Diabetes and Vascular Disease Research

Association between a gene variant near ataxia telangiectasia mutated and coronary artery disease in men

Stephan Schiekofer, Izabela Bobak, Marcus E Kleber, Winfried Maerz, Gottfried Rudofsky, Klaus A Dugi and Jochen G Schneider

>> Version of Record - Dec 16, 2013

OnlineFirst Version of Record - Nov 26, 2013

What is This?

Association between a gene variant near ataxia telangiectasia mutated and coronary artery disease in men

Stephan Schiekofer[1], Izabela Bobak[2], Marcus E Kleber[3,4], Winfried Maerz[4,5,6], Gottfried Rudofsky[7], Klaus A Dugi[7] and Jochen G Schneider[2,8]

Abstract

Objective: Type 2 diabetes is accompanied by increased mortality from coronary artery disease (CAD), but the mechanisms linking these conditions remain elusive. Hence, treatment of hyperglycaemia alone is not sufficient to avoid CAD in diabetes. Alternative views suggest that metabolic and vascular diseases share unifying cellular defects that could serve as targets for novel therapeutic strategies. Recently, a variant [single-nucleotide polymorphism (SNP); rs11212617] near the gene for ataxia telangiectasia mutated (ATM) has been associated with glycaemic response to metformin.

Materials and methods: We determined rs11212617 in 240 male patients who underwent elective coronary angiography.

Results: While the variant was not associated with glucose concentrations, the A allele was significantly associated with the presence of CAD (chi-square, $p = 0.003$), as well as with logarithmically transformed quantitative CAD indices [severe score (SS): 0.5 (0.4–0.6) vs 0.3 (0.2–0.5); extent score (ES): 2.63 (2.4–2.9) vs 1.94 (1.4–2.4), both $p < 0.05$, respectively]. Multivariate analysis revealed an independent association between the A allele with ES ($\beta = 0.17$, $p < 0.01$).

Conclusion: Our data suggest that ATM-dependent signalling might play a role in the development of atherosclerotic vascular disease, but larger studies are necessary to substantiate such a hypothesis.

Keywords

Vascular disease, metabolic syndrome, co-morbidities

Introduction

Type 2 diabetes represents a major challenge to health care because the numbers of affected individuals are on the rise not only in industrialised countries, and its micro- and macro-vascular complications can cause blindness, renal failure, amputations and increased cardiovascular morbidity and mortality.[1] Although type 2 diabetes is diagnosed by elevated blood glucose concentration, targeting hyperglycaemia as such has surprisingly yielded little to no benefit in reducing diabetic complications or even mortality.[2]

Therefore, the apparent incomplete understanding of the disease pathophysiology has led to the search for alternative clues tying different chronic conditions by unifying cellular mechanisms. Recently, an association between the C allele of the rs11212617 variant near the gene for ataxia telangiectasia mutated (ATM) has been demonstrated to affect the treatment response to metformin in human diabetes, possibly through modulating the activation of adenosine monophosphate–activated protein kinase (AMPK)

[1]Center for Geriatric Medicine at Bezirksklinikum Regensburg, Regensburg, Germany
[2]Department of Internal Medicine II, Saarland University Medical Center, Homburg, Germany
[3]Department of Internal Medicine II – Cardiology, University of Ulm Medical Centre, Ulm, Germany
[4]Mannheim Institute of Public Health, Social and Preventive Medicine, Medical Faculty Mannheim, Heidelberg University, Heidelberg, Germany
[5]Synlab Academy, Synlab Services GmbH, Mannheim, Germany
[6]Clinical Institute of Medical & Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

[7]Department of Internal Medicine I, University of Heidelberg, Heidelberg, Germany
[8]Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch, Luxembourg

Corresponding author:
Jochen G Schneider, Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Campus Belval, 7 Avenue Des Hauts Fourneaux, L-4362 Esch, Luxembourg.
Email: jg.schneider@outlook.com

by metformin.[3] ATM is a protein kinase that responds to DNA damage by phosphorylation of p53 and other targets but is also involved in insulin signalling.[4] Previous data showed that heterozygous mutations in ATM (up to 1.4%– 2% of the general population) can lead to higher mortality and earlier death from coronary artery disease (CAD).[5]

The aim of our study was to test for an association between the rs11212617 near the ATM gene with CAD in men undergoing coronary angiography.

Materials and methods

A total of 253 men, ages from 58 to 86 years, were recruited from the University Hospital Heidelberg. All individuals had diagnosed or suspected CAD and underwent elective coronary angiography. The presence, severity and the extent of CAD were assessed as described previously.[6] Patient characteristics and biochemical parameters were determined in a standard clinical research setting as described.[6] In all, 242 patients were enrolled and genotyped for the polymorphism at the locus near the ATM gene (rs11212617) using a TaqMan assay according to the manufacturer's protocol (Life Science Technologies, Darmstadt, Germany). From 11 patients, DNA, serum or other essential information was missing. The study was approved by the Ethics Committee of the University of Heidelberg, and all participants gave written consent to the study. Statistical methods comprised chi-square test, comparisons of means by *t*-tests and analysis of variance (ANOVA) using logarithmic transformation of non-parametric parameters, followed by post hoc testing against the one group as baseline, as well as multivariate regression analyses, including analysis of covariance (ANCOVA) according to the general linear model using logarithmically transformed parameters. Data are expressed as mean ± 95% confidence interval (CI), on logarithmic scale where appropriate. Statistical analyses were performed using SPSS, release 19 (IBM SPSS Statistics, Chicago, IL, USA).

Results

The allele frequencies were 0.52 for the A allele and 0.48 for the C allele with 114 heterozygous subjects (CA). The allele frequencies were in accordance with the Hardy–Weinberg equilibrium (chi-square, $p = 0.81$). The characteristics of the study population according to the genotype are displayed in Table 1. Because the minor allele (C allele) occurred in nearly 50% of the subjects, we decided to employ a general model at first instance with genotype-based testing which treats the three genotypes as separate categories, leaving an additional degree of freedom as compared to weigh out a dominant or recessive model.

Previous results suggested an association of the C allele of the rs11212617 variant near the ATM locus with a beneficial effect on HbA1c-lowering potential and a lower

HbA1c when analysed in a quantitative trait.[3] Although the effect on glucose-lowering potential could not be assessed in our cross-sectional cohort, we did not find a significant association between the rs11212617 variant and glucose concentration (data not shown). In contrast, we found a significant higher frequency of CAD presence among carriers of the A allele ($p = 0.003$ by chi-square test of independence). This association was further substantiated by significant higher logarithmically transformed quantitative CAD indices in presence of the A allele [severe score (SS): 0.5 (0.4–0.6) vs 0.3 (0.2–0.5); extent score (ES): 2.63 (2.4–2.9) vs 1.94 (1.4–2.4), both $p < 0.05$, respectively] and by an association between the mere A allele presence and the logarithmically transformed markers of CAD (Table 1).

Being aware of the fact that the associations were consistent but modest, we tested for independent effects employing a multivariate regression analysis using ES as dependent variable and several demographic and metabolic parameters known to be associated with atherosclerosis as confounding factors [age, body mass index (BMI), lipid and fasting glucose levels, smoking, previous myocardial infarction, hypertension and statin use]. The results revealed an independent association between the A allele and ES of CAD ($\beta = 0.17$, $p < 0.01$; Table 2) with an effect size at least equally powerful as compared to the influence of low-density lipoprotein (LDL) cholesterol or hypertension. Equal results were obtained for SS in multivariate analysis (data not shown). The distribution of CAD ES and SS (Table 1) suggested testing for a dominant model for AA/CA versus CC. This comparison yielded a nearly significant difference in SS of CAD [0.34 (0.2–0.4) vs 0.5 (0.4–0.5), $p = 0.06$] and a significant difference of ES [1.9 (1.48–2.32) versus 2.6 (2.31–2.79), $p = 0.01$] in an ANCOVA of logarithmically transformed scores according to the general linear model using the same confounding variables as indicated above.

Conclusion

The pathogenesis and pathophysiology of type 2 diabetes and its complications are despite the pandemic dimension of diabetes incompletely understood,[1] hence the contribution of isolated hyperglycaemia and/or its correction to CAD is not clear.[2] Targeting a cluster of different risk factors at a time, which account for the so-called metabolic syndrome, seems to be more effective in reducing vascular disease. The question becomes, however, whether these metabolic syndrome components are disparate or share a unifying, yet unknown underlying mechanism,[7] following Barabasi's basic concept of complex cellular networks underlying genotype-to-genotype relationships.[8]

The report on the association between the variant rs11212617 near the ATM gene and metformin treatment response suggests the participation of ATM, a DNA repair enzyme, in metabolic diseases.[3] While this finding is

Table 1. Clinical characteristics of patients according to rs11212617 genotype.

Factor	CC (*n* = 58)	CA (*n* = 114)	AA (*n* = 70)
Age (years)	60.0 (57.5–62.6)	62.8 (61.0–65.0)	60.7 (58.1–63.2)
BMI (kg/m^2)	27.1 (26.3–27.9)	27.8 (27.2–28.5)	27.4 (26.5–28.2)
Cholesterol (mg/dL)			
Total	212.7 (200.1–225.3)	203.3 (195.1–211.6)	203.1 (191.5–214.7)
HDL	41.2 (38.4–43.9)	40.0 (37.6–42.3)	38.8 (36.1–41.5)
LDL	146.2 (135.8–156.4)	138.0 (131.6–144.4)	140.7 (129.8–151.6)
VLDL[a]	1.29 (1.2–1.4)	1.3 (1.2–1.4)	1.3 (1.2–1.4)
Triacylglycerol[a]	2.11 (2.0–2.2)	2.1 (2.1–2.15)	2.0 (2.0–2.14)
Atherosclerosis risk index	5.3 (4.9–5.7)	5.6 (5.0–6.1)	5.7 (5.2–6.2)
Fasting plasma glucose (mg/dL)	111.7 (102.6–120.6)	113.5 (106.2–122.4)	115.3 (104.4–124.2)
Pack years[a]	3.2 (2.9–3.4)	3.1 (2.9–3.3)	3.1 (2.8–3.4)
Hs-CRP[a]	0.25 (0.12–0.38)	0.29 (0.18–0.38)	0.20 (0.07–0.31)
SS[a]	0.34 (0.2–0.46)	0.46 (0.36–0.54)	0.52 (0.41–0.64)*
ES[a]	1.9 (1.44–2.4)	2.5 (2.2–2.9)*	2.8 (2.4–3.2)**
Previous MI	38%	33%	35%
Statin use	38%	50%	48%
Hypertension	45%	54%	49%

BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; Hs-CRP: high sensitivity C-reactive protein; SS: severe score; ES: extent score; MI: myocardial infarction; CI: confidence interval; ANOVA: analysis of variance.
Values are mean ± 95% CI. Bold variables were entered into multivariate analysis.
[a]Log-transformed parameter ± 95% CI from univariate analyses.
*$p < 0.05$, **$p < 0.01$, ANOVA of log-transformed values.

Table 2. Multiple regression analysis result of variables with significant effect on CAD extent.

Independent variable	*T*	95% CI	*p* value
rs11212617	2.7	1.7 to 10.7	0.008
BMI	1.22	−0.378 to 1.6	0.2
Age	2.7	0.13 to 0.83	0.008
LDL cholesterol	1.5	−0.04 to 0.30	0.1
HDL cholesterol	−0.86	−0.78 to 0.32	0.4
Atherogenic index	−0.7	−6.9 to 3.3	0.5
Hs-CRP	−1.12	−0.89 to 0.23	0.2
Fasting glucose	0.26	−1.5 to 2.0	0.8
Smoking	2.1	0.015 to 0.34	0.03
Previous MI	4.9	11.3 to 26.2	<0.001
Hypertension	2.0	0.24 to 14.2	0.045
Statin	2.9	3.2 to 17.0	0.004

CAD: coronary artery disease; CI: confidence interval; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Hs-CRP: high sensitivity C-reactive protein; MI: myocardial infarction.
The dependent variable is ES (a.u.); $r^2 = 0.371$. *T* represents the estimated coefficient, divided by its own standard error. *T* values below −2 or above 2 are considered as useful predictors in the model.

seemingly unexpected, links between DNA-damage response and metabolic or vascular diseases in mice have been published before.[9,10]

These data prompted us to study the potential influence of this variant on CAD in humans. Our data show a significant correlation between the A allele of rs11212617 and SS and ES in men. This association was independent of other potential confounding factors in a multivariate analysis

(Table 2). Our results may actually corroborate a proposed beneficial effect of the C allele of rs11212617 on metformin's effect on lowering HbA1c,[3] by a possibly accompanied beneficial effect on vascular disease, but this hypothesis is very speculative and lacks a mechanistic explanation. However, besides an effect of the ATM kinase on AMPK activity,[3] other modes of action also qualify for an ATM-mediated influence on metabolic vascular disease, including

the involvement of reactive oxygen species (oxidative stress) and other stress-related signalling.[11] In fact, it seems that various effects differentially regulate/modulate the effects that link ATM to metabolic vascular diseases.[12]

Our study suffers from limitations such as the moderate subject number of males only and the lack of haplotype genotypic data. However, a recent study corroborates our findings. The authors showed a significant correlation between functional polymorphism (rs189037) in the ATM promoter region, with lower ATM expression associated with the degree of CAD as well as with diabetes mellitus prevalence in the Chinese cohort.[13]

In conclusion, the rs11212617 variant near the ATM gene is associated with CAD in men, which suggests that ATM activity may be associated with cardiovascular disease by complex underlying mechanistic network. The observed association and the missing underlying explanation warrant further basic research efforts and epidemiological evidence.

References

1. Virally M, Blickle JF, Girard J, et al. Type 2 diabetes mellitus: epidemiology, pathophysiology, unmet needs and therapeutical perspectives. *Diabetes Metab* 2007; 33: 231–244.
2. Skyler JS, Bergenstal R, Bonow RO, et al. Intensive glycemic control and the prevention of cardiovascular events: implications of the ACCORD, ADVANCE, and VA diabetes trials: a position statement of the American Diabetes Association and a scientific statement of the American College of Cardiology Foundation and the American Heart Association. *Diabetes Care* 2009; 32: 187–192.
3. Zhou K, Bellenguez C, Spencer CC, et al. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. *Nat Genet* 2011; 43: 117–120.
4. Derheimer FA and Kastan MB. Multiple roles of ATM in monitoring and maintaining DNA integrity. *FEBS Lett* 2010; 584: 3675–3681.
5. Su Y and Swift M. Mortality rates among carriers of ataxia-telangiectasia mutant alleles. *Ann Intern Med* 2000; 133: 770–778.
6. Schneider JG, Schiekofer S, Eynatten M, et al. The proline 12 alanine substitution in the PPARgamma2 gene is associated with increased extent of coronary artery disease in men. *Exp Clin Endocrinol Diabetes* 2009; 117: 519–521.
7. Semenkovich CF. Insulin resistance and atherosclerosis. *J Clin Invest* 2006; 116: 1813–1822.
8. Vidal M, Cusick ME and Barabasi AL. Interactome networks and human disease. *Cell* 2011; 144: 986–998.
9. Schneider JG, Finck BN, Ren J, et al. ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. *Cell Metab* 2006; 4: 377–389.
10. Mercer JR, Cheng KK, Figg N, et al. DNA damage links mitochondrial dysfunction to atherosclerosis and the metabolic syndrome. *Circ Res* 2010; 107: 1021–1031.
11. Mercer JR, Yu E, Figg N, et al. The mitochondria-targeted antioxidant MitoQ decreases features of the metabolic syndrome in ATM+/-/ApoE-/- mice. *Free Radic Biol Med* 2012; 52: 841–849.
12. Razani B, Feng C and Semenkovich CF. p53 is required for chloroquine-induced atheroprotection but not insulin sensitization. *J Lipid Res* 2010; 51: 1738–1746.
13. Li S, Zhang L, Chen T, et al. Functional polymorphism rs189037 in the promoter region of ATM gene is associated with angiographically characterized coronary stenosis. *Atherosclerosis* 2011; 219: 694–697.