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Genetic variants in the leukemia-associated Rho guanine nucleotide exchange factor (*ARHGEF12*) gene are not associated with T2DM and related parameters in Caucasians (KORA study)

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Abstract

Objective: The aim of our study was to determine the variant pattern of the leukemia-associated Rho guanine nucleotide exchange factor (*LARG*, or *ARHGEF12*) gene and investigate whether *LARG* variants are associated with diabetes mellitus type 2 (T2DM), the metabolic syndrome (MetS), or related parameters such as insulin sensitivity in German Caucasians.

Design: We analyzed single nucleotide polymorphisms (SNPs) in the *LARG* gene in the 55–74-year-old individuals of the population-based German Caucasian Cooperative Health Research in the region of Augsburg (KORA) survey 4 (S4).

Methods: Sequencing of Tyr1306Cys, which was of functional relevance in Pima Indians, in 48 randomly selected individuals and genotyping of 11 additional *LARG* SNPs in 1653 subjects were performed. Four linkage disequilibrium (LD) blocks ($r^2 \geq 0.8$) were established and each block was statistically analyzed for association with metabolic traits. The association with T2DM and the MetS was analyzed by logistic regression in 1462 subjects, and HOMA-IR (homeostasis model assessment of insulin resistance) as a measure of insulin sensitivity was analyzed by the Kruskal–Wallis test in 1346 fasting subjects.

Results: The polymorphism Tyr1306Cys, which was significantly associated with insulin sensitivity in Pima Indians, was not found in the KORA S4 population. Statistical analysis yielded no significant associations ($P > 0.05$) between the analyzed *LARG* variants and T2DM, the MetS, or related parameters such as insulin sensitivity.

Conclusions: Caucasian individuals and Pima Indians differ in their genetic variance pattern in the *LARG* gene region. There is no evidence in the Caucasian KORA study that variants of the *LARG* gene confer susceptibility for T2DM, insulin sensitivity, or the MetS.

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Introduction

Lifestyle factors and genetic background play causal roles for the development of type 2 diabetes mellitus (T2DM) (1). Obesity, hypertension, dyslipidemia, and T2DM as a result of insulin resistance and impaired insulin secretion are characteristic parameters of the metabolic syndrome (MetS) (2). Insulin action is partly regulated by RhoA, a member of the Rho family of GTPases. RhoA activation reduces skeletal muscle glucose transport by the repression of signals of insulin receptor substrate and phosphatidylinositol 3-kinase/serine–threonine kinase (3). *LARG* (leukemia-associated Rho guanine nucleotide exchange factor) is a member of

the Rho family of guanine nucleotide exchange factors (GEFs) (4), which are involved in the regulation of the GTP-dependent Rho protein cycle. Decreased *LARG* activity leads to decreased RhoA activity and thus to increased insulin sensitivity (5).

A genome screen in more than 1200 Pima Indians detected a linkage between the microsatellite marker D11S4464 on chromosome 11q23–24 and body mass index (BMI) (logarithm of odds (LOD) = 3.6) as well as T2DM (LOD = 1.7) (6). The *LARG* gene, spanning 152 kb and consisting of 40 exons, is located 3.5 mega bases away from this marker. Its synonymous name (HUGO Gene Nomenclature Committee) is *ARHGEF12* (Rho guanine nucleotide exchange factor 12). Kovacs *et al.*

identified variants in the *LARG* gene and detected that an A/G substitution in exon 38 (Tyr1306Cys) coding for an amino acid exchange was significantly associated with insulin-mediated glucose uptake in 322 nondiabetic Pima Indians (7). Moreover, the genetic variant caused altered protein expression in NIH3T3 mouse fibroblasts. The *LARG* (Cys1306) protein had a significantly reduced ($P=0.03$) activity when compared with *LARG* (Tyr1306) protein (7). The aim of our study was to determine the variant pattern of the *LARG* gene in the population-based KORA (Cooperative Health Research in the Region of Augsburg) survey 4 (S4) and to investigate whether *LARG* variants are associated with T2DM, the MetS, or related parameters such as insulin sensitivity in German Caucasians.

Subjects and methods

Subjects

KORA S4 is a population-based study of adults with German nationality and main address in the study region of Augsburg (8). This survey included a standardized interview and clinical investigation and was conducted under the same conditions as the previous three surveys within the World Health Organization (WHO) MONICA Augsburg project (9). An oral glucose tolerance test (OGTT) was performed in 1354 participants aged 55–74 years (10). Measurement of quantitative parameters important for T2DM and the MetS was performed according to standardized protocols (10). The WHO criteria (11) were used for the definition of diabetes, and the MetS was defined according to the International Diabetes Federation definition for European persons (12). Finally, 1462 subjects, consisting of 1226 nondiabetic individuals who had undergone an OGTT, and 236 subjects with T2DM, were included in statistical analysis for T2DM and the MetS. For the quantitative parameters, 1346 fasting subjects were analyzed. Subjects with diabetes mellitus type 1 ($n=18$ for the analysis of T2DM and the MetS or $n=8$ for the analysis of quantitative parameters respectively) were excluded. Of the individuals in the analyzed sample 51.5% were men, the average age was 64 years and the mean BMI was 28 kg/m². Men ($n=137$) and women ($n=99$) with T2DM had a higher mean age (65.07/65.09 years) and a higher mean BMI (29.88/32.27 kg/m²) compared with participants without T2DM (mean age, 63.98/63.81 years; mean BMI, 27.99/28.43 kg/m²). All participants gave written informed consent for genetic studies.

Sequencing

For sequencing, 10–50 ng genomic DNA sample of 48 randomly selected subjects from KORA S4 were amplified by PCR using HotStarTaq DNA polymerase (Qiagen) and

a DNA Engine Tetrad thermal cycler (BioRad). Sequencing was done with the Big Dye Terminators version 3.7 (Applied Biosystems, Foster City, CA, USA) on an ABI 3730 sequencer (ABI) according to manufacturer's protocol. Sequence analysis was performed using Informax Vector NTI Suite 9.0.0 (Invitrogen).

Genotyping

Eleven polymorphisms were selected by literature and according to linkage disequilibrium (LD) blocks ($r^2 \geq 0.8$) by the HapMap project (www.hapmap.org) (public release no. 21a). Polymorphisms with a minor allele frequency below 10% were not included in the genotyping process due to the low power to detect an association. The selected single nucleotide polymorphisms (SNPs) completely cover the common genetic variation in the *LARG* gene and flanking regions.

DNA samples were genotyped with the MassARRAY system using the iPLEX chemistry (Sequenom, San Diego, CA, USA). Primer extension products were loaded onto a 384 element chip using a nanoliter pipetting system (SpectroCHIP, SpectroPOINT Spotter, Sequenom). The samples were analyzed in a MALDI TOF MS (matrix-assisted laser desorption ionization time of flight mass spectrometer: Bruker Daltonik, Leipzig, Germany). The resulting mass spectra were analyzed automatically for peak identification via the SpectroTYPER RT 3.4 software (Sequenom). For quality reasons, 10% of the spectra were checked independently by two investigators.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was checked by Fisher's exact test, and the LD structure was calculated with the java linkage disequilibrium plotter (JLIn) program (<http://www.genepi.com.au/projects/jlin>). All further analyses were performed with SAS (Statistical Analysis System version 9.1, Cary, NC, USA) and parametric analyses were adjusted for age and sex and additional for BMI when quantitative parameters were analyzed. T2DM and the MetS were analyzed by logistic regression. Quantitative parameters with approximate normal distribution were analyzed by linear regression, and homeostasis model assessment of insulin resistance (HOMA-IR) as a measure of insulin sensitivity was calculated by the product of fasting glucose (mU/l) and fasting insulin (mmol/l) divided by 22.5 (13) and analyzed by nonparametric Kruskal–Wallis test. All regression analyses were performed comparing the homozygous or heterozygous minor allele carriers separately, with the homozygous major allele carriers as reference group. $P < 0.05$ was regarded as statistically significant. The power calculation was done with genetic power calculator software (14).

Results

First, 48 subjects (96 chromosomes) of KORA S4 were sequenced for the polymorphism Tyr1306Cys and only the A allele, which codes for Tyr1306, was detected. For all 11 genetic variants, genotyping was successful with an average success rate of 98.8% and every polymorphism fulfilled the criteria of HWE ($P \geq 0.05$). Four LD blocks ($r^2 \geq 0.8$) were established and for statistical analysis one representative SNP tagging all other polymorphisms of this block was analyzed. Three of these polymorphisms (rs538661, rs476636, and rs2276035) are located in introns, whereas one SNP (rs12806740) is localized in the 5' flanking region (Table 1).

The analyzed sample consisted of 1462 KORA S4 subjects including 236 individuals with T2DM and 799 individuals with the MetS. We did not detect significant associations between the *LAGG* polymorphism (rs12806740, rs538661, rs476636, rs2276035) and T2DM or the MetS (Table 2). Moreover, in our study, none of the tested quantitative parameters (fasting triglycerides, high- or low-density lipoprotein cholesterol, total cholesterol, percent body fat, waist to hip ratio, uric acid, fasting glucose, 2-h plasma glucose, fasting insulin, blood pressure) were significantly associated with the investigated polymorphisms (data not shown). There was also no significant association between HOMA-IR (lowest $P=0.49$) and the analyzed SNPs in 1346 fasting KORA S4 subjects. The median for HOMA-IR was 2.42 (Table 2).

For the power analysis, we assumed that the minor alleles were associated with higher risk for T2DM or the MetS according to a codominant genetic model. The power ($\alpha=0.05$) to detect a minimum OR (odds ratio) for T2DM of 1.3 in a global test was 88% for the polymorphisms rs12806740 or rs538661, and 65% for the polymorphisms rs476636 or rs2276035. The power to detect a minimum OR for the MetS of 1.3 was 100% for all four polymorphisms.

Discussion

Sequencing of the polymorphism Tyr1306Cys was performed and four representative polymorphisms in the *LAGG* gene were analyzed for association with T2DM, the MetS, and related parameters. The results of our study give no evidence for any association between polymorphisms in the *LAGG* gene and the analyzed parameters. In contrast to the findings of Kovacs *et al.*, who reported a strong association between the polymorphism Tyr1306Cys and insulin sensitivity during a hyperinsulinemic–euglycemic clamp in Pima Indians (7), this polymorphism is surprisingly monomorphic (only the AA genotype was detected) or has at least a very low minor allele frequency in German Caucasians. In the Pima study, the A allele was associated with lower insulin sensitivity (7). Regarding the results for T2DM, the negative association results of Kovacs *et al.* (7) could be replicated in this study. In both German Caucasians and Pima Indians, none of the genetic variants in the *LAGG* gene were associated with T2DM.

Different from the Pima study (7), the *LAGG* gene is not associated with insulin sensitivity in the German population, but the comparison is not completely adequate, because in the Pima study the gold standard (clamp) for insulin sensitivity was measured whereas in our study HOMA-IR was used.

The strengths of KORA S4 are its large size, intensive phenotyping, and population-based design. In particular, OGTT status is available from almost all subjects. The systematic genetic dissection of the *LAGG* gene and complete coverage of the whole gene locus strengthen our conclusions concerning the association between the gene and the various outcomes. Furthermore, the relative high power of the study fortifies our results for T2DM and the MetS.

Recently, genetic variants of another Rho guanine nucleotide exchange factor gene, *ARHGEF11*, located on chromosome 1, was found to be associated with insulin resistance or T2DM in Pima Indians or Amish populations respectively (15, 16). It will be interesting in this context to clarify in future studies whether this

Table 1 Characteristics of polymorphisms in the leukemia-associated Rho guanine (*LAGG*) gene and its 5' flanking region used for association analysis.

SNP and gene position	In LD ($r^2 \geq 0.8$) with the following SNPs	Major/minor allele	Minor allele frequency (nondiabetic/diabetic individuals)	Genotyping success rate (HWE P value ^a)
rs12806740 (5' flanking/-4325)	rs4539328 rs723937 rs2305008	G/A	0.37/0.37	0.98 (0.20/0.39)
rs538661 (Intron 12)	rs496248 rs503473	C/T	0.49/0.51	0.98 (0.51/0.37)
rs476636 (Intron 22)	rs2305011	A/G	0.14/0.12	0.98 (0.13/0.84)
rs2276035 (Intron 33)	rs7129944	G/A	0.14/0.16	0.98 (0.27/0.42)

Linkage disequilibrium (LD) was calculated by r^2 (correlation coefficient) between the 11 genotyped SNPs in 1462 subjects; four SNPs (bold) tag the other SNPs due to high LD ($r^2 \geq 0.8$). ^a Nondiabetic/diabetic individuals.

Table 2 Association results between polymorphisms in the leukemia-associated Rho guanine (*LARG*) gene and diabetes mellitus type 2 (T2DM) (World Health Organization definition), the metabolic syndrome (MetS) (International diabetes federation definition), and HOMA-IR.

SNP	P value*	N	Heterozygous vs reference		Homozygous minor allele vs reference	
			OR (95%CI)	P value [†]	OR (95% CI)	P value [†]
T2DM						
rs12806740	0.88	1432	0.89 (0.58/1.39)	0.62	0.93 (0.59/1.45)	0.74
rs538661	0.45	1431	1.22 (0.86/1.72)	0.26	1.25 (0.84/1.87)	0.27
rs476636	0.40	1433	0.66 (0.15/2.96)	0.59	0.55 (0.13/2.38)	0.42
rs2276035	0.29	1428	0.66 (0.22/1.95)	0.45	0.84 (0.29/2.44)	0.75
MetS						
rs12806740	0.73	1425	0.88 (0.64/1.21)	0.43	0.90 (0.65/1.25)	0.54
rs538661	0.42	1424	1.08 (0.83/1.40)	0.59	1.22 (0.90/1.65)	0.20
rs476636	0.58	1426	1.60 (0.61/4.14)	0.34	1.48 (0.58/3.78)	0.41
rs2276035	0.07	1421	0.55 (0.26/1.16)	0.11	0.71 (0.34/1.46)	0.35

SNP	P value [‡]	N	Median	25th/75th percentiles	
HOMA-IR					
rs12806740					
GG		517	2.50	1.63	3.83
GA	0.84	608	2.46	1.65	3.75
AA		192	2.47	1.69	3.87
rs538661					
TT	0.49	307	2.52	1.69	3.87
CT		665	2.41	1.63	3.76
CC		344	2.49	1.67	3.77
rs476636					
AA		974	2.49	1.68	3.77
AG	0.75	325	2.44	1.62	3.87
GG		18	2.22	1.42	3.74
rs2276035					
GG		955	2.48	1.64	3.80
GA	0.90	328	2.50	1.68	3.73
AA		30	2.42	1.68	4.39

Data for T2DM and MetS were analyzed by comparing heterozygous or homozygous subjects carrying the minor allele with the reference (homozygous for the major allele); odds ratios (OR) and 95% confidence intervals (95% CI) are shown; * global *P* value based on ANOVA; [†]*P* value based on logistic regression. HOMA-IR (homeostasis model assessment) was analyzed separately for each genotype; median (25th/75th percentiles) are shown; [‡]*P* value based on Kruskal–Wallis test.

nucleotide exchange factor plays a role in Caucasian populations.

In conclusion, there is neither evidence that the strongly associated polymorphism Tyr1306Cys in Pima Indians (7) existed in our study population nor was one of the analyzed parameters (T2DM, the MetS, HOMA-IR, and other quantitative traits) significantly associated with genetic variants in the *LARG* gene. Presumably, polymorphisms in the *LARG* gene do not play the same role in the different populations mainly because of the missing functional variant Tyr1306Cys in German Caucasians. This study strongly suggests that variants of the *LARG* gene do not confer susceptibility for T2DM, insulin sensitivity, or the MetS in German Caucasians.

In order to understand the interactions of genes, differences between populations, and the complexity of T2DM, future studies are necessary.

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References

- Illig T, Bongardt F, Schöpfer A, Müller-Scholze S, Rathmann W, Koenig W, Thorand B, Vollmert C, Holle R, Kolb H & Herder C. Significant association of the interleukin-6 gene polymorphisms

- A-174G and A-598G with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 5053–5058 (Erratum in *Journal of Clinical Endocrinology and Metabolism* 2004 **90** 6385).
- 2 Isomaa B. A major health hazard: the metabolic syndrome. *Life Sciences* 2003 **73** 2395–2411.
 - 3 Sowers JR. Insulin resistance and hypertension. *American Journal of Physiology. Heart and Circulatory Physiology* 2004 **286** H1597–H1602.
 - 4 Kourlas PJ, Strout MP, Becknell B, Veronese ML, Croce CM, Theil KS, Krahe R, Ruutu T, Knuutila S, Bloomfield CD & Caligiuri MA. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. *PNAS* 2000 **97** 2145–2150.
 - 5 Van den Berghe N, Barros LF, Van Mackelenbergh MG & Krans HM. *Clostridium botulinum* C3 exoenzyme stimulates GLUT4-mediated glucose transport, but not glycogen synthesis, in 3T3-L1 adipocytes – a potential role of rho? *Biochemical and Biophysical Research Communications* 1996 **229** 430–439.
 - 6 Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH & Knowler WC. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *American Journal of Human Genetics* 1998 **63** 1130–1138.
 - 7 Kovacs P, Stumvoll M, Bogardus C, Hanson RL & Baier LJ. A functional Tyr1306Cys variant in LARG is associated with increased insulin action *in vivo*. *Diabetes* 2006 **55** 1497–1503.
 - 8 Wichmann HE, Gieger C & Illig T, for the MONICA KORA Study group. KORA-gen – resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen* 2005 **67** S26–S30.
 - 9 Holle R, Happich M, Löwel H & Wichmann HE. KORA – a research platform for population based health research. *Gesundheitswesen* 2005 **67** S19–S25.
 - 10 Rathmann W, Haastert B, Icks A, Löwel H, Meisinger C, Holle R & Giani G. High prevalence of undiagnosed diabetes mellitus in southern Germany: target populations for efficient screening. The KORA survey 2000. *Diabetologia* 2003 **46** 182–189.
 - 11 World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Report of WHO consultation. Geneva: WHO, 1999.
 - 12 Alberti KG, Zimmet P & Shaw J. The IDF Epidemiology Task Force Consensus Group. The metabolic syndrome – a new worldwide definition. *Lancet* 2005 **366** 1059–1062.
 - 13 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–491.
 - 14 Purcell S, Cherny SS & Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003 **19** 149–150.
 - 15 Ma L, Hanson RL, Que LN, Cali AM, Fu M, Mack JL, Infante AM, Kobes S, Bogardus C, Shuldiner AR & Baier LJ. Variants in ARHGEF11, a candidate gene for the linkage to type 2 diabetes mellitus on chromosome 1q, are nominally associated with insulin resistance and type 2 diabetes mellitus in Pima Indians. *Diabetes* 2007 **56** 1454–1459.
 - 16 Fu M, Sabra MM, Damcott C, Pollin TI, Ma L, Ott S, Shelton JC, Shi X, Reinhart L, O'Connell J, Mitchell BD, Baier LJ & Shuldiner AR. Evidence that Rho guanine nucleotide exchange factor 11 (ARHGEF11) on 1q21 is a type 2 diabetes susceptibility gene in the Old Order Amish. *Diabetes* 2007 **56** 1363–1368.