at the Department of Clinical Chemistry, University of Freiburg, Germany. Samples collected before randomization and before the start of a dialysis session were used for measurements of apoA-IV and all blood parameters used in these analyses.

Statistical analysis

Statistical analysis was performed using PASW statistics version 18 (SPSS Inc., Chicago IL, USA) and R statistical software, version 2.8.1 (http://www.R-project.org, Vienna, Austria). For all tests, $P < 0.05$ was considered to be statistically significant. Baseline characteristics were stratified according to quartiles of apoA-IV. Univariate comparisons of continuous variables between quartiles of apoA-IV concentration were performed using one-way analysis of variance, or nonparametric Kruskal–Wallis tests in case of nonnormally distributed variables. A Mann–Whitney *U* test was applied for the comparison of apoA-IV changes during the first 6 months of the study between treatment groups. Categorical variables were compared using the chi-square test. As part of the descriptive analysis, the logistic regression analyses modelling comorbidities at baseline were adjusted for age and gender. Cox proportional hazards regression analyses were performed to calculate hazard ratio (HR) values for an increment of 10 mg dL^{-1} apoA-IV. The calculated models did not violate the proportional hazards assumption. Moreover, using nonlinear splines, models did not depart from linearity.

In the main model, Cox regression analyses were adjusted for potential confounders including age, gender, medication allocation (placebo or atorvastatin) and coronary heart disease at baseline (history of myocardial infarction, CABG, PTCA or angiographically documented coronary heart disease). In an additional model, we adjusted for all further variables and major comorbidities that were significantly associated with apoA-IV at baseline using a stepwise procedure. All variables in the final model of total mortality in addition to apoA-IV were included in the subsequent models with all other outcomes. As various parameters might have an influence on wasting and no final guidelines for its classification exist, we defined wasting in accordance with recommendations of an expert panel [24]. The hypothesis that apoA-IV might be influenced by nutritional and wasting parameters was first tested using BMI (kg m^{-2}) as a surrogate marker of wasting, stratifying the patient population into two groups: wasting (BMI $\leq$ 23 kg m^{-2}) and nonwasting (BMI > 23 kg m^{-2}). Only outcomes with $\geq$ 15 events are presented in the tables. To confirm the validity of this BMI stratification, an alternative extended wasting definition was also used according to a recent analysis of the same population [29] as described recently [24]. In these further analyses, patients were stratified into three groups: moderate wasting, if values were below the median levels of BMI (26.75 kg m^{-2}), serum albumin (3.8 g dL^{-1}), creatinine (6.8 mg dL^{-1}) and sCRP (5.1 mg L^{-1}); severe wasting if BMI, albumin and creatinine were below the median values, but sCRP levels were above the median; and nonwasting for the remaining patients. For all subsequent analyses, we combined patients in the moderate and severe wasting groups into a single group.

Results

Baseline analysis

Baseline apoA-IV concentration data were available for 1224 of the 1255 patients enrolled in the 4D Study. The mean apoA-IV concentration at baseline was 49.8 ± 14.2 mg dL^{-1}, which is about three times higher than in the general population. There was no significant difference in apoA-IV concentrations between the atorvastatin and placebo groups (49.6 ± 13.8 vs. 50.1 ± 14.5 mg dL^{-1}, $P = 0.86$). There was no effect of treatment on apoA-IV concentrations: during the first 6 month of follow-up, changes in apoA-IV concentrations were similar in the atorvastatin and placebo groups (−1.2 vs. −1.8 mg dL^{-1}, respectively, $P = 0.34$). Patient baseline characteristics according to quartiles of apoA-IV are shown in Table 1.

Table 1 *Baseline characteristics stratified by quartiles of apoA-IV*

	ApoA-IV quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
ApoA-IV range, mg dL^{-1}	≤39.81	39.82–48.74	48.75–58.47	>58.47	–
Number of patients, *n*	306	310	303	305	–
Age, years	67 (8)	66 (8)	66 (8)	64 (9)	<0.001
Female gender, *n* (%)	144 (47)	150 (48)	141 (47)	131 (43)	0.57
Body mass index, kg m^{-2}	27.6 (5.2)	27.8 (4.8)	27.4 (4.4)	27.4 (4.9)	0.71
Atorvastatin treatment, *n* (%)	146 (48)	158 (51)	149 (49)	151 (50)	0.88
Smoker and ex-smoker, *n* (%)	121 (40)	122 (39)	120 (40)	134 (44)	0.60
Systolic blood pressure, mmHg	144 (22)	147 (23)	147 (22)	145 (21)	0.08
Diastolic blood pressure, mmHg	76 (11)	76 (11)	76 (11)	76 (11)	0.92
Time receiving dialysis, months	7.1 (6.4)	8.2 (7.1)	8.5 (7.1)	8.8 (6.6)	0.004
Duration of diabetes, years	18.1 (9.0)	18.2 (8.6)	18.3 (8.7)	17.8 (8.7)	0.98
Ultrafiltration volume[a], kg	2.1 (1.2)	2.2 (1.5)	2.4 (1.3)	2.4 (1.2)	0.001
Laboratory parameters					
Glycated haemoglobin, %	6.6 (1.2)	6.7 (1.2)	6.7 (1.2)	6.8 (1.4)	0.40
Albumin, g dL^{-1}	3.7 (0.3)	3.8 (0.3)	3.8 (0.3)	3.9 (0.3)	<0.001
Sensitive C-reactive protein, mg L^{-1} (25th; 50th; 75th percentile)	16.3 (3.6;7.6;20.3)	11.7 (2.6;5.6;14.8)	9.0 (2.0;4.3;10.3)	7.0 (1.7;3.6;7.5)	<0.001
Phosphate, mmol L^{-1}	1.8 (0.5)	1.9 (0.5)	2.0 (0.5)	2.1 (0.5)	<0.001
Total cholesterol, mg dL^{-1}	213 (43)	217 (41)	222 (43)	226 (43)	<0.001
LDL cholesterol, mg dL^{-1}	123 (29)	123 (29)	128 (31)	129 (30)	0.003
HDL cholesterol, mg dL^{-1}	32 (10)	35 (12)	37 (13)	41 (15)	<0.001
Triglycerides, mg dL^{-1} (25th; 50th; 75th percentile)	264 (154;228;323)	278 (155;229;355)	259 (153;215;312)	253 (140;202;317)	0.096
Very low-density lipoprotein cholesterol, mg dL^{-1}	58 (31)	60 (35)	57 (34)	56 (36)	0.18
Haemoglobin, g dL^{-1}	10.8 (1.4)	10.9 (1.3)	10.9 (1.3)	10.9 (1.4)	0.86

Values are given as mean (standard deviation) or number of patients (%) if not indicated otherwise. To convert levels of: cholesterol to mmol L^{-1}, multiply by 0.0259; triglycerides to mmol L^{-1}, multiply by 0.0113; haemoglobin to mmol L^{-1}, multiply by 0.62; serum albumin to g L^{-1}, multiply by 10.
[a]The ultrafiltration volume was calculated based on body weight at randomization before and after dialysis.

Table 2 *Associations between apoA-IV and comorbidities at baseline*

	Total group (n = 1224)		
Comorbidity	No of events	OR (95% CI)[a]	P
Congestive heart failure[b]	435	0.81 (0.74–0.88)	<0.001
Coronary heart disease[c]	362	0.92 (0.84–1.00)	0.06
Angiographically defined coronary heart disease	259	0.87 (0.78–0.96)	0.007
Signs of ischaemia	376	0.96 (0.87–1.04)	0.31
ECG changes			
Signs of MI	170	0.89 (0.79–1.01)	0.07
Arrhythmia	232	0.84 (0.75–0.94)	0.002
Atrial fibrillation/flutter	113	0.81 (0.69–0.94)	0.006
Complete RBBB or LBBB	110	0.86 (0.74–1.00)	0.05
Stroke/TIA	216	0.97 (0.88–1.08)	0.61
Peripheral vascular disease	543	0.92 (0.85–1.00)	0.06

MI, myocardial infarction; RBBB, right bundle branch block; LBBB, left bundle branch block; ECG, electrocardiogram; TIA, transient ischaemic attack. Of note, different types of disease and interventions are not mutually exclusive.
[a]Logistic regression model adjusted for age and gender.
[b]Predominantly New York Heart Association class II.
[c]Coronary heart disease defined by a history of myocardial infarction, coronary artery bypass grafting, percutaneous coronary intervention or angiographically defined coronary heart disease.

The strongest association between apoA-IV and comorbidities at baseline was observed for congestive heart failure (Table 2); apoA-IV was inversely associated with the prevalence of congestive heart failure (odds ratio = 0.81 per 10 mg dL^{-1} increment in apoA-IV, $P < 0.001$). Furthermore, there were associations between apoA-IV and ECG abnormalities such as arrhythmia, atrial fibrillation/flutter and right or left bundle branch block which are known causes or consequences of congestive heart failure. Associations between apoA-IV and variables reflecting atherosclerotic disease were weaker than for congestive heart failure. No associations between apoA-IV and cerebrovascular disease were observed at baseline (Table 2).

Prospective follow-up and total mortality

The mean follow-up was 3.96 years in the group of patients receiving atorvastatin and 3.91 years in the placebo group. Of the 1224 patients with available apoA-IV values, 600 died, including 265 from cardiac causes. In total, there were 438 cardiac and 144 cerebrovascular cumulative events. These cardiovascular events were observed in 455 patients and if a patient experienced more than one event, the event which occurred first was considered in the analysis.

Table 3 shows the results from the main Cox regression model investigating the association between apoA-IV concentrations and relevant endpoints, adjusted for age, gender, medication (atorvastatin or placebo) and coronary heart disease. Each 10 mg dL^{-1} increase in apoA-IV concentration was associated with an 11% reduced risk of death during the observation period ($P = 0.001$). After stratifying for BMI, this association was only observed in patients with BMI > 23 kg m^{-2} (HR = 0.87, 95% CI 0.82–0.94; $P < 0.001$). No association was seen in those patients with BMI $\leq$ 23 kg m^{-2} (HR = 1.00; $P = 0.95$). Formal testing provided evidence of interactions between apoA-IV and BMI with a cut-off value of 23 kg m^{-2} for all major outcomes: all-cause mortality ($P = 0.06$), death from cardiac causes ($P = 0.02$), sudden cardiac death ($P = 0.02$), combined cardiac events ($P = 0.03$), combined cerebrovascular events ($P = 0.03$) and combined cardiovascular events ($P = 0.005$). Therefore, we performed all main analyses stratified by this BMI cut-off level.

As apoA-IV was significantly associated with several parameters and comorbidities at baseline, we performed the analyses in the total group based on these parameters using a stepwise procedure; age, coronary heart disease, congestive heart failure and arrhythmia at baseline, HDL cholesterol, phosphate, sCRP (log-transformed), albumin and ultrafiltration volume remained in the model. This slightly weakened the association between apoA-IV and total mortality (HR = 0.94, 95% CI 0.88–0.999, $P = 0.05$ and HR = 0.92, 95% CI 0.85–0.99, $P = 0.02$ for the total population and the group with BMI > 23 kg m^{-2}, respectively (Table S1). As sCRP and albumin were included in these analyses, we can exclude the possibility that the association between apoA-IV and outcomes is confounded by inflammation.

Table 3 *Association between apoA-IV and outcomes during the prospective follow-up*

	Total group (n = 1224)			BMI > 23 kg m^{-2} (n = 1038)			Nonwasting extended (n = 1032)		
Endpoints	No of events	HR (95% CI)	P	No of events	HR (95% CI)	P	No of events	HR (95% CI)	P
Death from all causes	600	0.89 (0.85–0.95)	0.001	478	0.87 (0.82–0.94)	<0.001	488	0.89 (0.84–0.96)	0.001
Death from cardiac causes	265	0.93 (0.85–1.02)	0.11	206	0.88 (0.80–0.98)	0.02	224	0.91 (0.82–0.998)	0.05
Sudden cardiac death	157	0.89 (0.79–1.00)	0.06	118	0.83 (0.72–0.95)	0.006	126	0.86 (0.76–0.98)	0.02
Combined cardiac events	438	0.98 (0.92–1.05)	0.62	355	0.95 (0.88–1.03)	0.19	376	0.95 (0.89–1.03)	0.20
Nonfatal myocardial infarction	142	1.09 (0.97–1.22)	0.14	124	1.04 (0.92–1.19)	0.50	124	1.05 (0.93–1.20)	0.41
Combined cerebrovascular events	144	0.88 (0.78–1.00)	0.05	124	0.84 (0.73–0.96)	0.01	119	0.92 (0.80–1.05)	0.21
Stroke (fatal and nonfatal)	99	0.90 (0.78–1.05)	0.18	84	0.89 (0.76–1.05)	0.17	80	0.93 (0.79–1.09)	0.37
Combined cardiovascular events	455	0.97 (0.91–1.04)	0.36	367	0.92 (0.85–0.997)	0.04	379	0.96 (0.89–1.03)	0.23

Cox model adjusted for age, gender and coronary heart disease at baseline and medication (atorvastatin or placebo); per 10 mg dL^{-1} increase in apoA-IV. Death from cardiac causes comprised fatal myocardial infarction, sudden cardiac death, death because of congestive heart failure, death because of coronary heart disease including during or within 28 days after an intervention; combined cardiac events was defined as death from cardiac causes (as above) as well as nonfatal myocardial infarction and nonfatal PTCA or CABG interventions; combined cerebrovascular events included fatal and nonfatal strokes (ischaemic, haemorrhagic and unclear types) and TIA/PRIND; combined cardiovascular events included death from cardiac causes (as above), nonfatal myocardial infarction and fatal and nonfatal stroke. Subcategories with few events (n < 15) are not listed in the Table.

PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass grafting; TIA, transient ischaemic attack; PRIND, prolonged reversible ischaemic neurological deficit.

These findings were confirmed using an alternative extended wasting definition by stratifying patients into either nonwasting or combined moderate and severe wasting groups. In addition to BMI, this definition uses serum albumin, creatinine and sCRP for stratification (see Methods). As shown in Fig. 1, 112 patients formerly included in the wasting group with BMI $\leq$ 23 kg m^{-2} were reclassified in the nonwasting group according to the extended wasting definition. Moreover, 53 and 65 patients were reclassified from the nonwasting group with BMI > 23 kg m^{-2} to the moderate and severe wasting groups, respectively, according to the extended wasting definition. Despite these changes, a significant association between apoA-IV and all-cause mortality was found in the nonwasting group (HR = 0.89, 95% CI 0.84–0.96, P = 0.001, n = 1032). No such associations were found in the extended combined wasting group (HR = 1.00, 95% CI 0.86–1.16, P = 0.99, n = 196). Further adjustment for apoA-IV-related parameters determined by a stepwise procedure revealed similar results compared to the group with BMI > 23 kg m^{-2} (Table S1).

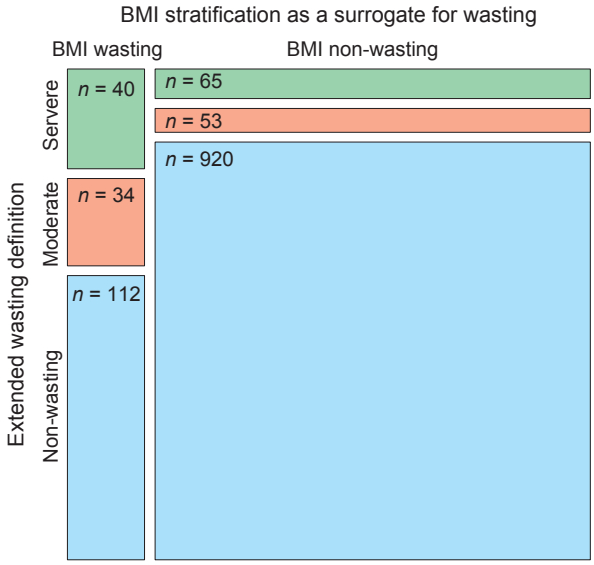

Fig. 1 *Stratification of study participants according to BMI and the extended wasting classification. This mosaic plot shows differences between groups according to the BMI stratification (wasting: BMI ≤ 23 kg m^{-2}; nonwasting: BMI > 23 kg m^{-2}) and the extended wasting classification (moderate wasting: BMI ≤ 26.75 kg m^{-2}, serum albumin ≤ 3.8 g dL^{-1}, sCRP ≤ 5.1 mg L^{-1}; severe wasting: BMI ≤ 26.75 kg m^{-2}, serum albumin ≤ 3.8 g dL^{-1}, sCRP > 5.1 mg L^{-1}; nonwasting: all remaining patients).*

Prospective follow-up and endpoint subgroups

Analyses of subgroups of endpoints revealed that the associations observed in the entire patient group were mainly because of patients with a BMI > 23 kg m^{-2} or those in the extended nonwasting group. In patients with BMI ≤ 23 kg m^{-2} and those in the extended combined wasting group, the associations often pointed even in the opposite direction (Table S2). Table 3 shows significant associations in patients with BMI > 23 kg m^{-2} between apoA-IV concentrations and death from cardiac causes (HR = 0.88, 95% CI 0.80–0.98, $P = 0.02$) or sudden cardiac death (HR = 0.83, 95% CI 0.72–0.95, $P = 0.006$). Adjustment for further potential confounders at baseline, for example inflammatory markers such as sCRP and albumin, had only marginal effects (Table S1).

When all cardiac events combined (including fatal and nonfatal events) were analysed, significant associations with apoA-IV concentrations in patients with BMI > 23 kg m^{-2} and in the extended nonwasting group (both HR = 0.95, $P = 0.19$ and $P = 0.20$, respectively) were no longer observed. This may be explained by the fact that atherogenic events such as fatal and nonfatal myocardial infarction or cardiovascular interventions, which were included in the overall group with cardiac events, were not associated with apoA-IV concentration.

All cerebrovascular events combined were associated with apoA-IV concentration: the risk decreased by 12% and 16% with each 10 mg dL^{-1} increase in apoA-IV in the entire group, and the group with BMI > 23 kg m^{-2}, respectively. However, the association with combined cerebrovascular events was no longer significant in the extended nonwasting group.

Discussion

In the present study, we investigated whether apoA-IV concentration is a risk predictor of fatal and nonfatal pre-specified and centrally adjudicated endpoints in patients with type 2 diabetes mellitus on long-term haemodialysis. The results demonstrate that low apoA-IV concentrations predict all-cause mortality and death from cardiac causes (i.e. sudden cardiac death). These associations were mainly because of patients with BMI above 23 kg m^{-2} and those in the nonwasting group according to the extended classification. However, there was no impact of apoA-IV on the risk of atherogenic events (i.e. fatal and nonfatal myocardial infarction or cardiovascular interventions).

The strongest association observed for baseline apoA-IV was an inverse correlation with prevalent congestive heart failure; this is a novel finding of the present study. We also observed associations between apoA-IV concentration and baseline cardiovascular conditions, including atherosclerotic diseases such as coronary heart disease. This finding is in line with those of earlier cross-sectional studies of atherosclerotic outcomes in the general population or in patients with kidney diseases [3, 10–15]. Whether this association with atherosclerotic endpoints is caused by low apoA-IV concentrations or whether it is pathogenetically driven by the association with congestive heart failure cannot be determined using cross-sectional data. However, prospective observation in the 4D Study did not provide evidence that low apoA-IV levels are associated with atherosclerotic endpoints such as fatal and nonfatal myocardial

infarction, PTCA and CABG. It is interesting that myocardial infarction is the cause of most deaths in diabetic patients in the general population [31]. By contrast, sudden cardiac death is the major cause of death in patients with diabetes and diabetic nephropathy undergoing haemodialysis. Endpoints caused by atherosclerotic events are less frequent in these patients [28, 32], which may explain our finding that low apoA-IV concentrations had a pronounced effect on total mortality and sudden cardiac death, but not on atherosclerotic endpoints. This finding in the follow-up observation is in agreement with those concerning baseline comorbidities, where the association between apoA-IV and congestive heart failure was stronger than that between apoA-IV and atherosclerotic outcomes of the heart and arterial vessels.

In the prospective follow-up of this study, we noted that the association between apoA-IV and adverse outcomes might have been modified by nutrition and wasting. For most of the outcomes shown in Table 3, the adverse effect of low apoA-IV levels could only be observed in the group without wasting (BMI > 23 kg m^{-2} or the extended nonwasting group); in the group with BMI ≤ 23 kg m^{-2} and the extended combined wasting group the correlation with most outcomes was in fact in the opposite direction. This implies that apoA-IV might have an important role in the early stages of protein–energy wasting, and might thus indicate the beginning of a worsening of nutritional status even in patients with normal BMI or those classified in the nonwasting group according to the extended wasting definition.

Atorvastatin did not have any effect on apoA-IV concentrations as evidenced by the similar and very minor changes among patients in both the atorvastatin and placebo groups during the first 6 months of the trial. This finding was not unexpected as statins have a strong effect on the LDL cholesterol pathway and only small effects on HDL cholesterol levels.

Our study is limited by the fact that the analysis was performed *post hoc* and that the 4D Study is based on a selected cohort of patients from Germany with type 2 diabetes mellitus undergoing haemodialysis. Whether the findings can be extrapolated to other populations and other disease conditions is yet to be determined. The main strengths of the 4D Study are the long-term follow-up design, the large number of patients and the high incidence of pre-specified and centrally adjudicated endpoints [28].

In conclusion, results from the 4D Study revealed that low apoA-IV concentrations are strongly associated with the risk of all-cause mortality and death from cardiac causes, especially sudden cardiac death. ApoA-IV was less strongly associated with atherogenic events such as fatal and nonfatal myocardial infarction as well as with cardiovascular interventions. These associations were particularly strong in well-nourished patients which points to potentially complex interactions between apoA-IV, protein–energy wasting and nutrition.

Conflict of interest statement

No conflicts of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Associations between apoA-IV and outcomes during the prospective follow-up in the total population and in both nonwasting groups using further adjustment.

Table S2. Association between apoA-IV and outcomes during prospective follow-up in the wasting groups.

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