Diabetologia (2008) 51:597–601
DOI 10.1007/s00125-008-0926-y

SHORT COMMUNICATION

Polymorphisms in the *TCF7L2*, *CDKAL1* and *SLC30A8* genes are associated with impaired proinsulin conversion

K. Kirchhoff · F. Machicao · A. Haupt · S. A. Schäfer · O. Tschritter · H. Staiger · N. Stefan · H.-U. Häring · A. Fritsche

Received: 6 November 2007 / Accepted: 17 December 2007 / Published online: 9 February 2008
© Springer-Verlag 2008

Abstract
Aims/hypothesis Variation within six novel genetic loci has been reported to confer risk of type 2 diabetes and may be associated with beta cell dysfunction. We investigated whether these polymorphisms are also associated with impaired proinsulin to insulin conversion.
Methods We genotyped 1,065 German participants for single nucleotide polymorphisms rs7903146 in *TCF7L2*, rs7754840 in *CDKAL1*, rs7923837 and rs1111875 in *HHEX*, rs13266634 in *SLC30A8*, rs10811661 in *CDKN2A/B* and rs4402960 in *IGF2BP2*. All participants underwent an OGTT. Insulin, proinsulin and C-peptide concentrations were measured at 0, 30, 60, 90 and 120 min during the OGTT. Insulin secretion was estimated from C-peptide or insulin levels during the OGTT using validated indices. We used the ratio proinsulin/insulin during the OGTT as indicator of proinsulin conversion.
Results In our cohort, we confirmed the significant association of variants in *TCF7L2*, *CDKAL1* and *HHEX* with reduced insulin secretion during the OGTT ($p<0.05$ for all). Variation in *SLC30A8, CDKN2A/B* and *IGF2BP2* was not associated with insulin secretion. The risk alleles of the variants in *TCF7L2*, *CDKAL1* and *SLC30A8* reduced proinsulin to insulin conversion ($p<0.05$ for all), whereas the risk alleles in *HHEX*, *CDKN2A/B* and *IGF2BP2* were not associated with reduced proinsulin to insulin conversion ($p>0.6$).
Conclusions/interpretation Diabetes-associated variants in *TCF7L2* and *CDKAL1* impair insulin secretion and conversion of proinsulin to insulin. However, both aspects of beta cell function are not necessarily linked, as impaired insulin secretion is specifically present in variants of *HHEX* and impaired proinsulin conversion is specifically present in a variant of *SLC30A8*.

Keywords Genetics of type 2 diabetes · Insulin secretion · Insulin synthesis

Abbreviations

GLP-1	glucagon-like peptide 1
PI/I	relative proportion of proinsulin to insulin
SNP	single nucleotide polymorphism

Introduction

Recent genome-wide association scans have found various new diabetes susceptibility genes including *TCF7L2*, *CDKAL1*, *HHEX*, *SLC30A8*, *CDKN2A/B* and *IGF2BP2* [1–3]. Impaired insulin secretion and impaired insulin sensitivity are the major pathogenic mechanisms leading to type 2 diabetes. Several of these novel risk loci for type 2 diabetes have been found to be associated mainly with impaired beta cell function [4–8]. Furthermore, a recently published study found associations between single nucleotide polymorphisms (SNPs) in *TCF7L2* and increased fasting proinsulin concentration, suggesting that, in addition

Electronic supplementary material The online version of this article (doi:10.1007/s00125-008-0926-y) contains supplementary material, which is available to authorised users.

K. Kirchhoff · F. Machicao · A. Haupt · S. A. Schäfer · O. Tschritter · H. Staiger · N. Stefan · H.-U. Häring · A. Fritsche (✉)
Department of Internal Medicine, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, Eberhard-Karls-Universität Tübingen, Otfried-Müller-Str. 10, 72076 Tübingen, Germany
e-mail: andreas.fritsche@med.uni-tuebingen.de

to insulin secretion, variation in *TCF7L2* might be involved in insulin synthesis and processing [9].

The conversion of proinsulin to insulin is one aspect of beta cell function. The relative proportion of proinsulin to insulin (PI/I) in the secretory granule represents an estimate of the efficiency of proinsulin processing [10]. A decrease in the PI/I ratio indicates an increase in the rate of proinsulin processing and vice versa. An elevated PI/I ratio has been observed in conditions with impaired beta cell function, such as type 2 diabetes [11] and impaired glucose tolerance [12].

The present study examined the association of SNPs in *TCF7L2* (rs7903146), *CDKAL1* (rs7754840), *HHEX* (rs7923837, rs1111875), *SLC30A8* (rs13266634), *CDKN2A/B* (rs10811661) and *IGF2BP2* (rs4402960) with proinsulin processing. We hypothesised that an impaired proinsulin to insulin conversion might be part of the mechanisms leading to impaired insulin secretion in carriers of these polymorphisms.

Methods

Participants The participants were selected from the ongoing Tübingen Family Study, which currently includes ~2,000 individuals at increased risk of diabetes [4, 8]. Less than 1% of participants are related to each other. Individuals on medication affecting glucose metabolism were excluded. Inclusion of the participants in the present study was based on availability of: (1) DNA samples for genotyping (n=1,650); and (2) complete OGTT data (glucose, insulin, C-peptide and proinsulin levels available for all time points during the OGTT, n=1,065). Data on C-peptide levels during IVGTT and OGTT from 545 participants (51%) included in the present study have been published previously [8].

The anthropometric characteristics of the study population are shown in Table 1. A positive family history for diabetes was reported by 750 participants (70%). All participants were genotyped for the following SNPs: *TCF7L2*, rs7903146; *CDKAL*, rs7754840; *HHEX*, rs7923837 and rs1111875; *SLC30A8*, rs13266634; *CDKN2A/B*, rs10811661; and *IGF2BP2*, rs4402960. Informed written consent was obtained from all participants and the local Ethics Committee approved the protocol.

Genotyping Genotyping was done using the TaqMan assay (Applied Biosystems, Forster City, CA, USA). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 7000 and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems). The overall genotyping success rate was 99.98%. Re-screening of 3.16% of participants generated 100% identical results. All SNPs were distributed according to Hardy–Weinberg equilibrium.

Oral glucose tolerance test After an overnight fast of 12 h, participants ingested a solution containing 75 g glucose at 08:00 hours. Venous blood samples were obtained at 0, 30, 60, 90 and 120 min and plasma glucose, insulin, C-peptide and proinsulin concentrations were determined.

Analytical procedures Blood glucose was determined using a bedside glucose analyser (glucose-oxidase method; Yellow Springs Instruments, Yellow Springs, CO, USA). Plasma insulin and proinsulin were determined by microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan, and IBL, Hamburg, Germany, respectively). The proinsulin assay has 0% cross-reactivity with human insulin and C-peptide. The insulin assay has 0% cross-reactivity with proinsulin.

Table 1 Anthropometric characteristics of the cohort

	Women		Men	
	Mean	25th–75th quartile	Mean	25th–75th quartile
Glucose tolerance (n)				
NGT/IGT/Diabetes	555/114/22		304/56/14	
Age (years)	39±1	29–49	40±1	27–50
BMI (kg/m^2)	27.52±0.27	22.37–31.28	27.02±0.29	23.15–29.25
Body fat (%)	32.8±0.4	26.0–39.0	22.3±0.4	17.0–26.5
BG 0 min (mmol/l)	5.11±0.03	4.72–5.33	5.23±0.04	4.79–5.50
BG 120 min (mmol/l)	6.45±0.08	5.11–7.22	6.31±0.12	4.78–7.17
Insulin sensitivity (AU)	17.1±0.4	8.9–22.6	18.1±0.6	9.4–25.5
Insulin 0 min (pmol/l)	5.3±0.2	32.0–73.0	6.5±0.3	28.8–68.0
Proinsulin 0 min (pmol/l)	60.7±1.8	2.0–7.0	54.7±2.2	2.0–8.2

Unless otherwise indicated, values are mean±SEM or geometric mean (25th–75th quartile). Insulin sensitivity was calculated by the method of Matsuda and DeFronzo: see ESM Table 1)

AU, arbitrary units; BG, blood glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance

Table 2 Association of diabetes susceptibility alleles with measures of insulin secretion, proinsulin conversion and insulin sensitivity during the OGTT

Gene/SNP (Genotype 1/2) Trait	11	12	22	*p* value	*p* value adjusted[a]
TCF7L2 rs7903146 (C/T)					
Female/male (*n*)	331/192	305/143	55/39	0.12	
BMI (kg/m^2)	27.65± 0.3	27.01±0.3	27.21±0.54	0.40	
BG 120 min (mmol/l, OGTT)	6.22±0.09	6.40±0.1	7.44±0.31	<0.0001	
Insulin sensitivity (AU)	16.93±0.48	18.10±0.53	17.02±1.15	0.13	
PI:I ratio 0	0.116±0.006	0.126±0.006	0.18±0.025	0.011	
AUC proinsulin:AUC insulin	0.053±0.002	0.054±0.002	0.065±0.006	0.019	
AUC C-peptide:AUC glucose (pmol:mmol)	316±5	298±5	278±11	0.0002	0.0008
CDKAL1 rs7754840 (A/C)					
Female/male (*n*)	325/163	293/177	73/34	0.29	
BMI (kg/m^2)	27.19±0.28	27.59±0.32	26.97±0.6	0.68	
BG 120 min (mmol/l, OGTT)	6.31±0.08	6.55±0.11	6.19±0.2	0.31	
Insulin sensitivity (AU)	17.56±0.5	17.1±0.52	18.45±1.06	0.22	
PI:I ratio 0	0.126±0.007	0.125±0.007	0.129±0.01	0.84	
AUC proinsulin:AUC insulin	0.053±0.002	0.054±0.002	0.063±0.004	0.038	
AUC C-peptide:AUC glucose (pmol:mmol)	309±4	301±5	306±11	0.21	0.04
SLC30A8 rs13266634 (C/T)					
Female/male (*n*)	362/194	273/143	56/37	0.61	
BMI (kg/m^2)	27.19±0.27	27.27±0.33	28.58±0.70	0.13	
BG 120 min (mmol/l, OGTT)	6.30±0.08	6.50±0.11	6.63±0.27	0.27	
Insulin sensitivity (AU)	17.79±0.46	17.20±0.54	16.31±1.32	0.13	
PI:I ratio 0	0.130±0.006	0.119±0.007	0.130±0.018	0.26	
AUC proinsulin:AUC insulin	0.057±0.002	0.052±0.002	0.050±0.005	0.0061	
AUC C-peptide:AUC glucose (pmol:mmol)	303±4	307±5	306±12	0.97	0.70
HHEX rs7923837 (G/A)					
Female/male (*n*)	274/138	329/187	88/49	0.64	
BMI (kg/m^2)	27.2±0.31	27.4±0.3	27.5±0.6	0.93	
BG 120 min (mmol/l, OGTT)	6.7±0.1	6.2±0.1	6.2±0.2	0.006	
Insulin sensitivity (AU)	17.66±0.54	17.53±0.51	16.42±0.84	0.67	
PI:I ratio 0	0.138± 0.008	0.120±0.006	0.110±0.009	0.59	
AUC proinsulin:AUC insulin	0.056±0.002	0.053±0.002	0.052±0.003	0.86	
AUC C-peptide:AUC glucose (pmol:mmol)	289±5	315±5	317±9	0.0003	0.0005
HHEX rs1111875 (C/T)					
Female/male (*n*)	242/122	340/194	109/58	0.68	
BMI (kg/m^2)	27.13±0.33	27.46±0.29	27.41±0.53	0.81	
BG 120 min (mmol/l, OGTT)	6.64±0.13	6.25±0.09	6.37±0.16	0.03	
Insulin sensitivity (AU)	17.85±0.59	17.43±0.48	16.49±0.83	0.45	
PI:I ratio 0	0.140±0.009	0.120±0.006	0.116±0.009	0.74	
AUC proinsulin:AUC insulin	0.056±0.002	0.053±0.002	0.053±0.003	0.98	
AUC C-peptide:AUC glucose (pmol:mmol)	287±5	314±5	318±9	0.0001	0.0002
CDKN2A/B rs10811661 (T/C)					
Female/male (*n*)	456/253	213/110	22/11	0.88	
BMI (kg/m^2)	27.40±0.24	27.12±0.36	28.44±1.30	0.50	
BG 120 min (mmol/l, OGTT)	6.41±0.08	6.48±0.12	5.66±0.25	0.09	
Insulin sensitivity (AU)	17.77±0.43	16.73±0.60	16.64±1.63	0.46	
PI:I ratio 0	0.128±0.006	0.125±0.007	0.088±0.013	0.43	
AUC proinsulin:AUC insulin	0.055±0.002	0.055±0.002	0.042±0.004	0.40	
AUC C-peptide:AUC glucose (pmol:mmol)	304±4	308±6	307±17	0.64	0.72
IGF2BP2 rs4402960 (G/T)					
Female/male (*n*)	293/182	320/168	78/24	0.01	
BMI (kg/m^2)	27.64±0.32	27.05±0.28	27.37±0.67	0.47	
BG 120 min (mmol/l, OGTT)	6.52±0.11	6.27±0.09	6.52±0.20	0.19	
Insulin sensitivity (AU)	17.35±0.52	17.56±0.50	17.11±0.99	0.78	
PI:I ratio 0	5.73±0.26	5.50±0.26	6.81±0.56	0.028	
AUC proinsulin:AUC insulin	0.055±0.002	0.054±0.002	0.055±0.004	0.98	
AUC C-peptide:AUC glucose (pmol:mmol)	305±5	306±5	297±11	0.68	0.64

Insulin sensitivity was calculated by method of Matsuda and DeFronzo: see ESM

[a] Adjusted for age and insulin sensitivity

AU, arbitrary units; BG, blood glucose

Calculations Insulin secretion in the OGTT was assessed by calculating the AUC for C-peptide divided by the AUC for glucose. For additional validated insulin secretion and sensitivity indices see Electronic supplementary material (ESM) Tables. Proinsulin to insulin conversion was determined by dividing AUC proinsulin by AUC insulin.

Statistical analyses Data are given as mean±SEM. Data that were not normally distributed were logarithmically transformed. A p value of <0.05 was considered statistically significant. All risk alleles show an additive inheritance pattern [3]; we therefore used an additive linear model for all analyses. The statistical software package JMP 4.0 (SAS Institute, Cary, NC, USA) was used.

Our study was sufficiently powered ($1-\beta>0.8$, $\alpha=0.05$) to detect effect sizes with Cohen's $d\geq0.19$ for the SNPs with the lowest minor allele frequencies. Power calculations were performed using G*power software (www.psycho.uni-duesseldorf.de/aap/projects/gpower/).

Results

Insulin secretion The risk alleles of rs7903146 in *TCF7L2*, rs7754840 in *CDKAL1* and rs7923837/rs1111875 in *HHEX* were associated with lower insulin secretion measured as AUC (C-peptide)/AUC (glucose) during the OGTT ($p<0.05$ for all; Table 2). Other validated indices for insulin secretion estimated from OGTT data showed similar results (ESM Tables 1–7). Insulin secretion based on measurement of C-peptide and insulin levels during the OGTT was not associated with polymorphisms in *SLC30A8*, *IGF2BP2* and *CDKN2A/B* ($p>0.5$).

Proinsulin/insulin ratio during the OGTT The risk alleles of rs7903146 in *TCF7L2*, rs7754840 in *CDKAL1* and rs13266634 in *SLC30A* were associated with a significantly higher AUC proinsulin/AUC insulin ratio ($p<0.05$ for all) (Table 2). The fasting and 30 min proinsulin/insulin ratios were significantly increased in carriers of the risk alleles in *TCF7L2*, whereas carriers of the risk alleles in *CDKAL1* had significantly increased proinsulin/insulin ratios at 30 min only and carriers of risk alleles in *SLC30A8* had increased proinsulin/insulin ratios at 60 and 90 min during the OGTT (ESM Tables 1, 2, 3). There were no significant differences in proinsulin/insulin ratios during the OGTT between the genotypes of rs7923837 and rs1111875 in *HHEX*, rs10811661 in *CDKN2A/B* and rs4402960 in *IGF2BP2*. Homozygote carriers of the risk allele in *IGF2BP2* merely had increased fasting proinsulin levels and proinsulin/insulin ratio (Table 2, ESM Table 7).

Discussion

In the present study, we found that common variants associated with increased risk of diabetes in *TCF7L2*, *CDKAL1* and *SLC30A8* are also associated with impaired conversion of proinsulin to insulin. However, we found no impact on proinsulin conversion for the diabetes-associated SNPs in *HHEX, CDKN2A/B* and *IGF2BP2*.

The mechanisms by which the variants in *TCF7L2*, *CDKAL1* and *SLC30A8* influence proinsulin processing are a matter of speculation. Recently, Loos et al. [9] demonstrated that the genes of proprotein convertases 1 and 2, which are key proteins in the conversion from proinsulin to insulin, exhibit binding sites for T cell transcription factor. Therefore, an interaction with these proprotein convertases may be a mechanism leading to increased proinsulin levels in carriers of the risk alleles of *TCF7L2*. Another possibility is that the impaired glucagon-like peptide 1 (GLP-1) action present in carriers of the risk alleles in *TCF7L2* leads to impaired proinsulin processing. Two recent studies [4, 5] have demonstrated that the risk alleles in *TCF7L2* are associated with impaired incretin effect, as the insulin response to oral glucose was lower than that to intravenous glucose. In addition, GLP-1 infusion led to lower insulin secretion in carriers of the risk alleles in *TCF7L2* [4]. As GLP-1 infusion is able to normalise reduced proinsulin conversion [13] and GLP-1 signalling is impaired in carriers of the risk alleles in *TCF7L2*, it is conceivable that the GLP-1 signalling defect also leads to impaired proinsulin processing.

The mechanism by which the variant in the *SLC30A8* gene affects proinsulin processing is also unclear. *SLC30A8* encodes the zinc transporter protein member 8 (ZnT-8), which is important for storage and maturation of insulin in the granules of the beta cell [14]. It might well be that a functional defect in ZnT-8 impairs proinsulin processing. While demonstrating impaired proinsulin processing in the risk allele of rs13266634 in *SLC30A8*, we found no effect of this variant on insulin secretion after an oral glucose load, a finding which is in agreement with our previously published results [8]. However, we did demonstrate an effect on insulin secretion in the IVGTT [8]. This finding suggests a defect in proinsulin processing that only leads to impaired insulin secretion under a strongly increased secretory demand, as is present during the IVGTT.

Variation in *HHEX* results in a marked impairment of insulin secretion, in response to both oral and intravenous glucose administration [8]. The present results show that this is not due to impaired proinsulin processing, at least during an oral glucose challenge. To our knowledge, no other beta cell defects are known to alter insulin secretion but not proinsulin conversion or vice versa.

In carriers of the risk allele in *IGF2BP2* we found increased fasting proinsulin levels but no differences in proinsulin conversion after glucose stimulation. Fasting levels of proinsulin and insulin are largely affected by different clearance rates and do not represent the proinsulin conversion in the beta cell [10, 13]. Therefore, we believe that variation in *IGF2BP2* has no major impact on proinsulin conversion.

In conclusion, our data suggest that impaired proinsulin processing is one possible mechanism that leads to impaired insulin secretion and increased risk of diabetes in carriers of the risk alleles in *TCF7L2* and *CDKAL1*. However, these two aspects of beta cell dysfunction, impaired insulin secretion and proinsulin processing, are not necessarily linked. Carriers of a variant in *SLC30A8* specifically showed impaired proinsulin conversion during oral glucose challenge, whereas carriers of variants in *HHEX* specifically exhibited impaired insulin secretion.

Acknowledgements We thank all study participants for their cooperation. We thank the International HapMap Consortium for the public allocation of genotype data. We gratefully acknowledge the technical assistance of A. Bury, A. Guirguis, H. Lutz, M. Weisser, R. Werner, E. Kollmar and B. Horrer. The study was supported by a grant from the Deutsche Forschungsgemeinschaft (KFO 114).

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

References

1. Sladek R, Rocheleau G, Rung J et al (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445:881–885
2. Scott LJ, Mohlke KL, Bonnycastle LL et al (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316:1341–1345
3. Zeggini E, Weedon MN, Lindgren CM et al (2007) Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 316:1336–1341
4. Schafer SA, Tschritter O, Machicao F et al (2007) Impaired GLP-1 induced insulin secretion in carriers of *TCF7L2* polymorphisms. Diabetologia 50:2443–2450
5. 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
6. Steinthorsdottir V, Thorleifsson G, Reynisdottir I et al (2007) A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 39:770–775
7. Pascoe L, Tura A, Patel SK et al (2007) Common variants of the novel type 2 diabetes genes, CDKAL1 and HHEX/DIE, are associated with decreased pancreatic z-cell function. Diabetes 56:3101–3104
8. Staiger H, Machicao F, Stefan N et al (2007) Polymorphisms within novel risk loci for type 2 Diabetes determine β-cell function. PloS ONE 2:e832
9. Loos RJF, Franks PW, Francis RW et al (2007) TCF7L2 polymorphisms modulate proinsulin levels and β-cell function in a British Europid population. Diabetes 56:1943–1947
10. Fritsche A, Madaus A, Stefan N et al (2002) Relationships among age, proinsulin conversion, and beta-cell function in nondiabetic humans. Diabetes 51(Suppl 1):S234–S239
11. Roder ME, Porte D Jr, Schwartz RS, Kahn SE (1998) Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 83:604–608
12. Larsson H, Ahrén B (1999) Relative hyperproinsulinemia as a sign of islet dysfunction in women with impaired glucose tolerance. J Clin Endocrinol Metab 84:2068–2074
13. Stumvoll M, Fritsche A, Stefan N, Hardt E, Häring H (2001) Evidence against a rate limiting role of proinsulin processing for maximal insulin secretion in subjects with impaired glucose tolerance and β-cell dysfunction. J Clin Endocrinol Metab 86:1235–1239
14. Chimienti F, Devergnas S, Favier A, Seve M (2004) Identification and cloning of a β-cell-specific zinc transporter, ZnT-8, localised into insulin secretory granules. Diabetes 53:2330–2337