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Short communication

Lack of association between the Trp719Arg polymorphism in kinesin-like protein-6 and cardiovascular risk and efficacy of atorvastatin among subjects with diabetes on dialysis: The 4D study

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ABSTRACT

Aims: We investigated whether KIF6 Trp719Arg genotypes affect cardiovascular outcomes and efficacy of statin therapy in patients with type 2 diabetes mellitus undergoing hemodialysis.
Methods and results: We conducted a post hoc analysis of the 4D-study, a randomized trial including 1255 patients. Patients were randomly assigned to double-blind treatment with either 20 mg of atorvastatin ($n = 619$) or placebo ($n = 636$) once daily and followed for 4 years (median). DNA was available for 1232 patients and we assessed KIF6 Trp719Arg genotypes by PCR and subsequent restriction digest. Carriers of the Arg719 allele showed no increased prevalence of cardiovascular disease. The incidence of cardiac death, MI, and stroke did not differ across KIF6 genotypes, irrespective of whether the patients were treated with atorvastatin or not.
Conclusion: In patients with type 2 diabetes mellitus on hemodialysis, KIF6 Trp719Arg genotypes were not associated with adverse cardiovascular outcomes during follow-up or with the efficacy of atorvastatin therapy.

1. Introduction

Atherosclerosis is a chronic disease driven by several risk factors [1] and one of the leading causes of morbidity and mortality in Western countries. One of these risk factors is LDL cholesterol (LDL-C) and statins are the most prominent drug class used for lowering LDL-C. The vast majority of intervention studies using a statin have shown a clear benefit, decreasing the rate of cardiovascular events by nearly one quarter per 1 mmol reduction in LDL-C [2]. In renal diseases abnormalities in lipoprotein metabolism are highly prevalent and are potential important risk factors for accelerated atherosclerosis [3]. The German Diabetes Dialysis Study [4D-Study (Die Deutsche Diabetes Dialyse Studie)] was a prospective, randomized, placebo-controlled trial investigating the effect of 20 mg atorvastatin on cardiovascular events in patients with type 2 diabetes mellitus (T2DM) receiving hemodialysis. Although the median LDL cholesterol level was significantly reduced by an average of 42% (53 mg/dL), no significant effect on the primary endpoint of combined cardiovascular events was seen [4]. The reason for this unexpected outcome is still not clear.

Recently, a variation of the kinesin-like protein 6 (KIF6), Trp719Arg, has been described which might explain some of the heterogeneity in the efficacy of statin therapy [5] on cardiovascular outcome. Two studies, CARE and WOSCOPS, showed that pravastatin significantly reduced coronary events only in carriers of the minor 719Arg allele. Data from the PROVE-IT TIMI 22 trial showed that this effect is not specific for pravastatin, but is also present for atorvastatin at a dose of 80 mg/day [6]. Recently, data from the Heart Protection Study [7] and the JUPITER trial [8] questioned the use of KIF6 genotypes to predict the efficacy of statins.

However, there have been no studies to data assessing the impact of KIF6 genotypes on cardiovascular outcomes and total mortality in T2DM patients receiving hemodialysis who represent a population at very high risk of adverse cardiovascular outcomes.

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2. Materials and methods

2.1. Study design and participants

Methods of the 4D-study have been described in detail elsewhere [4]. Briefly, the 4D-study was a randomized trial including 1255 patients with T2DM and less than two year previous treatment by hemodialysis. Patients were randomly assigned to a double-blinded treatment with either 20 mg of atorvastatin ($n = 619$) or placebo ($n = 636$) once daily and were followed up until the date of death, censoring, or the end of the study. The primary study endpoint was a composite of death from cardiac causes, myocardial infarction (MI) and stroke, whichever occurred first.

2.2. Laboratory procedures

All laboratory measurements of the 4D-study were performed at the Department of Clinical Chemistry, University of Freiburg, Germany. The KIF6 polymorphism (rs20455) was genotyped by polymerase chain reaction and FokI restriction fragment-length analysis (see Supplement).

2.3. Statistical analysis

We calculated descriptive statistics of patient characteristics by KIF6 genotype (means and standard deviations for continuous variables except for triglycerides, CRP, and NTproBNP (median with minimum and maximum)). For categorical variables we calculated absolute and relative frequencies. We used analysis of variance (ANOVA) or logistic regression to compare clinical and anthropometric characteristics and the history of cardiovascular events across KIF6 genotypes. Cox proportional hazards regression models were used to assess the effect of the KIF genotype on outcome and calculated hazard ratios (HR) and 95 percent confidence intervals (95% CI). We conducted all statistical tests 2-sided and considered p-values <0.05 as significant. All calculations were performed using SPSS for Windows (version 17.0.1).

3. Results

We genotyped 1232 patients and found a genotype distribution of 41.5% for homozygote carriers of the wild type allele Trp719 and of 13% for homozygote carriers of the rare variant Arg719. The genotypes were in Hardy–Weinberg equilibrium and within the range of published frequencies for Caucasians [6,9]. Patients' characteristics at baseline are shown in Table 1. There were no significant differences in the prevalence of cardiovascular risk factors between the groups of KIF genotypes. Supplementary Table 1 shows the results of the logistic regression analysis between KIF6 genotypes and their history of cardiac and vascular events. There was no association between KIF6 genotypes with the history of CAD, CHF, and stroke/TIA.

Of all 1232 patients analysed 628 were randomized to placebo and 604 to atorvastatin. During the mean follow-up of 4 years 604 patients died. Furthermore, cardiac death, MI, and stroke occurred in 308, 196, and 103 patients, respectively. Table 2 shows separately for placebo and atorvastatin treated patients the results of the time to event analysis of KIF6 genotypes for various endpoints. In the placebo group we found no effect of the KIF genotype on outcome. Likewise, in the atorvastatin group there was no association with the major events.

4. Discussion

This study investigated the effect of KIF6 Trp719Arg genotypes on components of cardiovascular disease, cardiovascular outcome and mortality during follow-up, and on the efficacy of atorvastatin in patients with type 2 diabetes mellitus on hemodialysis. The main findings are that KIF6 719Arg did not influence the cardiovascular outcomes or mortality during follow-up or the efficacy of atorvastatin within this cohort.

Association of KIF6 Trp719Arg genotypes with CAD were first published for the *Atherosclerosis Risk in Communities* (ARIC) study in 2007 [10] and were then replicated in 2008 in the *Cardiovascular Health Study* (CHS) [11], and the *Women's Health Study* (WHS) [12]. The results were supported by data from the placebo groups of the CARE, WOSCOPS, and PROSPER intervention studies (reviewed by Li et al. [13]). In a meta-analysis the authors calculated a risk ratio of 1.22 (95% CI 1.12–1.32; combined $p = 1.02 \times 10^{-6}$) for the minor allele affecting CAD events. Conflicting data were published from the *Ottawa Heart Genomics Study* [14], and from a huge replication study with 17,000 cases and 39,369 controls of European descent [9]. In both studies no association of KIF6 Trp719Arg genotypes with cardiovascular disease could be shown. Our results are in line with their observation. We found no association, irrespective of whether we analysed all CAD cases together or divided into subgroups like MI, CABG, PCI or CHD (data not shown).

The observation from previous studies that KIF6 Trp719Arg influences the efficacy of pravastatin and atorvastatin (CAR, WOSCOPS, PROSPER, and PROVE-IT) [13] has raised particular interest. Only carriers of the 719Arg allele showed a reduction in risk under statin therapy in the previous studies. This reduction was always independent from the cholesterol lowering effect. Based on these results the company Celera developed a pharmacogenetic assay (StatinCheck) based on KIF6 genotyping to predict whether or not a patient could benefit from statin therapy.

Actually, in the 4D study carriers of the KIF6 719Arg polymorphism did not differ from the 719Trp carriers in their response to treatment with atorvastatin. Is the effect of KIF6 719Arg dependent on the type and dose of the prescribed statin and therefore potentially not detectable in 4D? In PROVE IT-TIMI 22 the effect of 80 mg atorvastatin in comparison to 40 mg pravastatin was analysed [6], and no difference between the function of the hydrophilic and lipophilic statins was detected. In the other three published intervention studies the patients received 40 mg of pravastatin (CARE, WOSCOPS, PROSPER), a dose which is comparable to 20 mg atorvastatin. So, neither type nor dose of the statin explains the discrepancy.

Our results, showing that KIF6 genotypes do not influence the efficacy of statins, are supported by two other studies which have been published very recently: In the Heart Protection study [7], a secondary prevention study from the UK, 18.348 individuals taking 40 mg simvastatin or placebo for 5 years were analysed. Statin therapy significantly reduced the incidence of coronary and other major vascular events to a similar extent, irrespective of KIF6 genotype. Similar results were obtained in the JUPITER trial, a primary prevention study using 20 mg of rosuvastatin [8].

Potential limitations of our study need to be acknowledged. The study was conducted in a high risk population of dialysis patients. Therefore, it may be difficult to transfer results from patient groups with low or intermediate risk to our patients and vice versa. Both, in the 4D and in the AURORA trial, statins did not show the expected improvement in survival [4,15], although they decreased LDL cholesterol by 42%. Therefore our results concerning the missing effect of KIF6 polymorphism on the efficacy of atorvastatin should be repeated in further studies. Major strengths of our study include the uniform phenotyping in our study with information being provided by trained medical staff, the centrally

Table 1
Patients' baseline characteristics according to KIF6 genotype ($n = 1232$).

KIF6 genotype	Missing	T/T	T/C	C/C
n	23	511	561	160
Frequency (%)	–	41.5	45.5	13
Age (years)	67.5 ± 6.5	65.3 ± 8	65.7 ± 8.6	67 ± 8
Gender male (%) (*n*)	56.5 (13)	53 (271)	53.8 (302)	56.9 (91)
Atorvastatin (%) (*n*)	65.2 (15)	49.1 (251)	46.9 (263)	56.3 (90)
Ever smoking (%) (*n*)	47.8 (11)	40.3 (206)	37.6 (211)	49.4 (79)
Body mass index (kg/m^2)	28.3 ± 4.9	27.7 ± 5.1	27.2 ± 4.6	27.9 ± 4.5
Systolic BP[a] (mmHg)	146 ± 21	144 ± 22	147 ± 22	146 ± 20
Diastolic BP[a] (mmHg)	76 ± 13	75 ± 12	76 ± 11	75 ± 10
Diabetes since (years)	16.6 ± 10.2	18.1 ± 9.0	18.2 ± 8.7	18.0 ± 8.8
Dialysis since (month)	8.2 ± 7.6	8.0 ± 6.8	8.6 ± 6.9	8.2 ± 7.1
HbA1c (%)	6.5 ± 1.3	6.7 ± 1.3	6.8 ± 1.3	6.8 ± 1.2
TG (mg/dL)	193 (63–1090)	224 (56–1102)	212 (35–1074)	243 (45–1164)
LDL-C (mg/dL)	118 ± 31	125 ± 29	125 ± 31	128 ± 29
HDL-C (mg/dL)	37.2 ± 12.1	36 ± 13.2	36.7 ± 13.2	35.1 ± 13.2
CRP (mg/dL)	6.1 (0.3–26.8)	5.3 (0.2–305.7)	4.7 (0.3–237)	5.2 (0.4–105)
NT-pro BNP (pg/ml)	3617 (197–62,567)	3577 (118–86,217)	2993 (33–134,492)	3476 (210–200,032)
History of arrhythmia (%) (*n*)	13 (3)	19.4 (99)	17.1 (96)	23.8 (38)
CAD[b] (%) (*n*)	34.8 (8)	29.5 (151)	29.6 (166)	27.5 (44)
CHF[c] (%) (*n*)	39.1 (9)	35 (179)	35.1 (197)	36.9 (59)
Stroke/TIA (%) (*n*)	30.4 (7)	17.8 (91)	18.9 (106)	12.5 (20)
PVD[d] (%) (*n*)	56.5 (13)	41.5 (212)	44.9 (252)	51.9 (83)

[a] Blood pressure.
[b] Coronary artery disease (MI, CABG, PCI or CHD).
[c] Congestive heart failure.
[d] Peripheral vascular disease.

Table 2
HR for adverse outcome during follow-up according to KIF6 genotypes.

	HR (95% CI) *p* value					
	Unadjusted co-dominant	Adjusted co-dominant	Unadjusted dominant	Adjusted dominant	Unadjusted recessive	Adjusted recessive
Placebo						
All-cause mortality	0.984	0.918	1.203	1.055	0.898	0.834
	(0.831–1.166)	(0.771–1.093)	(0.866–1.671)	(0.751–1.480)	(0.718–1.122)	(0.661–1.054)
n = 315	0.856	0.339	0.269	0.758	0.343	0.128
Cardiac death	1.046	0.976	1.163	1.032	1.015	1.032
	(0.825–1.326)	(0.766–1.244)	(0.727–1.859)	(0.637–1.672)	(0.738–1.396)	(0.637–1.672)
n = 158	0.710	0.845	0.528	0.899	0.927	0.899
Myocardial infarction	0.834	0.807	0.709	0.578	0.827	0.833
	(0.621–1.120)	(0.601–1.083)	(0.358–1.404)	(0.2871–1.168)	(0.566–1.210)	(0.564–1.229)
n = 108	0.227	0.153	0.324	0.127	0.328	0.357
Stroke/TIA	0.935	0.906	0.581	0.563	1.064	1.023
	(0.593–1.473)	(0.558–1.471)	(0.180–1.876)	(0.167–1.900)	(0.580–1.953)	(0.536–1.951)
n = 44	0.771	0.691	0.363	0.354	0.840	0.945
Atorvastatin						
All-cause mortality	1.051	1.015	1.291	1.125	0.959	0.964
	(0.891–1.239)	(0.860–1.196)	(0.954–1.747)	(0.823–1.536)	(0.759–1.211)	(0.759–1.225)
n = 289	0.558	0.863	0.098	0.461	0.725	0.765
Cardiac death	1.011	0.967	1.257	1.085	0.902	1.085
	(0.025–1.326)	(0.762–1.226)	(0.817–1.936)	(0.692–1.702)	(0.649–1.254)	(0.692–1.702)
n = 144	0.927	0. 780	0.298	0.723	0.540	0.723
Myocardial infarction	0.937	0.969	0.937	0.860	0.908	1.012
	(0.691–1.269)	(0.714–1.314)	(0.510–1.723)	(0.460 1.608)	(0.596–1.385)	(0.653–1.568)
n = 88	0.673	0.839	0.834	0.636	0.654	0.957
Stroke/TIA	1.066	1.108	1.890	2.103	0.768	0.784
	(0.739–1.536)	(0.759–1.617)	(1.037–3.444)	(1.115–3.967)	(0.460–1.280)	(0.459–1.337)
n = 59	0.733	0.594	0.038	0.022	0.310	0.371

Models were adjusted for age, gender, BMI, systolic/diastolic blood pressure, smoking history, duration of dialysis, baseline LDL- and HDL-cholesterol, history of CAD, stroke, CHF, PVD, and logarithmically transformed concentrations of triglycerides, hsCRP, and NT-pro-BNP.

adjudicated endpoints and the relatively long follow-up in dialysis patients.

In conclusion, our post hoc analysis of the 4D study indicates that KIF6 Trp719Arg genotypes did not affect cardiovascular risk during follow-up nor the efficacy of atorvastatin treatment in patients with type 2 diabetes mellitus on hemodialysis. Further studies are needed to explain the findings of our study and to elucidate the underlying mechanisms. Until then, pharmacogenetic assays to predict statin response by KIF 6 genotype should be interpreted with caution.

Conflict of interest statement

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.07.126.

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