

A variant in the *KCNQ1* gene predicts future type 2 diabetes and mediates impaired insulin secretion

Running title: *KCNQ1* and type 2 diabetes

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Objective-Two independent genome wide association studies for type 2 diabetes in Japanese have recently identified common variants in the KCNQ1 gene to be strongly associated with type 2 diabetes. Here we studied whether a common variant in *KCNQ1* would influence BMI, insulin secretion and action and predict future type 2 diabetes in subjects from Sweden and Finland.

Research design and methods- Risk of type 2 diabetes conferred by *KCNQ1* rs2237895 was studied in 2,830 type 2 diabetes cases and 3,550 controls from Sweden (Malmö Case-Control) and prospectively in 16,061 individuals from the Malmö Preventive Project (MPP). Association between genotype and insulin secretion/action was assessed cross-sectionally in 3,298 non-diabetic subjects from the PPP-Botnia Study and longitudinally in 2,328 non-diabetic subjects from the Botnia Prospective Study (BPS). *KCNQ1* expression (n=18) and glucose-stimulated insulin secretion (n=19) was measured in human islets from non-diabetic cadaver donors.

Results. The C-allele of *KCNQ1* rs2237895 was associated with increased risk of type 2 diabetes in both the case-control (OR 1.23 [1.12-1.34], $p=5.6 \times 10^{-6}$) and the prospective (OR 1.14 [1.06-1.22], $p=4.8 \times 10^{-4}$) studies. Furthermore, the C-allele was associated with decreased insulin secretion (CIR $p=0.013$; DI $p=0.013$) in the PPP-Botnia study and in the BPS at baseline (CIR $p=3.6 \times 10^{-4}$; DI $p=0.0058$) and after follow-up (CIR $p=0.0018$; DI $p=0.0030$). C-allele carriers showed reduced glucose-stimulated insulin secretion in human islets ($p=2.5 \times 10^{-6}$).

Conclusion. A common variant in the *KCNQ1* gene is associated with increased risk of future type 2 diabetes in Scandinavians which partially can be explained by an effect on insulin secretion.

Recently, two independent genome wide association studies (GWASs) in Japanese have shown that single nucleotide polymorphisms (SNPs) in the *KCNQ1* gene (rs2074196, rs2237892, rs2237895, rs2283228 and rs2237897) are associated with type 2 diabetes (1; 2); We have previously replicated association of rs2074196 and rs2237892 reported by Yasuda et al. in Scandinavian subjects (1). Here we studied rs2237895 which is the only of the replicated variants by Unoki et al. in Danish population with a minor allele frequency >5% (43%) (2). *KCNQ1* encodes for a voltage-gated potassium channel which is highly expressed in cardiac muscle, pancreas, intestine and kidney. Mutations in the *KCNQ1* gene cause the long QT syndrome and deafness (3).

Here we studied whether rs2237895 increases risk of type 2 diabetes and/or affects insulin secretion and action in several Swedish and Finnish cross-sectional and prospective cohorts including a total of 28,067 individuals.

RESEARCH DESIGN AND METHODS

Study populations. Characteristics of the study participants are reported in Table 1.

Malmö case-control consisted of 2,830 diabetic cases from the Malmö Diabetes Registry (4) and 3,550 non-diabetic controls from the Malmö Diet and Cancer study (5) in Southern Sweden. All cases had Scandinavian origin, age at onset >35 years, C-peptide ≥ 0.3 nmol/l and no GAD Ab. Controls had fasting blood glucose <5.5 mmol/l and HbA1c <6.0% (6).

The Malmö Preventive Project (MPP) is a large population based prospective study from the city of Malmö, Sweden, consisting of 16,061 non-diabetic subjects, 2,063 of whom developed type 2 diabetes during a 24.8 year median follow-up period (7). Diagnosis of diabetes was confirmed from patient records or a fasting plasma glucose ≥ 7.0 mmol/l.

The PPP-Botnia Study (Prevalence, Prediction and Prevention of diabetes) is a population-based study from the Botnia region of Western Finland. 3,298 non-diabetic subjects (fasting plasma glucose <7.0 mmol/l and 2hr plasma glucose <11.1 mmol/l) were included in the current study.

The Botnia Study started in 1990 at the West coast of Finland aiming at identification of genes increasing susceptibility to type 2 diabetes in members from families with type 2 diabetes (8). The prospective part included 2,770 non-diabetic family members and/or their spouses (1,263 men and 1,507 women, mean age 45 years), 138 of whom developed type 2 diabetes during a 7.7 year (median) follow-up period (9). All subjects were given information about exercise and healthy diet and subjected at 2-3 years intervals to a new oral glucose tolerance test (OGTT). 2,328 non-diabetic individuals (fasting plasma glucose <7.0 mmol/l and 2hr plasma glucose <11.1 mmol/l) with available longitudinal measurements of insulin secretion and action were included in the current analyses.

Measurements. In MPP, fasting blood samples were drawn at baseline and follow-up visit for measurement of plasma glucose. In the PPP-Botnia study blood samples were drawn at 0, 30 and 120 minutes of the OGTT. In the BPS, blood samples were drawn at -10, 0, 30, 60 and 120 minutes of the OGTT both at baseline and at follow-up. Insulin sensitivity index (ISI) from the OGTT was calculated as $10,000/\sqrt{(P\text{-glucose}_{\text{fasting}} \times P\text{-insulin}_{\text{fasting}} \times \text{mean OGTT}_{\text{glucose}} \times \text{mean OGTT}_{\text{insulin}})}$ (10). The basal insulin resistance index (HOMA-IR) was calculated from fasting insulin and glucose concentrations (<http://www.dtu.ox.ac.uk>). Beta cell function was assessed as corrected insulin response during OGTT ($\text{CIR} = 100 \times \text{insulin}_{30} / [\text{glucose}_{30} \times (\text{glucose}_{30} - 3.89)]$) or as disposition index, i.e. insulin secretion

adjusted for insulin sensitivity ($DI=CIR \times ISI$) (11).

Genotyping. Genotyping was performed by an allelic discrimination method with a TaqMan assay on the ABI 7900 platform (Applied Biosystems, Foster City, CA, USA). We obtained an average genotyping success rate of 93.2%, and the average concordance rate, based on 2,944 duplicate comparisons using a KASPar competitive allele specific PCR system (Kbioscience, Hoddesdon, UK), was 99.9%. Hardy-Weinberg equilibrium was fulfilled in all studied populations ($p > 0.20$).

Glucose-stimulated insulin secretion. Islets from 27 human cadaver donors were provided by the Nordic network for clinical islets transplantation by the courtesy of Olle Korsgren, Uppsala University. The experimental protocol for isolation of islets was approved by the ethics committee of Uppsala University and performed in accordance with local institutional and Swedish national regulations.

Purified islets were collected under a stereomicroscope at room temperature. The islets were either directly subjected to Affymetrix analysis (see below) or preincubated for 30 min at 37° C in Krebs Ringer bicarbonate (KRB) buffer, pH 7.4, supplemented with 10 mmol/l N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 0.1% bovine serum albumin, and 1 mmol/l glucose. Each incubation vial contained 12 islets in 1.0 ml KRB buffer solution and treated with 95% O₂-5% CO₂ to obtain constant pH and oxygenation. After preincubation, the buffer was changed to a medium containing either 1 or 20 mmol/l glucose. The islets were then incubated for 1h at 37° C in a metabolic shaker (30 cycles per min). Immediately after incubation an aliquot of the medium was removed for measurement of insulin using a radioimmunoassay (Linco Research, Saint Charles, MO, USA). Glucose-stimulated insulin secretion is expressed as

fold change of insulin release from the islets by comparing release at 20 mmol/l with release using the 1 mmol/l glucose medium.

Expression of the KCNQ1 gene in human pancreatic islets. Total RNA was isolated with the AllPrep DNA/RNA Mini Kit (Qiagen GmbH, Hilden, Germany). RNA quality and concentration was measured using an Agilent 2100 bioanalyzer and Nanodrop ND-1000 equipment, respectively.

The microarrays were performed following the Affymetrix standard protocol. Briefly, total RNA 100-300 ng was processed following the GeneChip® Expression 3'-Amplification Reagents One-cycle cDNA synthesis kit instructions (Affymetrix Inc, Santa Clara, CA, USA) to produce double-stranded cDNA. This was used as a template to generate biotin-targeted cRNA following manufacturer's specifications. 15 µg of the biotin labeled cRNA was fragmented to strands between 35 and 200 bases in length, 10 µg of which was hybridized onto the GeneChip® Human Gene 1.0 ST whole transcript based assay overnight in the GeneChip® Hybridization oven 6400 using standard procedures. The arrays were washed and stained in a GeneChip® Fluidics Station 450. Scanning was carried out with the GeneChip® Scanner 3000 and image analysis was performed using GeneChip® Operating Software. The array data was summarized and normalized with Robust Multi-array Analysis (RMA) method using the software "Expression Console" (Affymetrix).

Statistical analyses. Variables are presented as mean ± standard deviation (SD), and if not normally distributed as median [interquartile range (IQR)]. The risk of developing type 2 diabetes is expressed as odds ratio (OR) using logistic regression analyses adjusted for age, gender and BMI. Since men and women in MPP were included at different time periods, we adjusted for this using inclusion period and an interaction term inclusion period x gender. Genotype-

phenotype correlations were studied using linear regression analyses adjusted for age, gender, and BMI (when appropriate). A robust variance estimate was used to adjust for within-pedigree dependence in BPS, treating each pedigree as an independent entity when calculating the variance of the estimates. Non-normally distributed variables (insulin, HOMA-IR, ISI, CIR and DI) were logarithmically (natural) transformed for analyses. For analysis of data from human pancreatic islets, one-way analysis of variance was used to assess association between genotype and phenotype (expression and insulin secretion) and Spearman correlation was used to assess association of *KCNQ1* mRNA level with insulin release. All statistical analyses were performed under an additive model with the Statistical Package for the Social Sciences version 16.0 (SPSS Inc., Chicago, IL) and STATA SE 10.1 (StataCorp LP, College Station, Texas). Meta-analyses were performed with metan using fixed effect models using the inverse variance method. Inter-study heterogeneity was tested by Cochran Q and the I-squared measure as implemented in STATA. Two-sided p-values of less than 0.05 were considered statistically significant.

RESULTS

Effect of *KCNQ1* rs2237895 on risk of type 2 diabetes and glucose levels. In the Malmö Case-Control study, the C-allele of *KCNQ1* rs2237895 was more frequent in cases than in controls (0.44 and 0.40, χ^2 $p=9.5 \times 10^{-6}$), yielding an age, gender and BMI adjusted odds ratio (OR) of 1.23 (95% confidence interval, CI, 1.12-1.34, p -value= 5.6×10^{-6}). The same SNP (C-allele) predicted future type 2 diabetes in the MPP (OR 1.14, 95%CI 1.06-1.22, $p=4.7 \times 10^{-4}$). In the MPP, non-diabetic carriers of the C-allele showed higher fasting plasma glucose levels both at baseline ($p=0.033$), and after the 25-year follow-up period ($p=1.2 \times 10^{-6}$) (Table 2).

Also in the PPP-Botnia study, the C-allele carriers showed elevated fasting plasma glucose concentrations ($p=0.0067$) (Table 2).

Effect of *KCNQ1* rs2237895 on BMI, insulin secretion and action. There was no effect of the SNP on BMI in any of the cohorts. The C-allele carriers of the PPP-Botnia study showed lower insulin response to glucose at 30 min during OGTT (CIR $p=0.013$; DI $p=0.013$, Table 2, Figure 1A and B). Also, in BPS, the C-allele was associated with reduced beta-cell function at baseline (CIR $p=3.6 \times 10^{-4}$; DI $p=0.0058$) and at follow-up (CIR $p=0.0018$; DI $p=0.0030$) (Table 2, Figure 1C and D). The *KCNQ1* rs2237895 had no effect on insulin sensitivity estimated as HOMA-IR or ISI during OGTT.

Expression of *KCNQ1* and glucose-stimulated insulin secretion in human pancreatic islets. We also analyzed *KCNQ1* mRNA from microarray data on pancreatic islets from 18 non-diabetic human cadaver organ donors in relation to genotype. There was no significant difference in *KCNQ1* expression in human islets between carriers of different genotypes ($p=0.65$). Information on glucose-stimulated insulin secretion at 1 mM and 20 mM of glucose was available for islets from 19 donors. Risk allele carriers showed lower glucose-stimulated insulin secretion measured as fold change of insulin release from the islets at 1 mM and 20 mM glucose, i.e. stimulation index ($p=2.5 \times 10^{-6}$) (Figure 1 E). These results remained unchanged when stimulation index was adjusted for the basal insulin secretion at 1 mM of glucose (Supplementary Figure 1, $p=4.7 \times 10^{-5}$ (available online at <http://diabetes.diabetesjournals.org>). We could not observe any correlation between *KCNQ1* expression and glucose-stimulated insulin secretion ($n=10$, $r=0.115$, $p=0.75$).

DISCUSSION

The key finding of the present study was that a common variant rs2237895 in the

KCNQ1 gene was associated with increased risk of future type 2 diabetes due to impairment of beta-cell function.

Variants in *KCNQ1* have been associated with type 2 diabetes predominantly in Asian subjects. Most of these studies had a case-control design which tends to overestimate the risk of a SNP because cases and controls usually represent two extremes of the distribution of glucose tolerance. This is to our knowledge the first population-based study using a prospective design showing that the SNP indeed increases risk of future type 2 diabetes and that this is due to a failing beta-cell function.

Although common variants in *KCNQ1* increase susceptibility to type 2 diabetes in both Asians and Europeans, the frequency of the risk allele (RAF) of most SNPs is much higher in Europeans than in Asians, (92-96% in Europeans compared to 59-69% in Japanese) which most likely explains why SNPs in this gene were not significantly associated with type 2 diabetes in the initial European GWASs. In this regard, SNP rs2237895 represents an exception, since the RAF was similar in Scandinavians (43%) and in Asians (36%, (1)). The ORs for type 2 diabetes were also quite similar across studies (1.23 in the current study, 1.31 in Asians (1), and 1.24 in Danes (2)).

We also provide compelling evidence that the risk C-allele is associated with deterioration of beta-cell function over time in the BPS, which thereby confirms and extends our previous observation of an association between the risk allele of another SNP (rs2237892) in *KCNQ1* and impaired insulin secretion ($p=0.024$) (1).

In analogy with Kir 6.2 (KCNJ11), *KCNQ1* is an ATP dependent potassium channel which is also expressed in human islets. It has been ascribed a role in insulin secretion, most likely through alterations in the membrane repolarization potential of the pancreatic beta-cells. Indeed, we found that

risk allele carriers had lower glucose-stimulated insulin secretion in human islets. We could not demonstrate a significant effect of the genotype on expression of the *KCNQ1* gene in human islets suggesting that the effect could be mediated through effects on splicing, translation or posttranslational modifications. However, we cannot rule out that this lack of effect is due to low power because of limited number of human islets.

In conclusion, we provide conclusive evidence that common variants in the *KCNQ1* gene increase risk of future type 2 diabetes by causing impaired beta-cell function.

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Table 1. Characteristics of the study participants

	Malmö Case Control		Malmö Preventive Project	PPP-Botnia Study	Botnia Prospective Study
	Cases	Controls			
Number (m/f)	2,830 (1,667/1,163)	3,550 (1,340/2,210)	16,061 (10,416/5,645)	3,298 (1,538/1,760)	2,328 (1,065/1,263)
Age (years)	57.9 ± 11.5	57.5 ± 6.0	45.5 ± 6.9	48.5 ± 15.9	45.5 ± 13.6
BMI (kg/m ²)	29.6 ± 5.5	25.1 ± 3.6	24.3 ± 3.3	26.2 ± 4.2	25.6 ± 3.9
Fasting P-glucose (mmol/l)	11.89 ± 4.34	4.78 ± 0.36	5.45 ± 0.56	5.16 ± 0.55	5.52 ± 0.57
HOMA-IR (pmolxmmol/l ²)	3.08 [2.38]	0.67 [0.46]	0.95 [1.16]	0.60 [0.50]	0.58 [0.40]
Corrected insulin response (mUxl ² /mmol ²)	-	-	149 [127]	157.7 [163.3]	112.2 [124.6]
Insulin sensitivity index (l/(mUxmmol))	-	-	165 [170]	144.9 [111.9]	147.6 [111.3]
Disposition index (l ³ /mmol ³)	-	-	23,393 [27,302]	22,535 [25,855]	15,717 [18,334]
Follow-up time	-	-	24.8 [4.7]	-	7.6 [5.2]
<i>KCNQ1</i> rs2237895 RAF	0.44	0.40	0.41	0.49	0.47

Data are mean ± SD or median [IQR], RAF – risk allele frequency. Baseline characteristics are shown for Malmö Preventive Project and Botnia Prospective Study.

Table 2. Metabolic effects of *KCNQ1* rs2237895 in non-diabetic individuals from the studied populations

	N	AA	Genotype		Additive model		
			AC	CC	Beta	SE	p-value
Malmö Preventive Project							
Baseline							
BMI	12,326	24.0 ± 3.1	24.0 ± 3.1	24.0 ± 3.0	-0.035	0.040	0.37
Fasting plasma glucose (mmol/l)	12,328	5.41 ± 0.55	5.42 ± 0.54	5.42 ± 0.55	0.015	0.007	0.033
2hr plasma glucose (mmol/l)	6,718	6.28 ± 1.58	6.36 ± 1.65	6.36 ± 1.67	0.050	0.026	0.052
Follow-up							
BMI	12,271	26.9 ± 3.9	26.8 ± 3.9	26.8 ± 3.9	0.023	0.051	0.65
Fasting plasma glucose (mmol/l)	12,327	5.44 ± 0.54	5.46 ± 0.55	5.50 ± 0.55	0.033	0.007	1.2x10⁻⁶
PPP-Botnia Study							
BMI	2,991	26.1 ± 4.0	26.2 ± 4.2	26.1 ± 4.3	0.033	0.102	0.75
Fasting plasma glucose (mmol/l)	2,994	5.13 ± 0.53	5.17 ± 0.56	5.20 ± 0.56	0.038	0.014	0.0067
2hr plasma glucose (mmol/l)	2,976	5.08 ± 1.53	5.17 ± 1.56	5.23 ± 1.65	0.074	0.038	0.053
Insulin sensitivity (ISI, l/mUxmmol)	2,838	145 [109]	144 [111]	144 [112]	0.001	0.013	0.95
Insulin resistance (HOMA-IR, pmolxmmol/l ²)	2,939	0.60 [0.50]	0.60 [0.50]	0.60 [0.50]	0.001	0.014	0.93
Insulin secretion (CIR, mUxI ² /mmol ²)	2,849	166 [180]	155 [158]	157 [161]	-0.048	0.019	0.013
Insulin secretion (CIR adjusted for ISI)					-0.051	0.019	0.0073
Disposition index (DI, I ³ /mmol ³)	2,814	23,464 [27,419]	22,366 [25,013]	21,866 [24,716]	-0.052	0.021	0.013
Botnia Prospective Study							
Baseline							
BMI	2,123	25.8 ± 3.9	25.4 ± 3.9	25.7 ± 3.9	-0.065	0.119	0.59
Fasting plasma glucose (mmol/l)	2,128	5.59 ± 0.58	5.49 ± 0.56	5.53 ± 0.58	-0.029	0.022	0.19
2hr plasma glucose (mmol/l)	2,128	6.10 ± 1.45	6.08 ± 1.45	6.27 ± 1.50	0.072	0.048	0.13
Insulin sensitivity (ISI, l/mUxmmol)	2,128	144 [106]	154 [114]	146 [109]	0.014	0.018	0.42
Insulin resistance (HOMA-IR, pmolxmmol/l ²)	2,128	0.61 [0.42]	0.57 [0.37]	0.58 [0.43]	-0.015	0.016	0.34
Insulin secretion (CIR, mUxI ² /mmol ²)	2,128	118 [131]	115 [126]	96 [117]	-0.089	0.025	3.6x10⁻⁴
Insulin secretion (CIR adjusted for ISI)					-0.083	0.024	7.2x10⁻⁴
Disposition index (DI, I ³ /mmol ³)	2,128	15,934 [19,260]	16,663 [19,049]	14,102 [16,413]	-0.075	0.027	0.0058
Follow-up							
BMI	2,068	26.8 ± 4.2	26.4 ± 4.0	26.8 ± 4.3	-0.051	0.136	0.71
Fasting plasma glucose (mmol/l)	2,128	5.29 ± 0.57	5.30 ± 0.55	5.33 ± 0.60	0.032	0.018	0.077
2hr plasma glucose (mmol/l)	2,128	5.77 ± 1.76	5.85 ± 1.67	6.00 ± 1.77	0.127	0.050	0.011

Insulin sensitivity (ISI, l/mUxmmol)	2,127	101 [94]	102 [85]	96 [93]	-0.003	0.019	0.89
Insulin resistance (HOMA-IR, pmolxmmol/l ²)	2,125	0.97 [0.87]	0.93 [0.76]	0.96 [0.90]	-0.010	0.018	0.58
Insulin secretion (CIR, mUxl ² /mmol ²)	2,128	160 [184]	142 [160]	130 [140]	-0.086	0.027	0.0018
Insulin secretion (CIR adjusted for ISI)					-0.087	0.027	0.0013
Disposition index (DI, l ³ /mmol ³)	2,127	15,563 [21,424]	14,322 [18,304]	13,299 [17,404]	-0.089	0.030	0.0030

Meta analysis

BMI							0.41
Fasting plasma glucose (mmol/l)							0.0078
2hr plasma glucose (mmol/l)							0.0021
Insulin sensitivity (ISI, l/mUxmmol)							0.58
Insulin resistance (HOMA-IR, pmolxmmol/l ²)							0.57
Insulin secretion (CIR, mUxl ² /mmol ²)							3.0x10⁻⁵
Insulin secretion (CIR adjusted for ISI)							2.7x10⁻⁵
Disposition index (DI, l ³ /mmol ³)							2.5x10⁻⁴

Beta (SE) from linear regression analysis adjusted for age, gender, BMI and within pedigree dependence (Botnia Prospective Study) denotes the effect size by each C allele (additive model) on phenotype. Meta analysis includes the PPP-Botnia Study and the Botnia Prospective Study at baseline.

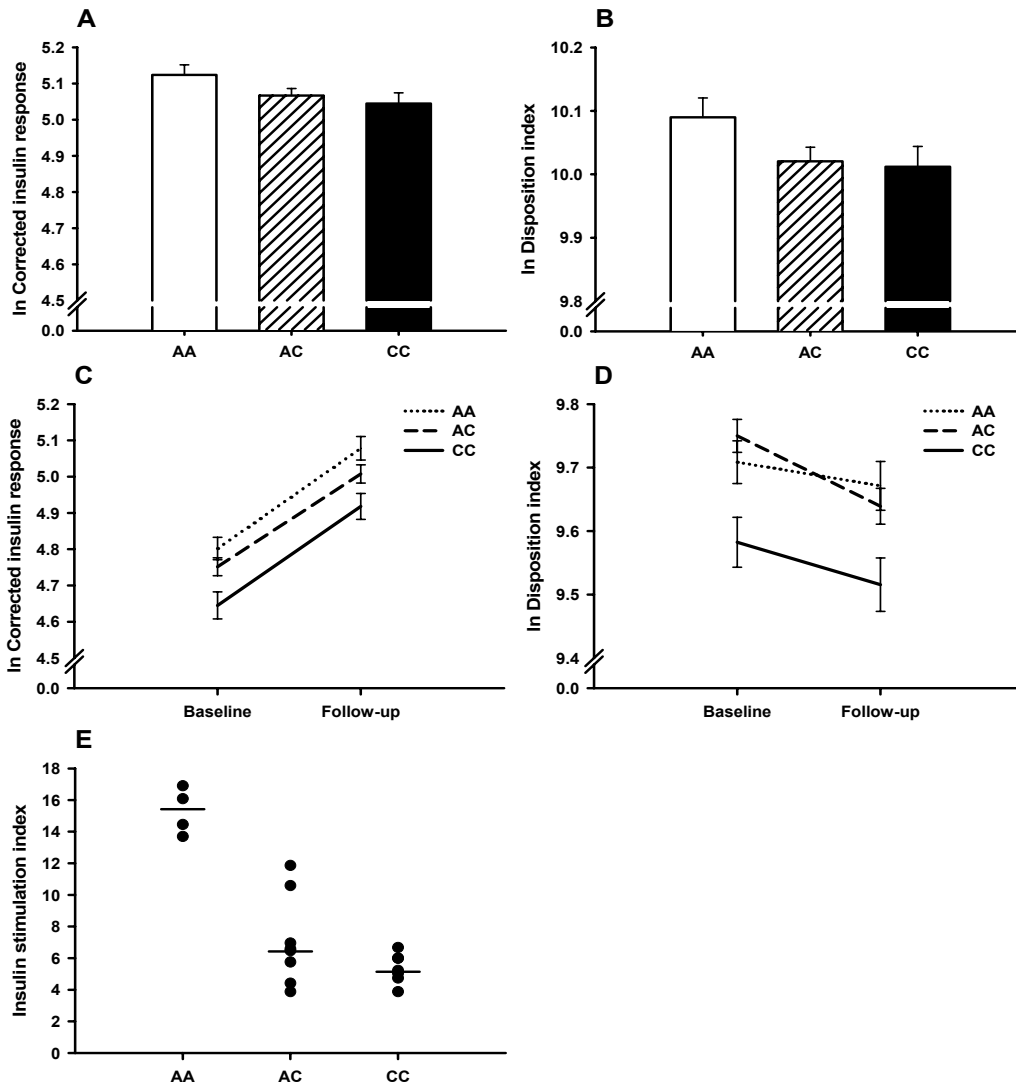


Figure 1. Effect of *KCNQ1* rs2237895 on beta-cell function.

Data are represented as the mean of the unadjusted logarithmic (natural) values for corrected insulin response and disposition index. Error bars denote standard error of the mean.

Panel A shows decline in corrected insulin response (CIR) with each C-allele ($p=0.013$) in the PPP-Botnia study.

Panel B shows decrease in disposition index (DI) with each C-allele ($p=0.013$) in the PPP-Botnia study.

Panel C shows CIR in different genotype carriers at baseline and at follow-up in the Botnia prospective study. The C-allele carriers had a lower CIR at baseline ($p=3.6 \times 10^{-4}$) which maintained lower at follow-up ($p=0.0018$).

Panel D shows DI in different genotype carriers at baseline and at follow-up in the Botnia prospective study. The C-allele carriers had lower DI at baseline ($p=0.0058$) which maintained lower at follow-up ($p=0.0030$).

Panel E shows glucose-stimulated insulin secretion as fold change in insulin release at high (20 mM) compared to low (1 mM) glucose concentration from human islets in different genotype carriers. C-allele carriers showed lower glucose-stimulated insulin secretion ($p=2.5 \times 10^{-6}$).