Diabetologia (2013) 56:2414–2423
DOI 10.1007/s00125-013-3021-y

ARTICLE

Parental history of type 2 diabetes, *TCF7L2* variant and lower insulin secretion are associated with incident hypertension. Data from the DESIR and RISC cohorts

Fabrice Bonnet · Ronan Roussel · Andrea Natali · Stéphane Cauchi · John Petrie · Martine Laville · Loïc Yengo · Philippe Froguel · Céline Lange · Olivier Lantieri · Michel Marre · Beverley Balkau · Ele Ferrannini · for the DESIR and RISC Study Groups

Received: 3 May 2013 / Accepted: 24 July 2013 / Published online: 14 August 2013

Abstract
Aims/hypothesis The relationship between insulin secretion and the incidence of hypertension has not been well characterised. We hypothesised that both a parental history of diabetes and *TCF7L2* rs7903146 polymorphism, which increases susceptibility to diabetes because of impaired beta cell function, are associated with incident hypertension. In a separate cohort, we assessed whether low insulin secretion is related to incident hypertension.
Methods Nine year incident hypertension was studied in 2,391 normotensive participants from the Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) cohort. The relationship between insulin secretion and 3 year incident hypertension was investigated in 1,047 non-diabetic, normotensive individuals from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) cohort. Insulin secretion during OGTT was expressed in relation to the degree of insulin resistance, as assessed by a hyperinsulinaemic–euglycaemic clamp.
Results In the DESIR cohort, a parental history of diabetes and the *TCF7L2* at-risk variant were both associated with hypertension incidence at year 9, independently of waist circumference, BP, fasting glucose, insulin levels and HOMA-IR at inclusion (p=0.02 for parental history, p=0.006 for

Members of the DESIR Study Group and the RISC investigators are listed in the electronic supplementary material (ESM).

Electronic supplementary material The online version of this article (doi:10.1007/s00125-013-3021-y) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

F. Bonnet (✉)
Service Endocrinologie-Diabétologie, CHU Rennes, Université Rennes 1, Inserm UMR 991, Rennes, France
e-mail: fabrice.bonnet@chu-rennes.fr

R. Roussel · M. Marre
AP-HP, Hôpital Bichat, Diabetology Endocrinology Nutrition, Université Paris Diderot, Paris, France

R. Roussel · M. Marre
Inserm U872, Paris, France

A. Natali · E. Ferrannini
Department of Internal Medicine, University of Pisa, Pisa, Italy

S. Cauchi · L. Yengo · P. Froguel
CNRS-UMR-8090, Institute of Biology, Lille 2 University, Pasteur Institute, Lille, France

J. Petrie
BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK

M. Laville
Centre de Recherche en Nutrition Humaine, CRNH Rhône-Alpes, Inserm U 870-INRA 1235, Lyon, France

P. Froguel
Genomic Medicine, Hammersmith Hospital, Imperial College London, London, UK

C. Lange · B. Balkau
Inserm, Centre for Research in Epidemiology and Population Health (CESP), U1018, Epidemiology of Diabetes, Obesity and Chronic Renal Disease Over the Lifecourse, Villejuif, France, Univ Paris-Sud, UMRS 1018, Villejuif, France

O. Lantieri
IRSA, La Riche, France

M. Marre
Inserm U695, Paris, France

TCF7L2). In the RISC cohort, a lower insulin secretion rate during the OGTT at baseline was associated with both higher BP and a greater risk of hypertension at year 3. This inverse correlation between the insulin secretion rate and incident hypertension persisted after controlling for baseline insulin resistance, glycaemia and BP ($p=0.007$).
Conclusions/interpretation Parental history of diabetes, *TCF7L2* rs7903146 polymorphism and a reduced insulin secretion rate were consistently associated with incident hypertension. A low insulin secretion rate might be a new risk factor for incident hypertension, beyond insulin resistance.

Keywords Hypertension · Insulin secretion · Parental history of diabetes · *TCF7L2* · Type 2 diabetes

Abbreviations

DESIR	Data from an Epidemiological Study on the Insulin Resistance Syndrome
GWAS	Genome-wide association study
HOMA2%B	HOMA of beta cell function
RISC	Relationship between Insulin Sensitivity and Cardiovascular Disease

Introduction

Hypertension and type 2 diabetes are strongly interrelated and might share common environmental risk factors such as sedentary behaviours, visceral adiposity and insulin resistance [1]. Elevated BP confers an increased risk for incident type 2 diabetes in the general population [2] and hypertension treatments might also modulate the risk of diabetes [3]. Fasting blood glucose, hyperinsulinaemia and HbA_{1c} have been associated with the development of hypertension in non-diabetic individuals [4–8]. However, the pathophysiology underlying the association between elevated glycaemia and incident hypertension has not been well characterised. In particular, the impact on incident hypertension of a parental history of diabetes or genetic predisposition to type 2 diabetes, which is mainly associated with beta cell dysfunction [9], is not known. Furthermore, although the epidemiological link between insulin resistance and hypertension is recognised, the relationship between insulin secretion and the incidence of hypertension and elevated BP has not been investigated in the general population.

The aim of our study was to assess, in the prospective Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) cohort, the impact of a genetic predisposition to type 2 diabetes on the development of hypertension. We analysed the impact of the *TCF7L2* rs7903146 polymorphism on incident hypertension, because this polymorphism has the largest effect on susceptibility to diabetes among the predisposing genes discovered to date [10]. As we observed an association of incident hypertension with the *TCF7L2* at-risk allele, we tested whether defects in insulin secretion predispose to 3 year incident hypertension in 1,047 non-hypertensive, non-diabetic participants from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) cohort. All of the RISC participants had an accurate evaluation at baseline of both insulin sensitivity by the hyperinsulinaemic–euglycaemic clamp and insulin secretion from an extended OGTT [11].

Methods

The DESIR cohort

We studied men and women aged 30–65 years, who participated in the 9 year follow-up study, DESIR. Participants were recruited from volunteers who were offered periodic health examinations, free of charge, by French social security in ten health examinations centres in western France [12, 13]. All participants signed an informed consent form and the protocol was approved by an ethics committee. Cases of hypertension were defined by treatment for hypertension or a resting systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg at one of the four 3-yearly examinations. After the exclusion of individuals with hypertension at baseline, we studied 2,391 participants with genotype data for *TCF7L2* rs7903146 (745 had incident hypertension during the follow-up).

Clinical assessment Two measures of BP, using a mercury sphygmomanometer, were taken in a supine position after 5 min rest; mean values were used. Weight and height were measured in lightly clad participants, and BMI was calculated. The examining physician noted the parental history of diabetes in a clinical questionnaire; treatment for diabetes and hypertension were recorded at each of the 3-yearly examinations. Smoking habits and alcohol intake were recorded in a patient-completed questionnaire

Biochemical measurements All biochemical measurements were from one of four health centre laboratories located in France at Blois, Chartres, La Riche and Orléans. The interlaboratory variability for normal and pathological values was assessed monthly. Fasting plasma glucose, measured by the glucose-oxidase method, was applied to fluoro-oxalated plasma using a Technicon RA100 analyser (Bayer Diagnostics, Puteaux, France) or a Specific or Delta device (Konelab, Evry, France). HbA_{1c} was determined by HPLC (L9100 ion-exchange analyser; Hitachi/Merck-VWR, Fontenay-sous-Bois, France) or immunoassay (DCA 2000; Bayer Diagnostics). Insulin was quantified by microparticle enzyme

immunoassay with an automated analyser (IMX; Abbott, Rungis, France). Glucose, HbA_{1c} and insulin have been standardised across laboratories and over the years of the study. Indices of insulin resistance (HOMA-IR) [14] and beta cell secretion (HOMA2%B) were computed using software downloaded from www.dtu.ox.ac.uk [15].

Genotyping TCF7L2 single nucleotide polymorphism rs7903146 genotyping was performed with the SNPlex Genotyping System (Applied Biosystems, Foster City, CA, USA) based on the oligonucleotide ligation assay combined with multiplex PCR target amplification [12].

The RISC cohort

RISC is a prospective observational cohort study whose rationale and methodology, as well as the characteristics of the individuals recruited, have been previously published [11, 16]. Clinically healthy men and women, aged 30–60 years, were recruited from the local populations of 19 centres in 14 European countries. Initial exclusion criteria were: treatment for obesity, hypertension, lipid disorders or diabetes, pregnancy, cardiovascular or chronic lung disease, weight change ≥5 kg in the last 6 months, cancer (in the last 5 years) and renal failure. Exclusion criteria after screening were: arterial BP ≥140/90 mmHg, fasting plasma glucose ≥7.0 mmol/l, 2 h plasma glucose (following a 75 g OGTT) ≥11.0 mmol/l, total serum cholesterol ≥7.8 mmol/l, serum triacylglycerol ≥4.6 mmol/l and ECG abnormalities. In the current investigation, we studied 1,047 healthy individuals (579 women and 468 men) who had an evaluation of both insulin sensitivity and insulin secretion at baseline and who had complete data at the 3 year follow-up. Volunteers were given detailed written information on the study as well as an oral explanation, and all signed consent forms. The protocol was approved by the ethics committee of each recruiting institution.

Height and body weight were measured and BMI was calculated. Alcohol and tobacco consumption were assessed using a standardised semi-quantitative questionnaire [17]. Information on physical activity was collected with the 7 day International Physical Activity Questionnaire, a previously validated assessment tool for international studies that provides a comprehensive evaluation of daily physical activity habits [18].

Blood pressure BP was measured in triplicate by trained study nurses using an OMRON 705CP BP monitor (Omron Healthcare GmbH, Hamburg, Germany) after 5 min of rest with participants sitting, according to a standard protocol; the median of these readings was used in this analysis for both baseline and follow-up examinations. Hypertension was defined as median systolic BP ≥140 mmHg, median diastolic BP ≥90 mmHg or treatment for hypertension in routine care at follow-up.

OGTT Blood samples were taken at fasting and 30, 60, 90 and 120 min into the OGTT, for central analysis of routine blood chemistry. Blood collected during the studies was separated into plasma and serum, aliquoted and stored at –20°C for central assays of glucose and insulin.

Glucose concentrations were measured with the glucose-oxidase technique. Plasma insulin and C-peptide were measured by a two-site time-resolved fluoroimmunoassay (AutoDELFIA insulin kit; Wallac Oy, Turku, Finland) using monoclonal antibodies, with the following assay characteristics (for insulin and C-peptide, respectively): sensitivity 1–2 and 5 pmol/l; within-assay variation 5% and 5%; and between-assay variation 5% and 3.5%.

Insulin sensitivity On a separate day within 1 month of the OGTT, participants underwent a hyperinsulinaemic–euglycaemic clamp. Exogenous insulin was administered as a primed continuous infusion at a rate of 240 pmol m^{-2} min^{-1} with a variable 20% dextrose infusion adjusted every 5–10 min to maintain the plasma glucose level within 0.8 mmol/l (±15%) of the target glucose level (4.5–5.5 mmol/l). The clamp procedure was standardised across centres [11]; the data from each clamp study were transferred to the coordinating centre, where they underwent quality-control scrutiny according to predefined criteria.

Insulin sensitivity is expressed as the ratio of the *M* value during the final 40 min of the 2 h clamp to the mean plasma insulin concentration measured during the same interval (M/I), normalised to fat-free mass and expressed in units of μmol min^{-1} (kg fat-free mass)$^{-1}$ (nmol/l)$^{-1}$.

Insulin secretion Beta cell function was assessed from the OGTT using a model describing the relationship between insulin secretion (calculated from C-peptide with the method of van Cauter et al [19]) and glucose concentration, previously described in detail [20, 21]. From the model-estimated beta cell dose–response, relating insulin secretion (in pmol m^{-2} min^{-1}) to glucose concentration, insulin secretion at 5 mmol/l glucose (the average basal glucose in participants with normal glucose tolerance) was estimated. This variable represents insulin secretion in basal conditions, if basal glucose were 5 mmol/l in each participant. Total insulin secretion was also determined using the model (integral during the OGTT, in nmol m^{-2}) [21]. We considered, for all statistical analyses, that the product of either total insulin secretion or insulin secretion at 5 mmol/l glucose with the M/I value from the hyperinsulinaemic–euglycaemic clamp to express the rate of insulin secretion, in relation to the concomitant degree of insulin resistance.

Statistical analysis

Data are expressed as mean ± SD or median (interquartile range) for variables with a skewed distribution, while categorical data are presented as percentages. Variables that were not symmetrically distributed were $\log_{10}$ transformed before analysis. Baseline characteristics, means and percentages, were compared using Student's t and χ^2 tests, respectively, according to incident hypertension.

For the DESIR cohort, as there was no significant interaction between sex and either parental history of diabetes or *TCF7L2* rs7903146 polymorphism on the risk of hypertension, we analysed men and women together. The relationships between both parental diabetes and *TCF7L2* rs7903146 genotype with incident hypertension were assessed by logistic regression analysis, with adjustment for sex, age and waist circumference. Further adjustments for fasting insulinaemia, fasting glycaemia and mean BP were made. We also tested for an interaction between HbA_{1c} >5.7% and *TCF7L2* variants on incident hypertension.

In the RISC cohort, BP levels at year 3 were compared according to quartiles of insulin secretion rate at baseline by ANOVA; we excluded individuals treated with antihypertensive medications at year 3 for this analysis.

A logistic regression analysis was used to test the association between the insulin secretion rate at baseline, assessed both as a continuous variable and stratified into quartiles, and incident hypertension at year 3 with adjustment for age, sex, recruitment centre, physical activity, waist circumference, fasting glycaemia and systolic and diastolic BP levels at baseline.

Statistical analyses used StatView (version 5.0, SAS Institute, Cary, NC, USA) and SAS version 9.2.

Results

The DESIR cohort

In the DESIR cohort, those who developed incident hypertension had higher blood pressure levels, fasting glucose, HbA_{1c}, BMI, waist circumference and insulin concentrations at baseline, as compared with those who remained normotensive (Table 1).

Fasting glycaemia but not HbA_{1c} levels at baseline remained associated with incident hypertension in a logistic regression analysis, after adjustment for sex and baseline age, waist circumference and mean BP. Neither the HOMA-IR nor the HOMA2%B indices were significantly related to the risk of incident hypertension in this multivariable model.

Parental history of diabetes A parental history of diabetes was associated with a higher incidence of hypertension at 9 years (36% vs 30%, p=0.02). This association persisted after controlling for potential confounders such as waist circumference and BMI (Table 2). Further adjustment for baseline fasting glycaemia, fasting insulinaemia, HOMA-IR and HOMA2%B did not alter the significant relationship (Table 2).

TCF7L2 rs7903146 polymorphism The *TCF7L2* rs7903146 variant conformed to Hardy–Weinberg equilibrium. BP levels at baseline did not differ according to the *TCF7L2* rs7903146 genotype. However, *TCF7L2* at-risk variants (CT+TT) were significantly associated with both higher systolic and diastolic BP at year 9 in univariate analysis (CT+TT vs CC genotype: 137±19 vs 135±19 mmHg, respectively, p=0.02 for systolic BP; 81±10 vs 80±10 mmHg, respectively, p=0.01 for diastolic BP).

The presence of the T allele was associated with an increased risk of incident hypertension at year 9, after controlling for sex

Table 1 Baseline characteristics of participants in the DESIR cohort according to incident hypertension (HTA) over the 9 year follow-up

Characteristic	Without incident HTA (n=1,646)	With incident HTA (n=745)	p value
Age (years)	44±9	49±9	0.0001
Men (%)	41	52	0.0001
BMI (kg/m^2)	23.4±3.0	24.8±3.3	0.0001
Waist circumference (cm)	78±10	84±10	0.0001
Smoking (%)	21	20	0.4
Systolic BP (mmHg)	121±9	127±7	0.0001
Diastolic BP (mmHg)	75±7	78±6	0.0001
Fasting glucose (mmol/l)	5.17±0.55	5.41±0.88	0.0001
HbA_{1c} (%)	5.4±0.4	5.5±0.6	0.0001
HbA_{1c} (mmol/mol)	35±4.4	37±6.0	0.0001
Fasting insulin (pmol/l)[a]	39.9 (20.8)	45.3 (23.3)	0.0001
HOMA-IR[a]	1.55 (0.91)	1.29 (0.73)	0.0001
HOMA2%B[a]	83.6 (25.7)	82.5 (25.2)	0.3

Data are shown as mean ± SD, median (interquartile range) or percentage

[a] Log transformation for statistical analysis

Table 2 Association between parental history of diabetes and incident hypertension in the DESIR cohort

	OR (95% CI)	*p* value
Unadjusted	1.30 (1.04, 1.64)	0.02
Adjusted for sex, age	1.45 (1.14, 1.84)	0.003
Adjusted for sex, age, BMI	1.41 (1.10, 1.80)	0.006
Adjusted for sex, age, waist circumference	1.41 (1.11, 1.81)	0.006
Adjusted for sex, age, waist circumference, HbA_{1c}	1.40 (1.09, 1.79)	0.008
Adjusted for sex, age, waist circumference, fasting glucose, fasting insulin	1.37 (1.07, 1.76)	0.01
Adjusted for sex, age, waist circumference, mean BP, smoking, HOMA-IR	1.37 (1.06, 1.77)	0.02
Adjusted for sex, age, waist circumference, fasting glucose, mean BP, smoking, alcohol intake	1.39 (1.04, 1.86)	0.03
Adjusted for sex, age, waist circumference, mean BP, smoking, alcohol intake, HOMA-IR	1.39 (1.04, 1.87)	0.03

and age, and the association persisted after further adjustment for waist circumference, BMI, mean BP, fasting glycaemia, insulinaemia, HOMA-IR and HOMA2%B at baseline (Table 3).

We further assessed whether baseline BMI or glycaemic status modified the effect of the *TCF7L2* at-risk allele on the risk of incident hypertension. There was no significant interaction between BMI (≥ or <27 kg/m^2) and *TCF7L2* on incident hypertension ($p_{interaction}$=0.25). However, there was a significant interaction between baseline HbA_{1c} above the median value (≥5.7%) and the effect of *TCF7L2* rs7903146 polymorphism on incident hypertension ($p_{interaction}$=0.03). After controlling for sex, age and waist circumference, the risk of hypertension was greater for individuals who carried the T allele and had HbA_{1c} ≥5.7% at baseline (OR 1.82; 95% CI 1.21, 2.74; p=0.004) than for those with the T allele but HbA_{1c} <5.7% (OR 1.11; 95% CI 0.90, 1.37; p=0.3).

The RISC cohort

Baseline glycaemia and hypertension at year 3 In univariate analysis, both fasting and 2 h glycaemia at baseline were significantly associated with incident hypertension at year 3 (Table 4). However, in a multivariable analysis, the association between both fasting glycaemia (OR 1.34; 95% CI 0.96, 1.86; p=0.09] and 2 h glycaemia (OR 1.03; 95% CI 0.92, 1.16; p=0.63) with the risk of hypertension at year 3 was no longer significant after controlling for sex, age, recruitment centre and waist circumference.

Baseline insulin secretion and hypertension at year 3 Individuals who developed hypertension at year 3 had, at baseline, reduced total insulin secretion during OGTT and also reduced basal beta cell function at 5 mmol/l glucose, as compared with those who remained normotensive (Table 4). This suggests that individuals with subtle alterations in insulin secretion, after accounting for the concomitant degree of insulin resistance, were at higher risk of developing hypertension at follow-up.

A lower insulin secretion rate at baseline (first vs fourth quartile of total insulin secretion during OGTT × M/I) was associated with higher BP at year 3 (systolic BP 123±14 vs 118±15 mmHg, p=0.0001; diastolic BP 78±9 vs 74±9 mmHg,

Table 3 Association between *TCF7L2* at-risk variants and incident hypertension (CT+TT vs CC as reference) in the DESIR cohort

	OR (95% CI)	*p* value
Unadjusted	1.15 (0.96, 1.36)	0.12
Adjusted for sex, age	1.23 (1.02, 1.47)	0.03
Adjusted for sex, age, BMI	1.22 (1.02, 1.47)	0.03
Adjusted for sex, age, waist circumference	1.22 (1.01, 1.47)	0.03
Adjusted for sex, age, waist circumference, HbA_{1c}	1.22 (1.02, 1.47)	0.03
Adjusted for sex, age, waist circumference, fasting glucose, fasting insulin	1.22 (1.01, 1.47)	0.03
Adjusted for sex, age, waist circumference, mean BP, smoking, HOMA-IR	1.31 (1.08, 1.59)	0.006
Adjusted for sex, age, waist circumference, fasting glucose, mean BP, smoking, alcohol intake	1.38 (1.11, 1.73)	0.004
Adjusted for sex, age, waist circumference, mean BP, smoking, alcohol intake, HOMA-IR	1.40 (1.12, 1.75)	0.003

Table 4 Baseline characteristics in the RISC cohort according to incident hypertension (HTA) over the 3 year follow-up

	Without incident HTA (n=881)	With incident HTA (n=166)	p value
Age (years)	43.9±8.2	47.7±8.1	<0.0001
Waist circumference (cm)	86±12	92±12	<0.0001
BMI (kg/m^2)	25.1±3.7	27.2±4.3	<0.0001
Smoker (%)	26.3	26.7	0.92
Physically inactive (%)	19.3	22.0	0.43
Systolic BP (mmHg)	116±12	128±10	<0.0001
Diastolic BP (mmHg)	74±8	80±7	<0.0001
Fasting glucose (mmol/l)	5.1±0.5	5.3±0.6	<0.0001
2 h glucose (mmol/l)	6.1±1.7	5.7±1.5	0.02
Fasting insulin (pmol/l)[a]	29.5 (21.0)	36.0 (25.7)	<0.0001
Clamp insulin sensitivity (M/I)[a]	134 (88)	114 (76)	<0.0001
Total insulin secretion[b] × M/I[a]	50.8 (29.7)	46.2 (25.8)	0.0005
Insulin secretion at 5 mmol[b] × M/I[a]	90.0 (72.7)	74.2 (71.5)	0.002

Data are shown as mean ± SD, median (interquartile range) or %

[a] Log-transformed for analysis

[b] Total insulin secretion during the OGTT at baseline and insulin secretion at 5 mmol/l are both expressed in relation to the M/I value (μmol min^{-1} [kg fat-free mass]$^{-1}$ [nmol/l]$^{-1}$) and multiplied by 10^{-2} for simplification of presentation

p<0.0001). A similar inverse association was observed for basal insulin secretion at a fixed normal glucose level of 5 mmol/l from the beta cell dose–response (first vs fourth quartile: systolic BP 125±13 vs 116±15 mmHg, p<0.0001; diastolic BP: 79±8 vs 73±9 mmHg, p<0.0001).

The incidence of hypertension at year 3 decreased progressively across the quartiles of both total insulin secretion and insulin secretion at 5 mmol/l at baseline (Fig. 1). Individuals with the lowest total insulin secretion rate during OGTT (quartile 1) had a higher incidence of hypertension as compared with those with the highest insulin secretion (quartile 4), after adjusting for other baseline risk factors (centre, age, sex, physical activity, waist circumference, smoking, alcohol intake, fasting glucose, systolic and diastolic BPs) (OR 2.02; 95% CI 1.06, 3.86; p=0.03).

Similarly, the inverse association between total insulin secretion rate during the OGTT, expressed as a continuous value, and the risk of incident hypertension at 3 years remained significant in a multivariable model after controlling for centre, age, sex, physical activity, waist circumference, smoking, alcohol intake, and systolic and diastolic BP levels at inclusion (OR per 1 SD log total insulin secretion 0.75; 95% CI 0.61, 0.93; p=0.007). The addition of fasting glucose and 2 h glucose levels into the model did not alter the significant relationship (OR 0.75; 95% CI 0.61, 0.92; p=0.007 and OR 0.75; 95% CI 0.61, 0.93; p=0.007, respectively).

Discussion

The main finding of this study is that a genetic predisposition to type 2 diabetes, and in particular the *TCF7L2* rs7903146 at-risk allele, was significantly associated with the incidence of hypertension after a 9 year follow-up in the general population; *TCF7L2* rs7903146 polymorphism conferred an increased risk of type 2 diabetes through alterations in insulin secretion capacity. Furthermore, we provide evidence, for the first time, that a lower insulin secretion rate during OGTT is a risk factor for the development of elevated BP in a non-diabetic cohort, even after taking into account insulin resistance, as assessed by the gold-standard method of the hyperinsulinaemic–euglycaemic clamp.

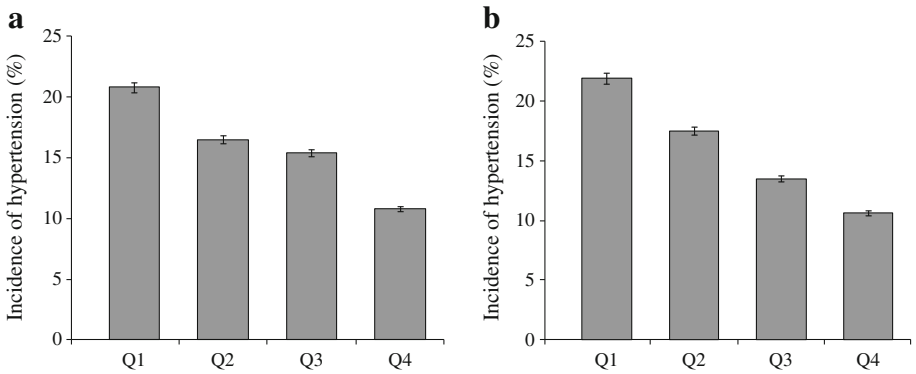

Fig. 1 Incidence of hypertension at year 3 according to the quartiles of (**a**) total insulin secretion rate × M/I during the OGTT (p for trend=0.02) and (**b**) basal insulin secretion rate at 5 mmol glucose × M/I at inclusion in the RISC cohort (p for trend=0.003). Error bars represent SEM

Previous evidence has shown that the offspring of individuals with type 2 diabetes have an increased risk of developing type 2 diabetes, with defects in early beta cell secretion [22–25]. It has also been suggested that these offspring have early autonomic dysfunction, even in the absence of glucose intolerance [26], and more often display metabolic abnormalities with high insulin levels and arterial hypertension [27, 28]. In a previous community-based cohort, parental diabetes appeared to be an independent predictor of longitudinal changes in both systolic and diastolic BPs in the offspring, regardless of race and sex [29]. Taken together, these observations and our results suggest that heritable factors related to type 2 diabetes increase the risk of various disorders, including hypertension.

Genetic susceptibility to type 2 diabetes seems to be more related to early beta cell dysfunction rather than to insulin resistance [30, 31]. We selected the *TCF7L2* rs7903146 polymorphism because this variant has the largest effect on type 2 diabetes susceptibility among the predisposing genes discovered to date [10, 32]. To our knowledge, this is the first report to study the longitudinal relationship of the *TCF7L2* genotype on incident hypertension in the general population. It has been demonstrated that the at-risk *TCF7L2* rs7903146 genotype is associated with impaired insulin secretion [33] and a reduced sensitivity of the beta cell to incretins [34]. The fact that the effect of the *TCF7L2* rs7903146 polymorphism on BP was independent of waist circumference, glycaemia or the insulin-resistance HOMA2-IR index underscores the potential role of insulin secretion defects on the evolution of BP over time. This is highlighted by our data on the relationship between both total and basal insulin secretion and 3 year incident hypertension in the RISC cohort. However, we suggest that the association of the *TCF7L2* genotype with incident hypertension might be related, at least in part, to reduced glucagon-like peptide-1 secretion in these individuals, as recent evidence has suggested that incretins might modulate BP [35]. The *TCF7L2* rs7903146 variant might then primarily affect incretin levels and, as a consequence, have independent effects on BP and on insulin secretion.

Our longitudinal results are in favour of a positive interaction between subtle elevations in glycaemia and genetic predisposition leading to defects in insulin secretion in the development of hypertension. Our results are consistent with recent genetic data showing an association between the *TCF7L2* rs7903146 variant and increased systolic BP in an endogamous ethnic group from India [36]. However, it should be noted that a recent large genome-wide association study (GWAS) did not report a significant association between the *TCF7L2* variant rs7903146 and BP, although a trend was observed for systolic BP (p=0.06) and pulse pressure (p=0.04) [37]. A regional plot around *TCF7L2* locus from the data publicly available of the ICBP GWAS study suggest that a signal near rs7903146 may modulate BP, although this variant does not appear to be a glycaemic signal (data not shown). The differences between our present study and the GWAS report might be related to the longitudinal follow-up used in our study, as opposed to the case–control design used in the GWAS. Indeed, we did not observe a significant association between the *TCF7L2* rs7903146 variant and the presence of hypertension in a cross-sectional analysis of the entire DESIR cohort at baseline (data not shown). Furthermore, in our study, we found that a large part of the association of the *TCF7L2* variant with hypertension was observed in people who already had baseline HbA_{1c} above the median value (≥5.7%), which might explain why the *TCF7L2* locus did not appear as being associated with hypertension in large cross-sectional GWAS that were not stratified on glucose. Our results need to be confirmed in other large prospective cohorts with long follow-ups.

The causal role of insulin in the development of hypertension has been much debated. It has been proposed that insulin resistance and/or hyperinsulinaemia promote the development of elevated BP over time [5, 6, 38, 39] with a positive association between insulin levels and incident hypertension [39, 40]. In these studies, the insulin secretion rate was not specifically assessed. Our findings confirm an association between fasting insulinaemia and BP but suggest a greater role for reduced insulin secretion in the pathogenesis of hypertension. Fasting insulin is a marker of insulin resistance which could be confounded by the presence of subtle defects in insulin secretion.

To our knowledge, the effects of insulin secretion on BP evolution have not been previously investigated, in contrast to the role of insulin resistance. In the present study, we found that a lower total insulin secretion rate was significantly associated with the incidence of hypertension in people without diabetes, and that this effect was independent of insulin sensitivity. This observation was confirmed when we assessed basal insulin secretion at a fixed normal glucose level of 5 mmol/l glucose, from the beta cell dose–response. Interestingly, in the present study, the inverse association between insulin secretion and incident hypertension was not related to weight gain over the follow-up (data not shown) and was also independent of insulin resistance, as assessed by the gold-standard method of the hyperinsulinaemic–euglycaemic clamp, suggesting alternative mechanisms.

A potential mechanism that might explain the increased hypertension risk with a reduced insulin secretion rate is the protective action exerted by insulin on the blood vessel. Haemodynamic actions of insulin have been suggested [41] and a small BP-lowering effect of insulin has been described in non-diabetic individuals [42]. Furthermore, a partial or complete deficiency in the insulin receptor and/or insulin-receptor substrate-1 in endothelial cells is associated with endothelial dysfunction and increased BP in mice [43, 44]. Together, these findings suggest that reduced activation of the

insulin pathway through diminished insulin secretion capacity, particularly in the presence of insulin resistance, might lead to endothelial dysfunction that favours the development of hypertension. Hyperinsulinaemia has to be interpreted as a compensation for enhanced insulin resistance. If this compensation is insufficient, the net effect at the cellular level, including probably the endothelium, is a down-tuned insulin signal.

Furthermore, recent evidence suggests that improving insulin secretion via the modulation of incretin concentrations can lower BP, even in non-diabetic hypertensive individuals [45, 46].

In parallel, subtle defects in beta cell function might facilitate the development of hypertension through possible repeated postprandial elevations in glucose levels over time. Glycaemic variability might contribute to increased BP through chronic induction of inflammation and enhanced oxidative stress [47, 48]. Oxidative stress has been shown to correlate with both glycaemic fluctuations and BP variability, suggesting possible alternative mechanisms to explain the increased incidence of hypertension in relationship to subtle defects in beta cell function [49]. In addition, the STOP-NIDDM trial found that acarbose treatment, which reduces postprandial hyperglycaemia, was associated with a significant reduction in the development of incident hypertension in those with impaired glucose tolerance, suggesting an impact of postprandial excursions on BP [50]. This point needs to be specifically investigated in another prospective study with continuous ambulatory assessment of glucose values.

Limitations of the present study include the absence of accurate measurements of both insulin resistance and beta cell function over time in the DESIR cohort, which precludes us from demonstrating that the association between parental history, the *TCF7L2* genotype and incident hypertension is directly related to defects in beta cell function. It is possible that the association observed between parental history and hypertension might be related, at least in part, to enhanced insulin resistance in the parents. The number of individuals with the at-risk *TCF7L2 rs7903146* genotype was limited in comparison with large genetic consortium studies and our results, albeit significant, need to be replicated in other populations. Furthermore, the absence of postprandial glucose levels did not allow us to investigate the exact role of this genotype in the development of elevated BP. The strengths of the present study are the use of two complementary cohorts specifically dedicated to the study of glucose metabolism, with a large number of normotensive participants at inclusion, aligned laboratory assays and continuous quality control of data. The RISC cohort is the largest cohort available with both systematic evaluation of insulin secretion and gold-standard measurements of insulin sensitivity by the glucose clamp technique, which provided the opportunity to clarify the role of alterations of insulin secretion in hypertension. For this important question to be addressed, a large number of individuals with high-quality measurements of glucose metabolism are required.

In conclusion, our study shows that a parental history of type 2 diabetes and at-risk variants in the *TCF7L2* rs7903146 genotype predispose to incident hypertension. The novel finding of the association between reduced insulin secretion and the development of elevated BP deserves further investigation through the study of the impact of treatments targeting insulin secretion and postprandial hyperglycaemia on BP. Our results suggest that defects in insulin secretion might be a new independent risk factor for the development of hypertension.

Acknowledgements For details of the DESIR Study Group and the RISC study and investigators, see the ESM.

Funding The DESIR study has been supported by Inserm contracts with CNAMTS, Lilly, Novartis Pharma and Sanofi-Aventis; and by Inserm (Réseaux en Santé Publique, Interactions entre les déterminants de la santé), Cohortes Santé TGIR, the Association Diabète Risque Vasculaire, the Fédération Française de Cardiologie, La Fondation de France, ALFEDIAM, ONIVINS, Ardix Medical, Bayer Diagnostics, Becton Dickinson, Cardionics, Merck Santé, Novo Nordisk, Pierre Fabre, Roche and Topcon.

The RISC study was supported by EU grant QLG1-CT-2001-01252, with additional support from AstraZeneca (Sweden). Activities of the European Group for the Study of Insulin Resistance (EGIR) are supported by an unrestricted research grant from Merck Serono, France.

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

Contribution statement FB was responsible for the conception of the study, analysed data, and wrote the manuscript; RR, BB, EF, CL, SC, LY, AN, ML, JP, PF, OL and MM contributed to the analysis and interpretation of the data and revised the manuscript critically. All authors approved the final version.

References

1. Ferrannini E, Cushman WC (2012) Diabetes and hypertension: the bad companions. Lancet 380:601–610
2. Conen D, Ridker PM, Mora S, Buring JE, Glynn RJ (2007) Blood pressure and risk of developing type 2 diabetes mellitus: the Women's Health Study. Eur Heart J 28:2937–2943
3. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL (2000) Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. N Engl J Med 342:905–912
4. Bjornholt JV, Erikssen G, Kjeldsen SE, Bodegard J, Thaulow E, Erikssen J (2003) Fasting blood glucose is independently associated with resting and exercise blood pressures and development of elevated blood pressure. J Hypertens 21:1383–1389
5. He J, Klag MJ, Caballero B, Appel LJ, Charleston J, Whelton PK (1999) Plasma insulin levels and incidence of hypertension in African Americans and whites. Arch Intern Med 159:498–503
6. Fagot-Campagna A, Balkau B, Simon D, Ducimetiere P, Eschwege E (1997) Is insulin an independent risk factor for hypertension? The Paris Prospective Study. Int J Epidemiol 26:542–550

7. Bower JK, Appel LJ, Matsushita K et al (2012) Glycated hemoglobin and risk of hypertension in the atherosclerosis risk in communities study. Diabetes Care 35:1031–1037
8. Britton KA, Pradhan AD, Gaziano JM et al (2011) Hemoglobin A1c, body mass index, and the risk of hypertension in women. Am J Hypertens 24:328–334
9. Gloyn AL, Braun M, Rorsman P (2009) Type 2 diabetes susceptibility gene TCF7L2 and its role in beta-cell function. Diabetes 58:800–802
10. Florez JC, Jablonski KA, Bayley N et al (2006) TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 355:241–250
11. Hills SA, Balkau B, Coppack SW et al (2004) The EGIR-RISC STUDY (The European Group for the Study of Insulin Resistance: Relationship between Insulin Sensitivity and Cardiovascular Disease Risk): I. Methodology and objectives. Diabetologia 47:566–570
12. Gautier A, Roussel R, Lange C et al (2011) Effects of genetic susceptibility for type 2 diabetes on the evolution of glucose homeostasis traits before and after diabetes diagnosis: data from the D.E.S.I.R. Study. Diabetes 60:2654–2663
13. Bonnet F, Marre M, Halimi JM et al (2006) Waist circumference and the metabolic syndrome predict the development of elevated albuminuria in non-diabetic subjects: the DESIR Study. J Hypertens 24:1157–1163
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
15. Antuna-Puente B, Disse E, Rabasa-Lhoret R, Laville M, Capeau J, Bastard JP (2011) How can we measure insulin sensitivity/resistance? Diabetes Metab 37:179–188
16. Bonnet F, Patel S, Laville M et al (2008) Influence of the ACE gene insertion/deletion polymorphism on insulin sensitivity and impaired glucose tolerance in healthy subjects. Diabetes Care 31:789–794
17. Bonnet F, Disse E, Laville M et al (2012) Moderate alcohol consumption is associated with improved insulin sensitivity, reduced basal insulin secretion rate and lower fasting glucagon concentration in healthy women. Diabetologia 55:3228–3237
18. Craig CL, Marshall AL, Sjostrom M et al (2003) International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35:1381–1395
19. Van Cauter E, Mestrez F, Sturis J, Polonsky KS (1992) Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 41:368–377
20. Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E (2002) Meal and oral glucose tests for assessment of beta-cell function: modeling analysis in normal subjects. Am J Physiol Endocrinol Metab 283:E1159–E1166
21. Mari A, Tura A, Natali A et al (2010) Impaired beta cell glucose sensitivity rather than inadequate compensation for insulin resistance is the dominant defect in glucose intolerance. Diabetologia 53:749–756
22. O'Rahilly S, Turner RC, Matthews DR (1988) Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. N Engl J Med 318:1225–1230
23. Meigs JB, Cupples LA, Wilson PW (2000) Parental transmission of type 2 diabetes: the Framingham Offspring Study. Diabetes 49:2201–2207
24. Bonadonna RC, Stumvoll M, Fritsche A et al (2003) Altered homeostatic adaptation of first- and second-phase beta-cell secretion in the offspring of patients with type 2 diabetes: studies with a minimal model to assess beta-cell function. Diabetes 52:470–480
25. Vauhkonen I, Niskanen L, Vanninen E, Kainulainen S, Uusitupa M, Laakso M (1998) Defects in insulin secretion and insulin action in non-insulin-dependent diabetes mellitus are inherited. Metabolic studies on offspring of diabetic probands. J Clin Invest 101:86–96
26. Frontoni S, Bracaglia D, Baroni A et al (2003) Early autonomic dysfunction in glucose-tolerant but insulin-resistant offspring of type 2 diabetic patients. Hypertension 41:1223–1227
27. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK, Ferrannini E (1989) Parental history of diabetes is associated with increased cardiovascular risk factors. Arteriosclerosis 9:928–933
28. Pontiroli AE, Monti LD, Pizzini A, Piatti P (2000) Familial clustering of arterial blood pressure, HDL cholesterol, and pro-insulin but not of insulin resistance and microalbuminuria in siblings of patients with type 2 diabetes. Diabetes Care 23:1359–1364
29. Srinivasan SR, Frontini MG, Berenson GS (2003) Longitudinal changes in risk variables of insulin resistance syndrome from childhood to young adulthood in offspring of parents with type 2 diabetes: the Bogalusa Heart Study. Metab Clin Exp 52:443–450, discussion 451–453
30. Florez JC (2008) Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: where are the insulin resistance genes? Diabetologia 51:1100–1110
31. Drong AW, Lindgren CM, McCarthy MI (2012) The genetic and epigenetic basis of type 2 diabetes and obesity. Clin Pharmacol Ther 92:707–715
32. Cauchi S, Meyre D, Choquet H et al (2006) TCF7L2 variation predicts hyperglycemia incidence in a French general population: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. Diabetes 55:3189–3192
33. Lyssenko V, Lupi R, Marchetti P et al (2007) Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest 117:2155–2163
34. Villareal DT, Robertson H, Bell GI et al (2010) TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. Diabetes 59:479–485
35. Yerram P, Whaley-Connell A (2012) Novel role for the incretins in blood pressure regulation. Curr Opin Nephrol Hypertens 21:463–468
36. Gupta V, Khadgawat R, Ng HK et al (2012) Association of TCF7L2 and ADIPOQ with body mass index, waist-hip ratio, and systolic blood pressure in an endogamous ethnic group of India. Genet Test Mol Biomark 16:948–951
37. Ehret GB, Munroe PB, Rice KM et al (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 478:103–109
38. Arnlov J, Pencina MJ, Nam BH et al (2005) Relations of insulin sensitivity to longitudinal blood pressure tracking: variations with baseline age, body mass index, and blood pressure. Circulation 112:1719–1727
39. Liese AD, Mayer-Davis EJ, Chambless LE et al (1999) Elevated fasting insulin predicts incident hypertension: the ARIC study. Atherosclerosis Risk in Communities Study Investigators. J Hypertens 17:1169–1177
40. Xun P, Liu K, Cao W, Sidney S, Williams OD, He K (2012) Fasting insulin level is positively associated with incidence of hypertension among American young adults: a 20-year follow-up study. Diabetes Care 35:1532–1537
41. Baron AD (1994) Hemodynamic actions of insulin. Am J Physiol 267:E187–E202
42. Heise T, Magnusson K, Heinemann L, Sawicki PT (1998) Insulin resistance and the effect of insulin on blood pressure in essential hypertension. Hypertension 32:243–248
43. Rask-Madsen C, Li Q, Freund B et al (2010) Loss of insulin signaling in vascular endothelial cells accelerates atherosclerosis in apolipoprotein E null mice. Cell Metab 11:379–389
44. Galkina EV, Butcher M, Keller SR et al (2012) Accelerated atherosclerosis in Apoe-/- mice heterozygous for the insulin receptor and the insulin receptor substrate-1. Arterioscler Thromb Vasc Biol 32:247–256
45. Mistry GC, Maes AL, Lasseter KC et al (2008) Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on blood pressure in nondiabetic

patients with mild to moderate hypertension. J Clin Pharmacol 48:592–598
46. Koren S, Shemesh-Bar L, Tirosh A et al (2012) The effect of sitagliptin versus glibenclamide on arterial stiffness, blood pressure, lipids, and inflammation in type 2 diabetes mellitus patients. Diabetes Technol Ther 14:561–567
47. Cherney DZ, Scholey JW, Sochett E, Bradley TJ, Reich HN (2011) The acute effect of clamped hyperglycemia on the urinary excretion of inflammatory cytokines/chemokines in uncomplicated type 1 diabetes: a pilot study. Diabetes Care 34:177–180
48. Monnier L, Mas E, Ginet C et al (2006) Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 295:1681–1687
49. Di Flaviani A, Picconi F, Di Stefano P et al (2011) Impact of glycemic and blood pressure variability on surrogate measures of cardiovascular outcomes in type 2 diabetic patients. Diabetes Care 34:1605–1609
50. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M (2003) Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA 290:486–494