Gene variants in the novel type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A and MTNR1B affect different aspects of pancreatic beta cell function.

Running title: Genes and insulin release during hyperglycemic clamps

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Objective - Recently results from a meta-analysis of genome wide association studies have yielded a number of novel type 2 diabetes loci. However, conflicting results have been published regarding their effects on insulin secretion and insulin sensitivity. In this study we used hyperglycemic clamps with three different stimuli to test associations between these novel loci and various measures of beta cell function.

Research design and methods - 336 participants, 180 normal glucose tolerant and 156 impaired glucose tolerant, underwent a two hour hyperglycemic clamp. In a subset we also assessed the response to GLP-1 and arginine during an extended clamp (n=123). All subjects were genotyped for gene variants in JAZF1, CDC123/CAMK1D, TSPAN8/LGR5, THADA, ADAMTS9, NOTCH2/ADAMS30, DCD, VEGFA, BCL11A, HNF1B, WFS1 and MTNR1B.

Results - Gene variants in CDC123/CAMK1D, ADAMTS9, BCL11A and MTNR1B affected various aspects of the insulin response to glucose (all p<6.9*10^-3). The THADA gene variant was associated with lower beta cell response to GLP-1 and arginine (both p<1.6*10^-3) suggesting lower beta cell mass as a possible pathogenic mechanism. Remarkably we also noted a trend towards an increased insulin response to GLP-1 in carriers of MTNR1B (p=0.03) which may offer new therapeutic possibilities. The other seven loci were not detectably associated with beta cell function.

Conclusions - Diabetes risk alleles in CDC123/CAMK1D, THADA, ADAMTS9, BCL11A and MTNR1B are associated with various specific aspects of beta cell function. These findings point to a clear diversity in the impact that these different gene variants may have on (dys-)function of pancreatic beta cells.
Genome wide association studies (GWAS) have revealed a large number of novel type 2 diabetes susceptibility loci (1-4). Most of the genes identified during the first wave of GWAS results are shown to affect beta cell function as indicated by lower insulin responses to oral (OGTT) or intravenous (IVGTT) glucose tolerance tests (5). By applying the hyperglycemic clamp methodology, considered the gold standard for measurements of beta cell function, we further refined the observed beta cell defects to defects in 1st but not 2nd phase glucose stimulated insulin secretion (GSIS) (6) or incretin stimulated secretion (7). This differentiation is of importance to help resolve the pathogenic mechanism of the diabetes loci identified by GWAS.

More recently the DIAGRAM consortium published at least six additional susceptibility loci, JAZF1, CDC123/CAMK1D, TSPAN8/LGR5, THADA, ADAMTS9, NOTCH2/ADAMS30, DCD, VEGFA, BCL11A, and NOTCH2/ADAMS30. Studies using OGTT’s have yielded conflicting results on the effects of these new loci on beta cell function and insulin sensitivity. Grarup et al. (9) reported beta cell dysfunction associated with gene variants in JAZF1, TSPAN8/LGR5, and CDC123/CAMK1D. The results for CDC123/CAMK1D have only been replicated by Sanghera et al in Asian Indians (10) but not by three other studies in Caucasians. All of the other three studies also failed to replicate the results for JAZF1 and TSPAN8/LGR5 (11-13). Furthermore gene variants in three other loci have been established as true type 2 diabetes susceptibility loci, HNF1B, WFS1, and MTNR1B (14-19). Although mutations in HNF1B are associated with beta cell defects in MODY it is unknown whether the type 2 diabetes associated common SNP is also associated with reduced beta cell function (14,15). It has been shown that WFS1 associates with reduced oral (11,13,20-22) but not intravenous glucose stimulated insulin secretion (22). Schäfer et al. (22) further demonstrated that the WFS1 gene affects GLP-1 stimulated insulin secretion during clamps. For the MTNR1B locus several studies have shown reduced insulin secretion in response to glucose (17-19,23,24).

In this study 180 normal and 156 impaired glucose tolerant (IGT) subjects originating from three independent studies in the Netherlands were genotyped for variants in JAZF1, CDC123/CAMK1D, TSPAN8/LGR5, THADA, ADAMTS9, NOTCH2/ADAMS30, DCD, VEGFA, BCL11A, HNF1B, WFS1, and MTNR1B. We tested whether these loci are associated with alterations in beta cell function as assessed by hyperglycemic clamp methodology with, in a subset, two additional secretagogues, namely GLP-1 and arginine. Arginine stimulation during hyperglycemia is a test of (near) maximal insulin secretion and has been proposed as a proxy for beta cell mass (25).

**RESEARCH DESIGN AND METHODS**

**Hypercglycaemic clamp cohorts.** Participants originated from three independent studies in the Netherlands (26-30). The clinical characteristics of the study sample are given in table 1. In short we recruited for this study 137 IGT subjects from the Hoorn study (26,29); 76 subjects (64 NGT/12 IGT) from Utrecht (27,28) and 123 twins and sibs (116 NGT/7 IGT) from the Netherlands Twin Register (NTR) (30). The NTR twin sample includes 66 monozygotic, 28 dizygotic twins and 29 of their non-twin sibs recruited from 50 families. Details of the three individual samples have previously been described (6,26-30).

**Hypercglycemic clamp procedure.** All participants underwent a hyperglycemic...
clamp at 10 mmol/l glucose for at least two hours (26,28-30). First phase insulin secretion was determined as the sum of the insulin levels during the first 10 minutes of the clamp. Second phase insulin secretion was determined as the mean of the insulin levels during the last 40 minutes of the second hour of the clamp (80-120 min). The insulin sensitivity index (ISI) was defined as the glucose infusion rate (M, µmol/min/kg) necessary to maintain the hyperglycemic clamp divided by the plasma insulin concentration (I, pmol/l) during the last 40 min of the second hour of the clamp (M/I). Mitrakou et al compared the insulin sensitivity index (ISI) determined with a hyperglycemic clamp with insulin sensitivity as determined using the euglycemic hyperinsulinemic clamp in the same subjects, and found a good agreement between the two methods (31). The disposition index (DI) was calculated by multiplication of first phase insulin secretion and ISI, in order to quantify insulin secretion in relation to the ambient insulin sensitivity (32,33).

Subjects from the NTR twin sample underwent a modification of the extended clamp using additional GLP-1 and arginine stimulation as described previously by Fritsche et al. (25). GLP-1 stimulated insulin release was measured as the mean incremental area under the curve (160 to 180 min) following GLP-1 stimulation (1.5 pmol kg\(^{-1}\) bolus for 1 min at t=120 followed by a continuous infusion of 0.5 pmol kg\(^{-1}\) min\(^{-1}\)). Arginine stimulated acute insulin release was measured by injecting a bolus of 5 grams arginine hydrochloride at t=180 as described previously (25). The acute insulin response to arginine was calculated as the mean incremental area under the curve from 182 to 185 min. 

**Genotyping.** Based on the available literature regarding the novel type 2 diabetes genes we selected gene variants in JAZF1 (rs864745), CDC123/CAMK1D (rs12779790), TSPAN8/LGR5 (rs7961581), THADA (rs7578597), ADAMTS9 (rs4607103), NOTCH2/ADAM30 (rs2641348) (8), the putative type 2 diabetes genes DCD (rs1153188), VEGFA (rs9472138) and BCL11A (rs10490072) (8), HNF1B (rs757210) (14,15), WFS1 (rs10010131) (16) and MTNR1B (rs10830963) (17-19). All SNPs were measured using either the Sequenom platform (Sequenom, San Diego, USA) or Taqman SNP genotyping assays (Applied Biosystems, Foster City, USA) in all individual subjects. The genotyping success rate was above 96% for all SNPs and samples measured in duplicate (~5%) were in complete concordance. All genotype distributions obeyed Hardy Weinberg equilibrium (p≥0.05) except for MTNR1B (p=0.01). SNP genotypes were recoded as 0, 1 or 2 with the 2 genotype as the at risk genotype reported in the original publications. **Statistics.** The effect of the gene variants on the beta cell responses was examined with linear regression assuming an additive model unless otherwise stated. To take into account the family relatedness (i.e. in the twin sample) empirical standard errors were used (using the generalized estimating equations (GEE)). The analyses of 1\(^{st}\) and 2\(^{nd}\) phase GSIS, GLP-1 and Arginine stimulated insulin secretion were adjusted for age, gender, BMI, study center, glucose tolerance status (NGT/IGT) and ISI. For the analysis of ISI and DI, ISI was removed from the covariates. All outcome variables were log-transformed prior to analysis. In addition to the analysis of the pooled data we also performed a random effects meta-analysis of the results obtained in the three separate cohorts using Comprehensive Meta-Analysis v2 software (www. Meta-analysis.com). A priori power calculations showed that the design used in this study would allow the detection of a difference in insulin secretion of approximately 15% (glucose) to 30% (GLP-1, Arginine) with 80% power (\(\alpha<0.05\))
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depending on the stimulus used and allele frequency of the SNPs. All data are given as estimated mean (95%-CI) unless otherwise stated. After correction for multiple hypothesis testing results were regarded significant at \( p \leq 0.008 \) (six tests).

Apart from the meta-analysis SPSS version 16.0 software (SPSS, Chicago, IL, USA) was used for all statistical analyses.

RESULTS
As previously shown second phase insulin secretion as measured with the hyperglycemic clamp was only slightly reduced in the subjects with IGT \((p>0.1)\) whereas all other measures of glucose stimulated insulin release and ISI were significantly lower \((\text{all } p<0.0001, \text{table 1})\)(28). Genotype distributions for each of the tested gene variants are given in table 2. Genotype distributions were comparable to other Caucasian populations.

First, no associations were found with insulin sensitivity with the sole exception of \(\text{THADA}\), where we noted a significantly lower insulin sensitivity index \((p=6.9\times10^{-3})\) in carriers of the T risk allele. Five loci, however, significantly affected beta cell function. These associations are shown in table 2 and will be briefly summarized below. Throughout, reported \( p \)-values represent the values obtained for the full model which includes the genotype of interest and age, gender, BMI, glucose tolerance status, family relatedness and insulin sensitivity (where appropriate) as covariates. A model without BMI yielded essentially the same results (data not shown).

A meta-analysis of the results in the three separate study samples instead of the analysis of the pooled data yielded virtually identical results (data not shown).

\(\text{THADA}\). Since the protective C/C genotype of the rs7578597 SNP is only present in three subjects we pooled the CC and CT genotype groups. The TT risk genotype was not significantly associated with 1\textsuperscript{st} phase GSIS \((p=0.77)\) but all other measures of beta cell function were reduced \((11 \text{ to } 37\%)\), though not always statistically significant; 2\textsuperscript{nd} phase insulin response \((p=0.019)\), disposition index \((p=0.039)\), GLP-1 \((p=1.6\times10^{-3})\) and arginine stimulated insulin response \(2.3\times10^{-4}\), table 2). As stated above we also noted a significantly lower insulin sensitivity index (ISI, \(p=6.9\times10^{-3}\)) in carriers of the at risk genotype.

\(\text{ADAMTS9}\). Analysis of rs4607103 in \(\text{ADAMTS9}\) provided evidence for an effect on 1\textsuperscript{st} phase GSIS. Carriers of the type 2 diabetes risk genotype ‘CC’ showed paradoxically a 40\% increased 1\textsuperscript{st} phase GSIS compared to the non-risk ‘TT’ reference genotype \((p=5.9\times10^{-3})\). This effect was similar in direction in both NGT and IGT subjects (Table 3). Furthermore, the risk allele carriers also showed a higher disposition index \((p=2.6\times10^{-3})\). Second phase GSIS, the response to GLP-1 or arginine and ISI were not significantly affected by the \(\text{ADAMTS9}\) genotype.

\(\text{BCL11A}\). Carriers of the rs10490072 ‘TT’ risk genotype of the \(\text{BCL11A}\) locus had on average a 16\% lower 1\textsuperscript{st} phase GSIS \((p=3.1\times10^{-3})\). The disposition index was also lower though not statistically significant \((p=0.010)\). Other measures of beta cell function and ISI were not significantly different (table 2).

\(\text{MTNR1B}\). The risk allele for \(\text{MTNR1B}\) was significantly associated with a decreased disposition index \((p=1.5\times10^{-3})\) but not other measures of glucose stimulated insulin
secretion. Although not statistically significant there were increased responses to GLP-1 (+30%, p=0.026) and arginine stimulation (+19%, p=0.037) in carriers of the risk allele for rs10830963.

**Other novel type 2 diabetes loci.** Gene variants in the JAZF1, TSPAN8/LGR5, DCD, NOTCH2/ADAM30, VEGFA, loci were not significantly associated with any of the beta cell measures or insulin sensitivity (Table 2).

**DISCUSSION**

The DIAGRAM consortium and others recently showed that JAZF1, CDC123/CAMK1D, TSPAN8/LGR5, THADA, ADAMTS9, NOTCH2/ADAM30, HNF1B, WFS1, MTNR1B and possibly also DCD, VEGFA, BCL11A should be added to the list of confirmed type 2 diabetes loci (8,14-19). In this study we have shown that gene variants in five of these loci are associated with measures of beta cell function obtained during hyperglycemic clamps, either in response to glucose alone and/or in combination with other beta cell secretagogues during hyperglycemia. In contrast to our previous work, which showed that most other known loci primarily affect 1st phase GSIS (6,7) (‘t Hart et al. unpublished results), the current set of loci also affected various other aspects of beta cell function.

**CDC123/CAMK1D, rs12779790.** Previously Grarup et al reported that the G risk allele of rs12779790 CDC123/CAMK1D was associated with a lower insulinogenic index, corrected insulin response (CIR) and area under the insulin/glucose curve during OGTTs (9). They also noted a lower disposition index in carriers of the G allele. The beta cell defect was confirmed in a study of subjects from Asian Indian origin (10). Three other studies in Caucasian whites failed to replicate the observation made by Grarup et al. However, in all three studies a similar, though not significant trend towards lower beta cell function could be observed (11-13). These results are in line with our observation of a lower insulin response to glucose stimulation. We also noted a trend towards a reduced insulin response after arginine stimulation (-32%, p=0.015). Arginine stimulation during hyperglycemia is a measure of (near) maximal insulin secretion and has been suggested as a proxy for beta cell mass. Given the putative role of CAMK1D in granulocyte function it seems plausible that this gene variant affects beta cell function by causing reduced beta cell mass due to enhanced apoptosis (34). Further research is, however, needed to verify this hypothesis.

**THADA, rs7578597.** We have shown that homozygous carriers of the risk allele have lower levels of various measures of beta cell function. This was not previously reported in any of the OGTT based studies although Stancakova et al showed some evidence for a reduced early phase insulin response (p=0.045) (13). THADA, encoding Thyroid Adenoma Associated protein, has been suggested to be involved in the death receptor pathway and apoptosis (35). Given the fact that the gene variant is associated with reduced response to arginine stimulation during the clamp this could imply that those subjects with the rs7578597 (T1187A) gene variant in THADA have a reduced beta cell mass due to increased apoptosis. Again further studies are needed to confirm our hypothesis of increased apoptosis and lower beta cell mass as the underlying disease mechanism. The THADA variant was the only variant associated with insulin sensitivity; this was however not corroborated by any of the other studies and may thus be a false positive association.

**ADAMTS9, rs4607103.** Remarkably we noted a significantly increased 1st phase GSIS and disposition index in carriers of the risk allele. The observed increased beta cell function was present in all separate samples and in NGT and IGT subjects when analyzed
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separately, arguing against a chance finding. Also Lyssenko et al. reported an increased DI during follow-up in carriers of the risk genotype (11). The other studies, however, did not report any changes in beta cell function or insulin sensitivity (9,10,12,13). Given these counterintuitive results and the unknown function of ADAMTS9 in type 2 diabetes susceptibility and / or beta cell function our data warrant further replication and studies into the disease mechanism.

**BCL11A, rs10490072.** For carriers of the risk allele in BCL11A we noted a significant reduction in 1st phase GSIS. Only Staiger et al. included BCL11A in their analyses and they did not corroborate our results (12). BCL11A, encoding B-cell CLL/lymphoma 11A, has been implicated in several blood related phenotypes and acts as a DNA-sequence specific transcriptional repressor, acting on genes like BCL6, COUP-TF and SIRT1 (36). Sirtuins, like SIRT1 have been implicated in several processes directly linked to type 2 diabetes (37) and one may speculate that BCL11A gene variants exert their effect via the regulation of SIRT1 expression.

**MTNR1B, rs10830963.** Recently the Melatonin receptor 1B gene has been identified as a novel type 2 diabetes and fasting plasma glucose gene (17-19). Also in this study the risk allele was associated with increased fasting plasma glucose levels (p=0.004). Several studies have shown that gene variants in this locus are associated with lower oral and intravenous glucose stimulated insulin secretion (38). Our results regarding the lower disposition index seem to corroborate these previous findings. Though not formally statistically significant due to the smaller sample size we, surprisingly, also noted increased insulin responses towards GLP-1 (+30%) and arginine stimulation (+19%). This seems to contradict the observed decreased insulin response to oral glucose during OGTT in MTNR1B carriers since it is known that the insulin response to oral glucose is in part mediated via the positive effects of incretins, like GLP-1 (39). In vitro short term exposure of beta cells and islets to melatonin results in a decreased insulin response to glucose and GLP-1 (38) but studies using INS-1E cells have also suggested that prolonged exposure to melatonin, in contrast to short term exposure, results in a potentiation of the response to GLP-1 (40). If replicated our results indicate that carriers of this gene variant may well benefit from treatment with GLP-1 agonists or DPP-IV inhibitors.

**WFS1** Previously it has been reported that WFS1 gene variants are associated with reduced insulin response to oral but not intravenous glucose (11,13,20-22). In line with those previous reports we also could not detect an effect of intravenous glucose. Furthermore, Schäfer et al demonstrated a reduced response to GLP-1 stimulation during hyperglycemic clamps (22). In this study with similar size and power we were unable to confirm this observation. Our data do not confirm previously reported beta cell defects in JAZF1 and TSPAN8 (9) which is in line with the other reports based on OGTTs (10-13).

One of the main limitations of the current study is the relatively small number of participants. Although this is the largest study applying the gold standard method for assessing beta cell function, the hyperglycemic clamp, we cannot exclude that we have missed subtle defects associated with the different gene variants especially given the fact that their effects on type 2 diabetes risk are also small. Furthermore we have applied a rather lenient correction for multiple hypotheses testing which means that some of the current findings may be spurious. Our results should therefore be regarded exploratory and we fully subscribe the need for replication but such replication is non-trivial because the hyperglycemic clamp methodology is demanding for both
researchers and participants. However, our current results clearly justify these investments.

A further limitation is the inclusion of a mix of normal and impaired glucose tolerant subjects. It is well known that subjects with IGT often have insulin resistance and/or insufficient beta cell function to maintain normal glucose homeostasis and are thus at high risk to develop type 2 diabetes. One may argue that the observed associations with decreased beta cell function are thus due to the known association with type 2 diabetes and the risk implied by the IGT state. However, our data analyzing separately NGT and IGT subjects showed that the direction of the effects for the gene variants we found associated was in general similar in both groups and not mainly driven by the IGT subjects arguing against this potential bias. Furthermore, we used a random effects meta-analysis approach to test whether the relationship between the genes and the outcome variables is homogeneous over the three cohorts. Also, this analysis yielded virtually identical results providing further evidence that our data are not influenced by the inclusion of the IGT subjects. However, although the associations we found are resistant to the above described analyses and present in both NGT and IGT subjects we cannot exclude that for other genes/loci this would not be the case.

In conclusion, we found novel associations between gene variants in \textit{THADA, ADAMTS9} \textit{and BCL11A} loci and various aspects of beta cell function. In carriers of the \textit{THADA} variant we observed decreases in both GLP-1 and arginine induced insulin release hinting at lower beta cell function and/or mass. Carriers of gene variants in \textit{ADAMTS9} and \textit{BCL11A} show alterations in 1st phase GSIS suggesting they may primarily affect processes involved in the rapid recruitment and release of insulin from insulin granules.

In addition to the above mentioned associations, we have confirmed that a gene variant in \textit{CDC123/CAMKID} is associated with reduced beta function and our data suggests it may do so via a reduced beta cell mass. Furthermore, our data suggest that carriers of the \textit{MTNR1B} risk allele may be more sensitive towards the stimulatory effects of GLP-1 which may offer therapeutic possibilities if confirmed.

These findings point to a clear diversity in the impact that these different gene variants may have on (dys)function of pancreatic beta cells and justify the use of the hyperglycemic clamp methodology, especially with additional secretagogues, to resolve the pathogenic mechanisms of these loci.

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|                      | Hoorn* | Utrecht* | NTR Twins* |
|----------------------|--------|----------|------------|
|                      | IGT    | NGT      | IGT        | NGT      | IGT     |
| N                    | 137    | 64       | 12         | 116      | 7       |
| Gender (M/F)         | 64/73  | 15/49    | 4/8        | 58/58    | 0/7     |
| Age (y)              | 60.5 ± 8.6 | 45.9 ± 6.4 | 49.5 ± 7.7 | 31.5 ± 6.5 | 31.2 ± 3.2 |
| BMI (kg/m²)          | 28.1 ± 4.0 | 25.8 ± 3.8 | 26.7 ± 4.1 | 24.2 ± 3.5 | 24.5 ± 3.3 |
| Fasting plasma glucose (mmol/l) | 6.3 ± 0.7 | 4.6 ± 0.4 | 5.1 ± 0.4 | 4.6 ± 0.4 | 4.6 ± 0.6 |
| 2-hr plasma glucose (mmol/l) | 8.8 ± 1.7 | 5.1 ± 1.0 | 8.5 ± 1.2 | 5.2 ± 1.1 | 8.1 ± 0.3 |
| Fasting plasma insulin (pmol/l) | 62 (46-91) | 30 (24-42) | 66 (42-78) | 34 (27-51) | 39 (29-60) |
| First-phase insulin response (pmol/l) | 587 (378-895) | 885 (644-1217) | 678 (461-909) | 814 (589-1162) | 795 (693-1210) |
| Second-phase insulin response (pmol/l) | 255 (176-354) | 260 (191-365) | 251 (186-307) | 218 (162-358) | 217 (210-434) |
| Insulin sensitivity index (µmol/min/kg/pmol/l) | 0.108 (0.068-0.164) | 0.190 (0.127-0.282) | 0.111 (0.082-0.256) | 0.227 (0.152-0.323) | 0.123 (0.109-0.183) |
| Disposition index (µmol/min/kg) | 65 (42-92) | 172 (103-238) | 72 (55-128) | 180 (140-234) | 138 (82-151) |
| GLP-1 stimulated insulin release (pmol/l) | n.a. | n.a. | n.a. | 1225 (734-2587) | 848 (577-1239) |
| Arginine stimulated insulin release (pmol/l) | n.a. | n.a. | n.a. | 2188 (1526-2973) | 1673 (1438-1908) |

Data are represented as means ± SD or median (interquartile range). N.a. not available.

* Original population from which the cohort originated (26,28-30)
| Gene                  | n   | First-phase insulin response (pmol/l) | Second-phase insulin response (pmol/l) | Insulin sensitivity index (µmol/min/kg/pmol/l) | Disposition index (µmol/min/kg) | n (GLP-1, Arg) | GLP-1 stimulated insulin release* (pmol/l) | Arginine stimulated insulin release* (pmol/l) |
|-----------------------|-----|--------------------------------------|----------------------------------------|-----------------------------------------------|-------------------------------|---------------|------------------------------------|-----------------------------------------------|
| **JAZF1, rs864745**   |     |                                       |                                        |                                               |                               |               |                                     |                                               |
| C/C                   | 73  | 727 (652-812)                        | 262 (236-292)                         | 0.141 (0.122-0.162)                           | 107 (95-121)                  | 26            | 1034 (799-1337)                     | 1728 (1495-1998)                              |
| C/T                   | 161 | 723 (672-778)                        | 239 (223-255)                         | 0.155 (0.142-0.170)                           | 111 (103-120)                 | 48            | 1374 (1122-1683)                    | 1992 (1727-2297)                              |
| T/T                   | 100 | 759 (686-841)                        | 263 (243-286)                         | 0.160 (0.145-0.177)                           | 124 (111-139)                 | 49            | 1200 (951-1514)                     | 2233 (1969-2532)                              |
| P                     | 0.54| 0.80                                 | 0.15                                  | 0.07                                          | 0.63                          | 0.018         |                                     |                                               |
| **CDC123/CAMKID, rs12779790** |     |                                       |                                        |                                               |                               |               |                                     |                                               |
| A/A                   | 212 | 755 (704-810)                        | 260 (245-275)                         | 0.155 (0.143-0.168)                           | 117 (109-127)                 | 74            | 1318 (1094-1588)                    | 2181 (1979-2403)                              |
| A/G                   | 110 | 713 (656-774)                        | 238 (220-258)                         | 0.153 (0.138-0.169)                           | 112 (101-123)                 | 48            | 1106 (881-1389)                     | 1817 (1588-2078)                              |
| G/G                   | 12  | 617 (478-797)                        | 200 (176-228)                         | 0.146 (0.108-0.198)                           | 94 (71-125)                   | 1             | 1142 (913-1428)                     | 1486 (1322-1671)                              |
| P                     | 0.10| 0.0049                               | 0.68                                  | 0.16                                          | 0.24                          | 0.015         |                                     |                                               |
| **TSPAN8/LGR5, rs7961581** |     |                                       |                                        |                                               |                               |               |                                     |                                               |
| T/T                   | 159 | 738 (687-793)                        | 253 (237-270)                         | 0.149 (0.135-0.164)                           | 113 (103-123)                 | 47            | 1253 (1028-1529)                    | 2094 (1860-2357)                              |
| T/C                   | 141 | 724 (668-784)                        | 247 (229-265)                         | 0.158 (0.142-0.175)                           | 113 (105-123)                 | 65            | 1222 (994-1503)                     | 2024 (1797-2280)                              |
| C/C                   | 34  | 738 (613-889)                        | 254 (219-295)                         | 0.160 (0.135-0.190)                           | 118 (97-142)                  | 11            | 1148 (796-1657)                     | 1710 (1362-2146)                              |
| P                     | 0.88| 0.84                                 | 0.34                                  | 0.72                                          | 0.73                          | 0.24          |                                     |                                               |
| **THADA, rs7578597**   |     |                                       |                                        |                                               |                               |               |                                     |                                               |
| C/C                   | 3   | 905 (484-1694)                       | 365 (317-421)                         | 0.125 (0.067-0.230)                           | 121 (80-182)                  | 0             | n.a.                               | n.a.                                          |
| C/T                   | 72  | 739 (662-825)                        | 271 (247-296)                         | 0.180 (0.160-0.204)                           | 127 (113-142)                 | 25            | 1783 (1352-2352)                    | 2605 (2236-3035)                              |
| T/T                   | 261 | 732 (689-778)                        | 244 (232-257)                         | 0.147 (0.137-0.158)                           | 110 (103-118)                 | 98            | 1120 (970-1292)                     | 1897 (1744-2064)                              |
| P                     | 0.77†| 0.019†                               | 0.0069†                               | 0.039†                                        | 0.0016†                       | 0.00023†      |                                     |                                               |
| **ADAMTS9, rs4607103** |     |                                       |                                        |                                               |                               |               |                                     |                                               |
| T/T                   | 20  | 549 (467-646)                        | 206 (172-246)                         | 0.136 (0.106-0.175)                           | 83 (69-99)                    | 7             | 777 (597-1011)                      | 1632 (1335-1994)                              |
| T/C                   | 119 | 725 (668-787)                        | 256 (238-274)                         | 0.152 (0.137-0.169)                           | 111 (101-123)                 | 47            | 1291 (1028-1621)                    | 1990 (1753-2260)                              |
| C/C                   | 187 | 767 (714-824)                        | 252 (237-268)                         | 0.157 (0.145-0.171)                           | 121 (112-130)                 | 69            | 1244 (1032-1498)                    | 2094 (1866-2350)                              |

\*p = 0.05
†p = 0.01
‡p = 0.001
Genes and insulin release during hyperglycemic clamps

| Gene          | SNP        | Genotype | Frequency (95% CI) | P     |
|--------------|-----------|----------|-------------------|------|
| NOTCH2/ADAM30 | rs2641348 | A/A      | 253 (692-782)     | 0.0059 |
|              |           | A/G      | 73 (661-841)      | 0.26 |
|              |           | G/G      | 10 (502-852)      | 0.32 |
|              |           |          | 0.18 (0.14-0.16)  | 0.0026 |
|              |           |          | 1223 (1100-1593)  | 0.38 |
| DCD          | rs1153188 | T/T      | 24 (670-982)      | 0.76 |
|              |           | T/A      | 120 (675-781)     | 0.33 |
|              |           | A/A      | 192 (678-790)     | 0.37 |
|              |           |          | 1336 (1151-1551)  | 0.89 |
| VEGFA        | rs9472138 | C/C      | 176 (674-774)     | 0.55 |
|              |           | C/T      | 131 (704-832)     | 0.29 |
|              |           | T/T      | 28 (578-835)      | 0.49 |
|              |           |          | 1096 (556-2161)   | 0.48 |
| BCL11A       | rs10490072| C/C      | 32 (703-934)      | 0.77 |
|              |           | C/T      | 126 (738-866)     | 0.80 |
|              |           | T/T      | 178 (637-737)     | 0.44 |
|              |           |          | 1311 (1139-1508)  | 0.55 |
| HNF1B        | rs757210  | C/C      | 118 (696-799)     | 0.0031 |
|              |           | C/T      | 145 (672-809)     | 0.39 |
|              |           | T/T      | 71 (634-782)      | 0.92 |
|              |           |          | 1174 (874-1577)   | 0.010 |
| WFS1         | rs10010131| C/C      | 118 (696-799)     | 0.38 |
|              |           | C/T      | 145 (672-809)     | 0.33 |
|              |           | T/T      | 71 (634-782)      | 0.35 |

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| Allele | N | Mean (95% CI) | Treated Mean (95% CI) | 1st Phase | Treated Mean (95% CI) | 2nd Phase | N | Mean (95% CI) | Treated Mean (95% CI) | 1st Phase | Treated Mean (95% CI) | 2nd Phase | P | 1st Phase | 2nd Phase | 3rd Phase | 4th Phase |
|--------|---|----------------|----------------------|-----------|----------------------|----------|---|----------------|----------------------|-----------|----------------------|----------|---|-----------|-----------|-----------|-----------|
| A/A    | 39 | 623 (527-737) | 258 (217-306) | 0.160 (0.128-0.200) | 99 (84-117) | 11 | 1564 (1155-2120) | 2311 (1773-3011) | 0.14 | 0.21 | 0.81 | 0.09 | 0.058 | 0.18 |
| A/G    | 176 | 751 (701-804) | 257 (243-272) | 0.149 (0.138-0.162) | 114 (106-123) | 66 | 1298 (1086-1551) | 2066 (1854-2303) | 0.010 | 0.27 | 0.22 | 0.0015 | 0.026 | 0.037 |
| G/G    | 119 | 749 (686-818) | 238 (221-257) | 0.158 (0.143-0.175) | 119 (108-131) | 46 | 1072 (848-1356) | 1900 (1663-2171) | 0.010 | 0.27 | 0.22 | 0.0015 | 0.026 | 0.037 |

Data are represented as estimated means (95% CI). Alleles identified as risk alleles for type 2 diabetes are indicated in bold. All variables were log-transformed before analysis. P-values were computed for additive models using linear generalized estimating equations (GEE) which takes into account the family relatedness when computing the standard errors. 1st and 2nd phase GSIS, GLP-1 and arginine stimulated insulin secretion were adjusted for study center, family relatedness, glucose tolerance status, age, gender, BMI and ISI. ISI and DI were adjusted for study center, family relatedness, glucose tolerance status, age, gender and BMI. *available for 123 subjects from the NTR twin sample. †P values are for the recessive model.
Table 3 Insulin response according to genotype in NGT and IGT subjects (genes with significant effects only).

| Gene              | n   | First-phase insulin response (pmol/l) | Second-phase insulin response (pmol/l) | Insulin sensitivity index (µmol/min/kg/pmol/l) | Disposition index (µmol/min/kg) | n   | First-phase insulin response (pmol/l) | Second-phase insulin response (pmol/l) | Insulin sensitivity index (µmol/min/kg/pmol/l) | Disposition index (µmol/min/kg) |
|-------------------|-----|--------------------------------------|---------------------------------------|-----------------------------------------------|--------------------------------|-----|--------------------------------------|---------------------------------------|-----------------------------------------------|---------------------------------|
|                   |     | Normal glucose tolerant |                           |                          |                           |     | Impaired glucose tolerant |                           |                          |                                |
| CDC123/CAMK1D, rs12779790 |     |                                      |                          |                          |                           |     |                                      |                           |                          |                                |
| C/C               | 109 | 888 (812-971)                        | 257 (239-276)            | 0.202 (0.182-0.225)     | 178 (160-199)            | 103 | 717 (610-843)                        | 249 (216-286)            | 0.109 (0.090-0.132)     | 79 (67-93)                       |
| C/T               | 66  | 792 (714-877)                        | 247 (226-269)            | 0.202 (0.177-0.230)     | 162 (146-181)            | 44  | 715 (584-875)                        | 208 (174-249)            | 0.102 (0.081-0.128)     | 77 (61-97)                       |
| T/T               | 5   | 720 (540-962)                        | 197 (164-237)            | 0.221 (0.166-0.295)     | 154 (122-193)            | 7   | 564 (363-877)                        | 186 (144-239)            | 0.096 (0.059-0.157)     | 60 (36-99)                       |
| P                 |     | 0.034                                | 0.14                    | 0.89                    | 0.13                    | 0.42| 0.0028                               | 0.44                    | 0.37                            |                                |
| THADA, rs7578597  |     |                                      |                         |                         |                         |     |                                      |                         |                                |                                |
| C/C               | 1   | 1109 (1005-1224)                     | 388 (355-424)            | 0.280 (0.247-0.318)     | 264 (240-290)            | 2   | 920 (359-2358)                       | 360 (270-478)            | 0.070 (0.035-0.138)     | 76 (46-124)                      |
| C/T               | 43  | 843 (728-976)                        | 272 (244-303)            | 0.249 (0.215-0.288)     | 192 (164-224)            | 29  | 730 (570-936)                        | 257 (209-316)            | 0.122 (0.092-0.162)     | 87 (68-112)                      |
| T/T               | 136 | 840 (776-910)                        | 243 (228-259)            | 0.190 (0.173-0.208)     | 164 (150-180)            | 125 | 710 (606-833)                        | 232 (202-266)            | 0.104 (0.087-0.125)     | 76 (64-90)                       |
| P                 |     | 0.91*                                | 0.057*                  | 0.0017*                 | 0.073*                  | 0.67*| 0.14*                               | 0.32*                   | 0.21*                           |                                |
| ADAMTS9, rs4607103|     |                                      |                          |                          |                           |     |                                      |                         |                                |                                |
| T/T               | 12  | 694 (581-830)                        | 207 (171-251)            | 0.182 (0.122-0.271)     | 137 (110-170)            | 8   | 487 (340-699)                        | 193 (137-272)            | 0.093 (0.068-0.127)     | 52 (37-73)                       |
| T/C               | 71  | 832 (759-912)                        | 245 (224-267)            | 0.204 (0.180-0.231)     | 169 (150-191)            | 54  | 699 (582-840)                        | 252 (215-296)            | 0.102 (0.082-0.128)     | 74 (62-89)                       |
| C/C               | 97  | 867 (788-955)                        | 259 (241-279)            | 0.204 (0.182-0.228)     | 176 (160-194)            | 94  | 747 (625-892)                        | 227 (196-263)            | 0.111 (0.092-0.135)     | 84 (69-102)                      |
| P                 |     | 0.11                                 | 0.054                   | 0.71                    | 0.12                    | 0.051| 0.75                                 | 0.22                    | 0.012                           |                                |
| BCL11A, rs10490072|     |                                      |                          |                          |                           |     |                                      |                         |                                |                                |
| C/C               | 18  | 976 (828-1151)                       | 230 (197-269)            | 0.213 (0.172-0.263)     | 210 (170-258)            | 14  | 740 (553-990)                        | 207 (159-268)            | 0.127 (0.091-0.177)     | 84 (60-119)                      |
| C/T               | 71  | 885 (799-979)                        | 261 (241-283)            | 0.191 (0.168-0.217)     | 175 (155-197)            | 55  | 815 (663-1000)                       | 231 (196-272)            | 0.101 (0.082-0.126)     | 85 (70-104)                      |
| T/T               | 91  | 785 (719-858)                        | 246 (227-267)            | 0.211 (0.188-0.236)     | 161 (146-177)            | 87  | 670 (570-787)                        | 238 (205-276)            | 0.108 (0.088-0.132)     | 74 (62-88)                       |
| P                 |     | 0.0066                               | 0.96                    | 0.59                    | 0.019                   | 0.10 | 0.33                                 | 0.73                    | 0.19                            |                                |
| MTNR1B, rs10830963|     |                                      |                          |                          |                           |     |                                      |                         |                                |                                |
| C/C               | 91  | 853 (778-936)                        | 243 (226-260)            | 0.212 (0.189-0.237)     | 177 (159-196)            | 96  | 762 (645-900)                        | 226 (195-263)            | 0.115 (0.094-0.140)     | 88 (74-104)                      |
| C/G               | 65  | 882 (802-970)                        | 267 (241-295)            | 0.188 (0.160-0.221)     | 172 (154-193)            | 48  | 732 (609-879)                        | 260 (218-309)            | 0.093 (0.075-0.114)     | 73 (61-87)                       |
| G/G               | 21  | 696 (593-818)                        | 241 (210-276)            | 0.228 (0.191-0.272)     | 157 (135-183)            | 14  | 509 (410-633)                        | 223 (175-284)            | 0.096 (0.066-0.141)     | 53 (40-70)                       |
Data are represented as estimated means (95% CI). Alleles identified as risk alleles for type 2 diabetes are indicated in bold. All variables were log-transformed before analysis. P-values were computed for additive models using linear generalized estimating equations (GEE) which takes into account the family relatedness when computing the standard errors. 1st and 2nd phase GSIS stimulated insulin secretion were adjusted for study center, family relatedness, age, gender, BMI and ISI. ISI and DI were adjusted for study center, family relatedness, glucose tolerance status, age, gender and BMI. * P values are for the recessive model.