

# Variants in the *SIRT1* Gene May Affect Diabetes Risk in Interaction With Prenatal Exposure to Famine

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**OBJECTIVE**—To investigate whether *SIRT1*, a nutrient-sensing histone deacetylase, influences fetal programming during malnutrition.

**RESEARCH DESIGN AND METHODS**—In 793 individuals of the Dutch Famine Birth Cohort, we analyzed the interaction between three *SIRT1* single nucleotide polymorphisms (SNPs) and prenatal exposure to famine on type 2 diabetes risk.

**RESULTS**—In the total population (exposed and unexposed), *SIRT1* variants were not associated with type 2 diabetes. A significant interaction was found between two *SIRT1* SNPs and exposure to famine in utero on type 2 diabetes risk ( $P = 0.03$  for rs7895833;  $P = 0.01$  for rs1467568). Minor alleles of these SNPs were associated with a lower prevalence of type 2 diabetes only in individuals who had been exposed to famine prenatally (odds ratio for rs7895833 0.50 [95% CI 0.24–1.03],  $P = 0.06$ ; for rs1467568 0.48 [0.25–0.91],  $P = 0.02$ ).

**CONCLUSIONS**—*SIRT1* may be an important genetic factor involved in fetal programming during malnutrition, influencing type 2 diabetes risk later in life.

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Fetal malnutrition may predispose to type 2 diabetes by altering gene expression profiles through epigenetic mechanisms (1–3). *SIRT1*, a nutrient-sensing histone deacetylase, is, in addition to deacetylation of histones, involved in glucose and insulin metabolism by regulating the expression of various transcription factors. Dietary factors influence the NAD<sup>+</sup>-to-NADH ratio regulating *SIRT1* activity (4). We hypothesized that genetic variants in *SIRT1* may interact with fetal malnutrition, influencing type 2 diabetes risk later in life. This was addressed in the Dutch Famine Birth Cohort.

**RESEARCH DESIGN AND METHODS**—The Dutch Famine Birth Cohort is composed of individuals born as term singletons in Amsterdam around

the famine in the Netherlands during World War II, as described in detail earlier (5). A total of 2,414 singletons were born between 1 November 1943 and 28 February 1947. A total of 810 of 1,423 invited subjects agreed to participate in the cohort study in 2002. The study was approved by the local medical ethics committee. All participants gave written informed consent. Prenatal exposure to famine was defined as a daily food ration for the pregnant mother of <1,000 calories during any 13-week period of gestation.

## Study parameters

The primary outcome measure of the current study was type 2 diabetes, which was defined as using antidiabetes medications or fasting glucose levels >7.0 mmol/L or 120-min glucose levels >11.0 mmol/L in

an oral glucose tolerance test (OGTT). Secondary outcome measures were glucose metabolism parameters (120-min glucose and insulin levels, area under the curve [AUC] for glucose and insulin) and BMI.

## Genotyping

DNA was available from 793 subjects for Taqman allelic discrimination assays. Three tagging single nucleotide polymorphisms (SNPs) were selected, covering most of the common variants of the *SIRT1* gene in whites (rs7895833, rs1467568, and rs497849, as described earlier) (6). Minor allele frequencies of the SNPs were 19, 36, and 22%, respectively. The SNPs were in Hardy-Weinberg equilibrium ( $\chi^2 < 3.1$ ; 1 df;  $P > 0.07$ ).

## Statistical methods

Associations of genetic variants and diabetes were investigated with binary logistic regression and associations with BMI, AUC, and 120-min levels of glucose and insulin with linear regression. Interactions were tested by creating interaction terms for each genetic variant with the exposure group. The minor allele was coded 1 and 0 for noncarriers. Unexposed subjects born before or conceived after the Dutch famine were coded 0 and 1 for exposed subjects.  $P < 0.05$  was considered significant.

**RESULTS**—A total of 337 subjects were exposed to famine in utero, and 456 subjects were born before or after the famine. In adulthood, BMI and type 2 diabetes prevalence were not different between subjects exposed and nonexposed to famine. A total of 117 of 791 individuals developed diabetes in adulthood (15.8% in the exposed and 14.1% in the unexposed group). Plasma glucose levels at 120 min of the OGTT were 0.37 mmol/L ( $P = 0.03$ ) higher for those exposed to famine in utero.

In the total population (exposed and unexposed), carriers of the minor allele of rs7895833 (GG and AG) and rs1467568 (AA and GA) had a higher BMI than noncarriers ( $\beta$  for rs7895833: 0.82 kg/m<sup>2</sup> [95% CI 0.09–1.56],  $P = 0.028$ ;  $\beta$  for

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rs1467568: 0.96 kg/m<sup>2</sup> [0.25–1.67], *P* = 0.008). There was no association between the *SIRT1* variants and diabetes and AUCs of glucose and insulin levels during OGTT.

Next, we investigated the interactions of the *SIRT1* SNPs with prenatal exposure to famine on type 2 diabetes risk and BMI at age 58 years. An interaction was found between prenatal exposure to famine and rs7895833 (*P* = 0.03) and rs1467568 (*P* = 0.01) but not with rs497849 on diabetes risk. In stratified analyses in subjects not prenatally exposed to famine, the SNPs and type 2 diabetes were not associated (Fig. 1A). In the exposed subjects, there was a borderline significant association between rs7895833 and diabetes and a significant association for rs1467568, with diabetes risk decreasing for minor allele carriers (Fig. 1A). We did not observe an interaction between any of the *SIRT1* variants and exposure to famine on BMI, glucose, and insulin values. However, minor allele carriers of rs7895833 tended to have lower 120-min glucose and insulin levels as well as lower AUC glucose/insulin values in the exposed

group, whereas such a trend was not evident in the unexposed subjects (data not shown).

In those prenatally exposed to famine, BMI was significantly higher in carriers of minor alleles of rs7895833 and rs1467568 (Fig. 1B). In subjects, who had not been exposed to famine prenatally, no significant association was found, although the direction of the effect was the same (Fig. 1B).

**CONCLUSIONS**—Our results show that an interaction between two SNPs in *SIRT1* (rs7895833 and rs1467568) and in utero exposure to malnutrition significantly influenced type 2 diabetes risk in adulthood. Minor allele carriers of these two genetic *SIRT1* variants who had been exposed to famine in utero had a 50% lower risk of developing diabetes than noncarriers but, surprisingly, had a higher BMI.

*SIRT1* is susceptible to intracellular fluctuations in the NAD<sup>+</sup>-to-NADH ratio and may influence type 2 diabetes risk by its known epigenetic effects and β-cell apoptosis (4,7,8). Recently, Sandovici et al.

(9) showed that suboptimal nutrition in rats during early development led to epigenetic silencing and reduced the expression of the transcription factor Hnf4a, which is required for pancreatic β-cell differentiation and glucose homeostasis. Valtat et al. (10) also showed that food restriction during gestation impaired glucose tolerance and decreased β-cell mass later in life. In line, we also found that exposure to famine in utero reduced glucose tolerance (5).

We previously reported an interaction between variants of the PPAR-γ2 gene and the IGF2BP2 gene and exposure to famine in utero on the prevalence of impaired glucose tolerance and type 2 diabetes (11,12). Whether *SIRT1* interacts with these genes on pathways influencing diabetes risk should be elucidated by additional studies.

Unexpectedly, associations between two *SIRT1* SNPs and BMI in subjects who had been exposed to famine were in the opposite direction as expected (13–15). Nevertheless, the *SIRT1* variants interacted with exposure to famine on diabetes risk independent of BMI.

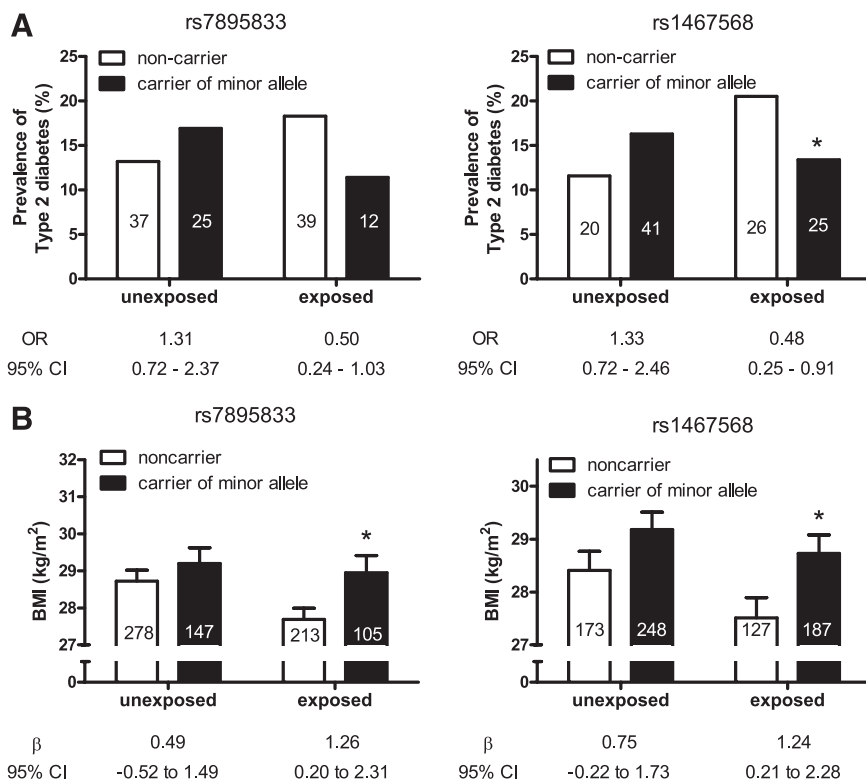
Interactions have been reported between *SIRT1* variants and niacin (6) and vitamin E (16) intake. It is possible that associations between *SIRT1* variants and diabetes risk can only be found when interactions with environmental factors are taken into account, such as fetal nutrition in our study.

Although our results are based on relatively small numbers and therefore have to be interpreted with caution, they support that *SIRT1* plays a role in the fetal programming of type 2 diabetes through fetal malnutrition.

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**Figure 1**—The relationship of the rs7895833 and rs1467568 *SIRT1* SNPs with the prevalence of diabetes (A) and BMI (B) according to prenatal exposure to famine. Odds ratios (ORs; A) and β-coefficients (β; B) are shown for carriers of the minor alleles. The number of subjects per group (i.e., diabetic patients for A) are shown inside each bar. \**P* < 0.05 vs. noncarriers.

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