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RESEARCH LETTER

Variation in the *HHEX–IDE* gene region predisposes to type 2 diabetes in the prospective, population-based EPIC-Potsdam cohort

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Keywords Diabetes mellitus, type 2 · EPIC · Incidence · Longitudinal studies · Polymorphism, single nucleotide · Prospective studies · Risk factor

Abbreviations

EPIC	European Prospective Investigation into Cancer and Nutrition
RR	relative risk
SNP	single nucleotide polymorphism

To the Editor: Type 2 diabetes mellitus is caused by a combination of genetic susceptibility and environmental exposures, particularly obesity, inactivity, specific dietary habits and smoking. However, to date, only a few genetic variants leading to type 2 diabetes have been clearly identified [1]. The recent availability of high-density genotyping arrays has presented the opportunity for genome-wide association studies. The first such study has identified several novel risk loci for type 2 diabetes, in particular, a non-synonymous single nucleotide polymorphism (SNP) in the gene for the zinc transporter solute carrier family 30 member 8 (*SLC30A8*; rs13266634), non-coding SNPs in a linkage disequilibrium block containing the genes for the hematopoietically expressed homeobox protein (*HHEX*), kinesin family member 11 (*KIF11*), and insulin-degrading enzyme (*IDE*) (rs1111875 and rs7923837), and SNPs in or near the hypothetical gene *LOC387761* (rs7480010) and the gene for exostosin 2 (*EXT2*; rs3740878, rs11037909 and rs1113132) [2]. For future application of these risk alleles in the assessment of the overall diabetes risk, it is important to validate and quantify their contribution in a prospective, population-based cohort. Thus, we investigated whether these polymorphisms are associated with diabetes incidence in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study.

In Potsdam, Germany, 27,548 volunteers, aged 35–65 years, from the general population, were recruited between 1994 and 1998 [3]. The baseline examination included anthropometric measurements, blood sampling, and a personal interview on lifestyle habits and medical history. Follow-up questionnaires have been administered every 2–3 years to obtain information on current medication and newly developed diseases, including diabetes. Informed consent was obtained from all study participants, and approval was given by the Ethics Committee of the State of Brandenburg, Germany.

Potentially incident cases of diabetes were identified in the full cohort in each follow-up questionnaire up to 31 August 2005 via self-reports of a diabetes diagnosis, diabetes-relevant medication or dietary treatment for diabetes. All potentially incident cases of diabetes were verified by questionnaires, mailed to the diagnosing physician, asking about the date and type of diagnosis, diagnostic

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Table 1 Association of novel genes with type 2 diabetes in the EPIC-Potsdam study

SNP/genotype	Subcohort *n* (%)[a]	External cases *n* (%)[a]	RR[b]	95% CI	*p* value
HHEX-KIF11-IDE rs1111875					
Co-dominant model					
G/G	772 (34.0%)	282 (41.1%)	1.00		
G/A	1,101 (48.6%)	307 (44.8%)	0.72	(0.57–0.91)	0.0062
A/A	394 (17.4%)	97 (14.1%)	0.52	(0.37–0.74)	0.0002
Additive model			0.72	(0.61–0.85)	<0.0001
HHEX-KIF-IDE rs7923837					
Co-dominant model					
G/G	885 (39.7%)	311 (45.1%)	1.00		
G/A	1,021 (45.8%)	291 (42.2%)	0.74	(0.59–0.93)	0.0106
A/A	324 (14.5%)	88 (12.7%)	0.71	(0.50–1.00)	0.0532
Additive model			0.81	(0.69–0.95)	0.0120
SLC30A8 rs13266634					
Co-dominant model					
C/C	1,089 (48.3%)	321 (47.1%)	1.00		
C/T	955 (42.3%)	297 (43.5%)	1.07	(0.86–1.34)	0.5457
T/T	212 (9.4%)	64 (9.4%)	1.21	(0.84–1.74)	0.3010
Additive model			1.09	(0.93–1.28)	0.2943
LOC387761 rs7480010					
Co-dominant model					
A/A	1,191 (52.7%)	384 (55.8%)	1.00		
A/G	895 (39.6%)	259 (37.6%)	0.96	(0.77–1.20)	0.7279
G/G	175 (7.7%)	45 (6.5%)	0.91	(0.59–1.40)	0.6630
Additive model			0.96	(0.81–1.14)	0.6168
EXT2 rs3740878					
Co-dominant model					
A/A	1,257 (55.8%)	371 (54.1%)	1.00		
A/G	843 (37.4%)	270 (39.3%)	1.15	(0.92–1.45)	0.2148
G/G	154 (6.8%)	45 (6.6%)	0.85	(0.53–1.36)	0.4977
Additive model			1.02	(0.86–1.22)	0.7768
EXT2 rs11037909					
Co-dominant model					
T/T	1,246 (55.3%)	368 (53.9%)	1.00		
T/C	848 (37.7%)	269 (39.4%)	1.15	(0.91–1.44)	0.2338
C/C	158 (7.0%)	46 (6.7%)	0.82	(0.52–1.28)	0.3800
Additive model			1.01	(0.85–1.19)	0.9352
EXT2 rs1113132					
Co-dominant model					
C/C	1,264 (55.9%)	374 (54.5%)	1.00		
C/G	846 (37.4%)	271 (39.5%)	1.17	(0.93–1.46)	0.1887
G/G	150 (6.6%)	41 (6.0%)	0.79	(0.49–1.26)	0.3181
Additive model			1.01	(0.85–1.20)	0.9005

[a] The case–cohort comprised 3,049 individuals, with 2,322 belonging to the subcohort (74 cases and 2,248 non-cases) and 727 external cases. Deviations from these numbers are due to missing genotypes

[b] RRs adjusted for age, sex, BMI and waist circumference

tests and treatment of diabetes. Only subjects with type 2 diabetes (code E11 according to the International Classification of Diseases, 10th revision; available from http://www.who.int/classifications/apps/icd/icd10online/, last accessed in June 2007) diagnosed by a physician and a diagnosis date after the baseline examination were considered as confirmed incident cases of type 2 diabetes and included in the study. Within a mean follow-up time of 7.1±1.8 years, 849 subjects were confirmed with incident type 2 diabetes [4].

We designed a prospective case–cohort study within the EPIC-Potsdam study involving all incident cases and a random sample of 2,500 subjects from the EPIC-Potsdam study population (subcohort). The following exclusion criteria were used: (1) a history of diabetes at baseline (self-reported diagnosis, medication or dietary treatment);

(2) self-reported diabetes during follow-up but without confirmation by a physician; (3) missing information on anthropometric measurements at baseline; (4) no blood sample available at baseline; and (5) incomplete follow-up data. After the exclusion of those who met the criteria, 2,322 individuals remained in the subcohort, 74 of whom developed incident diabetes during follow-up. There were 727 incident cases identified in the remainder of the total cohort and these were used as 'external' cases for analyses. By randomly selecting the subcohort with this type of research design the results are expected to be generalisable to the entire cohort without the need for genotyping the entire cohort.

Genotyping was performed using TaqMan technology (Applied Biosystems, Foster City, CA, USA) on 384 well plates. Genotyping error was ≤0.5%. Complete genotype information was available for 2,938 participants (96.4%) for rs13266634, 2,953 (96.9%) for rs1111875, 2,920 (95.8%) for rs7923837, 2,949 (96.7%) for rs7480010, 2,940 (96.4%) for rs3740878, 2,935 (96.3%) for rs11037909, and 2,946 (96.6%) for rs1113132. The genotype distributions were in agreement with Hardy–Weinberg equilibrium ($p>0.05$).

Relative risks (RRs) for associations between SNPs and risk of type 2 diabetes were calculated as hazard rate ratios stratified by age using Cox proportional hazards regression modified according to the Barlow method to account for the case–cohort design. Age was the underlying time variable in the counting processes, with entry defined as the subject's age at the time of recruitment, and exit defined as age at the diagnosis of diabetes or censoring.

We found evidence for an association between the *HHEX–KIF11–IDE* locus and type 2 diabetes independent of body fat. For rs1111875, the RRs for genotypes G/A and A/A compared with G/G were 0.72 (95% CI 0.57–0.91) and 0.52 (95% CI 0.37–0.74) after adjustment for age, sex, BMI and waist circumference (Table 1). Similarly, the minor A allele of rs7923837 was significantly inversely associated with diabetes risk (adjusted RR for the additive genetic model 0.81, 95% CI 0.69–0.95). SNPs within or near *SLC30A8*, *EXT2* and the hypothetical gene *LOC387761* were not significantly associated with type 2 diabetes in our cohort.

These data indicate that the *HHEX–KIF11–IDE* linkage disequilibrium block is a major type 2 diabetes locus in a large cohort from the general population of Potsdam, Germany. In addition, its effect is independent of adiposity, suggesting that it affects insulin sensitivity or secretion. We did not observe a significant association between the *SLC30A8* SNP rs13266634 and diabetes risk. However, we had insufficient power to detect the modest effect observed in recent genome-wide association studies [2, 5–8]: assuming an unmatched case–control design and an α-level of 0.05, our power to detect the previously reported OR of 1.12 for an additive genetic effect [5] was only 0.45. In contrast to the findings of Sladek et al. [2] we did not identify *EXT2* and the hypothetical gene *LOC387761* as diabetes loci; however, our results are in agreement with those of recent large-scale case–control studies [5–8].

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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