Research Article

Prostaglandin E synthase 2 (*PTGES2*) *Arg298His* polymorphism and parameters of the metabolic syndrome

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The prostaglandin E synthase 2 (*PTGES2*) gene maps to a locus linked to obesity and is involved in the synthesis of the antilipolytic compound prostaglandin E_2. In a recent study, we found an association of the minor *PTGES2 Arg298His* allele and lower risk of type 2 diabetes mellitus in the European Investigation into Cancer and Nutrition (EPIC) and Cooperative Health Research in the Augsburg Region (KORA) cohorts. Here, we employed our Metabolic Intervention Cohort Kiel (MICK) to assess the influence of the *PTGES2 Arg298His* polymorphism on a wider scale of parameters of the metabolic syndrome and postprandial metabolism. In comparison to subjects homozygous for the Arg allele, carriers of the His-allele showed significantly lower fasting insulin (geometric mean ± SEM: 11.8 μU/mL, 11.41–12.25 versus 13.0, 12.71 – 13.33; $p = 0.023$), lower postprandial insulin levels after an oral glucose tolerance test (area under the curve 77.2, 74.07 – 80.52 versus 81.2, 78.8 – 83.63; $p = 0.023$) and lower homeostasis model assessment (HOMA)-insulin-resistance (3.030, 2.909 – 3.157 versus 3.346, 3.257 – 3.438; $p = 0.041$) and HOMA-β-cell-function (107.2, 104.04 – 110.52 versus 117.2, 114.65 – 119.71; $p = 0.019$). Adjustment for body mass index (BMI) resulted in a loss of these significant differences. BMI tended to show lower values in His-allele carriers, ($p = 0.067$). In conclusion, risk-reducing effects of the minor His allele of the *PTGES2 Arg298His* polymorphism could be mediated partly by lowered BMI.

Keywords: Metabolic syndrome / Metabolic Intervention Cohort Kiel / Polymorphism / Prostaglandin E synthase 2

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1 Introduction

Prostaglandin E_2 (PGE_2) is produced in various organs and tissues and shows a broad range of bioactivities including smooth muscle dilatation/contraction, sodium excretion, body temperature regulation, induction of pain, inflammation-associated bone resorption, inhibition of immune responses and antilipolytic properties [1, 2]. Biosynthesis of PGE_2 from arachidonic acid is catalyzed by phospholipase A2, cyclooxygenase, and prostaglandin E synthase (PTGES). Three forms of PTGES that convert PGH2 to PGE2 are described [2]: microsomal PTGES (PTGES1), cytosolic PTGES (cPTGES) and membrane-associated PTGES (PTGES2). Whereas the physiological role of cPTGES is still uncertain [2], PTGES1 seems to be mainly involved in inflammation [3–5]. In contrast, PTGES2 is not induced by inflammatory stimuli and constitutively expressed in various tissues that show comparatively low PTGES1 expression [6, 7]. These findings imply an important role of PTGES2 in total PGE_2 production. Interestingly, the *PTGES2* gene maps close to the chromosomal band 9q34.13 [8], which shows a strong linkage signal for obesity

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Abbreviations: **BMI**, body mass index; **EPIC**, European Investigation into Cancer and Nutrition; **KORA**, Cooperative Health Research in the Augsburg Region; **MICK**, Metabolic Intervention Cohort Kiel; **oGTT**, oral glucose tolerance test; **oMTT**, oral metabolic tolerance test; **PGE_2**, prostaglandin E_2; **PTGES2**, prostaglandin E synthase 2; **SNP**, single nucleotide polymorphism

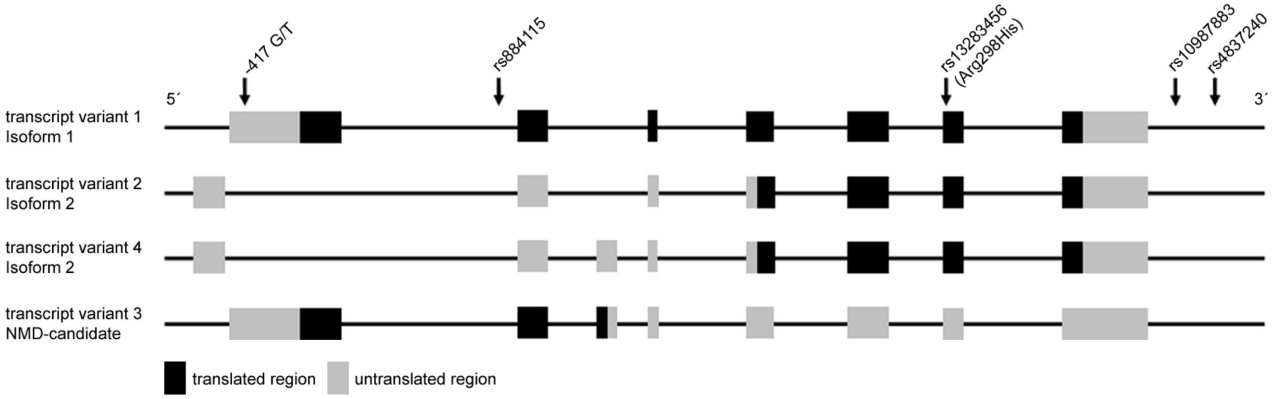

Figure 1. Polymorphisms, mRNA and protein identifiers of *PTGES2*. Four isoforms of PTGES2 are described, from top to bottom: Isoform 1 (NP_079348) based on transcript variant 1 (NM_025072), is the main isoform encoding a 377 AA protein to which this report and other publications refer. Isoform 2 (NP_945178, NP_945176) based on transcript variants 2 (NM_198940) and 4 (NM_198938), is not yet investigated. The transcript variant of isoform 3 is a possible nonsense-mediated mRNA decay (NMD) candidate. Information is based on NCBI Gene database entry.

[9]. Furthermore, PGE_2 is a potent antilipolytic agent in human adipose tissue [10–14]. Considering the chromosomal localization of *PTGES2* and its role in PGE_2 synthesis, we propose *PTGES2* as a candidate gene for risk factors of the metabolic syndrome. In an association study of the European Investigation into Cancer and Nutrition (EPIC) Potsdam and Cooperative Health Research in the Augsburg Region (KORA) cohorts [15], five single nucleotide polymorphisms (SNP) within the *PTGES2* gene region (Fig. 1) have been examined. The rare allele of the *Arg298His* polymorphism (dbSNP: rs13283456), which lies in exon 6 of the main *PTGES2* transcript (NM_025072, NP_079348), was significantly associated with a reduced risk of type 2 diabetes mellitus, which was also confirmed by haplotype analysis. To further investigate these findings we employed our Metabolic Intervention Cohort Kiel (MICK) to assess the influence of the *PTGES2 Arg298His* polymorphism on a wider scale of parameters of the metabolic syndrome and postprandial metabolism.

2 Materials and methods

2.1 MICK

Male subjects aged 45 to 65 years (716) were recruited from the resident register of Kiel, Germany, between January 2003 and March 2004. Exclusion criteria from the study were known diabetes type 1 or 2, diseases affecting nutrient digestion or metabolism, intake of lipid-lowering drugs or hormones, operation on the intestine within the past 3 months, hypo- or hyperthyreosis, chronic renal disease, hepatitis, cholestasis, alcoholism or cancer. Blood pressure, body weight, height, waist and hip circumference were determined at recruitment by means of standardized procedures. Impaired glucose metabolism and unknown type 2 diabetes mellitus were diagnosed based on fasting glucose levels at two different occasions and a standardized oral glucose tolerance test (oGTT) [16]. Of all participants, 124 men were diagnosed with impaired glucose regulation (fasting glucose >110 mg/dL determined on two different occasions or postprandial glucose >140 mg/dL); this number does not include type 2 diabetics. Forty-nine men were diagnosed with type 2 diabetes mellitus (fasting glucose >126 mg/dL determined on two different occasions and/or postprandial glucose >200 mg/dL). Furthermore, 541 subjects were classified having normal glucose metabolism and 2 subjects could not be diagnosed. All study participants had given informed consent and the genotype assessment was approved by the local ethics committee.

2.2 Laboratory parameters

Serum and plasma was separated from whole blood by centrifugation and stored at –70°C for further analyses. Serum cholesterol, serum HDL cholesterol, serum LDL cholesterol, plasma glucose and triglycerides were determined using Konelab System Reagents for Clinical Chemistry with a Kone Lab 20i analyzer (Thermo Fisher Scientific, Germany) according to the manufacturers' instructions and samples were measured in duplicate. Methods can be reviewed in [17]. Insulin was measured by radioimmunoassay [18] according to the manufacturers' instructions (Adaltis, Germany).

2.3 Oral metabolic tolerance test in MICK

A minimum of 3 days after the oral glucose tolerance test (oGTT) participants visited the department after a 12-h-overnight fast for an oral metabolic tolerance test (oMTT). An intravenous catheter equipped with disposable obtura-

Table 1. Primer and probe sequences for TaqMan SNP genotyping

	Primer forward	Primer revers	Probe 1 (VIC)	Probe 2 (FAM)
rs13283456 (Arg298His)	CCTCGCGCACGTTGTC	GCTGGGCTGGGAGTGG	CCAGGCACCGCCTC	CCAGGCACCACCTC

Sequences are given in 5′ to 3′ orientation.

tors was inserted into a forearm vein for blood sampling and a fasting blood sample (0 h) was obtained. Following this, the subjects drank 500 mL of a standardized high-fat mixed meal which contained 30 g of protein (11.9 kcal%), 75 g of carbohydrate (29.6 kcal%; 93% saccharose, 7% lactose), 58 g of fat (51.6 kcal%; 65% saturated, 35% unsaturated fatty acids), 10 g of alcohol (6.9 kcal%), 600 mg cholesterol and 30 000 IU retinylpalmitate. The total energy content was 1017 kcal (4255 kJ). The test meal was ingested within 15 min following drawing of the fasting blood sample. Blood withdrawal was repeated at 30 min, and 1, 2, 3, 4, 5, 6, 7, 8 and 9 h post-ingestion of oMTT. Subjects were allowed to walk or sit, but not to eat or exercise during the test. Intake of water *ad libitum* was permitted. Collected blood samples were processed as described above and used to determine serum triglycerides, insulin and glucose.

2.4 Genetic analyses

DNA was isolated from buffy coat (100 μL) using E.Z.N.A.® Blood DNA MiniKits by reversible binding of nucleic acids to a HiBind® column (Peqlab Biotechnologie, Erlangen, Germany; now distributed by Omega Bio-Tek, Doraville, GA, USA) according to the manufacturers instructions. Genotyping was performed with the TaqMan system [19] (ABI, Foster City, CA, USA) and fluorescence was measured with ABI Prism 7900 HT sequence detection system. 21 DNA failed genotyping. Sequences of primers and assay probes are shown in Table 1.

2.5 Statistical analyses

Statistics were computed with the Statistics Package for the Social Sciences 11.5 (SPSS, Chicago, IL, USA). Allele and genotype frequencies were determined by gene counting and the study population was tested for the distribution of genotypes according to Hardy-Weinberg-equilibrium (HWE) with a χ^2 test. Normally distributed variables are expressed as mean and SEM, variables with positively skewed distributions were log transformed and expressed as geometric mean with 95% confidence interval. Unadjusted differences in anthropometric variables according to genotype were analyzed by unpaired *t*-test, in case of variables with a skewed distribution based on log-transformed data. Modeled genotype differences in anthropometric, and blood pressure measurements were adjusted for age and, if appropriate, body mass index (BMI), using the General Linear Model procedure in SPSS. Modeling was not applied if the linear model showed a significant lack of fit. Significance level was set at $p < 0.05$.

3 Results

A total of 716 male subjects from MICK were genotyped for the *PTGES2 Arg298His* polymorphism, of which 21 failed to produce a genotyping result. The minor allele frequency was 0.17, and observed genotype frequencies were tested in compliance with Hardy-Weinberg equilibrium ($p = 0.4702$). Because of the low number of His homozygotes ($n = 18$) analyses were performed with a combined group of His homo- and heterozygotes (Arg/His + His/His) versus Arg homozygotes (Arg/ Arg). As shown in Table 2, significant associations of genotype groups with waist to hip ratio (WHR), blood pressure, total cholesterol, HDL, LDL, fasting and postprandial triglyceride levels, and postprandial glucose levels were not found. *PTGES2 298His* carriers show significant lower fasting insulin level ($p = 0.023$) whereas fasting glucose levels did not differ. Accordingly, homeostasis model assessment (HOMA)-IR and HOMA-β-cell function [20] are lower in His carriers ($p = 0.041$ and 0.019, respectively). The area under the curve of postprandial insulin course after an oGTT ($p = 0.023$) and ppHOMA-IR and ppHOMA-β-cell function ($p = 0.041$ and 0.019, respectively) were lower as well in comparison to homozygous Arg-allele carriers. After an oMTT, however, no differences were found. Additionally, BMI and waist circumference are lowered in His-allele carriers, but these differences reached no significance ($p = 0.067$ and 0.071, respectively). Computation of adjusted means for age, and if applicable, BMI, by univariate analysis of variance resulted in the loss of any significant differences between groups.

4 Discussion

We chose the prostaglandin E synthase 2 (*PTGES2*) gene as possible candidate gene for parameters of the metabolic syndrome, based on its chromosomal location linked to obesity [9] and its role in prostaglandin E_2 synthesis, a metabolite, which acts antilipolytic, and results in hypertrophy of adipocytes [14]. Furthermore, we found a decreased

risk of type 2 diabetes mellitus in carriers of the minor *PTGES2 298His* allele in two study populations (EPIC, KORA). Here, we performed an association study in the MICK to assess the influence of the *PTGES2 Arg298His* polymorphism on a wider scale of parameters of the metabolic syndrome and postprandial metabolism.

Our study revealed lower fasting insulin levels, lower postprandial insulin course after oGTT and lower HOMA-IR and HOMA-β-cell function for His-allele carriers in comparison to homozygous 298Arg carriers in an unadjusted model. At the same time, there was no significant difference in glucose levels. Adjustment for age and BMI resulted in a loss of significant differences between genotype groups. Therefore, higher insulin sensitivity of His-allele carriers could be mediated by their lower body weight. This hypothesis was only partially verified, as dif-

Table 2. Anthropometric and metabolic variables according to *PTGES2 Arg298His* genotypes

	Unadjusted			Adjusted		
	Arg/Arg	Arg/His + His/His	p[c)]	Arg/Arg	Arg/His + His/His	p
Subjects [n]	473	222		473	222	
Age [years][a)]	58.83 (0.252)	59.26 (0.362)	0.340	–	–	–
BMI [kg*m^{-2}][a,d)]	27.61 (0.192)	27.00 (0.261)	0.067	n/a	n/a	n/a
WHR[a,d)]	0.993 (0.003)	0.990 (0.004)	0.576	0.993 (0.003)	0.990 (0.004)	0.534
Waist circumference [cm][a,d)]	100.8 (0.54)	99.1 (0.77)	0.071	100.31 (0.24)	100.17 (0.36)	0.750
Systolic BP [mmHg][a,e)]	129.68 (0.800)	129.24 (1.291)	0.765	129.37 (0.760)	130.04 (1.112)	0.624
Diastolic BP [mmHg][a,e)]	80.89 (0.491)	79.69 (0.752)	0.173	80.68 (0.459)	80.13 (0.672)	0.507
Total cholesterol [mg*dl^{-1}][a,e)]	226.6 (1.88)	227.0 (2.84)	0.925	226.6 (1.90)	226.8 (2.79)	0.957
HDL [mg*dl^{-1}][a,e)]	53.08 (0.683)	53.80 (0.964)	0.552	n/a	n/a	n/a
LDL [mg*dl^{-1}][a,e)]	144.23 (1.529)	143.13 (2.191)	0.682	144.27 (1.517)	143.29 (2.217)	0.714
Fasting triglycerides [mg*dl^{-1}][b,e)]	124.6 (121.75-127.53)	120.6 (116.71-124.64)	0.422	123.7 (121.021-126.418)	123.0 (119.19-127.00)	0.890
Fasting Insulin [μU*mL^{-1}][b,e,f)]	13.0 (12.71-13.33)	11.8 (11.41-12.25)	0.023*	12.8 (12.57-13.04)	12.2 (11.90-12.56)	0.160
Fasting glucose [mg*dl^{-1}][b,e,f)]	104.1 (103.51-104.78)	104.0 (103.09-104.89)	0.885	103.8 (103.24-104.40)	104.6 (103.78-105.47)	0.432
ppTG AUC [mg*h*dl^{-1}][b,e)]	1562.4 (1529.55-1596.05)	1519.9 (1473.34-1567.97)	0.463	1554.0 (1522.29-1586.28)	1544.1 (1498.53-1591.05)	0.862
ppΔTG AUC [mg*h*dl^{-1}][b,e)]	420.1 (407.24-433.39)	407.3 (386.31-429.34)	0.592	n/a	n/a	n/a
ppInsulin AUC oMTT [μU*h*mL^{-1}][b,e)]	169.5 (164.83-174.37)	165.2 (158.84-171.76)	0.597	166.7 (162.96-170.52)	170.7 (165.09-176.51)	0.557
ppGlucose AUC oMTT [mg*h*dl^{-1}][b,e)]	515.6 (511.65-519.61)	516.9 (511.89-522.04)	0.844	513.6 (510.12-517.03)	520.8 (515.71-525.89)	0.241
ppInsulin AUC oGTT [μU*h*mL^{-1}][b,e)]	81.2 (78.80-83.63)	77.2 (74.07-80.52)	0.023*	80.1 (78.01-82.16)	79.8 (76.78-82.91)	0.941
ppGlucose AUC oGTT [mg*h*dl^{-1}][b,e)]	456.0 (451.40-460.61)	453.4 (447.27-459.59)	0.743	454.0 (449.95-458.16)	457.3 (451.28-463.39)	0.656
HOMA IR [μU*mL^{-1}* mmol*l^{-1}][b,e)]	3.346 (3.257-3.438)	3.030 (2.909-3.157)	0.041*	3.284 (3.217-3.353)	3.158 (3.063-3.256)	0.285
HOMA BCF [μU*l* mmol^{-1}*mL^{-1}][b,e)]	117.2 (114.65-119.71)	107.2 (104.04-110.52)	0.019*	115.9 (113.70-118.16)	109.4 (106.32-112.50)	0.090

a) Arithmetic mean (SEM).
b) Geometric mean (± SEM).
c) Unpaired *t*-test; two-sided significance.
d) Adjusted by age.
e) Adjusted by age and BMI.
f) Mean of two independent measurements (BP = blood pressure, pp = postprandial, TG = triglycerides, AUC = area under the curve, oMTT = oral metabolic tolerance test, oGTT = oral glucose tolerance test, n/a not applicable due to lack of fit, WHR = waist to hip ratio).

ferences in BMI between genotype groups revealed only borderline significance in the present study, significant differences in KORA and no significant differences in EPIC (accepted by *Journal of Clinical Endocrinology & Metabolism*). Based on these findings, a unifying hypothesis regarding functional consequence of the *PTGES2 Arg298His* SNP on BMI and insulin sensitivity is not feasible. Assuming a partial loss of function of the *PTGES2 298His* variant, reduced production of the antilipolytic compound PGE_2 in insulin target cells may have caused higher rates of lipolysis and/or lower turnover rates of insulin-stimulated phosphoinositide, which increases insulin sensitivity. Since we found a significant association between the *PTGES2 Arg298His* and β-cell function, an influence of the SNP on insulin sensitivity has to be also taken into account. A functional link between PGE_2 and β-cell dysfunction or impaired insulin secretion via the Akt (protein kinase B) pathway has been provided by cell culture experiments *in vitro* and *ex vivo* as well in animal studies and transgenic approaches [21, 22]. In future studies, connections between PTGES2 Arg298His and lipolysis, insulin sensitivity, and/or insulin secretion should be tested by functional approaches *in vitro*.

In conclusion, risk-reducing effects of the minor His allele of the *PTGES2 Arg298His* polymorphism could be mediated in part by lower BMI.

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