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Original Contribution

SIRT1 genetic variation and mortality in type 2 diabetes: interaction with smoking and dietary niacin

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ABSTRACT

SIRT1 protects cells against oxidative stress and aging. Its activity may be modulated by dietary niacin (vitamin B3) intake. We studied the association of *SIRT1* genetic variation with mortality in subjects with increased oxidative stress (type 2 diabetes and smokers) in relation to dietary niacin. In 4573 participants from the Rotterdam Study, including 413 subjects with prevalent and 378 with incident type 2 diabetes, three *SIRT1* tagging SNPs were genotyped and all-cause mortality was studied (average follow-up12 years). We found no association between *SIRT1* variation and mortality in the total population or in smokers. In subjects with prevalent type 2 diabetes, homozygous carriers of the most common *SIRT1* haplotype, 1, had 1.5 times (95%CI 1.1–2.1) increased mortality risk compared to noncarriers. This risk further increased among smokers and those with low niacin intake. In the lowest tertile of niacin intake, mortality risk was increased 2.3 (95%CI 1.1–4.9) and 5.7 (95%CI 2.5–13.1) times for heterozygous and homozygous carriers of haplotype 1. Subjects with incident diabetes showed similar findings but only when they smoked. We conclude that in subjects with type 2 diabetes, *SIRT1* genetic variation influences survival in interaction with dietary niacin and smoking. Correction of niacin deficiency and SIRT1 modulators may prolong the life span of patients with diabetes.

The silent information regulator Sir2 (an NAD^+-dependent[1] histone deacetylase) protein is related to longevity in lower organisms such as yeast, flies, and worms [1,2]. Sir2 has also been implicated in life-span extension during caloric restriction in these organisms [3–5]. The protein is highly conserved across species and humans have seven sirtuins (*SIRT1–7*) [6]. Mammalian SIRT1 is most homologous to yeast Sir2 and has been studied most extensively. SIRT1 may protect cells against oxidative and genotoxic stress by binding to and deacetylating a large number of substrates, such as tumor suppressor p53 and forkhead transcription factors FOXOs [2]. Accordingly, SIRT1 plays a protective role in many cell types under various conditions of increased stress [7–13]. By regulating other transcription factors, SIRT1 is also involved in glucose and fat metabolism [14–18].

During the deacetylation reaction, SIRT1 consumes NAD^+ and produces nicotinamide, which is a potent SIRT1 inhibitor [7,19]. SIRT1 activity can thus be modulated by pathways that influence NAD^+ and/or nicotinamide levels [9,20–22]. Nicotinamide and its acid form, nicotinic acid, are together known as vitamin B3 or niacin. Both serve as a precursor for the generation of cellular NAD^+, whereas only nicotinamide inhibits *SIRT1* activity.

Only a few human genetic association studies on *SIRT1* have been published so far. Variation in the *SIRT1* gene was not associated with longevity in a case–control study comparing long-lived individuals with younger subjects [23] and all-cause mortality was not influenced by *SIRT1* genetic variation in the Leiden 85-Plus study [24]. Among healthy nondiabetic offspring of type 2 diabetic patients, carriers of *SIRT1* polymorphisms had increased whole-body energy expenditure [25].

In light of the role of *SIRT1* in protection against oxidative stress, we hypothesized that a chronic increase in oxidative stress is required to observe an association of genetic variation of *SIRT1.* Diabetes and smoking are well-known conditions with increased oxidative stress. Moreover, hyperglycemia in diabetes leads to activation of the DNA repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1), which also uses NAD^+ and produces nicotinamide and thus could reduce SIRT1 activity [12,26–28]. SIRT1 expression in diabetes may also be decreased owing to a redox imbalance leading to a lower NAD^+-to-NADH ratio [29]. In endothelial progenitor cells (EPCs), SIRT1 expression and activity were reduced by treatment with high glucose, and SIRT1 was a critical modulator of EPC dysfunction during alteration of glucose

Abbreviations: BMI, body mass index; LD, linkage disequilibrium; PARP-1, poly(ADP-ribose) polymerase-1; NAD^+, nicotinamide adenine dinucleotide; SIRT, silent information regulator 2; SNP, single nucleotide polymorphism.

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metabolism [30]. Cigarette smoke was shown to decrease SIRT1 levels and activity in rat lungs, possibly by oxidative stress [31], and SIRT1 levels are reduced in macrophages and lungs of smokers and patients with chronic obstructive pulmonary disease [32]. Hence, in subjects with diabetes and in smokers SIRT1 expression and/or activity may be decreased, whereas there is a high need for SIRT1 protection against oxidative stress.

In the large cohort of elderly subjects in the Rotterdam Study we assessed the association of variation in the *SIRT1* gene with mortality in persons with type 2 diabetes mellitus and in cigarette smokers, according to dietary niacin intake.

Research design and methods

The Rotterdam study

The Rotterdam Study is a prospective, population-based cohort study of 7983 persons ages 55 years and older from a district of Rotterdam, The Netherlands. The study was designed to investigate the incidence and determinants of chronic disabling diseases. The rationale and design have been described previously [33,34]. Informed consent was obtained from each participant, and the Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study.

At baseline (1990–1993), all participants were interviewed and subsequently underwent extensive physical examination. Three follow-up examinations following the same protocol took place in 1993–1994, 1997–1999, and 2002–2004. In addition, the cohort has been continuously monitored for major disease outcomes and mortality through computerized linkage of the study database to general practitioners' medical files. Procedures on follow-up of all-cause mortality and coding of cardiovascular disease mortality were described previously [35]. Follow-up data on overall mortality were available until September 1, 2006. Cardiovascular disease-specific mortality was based on data until January 1, 2004.

Assessment of diabetes mellitus type 2

Prevalent diabetes was defined as use of antidiabetic medication and/or abnormal nonfasting glucose and/or an abnormal oral glucose tolerance test. A nonfasting or postload glucose level of 11.1 mmol/L or over was considered abnormal.

During follow-up, incident cases of diabetes were diagnosed by use of information from the general practitioners, the pharmacies' databases, and our follow-up examinations. Based on guidelines of the American Diabetes Association and the WHO we defined incident diabetes as follows: fasting plasma glucose level ≥7.0 mmol/L and/or random (nonfasting) plasma glucose level ≥11.1 mmol/L and/or use of oral antidiabetic medication and/or use of insulin (but not type 1 diabetes) and/or treatment by diet and registered by a general practitioner as having diabetes.

Assessment of dietary variables and covariates

At baseline, dietary intake was assessed through an interview by a trained dietician, using an extensive, validated semiquantitative food-frequency questionnaire (SFFQ) [36].

The amounts of food and drink intake indicated on the SFFQ were converted to energy intake and nutrient intake by means of the computerized *Dutch Food Composition Table*. For the current study, we used data on dietary intake of niacin (vitamin B3) (in mg/day), pyridoxine (vitamin B6), riboflavin (vitamin B2), and vitamins C and E and total energy intake (in kcal/day) and total protein intake (g/day), as well as data on the use of B-vitamin and multivitamin supplements. Dietary niacin intake was analyzed using the residual method to obtain sex-specific age- and energy-adjusted tertiles [37]. At baseline, smoking status was assessed during the interview and subjects were classified as current smokers or nonsmokers of cigarettes.

Measurements

Height (cm) and weight (kg) were measured at the initial examination, in standing position wearing indoor clothes without shoes. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared (kg/m^2). Two standardized blood pressure measurements were taken by using a random-zero sphygmomanometer, with the participant in sitting position, and averaged. Serum total cholesterol level was determined by an enzymatic procedure [38].

Genotyping

Three tagging single-nucleotide polymorphisms (SNPs) were selected from the HapMap database (http://www.hapmap.org) that covered most of the common (minor allele frequency >10%) variations of the *SIRT1* gene in Caucasians: rs7895833, rs1467568, and rs497849. Genotyping of the *SIRT1* SNPs was performed by TaqMan on genomic DNA isolated from peripheral leukocytes by standard salting-out procedures. The PCR mixture included 2 ng of genomic DNA in a 2-μl volume and the following reagents: FAM and VIC probes (200 nM), primers (0.9 μM), 2× TaqMan PCR Master Mix (ABgene, Epsom, UK). Reagents were dispensed in a 384-well plate using the Deerac Equator NS808 (Deerac Fluidics, Dublin, Ireland). PCR cycling reactions were performed in 384-well PCR plates in an ABI 9700 PCR system (Applied Biosystems, Foster City, CA, USA) and consisted of initial denaturation for 15 min at 95°C and 40 cycles of denaturation for 15 s at 95°C and annealing and extension for 60 s at 60°C. Results were analyzed by the ABI TaqMan 7900HT using the sequence detection system 2.22 software (Applied Biosystems). To confirm the accuracy of the genotyping results, 332 (5%) randomly selected samples were regenotyped with the same method. No inconsistencies were observed. All primers and probes used are available on request.

Population for analysis

The study sample for the current study comprised 4573 independently living participants, with complete dietary data and genotyping information. A total of 1067 of these subjects were smokers at baseline, whereas 413 subjects had diabetes mellitus at baseline (prevalent diabetes) and 378 subjects developed diabetes mellitus at follow-up (incident diabetes). Only subjects with minimal follow-up time of 1 year after the diagnosis of type 2 diabetes were studied to avoid interference from mortality due to underlying comorbid conditions. Of the subjects with prevalent diabetes 97 were smokers at baseline and of those with incident diabetes, 87.

Statistical analyses

Hardy–Weinberg equilibrium of the *SIRT1* SNPs was tested with the GENEPOP-package [39]. We inferred four common (frequencies >10%) multimarker haplotypes from these SNPs using the program Phase [40]. Haplotype alleles were numbered in order of decreasing frequency in the population (Fig. 1). Subjects were grouped according to genotype. Groups were based on allele copy number (0, 1, and 2, corresponding to noncarriers, heterozygote carriers, and homozygote carriers, respectively, of the most common haplotype alleles).

Cumulative survival was determined with Cox proportional hazards analyses. The 95% confidence intervals (95%CIs) of the hazard ratios (HRs) were calculated as the exponent of the regression coefficient and its standard error. The analyses were adjusted for age and sex and BMI. We performed separate analyses in subjects with