Short Communication

Whole-grain consumption and transcription factor-7-like 2 (*TCF7L2*) rs7903146: gene–diet interaction in modulating type 2 diabetes risk

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Whole grains are known to influence postprandial glucose response and insulin demand and are inversely associated with diabetes risk. Genetic variation of the transcription factor-7-like 2 encoding gene (*TCF7L2*) is assumed to promote an early insulin secretory defect and has been consistently attributed to the risk of developing type 2 diabetes. The present study examined the hypothesis that the protective effect of whole grains might be attenuated in the presence of the rs7903146 risk-conferring T-allele. We employed a case–cohort study of 2318 randomised individuals and 724 incident type 2 diabetes cases from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort. Multivariate Cox regression was used to estimate relative risks of diabetes including product terms testing for the genotype-specific effect modification of dietary whole grain. Dietary intake of whole grains was assessed by a validated FFQ. The *TCF7L2* rs7903146 T-allele was associated with type 2 diabetes (hazard ratio = 1·51; 95 % CI 1·21, 1·87) and modified the inverse association between whole-grain intake and diabetes risk (*P* = 0·016 for interaction). While whole-grain intake was inversely associated with diabetes risk among rs7903146 CC homozygote carriers (hazard ratio for 50 g portion per d = 0·86; 95 % CI 0·75, 0·99), the T-allele negated the protective effect of whole-grain intake (hazard ratio among T-allele carriers for 50 g portion per d = 1·08; 95 % CI 0·96, 1·23). These data provide evidence that the beneficial effect of whole-grain intake on diabetes risk is modified by *TCF7L2* rs7903146.

Type 2 diabetes: Dietary whole grains: *TCF7L2* polymorphisms: Gene–diet interaction: Prospective cohort studies

The consumption of whole grains has consistently been found to be inversely associated with type 2 diabetes risk in prospective studies[1,2]. Whole grains may lead to a prolongation and reduction of intestinal glucose absorption and thus to a lower postprandial insulin secretory demand. However, it is reasonable to assume that the effect of whole grains to reduce type 2 diabetes risk may depend on variation in the genes involved in the process of insulin secretion in the pancreatic β-cells. The transcription factor-7-like 2 (*TCF7L2*) gene is a major susceptibility locus for type 2 diabetes[3,4] and polymorphisms in this gene are suggested to lead to diabetes by means of a reduced insulin secretion[5]. Thus, we hypothesised that variation in the *TCF7L2* gene might influence the association between the intake of whole grains and type 2 diabetes risk, and evaluated this potential association in the large, population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort study.

Experimental methods

Study population

The EPIC-Potsdam study comprises 27 548 subjects from the general population of the Potsdam area in Northern Germany, mainly aged 35–65 years at recruitment (1994–8)[6,7]. We designed a prospective case–cohort study of 849 incident type 2 diabetes cases identified during a mean follow-up time of 7·1 years and 2500 randomly selected individuals (subcohort) from all participants of the EPIC-Potsdam study. Identification and verification of incident cases have been described in detail before[1]. From the subcohort, 2322 subjects remained for analysis after exclusion of participants with a history of diabetes at baseline, with self-reported diabetes during follow-up but without physicians' confirmation, and those without relevant baseline information, blood samples, and fully obtained follow-up data. Furthermore,

individuals reporting implausible energy intake (cutpoints of less than 3350 kJ/d or more than 25 100 kJ/d) were excluded from analysis, resulting in a final subcohort population of 2318 subjects. Of the 849 incident cases, 798 remained for analysis after similar exclusion criteria. Because the subcohort is representative of the full cohort at baseline, seventy-four incident cases belonged to the subcohort and 724 cases were so called 'external cases' identified in the remainder of the full cohort. Informed consent was obtained from all study participants, and approval was given by the Ethical Committee of the State of Brandenburg, Germany.

Dietary assessment

Frequency of intake and the portion size of 148 foods consumed during the 12 months before examination were measured with a validated FFQ. Information on frequency of intake and portion size was used to calculate the amount of food items in grams consumed on average per d. We calculated total whole-grain intake as the sum of whole-grain bread, whole-grain bread rolls, and whole-grain cereals and considered 50 g as one portion, equalling about one slice of bread or one roll in this population. The validity and reproducibility of the FFQ have been described previously[8,9].

Genotyping

Genotyping was performed with the TaqMan System (Applied Biosystems, Foster City, CA, USA) using 5 ng of whole-genome amplified DNA per single nucleotide polymorphism as described before[10]. An average genotyping success rate of 98·1 % was achieved. Replicate quality-control samples (6 %) were included and genotyped with ≥99 % concordance.

Statistical analysis

Hazard ratios were computed using a weighted Cox proportional hazards model, modified for the case–cohort design according to the Barlow method[11]. To determine the interaction effect between *TCF7L2* rs7903146, whole-grain intake and diabetes risk, we modelled whole-grain intake as a continuous variable (per 50 g/d portion), calculated genotype × whole-grain intake product terms in a multivariate adjusted Cox model, and performed linear hypothesis tests about the regression coefficients. In the model, genotype CT + TT, 'CC × whole grains' and 'CT + TT × whole grains' product terms, and additional covariates were included. The product terms are not the classic interaction terms, but allow estimation of the effect of nutrient intake in the genotype groups separately. A parameter of a gene–nutrient product term that is significantly different from zero can be interpreted as a significant nutrient effect in the carriers of the corresponding genotype. The power to detect a genotype-specific environmental effect is somewhat higher than that of a subgroup analysis with separate models for each genotype. We used information on covariates obtained from the baseline examination, including age, sex, waist circumference, BMI, sports activity (0, 0·1–4·0, >4·0 h/week), occupational activity (low, medium, high), educational attainment (less than high school, high school, more than high school), cigarette smoking (never, past, current <twenty cigarettes/d, current ≥twenty cigarettes/d), alcohol intake (0, 0 ≥ 5, 5 ≥ 10, 10 ≥ 20, 20 ≥ 40, and >40 g/d), red meat (per 150 g/d), processed meat (per 50 g/d), low-fat dairy (per 150 g/d), butter (per 20 g/d), margarine and vegetable oil (per 20 g/d) consumption, coffee intake (per 150 ml/d) and total energy intake (kJ/d). The statistical analyses were performed with SAS release 9.1 (SAS Institute, Cary, NC, USA).

Results

Cox proportional hazard ratios of *TCF7L2* single nucleotide polymorphisms located in the 64·6 kb linkage-disequilibrium region of the gene with type 2 diabetes are shown in Table 1. Among those *TCF7L2* polymorphisms, rs7903146 C > T showed the strongest association with type 2 diabetes risk (hazard ratio for T-allele carriers = 1·51; 95 % CI 1·21, 1·87) when we adjusted for age, sex, BMI and waist circumference.

We then evaluated whether *TCF7L2* rs7903146 and whole grain interact in the prediction of type 2 diabetes by including the product terms of genotype × whole-grain intake into the model. Whole-grain intake was significantly inversely associated with type 2 diabetes risk in rs7903146 C homozygotes (hazard ratio per 50 g/d portion = 0·86; 95 % CI 0·75, 0·99) after adjustment for age, sex, anthropometry, lifestyle and dietary confounders (Fig. 1). However, no association of whole-grain intake with diabetes risk was seen among T-allele carriers (hazard ratio per 50 g/d portion = 1·08; 95 % CI 0·96, 1·23). The difference between genotype-specific hazard ratios was statistically significant ($P = 0.016$). These associations were similar in crude models only adjusted for age (data not shown). The exclusion of study subjects (*n* 178) with baseline glucose concentrations greater then

Table 1. Association of transcription factor-7-like 2 (*TCF7L2*) single nucleotide polymorphisms with type 2 diabetes risk*

	Genotype *n*†			Minor allele carriers		
	11	12	22	Hazard ratio	95 % CI	*P*
rs7901695	359/1198	315/832	93/175	1·44	1·16, 1·79	0·001
rs7903146	376/1249	319/821	78/155	1·51	1·21, 1·87	0·0002
rs11196205	221/657	350/1074	204/479	1·24	0·98, 1·58	0·08
rs12255372	389/1255	317/823	66/131	1·36	1·10, 1·68	0·005

11, Homozygote major allele carriers; 12, heterozygotes; 22, homozygote minor allele carriers.
* Hazard ratios and 95 % CI adjusted for age, sex, BMI and waist circumference.
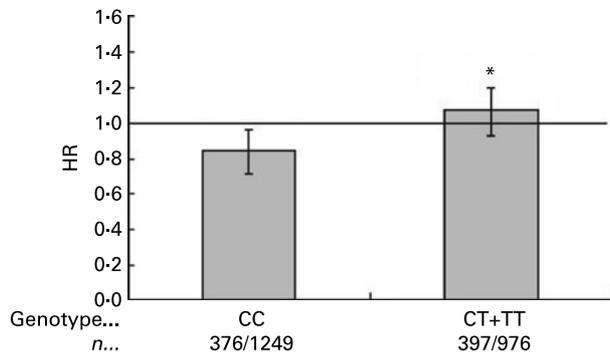† Number of incident cases and non-diabetic subjects from the subcohort.

Fig. 1. Hazard ratios (HR) of type 2 diabetes for whole grains (50 g/d portion) according to transcription factor-7-like 2 (*TCF7L2*) rs7903146 genotype. HR for whole grains were adjusted for age, sex, BMI, waist circumference, educational attainment, occupational activity, sports activity, cigarette smoking, consumption of alcohol, red meat, processed meat, low-fat dairy, butter, margarine and vegetable fat, coffee, and total energy intake (kJ/d). Vertical bars represent 95 % CI. *n*, Number of incident cases and non-diabetic subjects from the subcohort. For the CC genotype, HR = 0·86 (95 % CI 0·75, 0·99); for the CT+TT genotype, HR = 1·08 (95 % CI 0·96, 1·23). *HR for the CT+TT genotype was significantly different from that for the CC genotype (*P* < 0·016).

1260 mg/l in our analyses only produced minor changes in risk ratios and the difference between genotype groups remained significant (data not shown).

Discussion

In the US Diabetes Prevention Program, there was no apparent difference in diabetes-free survival under an intensive lifestyle intervention between *TCF7L2* rs7903146 genotypes[12], allowing the assumption that behavioural intervention can mitigate the risk conferred by the genetic background. However, the lifestyle intervention aimed at a reduction of body weight by increasing physical activity and by modifying diet, in particular fat intake, and succeeded primarily by improving insulin sensitivity. However, genetic variation in *TCF7L2* is specifically associated with impaired insulin secretion[5]. In the present study, we found that the protective effect of whole-grain intake on diabetes risk exclusively applied to CC genotype carriers of rs7903146, whereas subjects carrying the T-allele seemed to exhibit no benefit from whole-grain consumption. This suggests that the detrimental effect of the risk allele might mitigate the beneficial effect of whole grains on type 2 diabetes risk. The beneficial effect of whole grains may at least partly be related to their high fibre content. Proposed mechanisms, for soluble fibres in particular, involve delayed gastric emptying, inhibition of α-amylase, and a reduction of intestinal transit times, leading to a prolongation and reduction of intestinal glucose absorption and thus to a lower postprandial insulin secretory demand. Fibres in whole grains may also stimulate gastrointestinal hormone secretion, in particular gastric inhibitory polypeptide and glucagon-like peptide-1 (GLP-1)[13,14]. Because the insulin-secretory defect of the *TCF7L2* variant may arise from reduced GLP-1 efficiency rather then GLP-1 secretion[15,16], the beneficial effects of fibre-rich whole grains might be diminished in the context of a genetically disturbed GLP-1 action.

In conclusion, we found that the inverse association between whole-grain consumption and type 2 diabetes risk might depend on variation in *TCF7L2*.

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