The *CTRB1/2* locus affects diabetes susceptibility and treatment via the incretin pathway

Running title: The *CTRB1/2* locus: diabetes risk and treatment

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Abstract

The incretin hormone glucagon-like-peptide 1 (GLP-1) promotes glucose homeostasis and enhances beta-cell function. GLP-1 receptor agonists (GLP-1 RA) and dipeptidyl peptidase-4 (DPP-4) inhibitors, which inhibit the physiological inactivation of endogenous GLP-1, are used for the treatment of type 2 diabetes. Using the Metabochip we identified three novel genetic loci with large effects (30-40%) on GLP-1 stimulated insulin secretion during hyperglycemic clamps in non-diabetic Caucasian individuals (TMEM114; CHST3 and CTRB1/2; n=232, all p≤8.8*10^-7). rs7202877 near CTRB1/2, a known diabetes risk locus, also associated with an absolute 0.51±0.16% (5.6±1.7 mmol/mol) lower A1C response to DPP-4 inhibitor treatment in G allele carriers but there was no effect on GLP-1 RA treatment in type 2 diabetes patients (n=527). Furthermore, in pancreatic tissue we show that rs7202877 acts as expression quantitative trait locus for CTRB1 and CTRB2, encoding chymotrypsinogen, and increases fecal chymotrypsin activity in healthy carriers. Chymotrypsin is one of the most abundant digestive enzymes in the gut where it cleaves food proteins into smaller peptide fragments.

Our data identify chymotrypsin in the regulation of the incretin pathway, development of diabetes and response to DPP-4 inhibitor treatment.
The incretin hormone glucagon-like peptide-1 (GLP-1) is released after a meal by L-cells in the distal parts of the gastrointestinal tract and it potentiates glucose dependent insulin secretion by the pancreas, which is known as the incretin effect. Furthermore it reduces glucagon secretion and exerts beneficial effects on gut motility, satiety and food intake (reviewed in (1;2)). Based on these functions and the impaired incretin effect in type 2 diabetes patients, two novel incretin-based classes of glucose lowering drugs have been developed for treatment of type 2 diabetes (1). These include injectable GLP-1 receptor agonists (GLP-1 RA), which provide pharmacological levels of GLP-1, and dipeptidyl peptidase (DPP)-4 inhibitors, which inhibit the physiological inactivation of endogenous GLP-1. The enzyme DPP-4 rapidly inactivates endogenous GLP-1 by cleavage of the two N-terminal aminoacids (1-4). In addition to their direct insulinotropic effects on the pancreas, injectable GLP-1 RAs, like endogenous GLP-1, lower blood glucose by inhibiting glucagon secretion and slow down gastric emptying in a glucose-dependent manner. Also, GLP-1 RAs promote satiety and weight loss. All these actions contribute to their therapeutic efficacy (1-4). Similarly, oral DPP-4 inhibitors lower blood glucose by stimulating insulin and inhibiting glucagon secretion but are considered weight neutral (1;4).

Importantly, the treatment response to compounds of either drug class varies widely and it is conceivable that both environmental and genetic factors may underlie these differences.

Previously, we showed that during hyperglycemic clamps the insulin response to intravenous GLP-1 stimulation was affected by genetic factors (5) with an estimated heritability of 0.53 (0.33-0.70)(6). These findings indicate that genetic factors exert substantial effects on GLP-1 induced insulin response and, as a consequence, may affect an individual’s response to the GLP-1 based therapies.
In the present study the Metabochip (200k) was used to identify genetic variants affecting GLP-1 induced insulin secretion during hyperglycemic clamps in 232 non-diabetic participants from two independent populations, the Netherlands Twin Register (NTR) and the German Tübingen cohort (7;8). Subsequently, the influence of associated SNPs was tested on response to GLP-1 RA and DPP-4 inhibitor treatment in type 2 diabetes patients (n=527) from the Netherlands (DCS West-Friesland (9)) and the UK (GoDARTS (10)). Finally gene expression in pancreas and pancreatic islets and functional tests in healthy volunteers were carried out to further elucidate potential underlying mechanisms.

Our studies identify rs7202877 near *CTRB1* and *CTRB2* which both encode the digestive enzyme chymotrypsin in the regulation of the incretin pathway, development of diabetes and response to DPP-4 inhibitor treatment.

**Research Design and Methods**

*Study subjects and experimental procedures*

*Hyperglycemic clamp cohort.* We included 232 Caucasian subjects from two independent studies in the Netherlands and Germany. There were 126 subjects from the Netherlands Twin Register (NTR) including 69 monozygotic and 29 dizygotic twins as well as 28 of their non-twin sibs recruited from 54 families (120 normal glucose tolerant (NGT); 6 impaired glucose tolerant (IGT) (7). The German cohort consisted of 73 participants with NGT and 33 participants with IGT who were all unrelated (8). Clinical characteristics of both cohorts are given in Supplemental Table 1.
All subjects underwent a modified hyperglycemic clamp with glucose and GLP-1 as previously described (11). Briefly, subjects received a priming infusion of glucose to acutely raise blood glucose levels to 10 mmol/l. Variable glucose infusions were used to keep glucose levels constant throughout the whole procedure. After the second hour insulin secretion was further stimulated using ivGLP-1 infusion (4.5 pmol/kg bolus for 1 min at t = 120 followed by a continuous infusion of 1.5 pmol · kg⁻¹ · min⁻¹ for one hour). In the Dutch NTR twin cohort, slightly lower GLP-1 concentrations were used (1.5 pmol/kg and 0.5 pmol · kg⁻¹ · min⁻¹, respectively). The near maximal insulin response was tested by injecting a bolus of 5 grams arginine hydrochloride at t=180 min. First and second phase glucose stimulated insulin secretion (GSIS), insulin sensitivity index (ISI) and the disposition index (DI) were calculated as described previously (7). GLP-1 stimulated insulin secretion was measured as the incremental area under the curve during the last 20 minutes of the glucose + GLP-1 stimulation (t=160 to 180). In order to increase the power and robustness of the analysis of glucose stimulated insulin secretion in carriers of SNPs associated with GLP-1 stimulated insulin secretion we included another 216 subjects from two Dutch cohorts (Hoorn and Utrecht study cohorts) who underwent an identical hyperglycemic clamp but without the additional GLP-1 stimulation. Details of these cohorts can be found in reference 7 and Supplemental Table 1.

**Pharmacogenetic study cohort.** Type 2 diabetes patients participating in this study were from the Dutch Diabetes Care System West-Friesland (DCS, n=7515) (9) and the UK Genetics of Diabetes Audit and Research Tayside Scotland cohort (GoDARTS, n=8000+) (10). Patients are included in the current study if they were Caucasian and treated with either a GLP-1 RA or a DPP4-inhibitor for at least three
months (n=527). In DCS 22 patients were treated with a GLP-1 RA and another 49 patients with a DPP4-inhibitor. In GoDARTS 151 patients were treated with a GLP1-RA and another 305 patients were treated with a DPP4-inhibitor. Patient characteristics of both study cohorts are depicted in Supplemental Table 2, 7. Baseline A1C was defined as the measurement closest to the initiation of treatment (maximum 12 (DCS) or 6 (GoDARTS) months before). On treatment A1C was defined as the minimum A1C measure between 3 and 24 (DCS) or 18 (GoDARTS) months after therapy initiation.

*Metabochip genotyping and quality control*

All subjects participating in the hyperglycaemic clamp study were genotyped using the Metabochip (www.Illumina.com). The Metabochip was designed for replication (n=~66.000 SNPs) and fine mapping of regions on the genome (n=~120000), which have previously been associated with cardiovascular and/or metabolic diseases including type 2 diabetes (12). Our analyses focused on the 66000 SNPs for replication / follow-up. GenomeStudio software (Illumina inc. USA) was used to visualize the data and SNP calling. For quality control we used a cut-off for the genotyping call rate of 95%, a MAF in the combined cohort of 0.05 and the p-value cut-off for Hardy-Weinberg equilibrium was set at $10^{-4}$. In total 13.0% (8465) of the SNPs on the Metabochip were monomorphic or had a MAF<0.05 while another 5.4% (3526) of the SNPs failed the other QC criteria. In total 53354 SNPs passed quality control which is comparable to previous reports (12). We computed the genomic inflation factor ($\lambda$) as previously described (12;13). Given the low $\lambda$ of 1.014 (Supplemental Figure 1) the test statistics were not further adjusted.
The top loci ($p \leq 1 \times 10^{-4}$) were visually inspected for SNPs with a low number of homozygous mutant alleles ($n < 5$) and these SNP were subsequently reanalyzed using a carrier model (11 vs 12 + 22). The Sequenom MassARRAY iPLEX Gold platform (sequenom Inc, USA) was used for validation genotyping of our top SNPs and one proxy for each ($r^2 > 0.9$) and for two samples that failed QC on the Metabochip (concordance >99.9%). Taqman SNP genotyping assays (Applied Biosystems, USA) were used to genotype subjects participating in the other studies except for the GoDARTS pharmacogenetic study where data were extracted from a GWAS and Metabochip dataset (14).

**Gene expression studies** Human cadaveric donor pancreata ($n=35$) were procured through a multi-organ donor program. Tissue samples from the pancreata were used in the study if research consent was present and pancreata could not be used in clinical beta-cell therapy programs according to national laws. Tissue samples were snap frozen in liquid nitrogen and stored at −80°C. Tissues were first incubated overnight in RNAlater-ICE (Life technologies, USA) and subsequently RNA was isolated using Trizol reagent (Life technologies, USA). Gene expression was quantified using taqman gene expression assays (Life technologies, USA). The beta-2-microglobulin ($B2M$, Hs00984230_m1) gene was used a reference but similar data were obtained when using other housekeeping genes such as GAPDH or actin B. $CTRB$ gene expression was tested using a probe detecting both copies of the gene (Hs00157181_m1) while $BCAR1$ was detected with probe Hs01547079_m1. Expression levels were given as arbitrary units relative to the expression of B2M. Islets from cadaver donors ($n=45$) were provided by the Nordic Islet Transplantation Program (http://www.nordicislets.org). Purity of islets was assessed as described.
previously (15). RNA-seq libraries were prepared using the standard Illumina mRNA-Seq protocol and sequencing was performed in a Illumina HiSeq 2000 machine. The FASTQ output files generated were then aligned to the human reference genome (UCSC hg19) with TopHat (16). The annotated GTF transcript and fasta genome files were from UCSC and were downloaded from http://cufflinks.cbcb.umd.edu/igenomes.html. Gene expression was then considered as the normalized sum of expression of all its exons. The htseq-count script (http://www-huber.embl.de/users/anders/HTSeq/doc/count.html) was used by counting uniquely mapped reads in each exon using the mode "intersection-nonempty". Gene expression normalization was then performed using the TMM method (17), and further normalization was applied by adjusting the expression to gene length.

*Chymotrypsin activity study* In order to quantify chymotrypsin activity in carriers and non-carriers of rs7202877 near *CTRB1/2* we used stool samples from 40 carriers of the G allele and 40 age, gender and BMI matched non-carriers which are all unrelated. They are non-diabetic German subjects at increased risk for type 2 diabetes (positive family history, overweight) participating in an ongoing intervention study using different types of life style interventions. Clinical characteristics of this study group are given in Supplemental Table 3. A fresh stool sample was collected prior to participation in the intervention study and immediately frozen at -80 degrees Celsius. Fecal chymotrypsin activity was measured using a standard procedure first described by Kaspar et al (18). Chymotrypsin activity is expressed as U/g stool at 25 degrees Celsius. Sensitivity of chymotrypsin activity for proteolysis by human DPP4 has been tested after a 30 minute pre-incubation of the stool suspension (10ul) with or without
0.1U DPP4 (BPS Biosciences, USA) in a total volume of 40ul of 50mM Tris buffer (pH=7.5) at 37 degrees Celsius.

Statistics
If necessary outcome variables were log transformed prior to analysis to obtain normality. To take into account the family relatedness in the twin sample we used linear generalized estimating equations assuming additive models (GEE) in Bioconductor R (GEEpack, www.bioconductor.org). The analyses of glucose and GLP-1 stimulated insulin secretion were adjusted for age, sex, BMI, study center, glucose tolerance status (NGT/IGT), and ISI. For the analysis of ISI and DI, ISI was removed from the covariates. Additional adjustment for the prevailing insulin levels did not alter our results (data not shown). For the monozygotic (MZ) twin pairs we used the mean of the individual beta cell responses and covariates for both twins in a pair. This approach yields similar results compared to the inclusion of one randomly chosen twin from a MZ pair. GEE (SPSS inc, USA) was also used to calculate the estimated means adjusted for the above mentioned covariates. We computed the genomic inflation factor (Supplemental Figure 1) using SNPs included for the QT interval as previously described (12;13). Quantile–quantile plots were constructed using Bioconductor R (SNPmatrix www.bioconductor.org). LocusZoom was used to generate regional association plots of the loci of interest (19). The study wide significance threshold was set at p<9*10^{-7} based on the number of SNPs tested in this study (53k).

Efficacy of GLP-1 based treatment in type 2 diabetes patients was tested by linear regression analysis. The difference between baseline and the lowest on treatment A1C measurement or the lowest on treatment A1C itself was used as the dependent
variable. Age, gender, BMI and estimated glomular filtration rate (eGFR) were obtained at baseline. In order to take into account the possible effects of other glucose lowering medication, which in some cases was discontinued at initiation of GLP-1 based therapy, we used “stopped medication” (yes/no) as additional covariate. In addition to the above mentioned covariates, time between baseline and treatment A1C, diabetes duration and dose of GLP-1 based drugs were considered as other potential covariates. For DPP4-inhibitors users a stepwise regression analysis showed significant effects of baseline A1C, time between baseline and treatment A1C, stopped other medication and BMI as significant covariates in the model and these were subsequently included together with the genotype. BMI was no longer a significant predictor in those using a GLP-1 receptor agonist and was thus dropped from the model. For meta-analysis of the results obtained in the two separate cohorts we used CMA v2 software (www.meta-analysis.com).

A paired t-test was used to compare differences in fecal chymotrypsin activity between age, gender and BMI matched carriers and non-carriers of rs7202877. Fecal chymotrypsin activity was logarithmically transformed prior to analysis to obtain normality. For the above statistical analyses SPSS v20 (SPSS inc, USA) was used and a p≤0.05 was regarded significant unless stated otherwise.

RESULTS

GLP-1 stimulated insulin secretion

Three loci showed genome or study wide significance for association with the magnitude of GLP-1 stimulated insulin secretion (all p≤8.8*10^{-7}, Table 1). The regional plots for each locus are given in Supplemental Figure 2). To check the robustness of the associations we also analyzed the data from Dutch and German
subjects separately and found that the effect sizes and directions were comparable (all \( p \leq 1 \times 10^{-3} \)).

At chromosome 10q22.1 several SNPs showed evidence for association, with rs4148941 being genome wide significant. rs4148941 is located in the 3' untranslated region of the second exon of the CHST3 gene. However, the LD block also includes the nearby genes SPOCK2 and ASCC1. Carriers of the C allele at rs4148941 (MAF=0.39) had a 32 percent reduced GLP-1 stimulated insulin secretion (\( p = 3.9 \times 10^{-8} \)). Our second signal is represented by rs7202633, which is located on chromosome 16p13.2, 84kb upstream of TMEM114 (\( p = 2.0 \times 10^{-7} \)). Homozygous carriers of the minor allele of rs7202633 (MAF = 0.44) had an almost two fold increased response during stimulation with GLP-1. Finally the third most significant locus was tagged by rs7202877 near CTRB1, CTRB2 and BCAR1 (MAF=0.11) at chromosome 16q23.1, which has recently been identified as a locus that protects from type 2 diabetes (13). Subjects carrying the G allele showed a 33 percent increased GLP-1 stimulated insulin secretion (\( p = 1.9 \times 10^{-6} \)). To check whether these SNPs also associate with other measures of beta-cell function we also examined the effect of these SNPs on response to glucose and arginine stimulated insulin secretion. rs7202633 near TMEM114 also showed an increased response to arginine stimulation during hyperglycemia (\( p = 5.6 \times 10^{-4} \)) but none of these SNPs was significantly associated with glucose stimulated insulin secretion (all \( p > 0.01 \), Supplemental Table 4).

**GLP-1 RA and DPP-4 inhibitor treatment study in type 2 diabetes patients**

In total 527 patients were included, from the Dutch DCS West-Friesland and the UK GoDARTS studies (Supplemental Table 2). In both cohorts, patients carrying the G allele of rs7202877 near CTRB1/2 showed a significantly smaller decrease in A1C
levels after DPP-4 inhibitor treatment compared to TT carriers (n=354, Table 2, Supplemental Tables 5 - 7). Meta-analysis of both cohorts showed that carriers of the G allele had an absolute 0.51±0.16 % (5.6 ±1.7 mmol/mol) smaller decrease in A1C after adjustment for potential confounders (p=0.0015, Table 2). None of the other two loci showed significant association with DPP-4 inhibitor treatment response. In T2D patients treated with a GLP-1 RA (n=173) none of the SNPs showed a significant association, although rs4148941, located in CHST3, showed a trend towards lower treatment response in C allele carriers (0.24±0.13% (2.6±1.4 mmol/mol); p=0.08, Table 2, Supplemental Tables 5 and 6). In contrast to those treated with a DPP-4 inhibitor, carriers of the rs7202877 G allele treated with a GLP-1 RA showed a response comparable to carriers of the TT genotype (p=0.58, Table 2). There were no significant SNP x drug interactions.

Given the results described above and the overlap with genetic loci for type 1 (20) and type 2 (13) diabetes we focused our next steps on rs7202877 at chromosome 16q23.1. rs7202877 is located approximately 6 kb from the start of both CTRB1 and CTRB2, which both encode for the digestive enzyme chymotrypsinogen, and approximately 54 kb from BCAR1 encoding breast cancer anti-estrogen resistance-1 (Supplemental Figure 3).

**Gene expression in pancreas and islets.**

In order to further elucidate the potential working mechanism we examined whether rs7202877 acted as an expression quantitative trait locus (eQTL) by examining if it was associated with gene expression in RNA isolated from whole pancreas and isolated pancreatic islets. In 35 whole pancreata the minor G allele at rs7202877
increases mRNA expression of CTRB1/2 (p=0.01) but not BCAR1 (Figure 1). We next tested whether this SNP also acts as an expression quantitative trait locus (eQTL) in isolated islets from normal (n=24) and hyperglycemic (n=21) subjects. Using RNAseq data we confirmed the observation from whole pancreatic tissue showing that the SNP also acts as an eQTL for CTRB1 and CTRB2 but not BCAR1 in islets which was independent from A1C level, age, gender and BMI (p≤0.05, Figure 1).

**Chymotrypsin activity measurements**

Given the enhanced expression of both CTRB genes, we tested whether rs7202877 was associated with altered chymotrypsin activity. In an analysis of 40 carriers of the G allele, compared to 40 age, gender and BMI-matched non-carriers, the rs7202877 G allele was associated with an increased chymotrypsin activity in stool samples (p=0.023, Figure 2). Furthermore chymotrypsin has been identified as a target for DPP-4 in mice (21). A 30 min pre-incubation of the stool samples with 0.1U DPP-4 resulted in a small but significantly lower chymotrypsin activity in TT carriers but not in carriers of the G allele (-5.3%, p=0.01 and -2.1%, p=0.26 respectively; Figure 2).

**DISCUSSION**

In a unique sample of deeply phenotyped subjects who received GLP-1 during a hyperglycemic clamp to measure insulin secretion, we identified three loci that influence GLP-1 stimulated insulin secretion. The loci on chromosomes 10q22.1 (in CHST3 and near ASCC1, SPOCK2) and 16p13.2 (near TMEM114) have not previously been implicated in beta-cell function, type 2 diabetes susceptibility or related phenotypes. However, in publically available gene expression data from the
MuTHER consortium rs4148941 acts as eQTL for CHST3 in lymphoblast cell lines (LCL, p=5*10^{-51}) and SPOCK2 in both adipose tissue (p=1*10^{-21}) and LCL (p=3*10^{-4})(22). Given the additional trend towards association with GLP-1 RA treatment response in diabetic patients further research is warranted to establish its role in diabetes development and treatment.

Previously, the G allele of rs7202877 near CTRB1/2 on chromosome 16q23.1 has been identified as a risk factor in a GWAS for type 1 diabetes (20) and as a protective factor for development of type 2 diabetes (13). We now report positive effects of the G allele of rs7202877 on GLP-1 stimulated insulin secretion which likely explains the protective effect of this allele on type 2 diabetes susceptibility. Furthermore we observed decreased glucose reduction in patients treated with DPP-4 inhibitors. Our observations are further supported by the fact that the SNP increases gene expression of CTRB1/2 in islets and pancreata and enzyme activity in stool, placing CTRB1/2 in a key role of the enteroendocrine system.

rs7202877 is located in an intergenic region between CTRB1 and CTRB2 encoding chymotrypsinogen B1 and B2 respectively (Supplemental Figure 3). In an in silico functional analysis using the regulome database there is no known functional effect of rs7202877, indicated by a regulomeDB score of 5 (www.regulomedb.org) (23). At least 50 SNPs are in high LD (r^2>0.8) with rs7202877, some of which might affect gene expression but require further detailed study. The linkage disequilibrium block also encompasses the breast cancer anti-estrogen resistance protein 1 gene (BCAR1); for which it has been shown that rs7202877 acts as an eQTL in blood (13) although this was not confirmed in the MuTHER consortium data(22). BCAR1 is ubiquitously expressed in many tissues including the pancreas. However, given that the SNP acts
as eQTL for CTRB1/2 but not BCAR1 in the pancreas and alters chymotrypsin activity in the gut, CTRB1 and CTRB2 are the most likely candidate genes in this region although a role of BCAR1 can not be excluded (24). The chymotrypsinogens (EC 3.4.21.1) belong to a family of serine proteases that are secreted by the pancreas into the gastrointestinal tract as inactive precursors, which are then activated by proteolytic cleavage with trypsin. Chymotrypsin cleaves food proteins into smaller peptides suitable for further digestion. Chymotrypsinogens are highly expressed in the exocrine pancreas but expression in beta cells has also been shown (25) which is confirmed in this study and in INS1E cells. Interestingly, in a study in mice chymotrypsin has been identified as a target for DPP-4 (21). A peptide fragment (VPAlQPVLTG) containing the DPP-4 H₂N-Xaa-Pro consensus motif was found at 10-fold higher level in gut tissue from DPP-4⁻⁻ versus DPP-4⁻/+ mice. Our in vitro findings, showing reduced chymotrypsin activity after pre-incubation with DPP-4, confirm this observation in humans and may add to the observed differences in chymotrypsin activity.

As previously shown, altered exposure of intestinal L-cells to nutrients, resulting from changes in food composition, point of entry (after gastric bypass), rate of gastric emptying and gut motility, may affect GLP-1 secretion (1;4;26). It could be hypothesized that the observed increase in fecal chymotrypsin activity may be associated with alterations in L-cell stimulation and/or gastric emptying as a result of increased nutrient digestion. However, incretin secretion during OGTT in non-diabetic subjects was not significantly different (n=457; supplemental table 8). It has been shown that oral treatment with chymotrypsin inhibitors slows gastric emptying and acutely reduces glucose and insulin levels after a meal in type 2 diabetes patients (27). Prolonged use of these inhibitors in a phase IIa cancer chemoprevention trial
significantly reduced glucose levels in non-diabetic cancer patients (28). Interestingly, various vegetables, including potatoes and soybeans, contain high levels of trypsin and chymotrypsin inhibitors and high intake of soy has been associated with reduced risk of diabetes (29). These results support the observation that rs7202877 genotype, enhancing pancreatic chymotrypsin expression and enzyme activity in the gut, alters diabetes risk.

The divergent effect of rs7202877 on treatment response to DPP-4 inhibitors and GLP-1 RA is intriguing. At present our study design and sample size, especially regarding GLP-1 RA treated subjects (~50% power to detect an effect with similar size to the DPP-4 inhibitor group), does not allow us to establish the robustness of the difference in treatment response between GLP-1 RA and DPP-4 inhibitors and/or whether these findings have clinical consequences. To this end, a well-powered genotype selected prospective clinical trial would be required. It is, however, of note that the G allele at rs7208277 has opposing effects on type 1 and type 2 diabetes risk (13;20;20) which may hint at divergent effects at the level of the pancreas and other tissues, most likely the gut, differently affecting diabetes type 1 and type 2 susceptibility and GLP-1 based treatment responses but this requires further exploration.

Type 2 diabetes, of which pancreatic endocrine dysfunction is a hallmark, has also been associated with pancreatic exocrine dysfunction (30). Furthermore, GLP-1-based therapies exert insulinotropic effects but their use has also been linked to pancreatic exocrine disturbances including pancreatitis and pancreatic cancer (31-33). In this respect, the present finding is of interest as it identifies a genetic factor encoding a pancreatic digestive enzyme at the cross-road of pancreatic endocrine function and the response to GLP-1 based therapies. It is of further note that there is suggestive
evidence from the PanScan consortium that SNPs, including rs7202877, near the
*CTRB1/CTRB2* region are linked to pancreatic neoplasms (34). Studies into the
interrelationship of these genetic factors and phenotypic defects is mandatory to help
increasing our insight into the complex pathophysiology of diabetes and its co-
morbidities as well as our understanding of the regulation and physiological function
of the incretin pathway.

Limitations of this study include: Due to the unique and comprehensive deep
phenotypes the sample sizes are relatively small, although results are replicated and
size and directionally consistent in all independent cohorts. A further limitation is that
most studies presented here only involve non-diabetic individuals and thus our results
do not necessarily represent the situation in diabetic states. The power in our
pharmacogenetic studies is limited and larger better powered studies are needed to
check the robustness and clinical utility of our associations. Finally, the Metabochip
focuses on loci previously implicated in metabolic and cardiovascular phenotypes and
although this increases power it is likely that other loci exist that are not captured with
this chip.

In conclusion, several loci are associated with GLP-1 stimulated insulin secretion in
healthy non-diabetic volunteers. One of the loci, *CTRB1/2* on chromosome 16q23.1
was further associated with a reduced response to treatment with DPP-4 inhibitors in
type 2 diabetic patients. Carriers of the G allele at rs7202877 additionally showed
increased chymotrypsin mRNA expression and activity in pancreas and feces,
respectively. These results highlight the importance of chymotrypsin in the incretin
response and regulation of glucose homeostasis and may provide novel targets for preventive and therapeutic strategies in the combat of diabetes.
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Authors’ contribution
LMtH, MD, AF, NvL, DIB, GN, EMWE, ERP and EJCdG researched data and wrote the manuscript. JJH, LAD, TWvH, APG, SAH-S, AMCS-B., GW, MA, BG, JF, FC, JD, JJH-D, QH and FM researched data. PES, JMD, MHHK, RJH, LG, EJPdK, H-UH, TH, OP, CNAP contributed to the discussion and reviewed/edited the manuscript.

Ethics approval and participant consent
All studies were approved by the local Medical Ethics Review Board; all subjects gave written informed consent and the studies were performed according to the principles of the declaration of Helsinki.

Duality of interest statement
AF, GN, JMD, EJPdK, ERP, and DM have indicated that they have a potential duality of interest as they serve on advisory boards or act as consultant or speaker or have received grants from (pharmaceutical) companies who may either gain or lose from this publication. These companies include Abbott, Amylin, Astra-BMS, Boehringer Ingelheim, Eli Lilly, Merck Sharp & Dohme (MSD), Novo Nordisk, Sanofi and Poxel
Pharma. None of these companies was involved in this work or during the writing process. MA is currently employed at Unilever Research and Development, Vlaardingen, the Netherlands and RJH is currently employed by Eli Lilly & company in Indianapolis, IN, USA. However, the work as described in this manuscript was done for their previous employer, VU University Medical Center Amsterdam, The Netherlands. LMtH, AF, NvL, LAD, JF, FC, APG, CNAP, TWvH, SAH-S, AMCS-B, JJH-D, QH, JD, BG, TH, FM, GW, MHHK, JJH, H-UH, OP, LG, EJCdG, PES, DIB, and EMWE declare that they have no duality of interest regarding this publication.
References

1. Baggio, LL, Drucker, DJ: Biology of incretins: GLP-1 and GIP. *Gastroenterology* 132:2131-2157, 2007

2. Holst, JJ: The physiology of glucagon-like peptide 1. *Physiol Rev* 87:1409-1439, 2007

3. DeFronzo, RA, Okerson, T, Viswanathan, P, Guan, X, Holcombe, JH, MacConell, L: Effects of exenatide versus sitagliptin on postprandial glucose, insulin and glucagon secretion, gastric emptying, and caloric intake: a randomized, cross-over study. *Curr Med Res Opin* 24:2943-2952, 2008

4. Nauck, MA: Incretin-based therapies for type 2 diabetes mellitus: properties, functions, and clinical implications. *Am J Med* 124:S3-18, 2011

5. Mussig, K, Staiger, H, Machicao, F, Haring, HU, Fritsche, A: Genetic variants affecting incretin sensitivity and incretin secretion. *Diabetologia* 53:2289-2297, 2010

6. Simonis-Bik, AM, Eekhoff, EM, de moor, MH, Kramer, MH, Boomsma, DI, Heine, RJ, Dekker, JM, Maassen, JA, ’t Hart, LM, Diamant, M, de Geus, EJ: Genetic influences on the insulin response of the beta cell to different secretagogues. *Diabetologia* 52:2570-2577, 2009

7. ’t Hart, LM, Simonis-Bik, AM, Nijsps, G, Van Haeften, TW, Schafer, SA, Hoving-Duistermaat, JJ, Boomsma, DI, Groenewoud, MJ, Reiling, E, van Hove, EC, Diamant, M, Kramer, MH, Heine, RJ, Maassen, JA, Kirchhoff, K, Machicao, F, Haring, HU, Slagboom, PE, Willemsen, G, Eekhoff, EM, de Geus, EJ, Dekker, JM, Fritsche, A: A Combined Risk Allele Score of Eight Type 2 Diabetes Genes Is Associated With Reduced First Phase Glucose Stimulated Insulin Secretion During Hyperglycemic Clamps. *Diabetes* 59:287-293, 2010

8. Fritsche, A, Madaus, A, Renn, W, Tschritter, O, Teigel, A, Weisser, M, Maerker, E, Machicao, F, Haring, H, Stumvoll, M: The prevalent gly1057asp polymorphism in the insulin receptor substrate-2 gene is not associated with impaired insulin secretion. *J Clin Endocrinol Metab* 86:4822-4825, 2001

9. Zavrelova, H, Hoekstra, T, Alssema, M, Welschen, LM, Nijsps, G, Moll, AC, de Vet, HC, Polak, BC, Dekker, JM: Progression and regression: distinct developmental patterns of diabetic retinopathy in patients with type 2 diabetes treated in the diabetes care system west-friesland, the Netherlands. *Diabetes Care* 34:867-872, 2011

10. Morris, AD, Doyle, DIR, MacAlpine, R, EmисlieSmith, A, Jung, RT, Newton, RW, MacDonald, TM: The diabetes audit and research in Tayside Scotland (DARTS) study: electronic record linkage to create a diabetes register. *British Medical Journal* 315:524-528, 1997

11. Fritsche, A, Stefan, N, Hardt, E, Schutzenauer, S, Haring, H, Stumvoll, M: A novel hyperglycaemic clamp for characterization of islet function in humans: assessment of three different secretagogues, maximal insulin response and reproducibility. *Eur J Clin Invest* 30:411-418, 2000

12. Voight, BF, Kang, HM, Ding, J, Palmer, CD, Sidore, C, Chines, PS, Burtt, NP, Fuchsberger, C, Li, Y, Erdmann, J, Frayling, TM, Heid, IM, Jackson, AU, Johnson, T, Kilpelainen, TO, Lindgren, CM, Morris, AP, Prokopenko, I, Randall, JC, Saxena, R, Soranzo, N, Speliotes, EK, Teslovich, TM, Wheeler, E, Maguire, J, Parkin, M, Potter, S, Rayner, NW, Robertson, N, Stirrups, K, Winckler, W, Sanna, S, Mulas, A, Nagaraja, R, Cucca, F, Barroso, I, Deloukas, P, Loos, RJ, Kathiresan, S, Munroe, PB, Newton-Cheh, C, Pfeufer, A, Samani, NJ, Schunkert, H, Hirschhorn, JN, Altshuler, D, McCarthy, MI, Abecasis, GR, Boehnke, M: The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS Genet* 8:e1002793, 2012

13. Morris, AP, Voight, BF, Teslovich, TM, Ferreira, T, Segre, AV, Steinthorsdottir, V, Strawbridge, RJ, Khan, H, Grallert, H, Mahajan, A, Prokopenko, I, Kang, HM, Dina, C, Esko, T, Fraser, RM, Kanoni, S, Kumar, A, Lagou, V, Langenberg, C, Luan, J, Lindgren, CM, Muller-Nurasyid, M, Pechlivanis, S, Rayner, NW, Scott, LJ, Wiltshire, S,
Yengo, L, Kinnunen, L, Rossin, EJ, Raychaudhuri, S, Johnson, AD, Dimas, AS, Loos, RJ, Vedantam, S, Chen, H, Florez, JC, Fox, C, Liu, CT, Rybin, D, Couper, DJ, Kao, WH, Li, M, Cornelis, MC, Kraft, P, Sun, Q, van Dam, RM, Stringham, HM, Chines, PS, Fischer, K, Fontanillas, P, Holmen, OL, Hunt, SE, Jackson, AU, Kong, A, Lawrence, R, Meyer, J, Perry, JR, Platou, CG, Potter, S, Rehnberg, E, Robertson, N, Sivalaparatham, S, Stancakova, A, Stirrups, K, Thorleifsson, G, Tikkanen, E, Wood, AR, Almgren, P, Atalay, M, Benediktsson, R, Bonnycastle, LL, Burtt, N, Carey, J, Charpentier, G, Crenshaw, AT, Doney, AS, Dorkhan, M, Edkins, S, Emilsson, V, Eury, E, Forsen, T, Gertow, K, Gigante, B, Grant, GB, Groves, CJ, Guiducci, C, Herder, C, Hreidarsson, AB, Hui, J, James, A, Jonsson, A, Rathmann, W, Klopp, N, Kravic, J, Krjutskov, K, Langford, C, Leander, K, Lindholm, E, Lobbens, S, Mannisto, S, Mirza, G, Muhleisen, TW, Musk, B, Parkin, M, Palladis, L, Saramies, J, Sennblad, B, Shah, S, Sigurthsdottir, G, Silveira, A, Steinbach, G, Thorand, B, Trakalo, J, Veglia, F, Wennauer, R, Winckler, W, Zabaneh, D, Campbell, H, Van, DC, Uitterlinden, AG, Hofman, A, Sijbrands, E, Abecasis, GR, Owen, KR, Zeggini, E, Trip, MD, Forouhi, NG, Syvanen, AC, Eriksson, JB, Weyrich, S, Nothen, MM, Dorkhan, M, Palma, CN, Lyssenko, V, Tuomilehto, J, Chan, J, Flint, J, Aulchenko, Y, Almgren, P, Wei, J, Hattersley, AT, Doney, AS, Colhoun, H, Morris, AD, Walker, NM, Rich, SS: Genome-wide association study and meta-analysis of type 2 diabetes. *Diabetes* 64:1105-1111, 2009

11.  Zhou, K, Bellenguez, C, Spencer, CC, Bennett, AJ, Coleman, RL, Tavendale, R, Hawley, DA, Donnelly, LA, Schofield, C, Groves, CJ, Burch, L, Carr, F, Strange, A, Freeman, C, Blackwell, JM, Bramon, E, Brown, MA, Casas, JP, Corvin, A, Craddock, N, Deloukas, P, Dronov, S, Duncanson, A, Edkins, S, Gray, E, Hunt, S, Jankowski, J, Langford, C, Markus, HS, Mathew, CG, Plomin, R, Rautanen, A, Sawyer, SJ, Samani, NJ, Trombath, R, Viswanathan, AC, Wood, NW, Harries, LW, Hattersley, AT, Doney, AS, Colhoun, H, Morris, A, Sutherland, C, Hardie, DG, Peltonen, L, McCarthy, MI: Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet* 44:981-990, 2012

14.  Zhou, K, Bellenguez, C, Spencer, CC, Bennett, AJ, Coleman, RL, Tavendale, R, Hawley, DA, Donnelly, LA, Schofield, C, Groves, CJ, Burch, L, Carr, F, Strange, A, Freeman, C, Blackwell, JM, Bramon, E, Brown, MA, Casas, JP, Corvin, A, Craddock, N, Deloukas, P, Dronov, S, Duncanson, A, Edkins, S, Gray, E, Hunt, S, Jankowski, J, Langford, C, Markus, HS, Mathew, CG, Plomin, R, Rautanen, A, Sawyer, SJ, Samani, NJ, Trombath, R, Viswanathan, AC, Wood, NW, Harries, LW, Hattersley, AT, Doney, AS, Colhoun, H, Morris, A, Sutherland, C, Hardie, DG, Peltonen, L, McCarthy, MI: Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet* 44:981-990, 2012

16.  Trapnell, C, Pachter, L, Salzberg, SL: TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25:1105-1111, 2009

17.  Robinson, MD, Oshlack, A: A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol* 11:R25, 2010

18.  Kaspar, P, Moller, G, Wahlefeld, A, Stachler, F: A new photometric method for the determination of chymotrypsin in stool. *Fresenius Z Anal Chem* 311:391-392, 1982

19.  Pruim, RJ, Welch, RP, Sanna, S, Teslovich, TM, Chines, PS, Gliedt, TP, Boehnke, M, Abecasis, GR, Willer, CJ: LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 26:2336-2337, 2010

20.  Barrett, JC, Clayton, DG, Concannon, P, Akolkar, B, Cooper, JD, Erlich, HA, Julier, C, Morahan, G, Nerup, J, Nierars, C, Plagnol, V, Pociot, F, Schuijlenburg, H, Smyth, DJ, Stevens, H, Todd, JA, Walker, NM, Rich, SS: Genome-wide association study and meta-
analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 41:703-707, 2009

21. Tinoco, AD, Tagore, DM, Saghatelian, A: Expanding the dipeptidyl peptidase 4-regulated peptidome via an optimized peptidomics platform. *J Am Chem Soc* 132:3819-3830, 2010

22. Grundberg, E, Small, KS, Hedman, AK, Nica, AC, Buil, A, Keildson, S, Bell, JT, Yang, TP, Meduri, E, Barrett, A, Nisbett, J, Sekowska, M, Wilk, A, Shin, SY, Glass, D, Travers, M, Min, JL, Ring, S, Ho, K, Thorleifsson, G, Kong, A, Thorsteindottir, U, Ainali, C, Dimas, AS, Hassanali, N, Ingle, C, Knowles, D, Krestyaninova, M, Lowe, CE, di, MP, Montgomery, SB, Parts, L, Potter, S, Surdulescu, G, Tsaprouni, L, Tsoka, S, Bataille, V, Durbin, R, Nestle, FO, O'Rahilly, S, Soranzo, N, Lindgren, CM, Zondervan, KT, Ahmadi, KR, Schadt, EE, Stefansson, K, Smith, GD, McCarthy, MI, Mccarthy, P, Deloukas, P, Dermitzakis, ET, Spector, TD: Mapping cis- and trans-regulatory effects across multiple tissues in twins. *Nat Genet* 44:1084-1089, 2012

23. Boyle, AP, Hong, EL, Hariharan, M, Cheng, Y, Schaub, MA, Kasowski, M, Karczewski, KJ, Park, J, Hitz, BC, Weng, S, Cherry, JM, Snyder, M: Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res* 22:1790-1797, 2012

24. Mercader, JM, Puiggros, M, Segre, AV, Planet, E, Sorianello, E, Sebastian, D, Rodriguez-Cuenca, S, Ribas, V, Bonas-Guarch, S, Draghici, S, Yang, C, Mora, S, Vidal-Puig, A, Dupuis, J, Florez, JC, Zorzano, A, Torrents, D: Identification of novel type 2 diabetes candidate genes involved in the crosstalk between the mitochondrial and the insulin signaling systems. *PLoS Genet* 8:e1003046, 2012

25. Eizirik, DL, Sammeth, M, Bouckenooghe, T, Bottu, G, Sisino, G, Igoillo-Esteve, M, Orts, F, Santin, I, Coll, ML, Barthson, J, Bouwens, L, Hughes, L, Gregory, L, Lunter, G, Marselli, L, Marchetti, P, Marchetti, MI, Cnop, M: The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. *PLoS Genet* 8:e1002552, 2012

26. Pournaras, DJ, Aasheim, ET, Bueter, M, Ahmed, AR, Welbourn, R, Olbers, T, le Roux, CW: Effect of bypassing proximal gut on gut hormones involved with glycemic control and weight loss. *Surg Obes Relat Dis* 2012

27. Schwartz, JG, Guan, D, Green, GM, Phillips, WT: Treatment with an oral protease inhibitor slows gastric emptying and acutely reduces glucose and insulin levels after a liquid meal in type II diabetic patients. *Diabetes Care* 17:255-262, 1994

28. Armstrong, WB, Kennedy, AR, Wan, XS, Taylor, TH, Nguyen, QA, Jensen, J, Thompson, W, Lagerberg, W, Meyskens, FL, Jr.: Clinical modulation of oral leukoplakia and protease activity by Bowman-Birk inhibitor concentrate in a phase IIa chemoprevention trial. *Clin Cancer Res* 6:4684-4691, 2000

29. Villegas, R, ao, YT, ang, G, i, HL, lasy, TA, heng, W, hu, XO: Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health Study. *American Journal of Clinical Nutrition* 87:162-167, 2008

30. Larger, E, Philippe, MF, Barbot-Trystram, L, Radu, A, Rotaru, M, Nobecourt, E, Boitard, C: Pancreatic exocrine function in patients with diabetes. *Diabet Med* 2012

31. Cure, P, Pileggi, A, Alejandro, R: Exenatide and rare adverse events. *N Engl J Med* 358:1969-1970, 2008

32. Gale, EA: GLP-1-based therapies and the exocrine pancreas: more light, or just more heat? *Diabetes* 61:986-988, 2012

33. Singh, S, Chang, HY, Richards, TM, Weiner, JP, Clark, JM, Segal, JB: Glucagonlike Peptide 1-Based Therapies and Risk of Hospitalization for Acute Pancreatitis in Type 2 Diabetes Mellitus: A Population-Based Matched Case-Control Study. *JAMA Intern Med* 1-6, 2013

34. PanScan consortium. Pancreatic Cancer Cohort Consortium and Pancreatic Cancer Case-Control Consortium (PanScan). 2013. http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/analysis.cgi?study_id=phs000206.v3.p2&pha=2889.
Legend to Figure 1. Gene expression according to rs7202877 genotype in 35 whole pancreata and 45 human islet samples. Gene expression of *CTRB1/2* and *BCAR1* was measured using Taqman gene expression assays (pancreas) or RNAseq (Islets). A. *CTRB1/2* and *BCAR1* gene expression in whole pancreas in TT (n=26) and TG + GG (n=9) carriers adjusted for age, gender and BMI. B. Gene expression in islets from 27 TT carriers and 18 TG + GG carriers adjusted for age, gender, BMI and A1C.

Legend to Figure 2. Fecal chymotrypsin activity measurements according rs7202877 genotype. Chymotrypsin activity in stool samples was measured with the method of Kaspar et al. (18). A. Mean chymotrypsin activity (Act. ± SEM) of healthy volunteers with TT (n=40) or TG + GG genotype (n=40) at rs7202877 near *CTRB1/2*. B. Relative chymotrypsin activity (% Act. ± SEM) in stool samples pre-incubated with or without human DPP-4 (0.1 U) for 30 min at 37 degrees Celsius. The samples without DPP-4 pre-incubation were used a reference. TT genotype (n=15); TG+GG genotype (n=14).
Table 1. Significantly associated SNPs for GLP-1 stimulated insulin secretion during hyperglycemic clamps.

| SNP       | Chr | Nearest Gene | Alleles (MAF)* | 11  | 12  | 22  | P_add  | P_dom  | P_rec |
|-----------|-----|--------------|----------------|-----|-----|-----|--------|--------|-------|
| rs4148941 | 10  | CHST3        | A/C (0.39)     | 70  | 104 | 25  | 4.9*10^-5 | 3.9*10^-8 | 0.57 |
|           |     |              |                | 2253 (2026-2506) | 1481 (1324-1657) | 1659 (1373-2004) |
| rs7202633 | 16  | TMEM114      | A/T (0.44)     | 55  | 110 | 32  | 2.0*10^-7 | 3.5*10^-5 | 3.7*10^-5 |
|           |     |              |                | 1324 (1138-1541) | 1823 (1639-2027) | 2410 (2058-2822) |
| rs7202877 | 16  | CTRB1/CTRB2  | T/G (0.11)     | 156 | 36  | 4   | 8.8*10^-7 | 1.9*10^-6 | 0.017 |
|           |     |              |                | 1612 (1473-1765) | 2424 (2073-2834) | 2638 (1876-3710) |

Insulin secretion data are given as estimated means calculated with linear regression analysis (GEE) adjusted for age, gender, BMI, glucose tolerance status, study center and ISI. P-values are calculated using GEE assuming different genetic models with adjustment as described above. Genotype counts for carriers of the homozygous wild-type (11); heterozygous (12) and the mutant (22) allele are given. * Alleles at each locus are given as major allele / minor allele. MAF = minor allele frequency in this study population.
| SNP; Nearest gene(s) | DCS West-Friesland | GoDARTS | Meta-analysis |
|----------------------|--------------------|---------|--------------|
|                      | GLP-1 RA (n=22)    | GLP-1 RA (n=151) | GLP-1 RA (n=173) | p-value | DPP-4 inhibitors (n=354) | p-value |
| rs4148941; CHST3     | 0.41±0.45%         | 0.22±0.14% | -0.07±0.10%   | A/C (0.38) | 0.24±0.13% | 0.08 | -0.07±0.09% | 0.43 |
|                      | 4.5±4.9            | 2.4±1.5   | -0.8±1.1      | (0.38)    | 2.6±1.4    |       | -0.8±1.0    |       |
| rs7202633; TMEM114   | 0.43±0.45%         | 0.03±0.15% | 0.00±0.11%   | A/T (0.43) | 0.07±0.14% | 0.62 | 0.02±0.10% | 0.87 |
|                      | 4.7±4.9            | 0.3±1.6   | 0.0±1.2       | (0.43)    | 0.8±1.5    |       | 0.2±1.1     |       |
| rs7202877; CTRB1/2   | -1.33±0.74%        | 0.45±0.18%** | 0.49±2.0     | T/G (0.09) | -0.15±0.27% | 0.58 | 0.51±0.16% | 0.0015 |
|                      | -14.5±8.1          | 0.3±3.2   | 4.9±2.0       | (0.09)    | -1.6±3.0   |       | 5.6±1.7     |       |

Data are presented as A1C in % or mmol/mol adjusted for pre-treatment A1C, stopped other baseline medication, BMI and time between measures or pre-treatment A1C, stopped other baseline medication and time between measures for DPP-4 inhibitors and GLP-1 RA respectively. MAF minor allele frequency, with the effect allele in bold. * p≤0.05; ** p≤0.01.
Gene expression according to rs7202877 genotype in 35 whole pancreata and 45 human islet samples. Gene expression of CTRB1/2 and BCAR1 was measured using Taqman gene expression assays (pancreas) or RNAseq (Islets). A. CTRB1/2 and BCAR1 gene expression in whole pancreas in TT (n=26) and TG + GG (n=9) carriers adjusted for age, gender and BMI. B. Gene expression in islets from 27 TT carriers and 18 TG + GG carriers adjusted for age, gender, BMI and A1C.
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Supplemental appendix

Supplement to ‘t Hart L.M., Fritsche, A, Nijpels G et al. The CTRB1/2 locus affects diabetes susceptibility and treatment via the incretin pathway.

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### Supplemental Table 1. Clinical characteristics of the hyperglycemic clamp study groups.

|                        | NTR twin cohort | German cohort | Hoorn cohort | Utrecht cohort |
|------------------------|-----------------|---------------|--------------|----------------|
| n (NGT/IGT)            | 120/6           | 73/33         | 0/140        | 64/12          |
| Age (yrs)              | 31.5 ± 6.3      | 39.2 ± 12.8   | 60.4 ± 8.7   | 46.2 ± 6.6     |
| Gender (M/F)           | 60/66           | 48/58         | 69/71        | 19/57          |
| BMI (kg/m$^2$)         | 24.1 ± 6.4      | 25.7 ± 5.5    | 28.1 ± 4.0   | 25.9 ± 3.8     |
| Fasting glucose (mmol/l)| 4.6 ± 0.4     | 5.1 ± 0.7     | 6.3 ± 0.7    | 4.6 ± 0.5      |
| 2-hr glucose (mmol/l)  | 5.4 ± 1.2       | 6.6 ± 2.0     | 8.8 ± 1.7    | 5.6 ± 1.6      |
| Fasting Insulin (mmol/l)| 33 (27-51)    | 47 (30-67)    | 62 (46-90)   | 36 (24-54)     |
| 1$^{\text{st}}$ phase Glucose SIS (pmol/l) | 823 (613-1168) | 646 (461-1148) | 586 (375-889) | 852 (594-1154) |
| 2$^{\text{nd}}$ phase Glucose SIS (pmol/l) | 218 (166-365) | 228 (149-339) | 254 (172-350) | 260 (191-350) |
| GLP-1 SIS (pmol/l)     | 1225 (724-2597) | 2633 (1512-3992) | n.d. | n.d. |
| Arginine SIS (pmol/l)  | 2115 (1554-3001) | 2222 (1552-3289) | n.d. | n.d. |
| Insulin sensitivity Index (μmol·kg$^{-1}$·min$^{-1}$·pmol/l$^{-1}$) | 0.22 (0.14-0.31) | 0.12 (0.08-0.19) | 0.11 (0.07-0.17) | 0.18 (0.12-0.28) |

Data are means ± SD or median with interquartile range or n. SIS stimulated insulin secretion. n.d. not determined.
Supplemental Table 2. Clinical characteristics of the pharmacogenetic study groups.

|                      | DCS West-Friesland (n=71) | GoDARTS (n=456) |
|----------------------|---------------------------|-----------------|
|                      | GLP-1 RA                  | DPP4-           | GLP-1 RA      | DPP4-           |
|                      | 65.0 ± 10.8 *             | inhibitors      | 65.0 ± 9.7 *  | inhibitors      |
| Age (yrs)            | 58.3 ± 5.8                | 58.6 ± 8.2      | 65.0 ± 9.7    | *               |
| Gender (M/F)         | 9/13                      | 19/30           | 85/66         | 160/145         |
| BMI (kg/m^2)         | 41.6 ± 6.4                | 38.6 ± 5.7      | 33.3 ± 5.9    | *               |
| Diabetes duration (yrs) | 9.0 ± 7.6                 | 6.9 ± 3.9       | 9.6 ± 4.9     | 10.0 ± 4.9      |
|                      | vildagliptin/sitagliptin/other | 20/28/1         | 11/280/14     |
|                      | liraglutide/exenatide     | 18/4            | 83/68         | *               |
|                      |                           |                 |               |
| Time period (days)   | 591 ± 244                 | 772 ± 214 *     | 206 ± 120     | 204 ± 120       |
| Stopped other        |                           |                 |               |
| medication (% stopped) | 14%                      | 24%             | 46%           | 15% *           |
|                      |                           |                 |               |
| Pre-treatment HbA1c  | 7.9 ± 1.1; 7.3 ± 1.1; 9.5 ± 1.4; 8.8 ± 1.1; |                 |               |
| (%; mmol/mol)        | 63 ± 12; 56 ± 12 *; 80 ± 15; 73 ± 12 * |                 |               |
| Treatment HbA1c (%)  | 7.4 ± 1.3; 7.0± 1.1; 8.0 ± 1.4; 8.0± 1.4; |                 |               |
| mmol/mol             | 57 ± 14; 53 ± 12; 64 ± 15.3; 64 ± 15.3 |                 |               |
| Delta HbA1c (%)      | -0.59 ± 1.22; -0.27 ± 1.26; -1.47 ± 1.63; -0.82 ± 1.41; |
| mmol/mol             | -6.4 ± 13.3; -3.0 ± 13.8; -16.1 ± 17.8; -9.0 ± 15.4* |

Data are means with SD or n. Time period is defined as the number of days between baseline and lowest on treatment HbA1c. Stopped other medication reflects the percentage of participants that stopped other glucose lowering medication at or after the introduction of GLP-1 RA or DPP-4 inhibitor. * p<0.05 for GLP-1 RA vs DPP4 inhibitor group.
### Supplemental Table 3. Clinical characteristics of the chymotrypsin activity study.

|                  | Chymotrypsin activity study |
|------------------|-----------------------------|
|                  | All            | TT             | TG+GG          |
| Age (yrs)        | 42.6 ± 12.9    | 42.6 ± 12.9    | 42.6 ± 13.1    |
| Gender (M/F)     | 38/42          | 19/21          | 19/21          |
| BMI (kg/m²)      | 31.7 ± 4.7     | 31.5 ± 4.2     | 31.9 ± 5.2     |
| Fasting plasma glucose (mmol/l) | 5.4 ± 0.6 | 5.4 ± 0.6 | 5.4 ± 0.6 |
| Fasting plasma insulin (pmol/l) | 62 | 63 | 62 |
|                   | (46-93)        | (44-105)       | (46-88)        |
| Fasting GLP-1 (pmol/l) | n.a. | n.a. | n.a. |
| Fasting GIP (pmol/l) | n.a. | n.a. | n.a. |
| Fasting glucagon (pmol/l) | n.a. | n.a. | n.a. |
| Fecal chymotrypsin activity (U/g) | 14.5±9.1 | 12.6±7.2 | 16.5±10.4 * |

Data are means ± SD or median (inter quartile range). n.a. not available. * P<0.05 for TG+GG versus TT genotypes.

### Supplemental Table 4. Effects of SNPs associated with GLP-1 stimulated insulin secretion on other measures of beta cell function.

| SNP            | 1<sup>st</sup> phase GSIS | 2<sup>nd</sup> phase GSIS | Insulin sensitivity index | Disposition index | Arginine SIS |
|----------------|-----------------------------|-----------------------------|---------------------------|-------------------|--------------|
| rs4148941      | -0.025 (0.021) 0.24         | -0.035 (0.017) 0.42         | +0.013 (0.025) 0.60       | -0.004 (0.023) 0.86 | -0.052 (0.034) 0.12 |
| rs7202633      | +0.016 (0.015) 0.27         | +0.012 (0.013) 0.36         | +0.004 (0.017) 0.80       | +0.021 (0.017) 0.20 | +0.0654 (0.019) 5.6*10<sup>-4</sup> |
| rs7202877      | +0.033 (0.024) 0.17         | +0.016 (0.022) 0.47         | +0.006 (0.031) 0.85       | +0.022 (0.026) 0.40 | +0.073 (0.035) 0.038 |

Data are beta’s ± SE and p-values as obtained using SPSS GEE using additive (rs7202633), dominant (rs4148941) or carrier versus non-carriers genetic models (rs7202877). 1<sup>st</sup> and 2<sup>nd</sup> phase GSIS, Insulin Sensitivity Index and Disposition Index were measured in an extended cohort (n=448) as described in detail on page 2 and Supplemental table 1. Data are adjusted for age, gender, BMI, glucose tolerance status, study center and insulin sensitivity index (where appropriate). GSIS = glucose stimulated insulin secretion; Arginine SIS = arginine stimulated insulin secretion.
**Supplemental Table 5A.** Decrease in HbA1c (%) from baseline in response to GLP-1 RA treatment in Dutch type 2 diabetes patients.

| SNP          | GLP-1 RA |   |   | p-value |
|--------------|----------|---|---|---------|
|              | 11       | 12 | 22|         |
| rs4148941    | 8; -0.82±0.47 | 12; -0.56±0.38 | 2; +0.19±0.96 | 0.64 |
| rs7202633    | 5; -1.14±0.59 | 14; -0.44±0.35 | 3; -0.37±0.75 | 0.59 |
| rs7202877    | 19; -0.41±0.27 | 3; -1.73±0.68 | 0 | 0.09 |

In total 22 patients with type 2 diabetes participated in a study investigating the effects of the above mentioned gene variants on response to GLP-1 RA treatment (liraglutide n=18/ exenatide n=4). Data are n and estimated means ± SE corrected for pre-treatment HbA1c, stopped other baseline medication and time between measures.

**Supplemental Table 5B.** Decrease in HbA1c (%) from baseline in response to DPP4-inhibitor treatment in Dutch type 2 diabetes patients.

| SNP          | DPP4 inhibitors |   |   | p-value |
|--------------|-----------------|---|---|---------|
|              | 11       | 12 | 22|         |
| rs4148941    | 24; -0.22±0.22 | 18; -0.29±0.25 | 7; -0.40±0.41 | 0.93 |
| rs7202633    | 14; -0.25±0.30 | 26; -0.38±0.21 | 9; -0.02±0.38 | 0.70 |
| rs7202877    | 39; -0.43±0.16 | 10; +0.34±0.33 | 0 | 0.041 |

In total 49 patients with type 2 diabetes participated in a study investigating the effects of the above mentioned gene variants on response to DPP4 inhibitor treatment (vildagliptin n=20/sitagliptin n=28/saxagliptin n=1). Data are n and estimated means ± SE corrected for pre-treatment HbA1c, stopped other baseline medication, time between measures and BMI.
**Supplemental Table 6A.** Decrease in HbA1c (%) from baseline in response to GLP-1 RA treatment in UK type 2 diabetes patients

| SNP         | 11       | 12       | 22       | p-value |
|-------------|----------|----------|----------|---------|
| rs4148941   | 61; -1.63±0.16 | 64; -1.45±0.15 | 26; -1.16±0.24 | 0.15    |
| rs7202633   | 45; -1.54±0.19 | 77; -1.38±0.14 | 26; -1.53±0.25 | 0.85    |
| rs7202877   | 125; -1.49±0.11 | 23; -1.46±0.27 | 0         | 0.92    |

In total 151 patients with type 2 diabetes participated in a study investigating the effects of the above mentioned gene variants on response to GLP-1 RA (liraglutide n=83/exenatide n=68). Data are n and estimated means ± SE corrected for pre-treatment HbA1c, stopped other baseline medication and time between measures.

**Supplemental Table 6B.** Decrease in HbA1c (%) from baseline in response to DPP-4 inhibitor treatment in UK type 2 diabetes patients.

| SNP         | 11       | 12       | 22       | p-value |
|-------------|----------|----------|----------|---------|
| rs4148941   | 117; -0.77±0.11 | 138; -0.84±0.10 | 50; -0.92±0.17 | 0.46    |
| rs7202633   | 95; -0.88±0.12 | 159; -0.78±0.10 | 45; -0.92±0.18 | 0.99    |
| rs7202877   | 244; -0.90±0.07 | 57; -0.45±0.16 | 0         | 0.014   |

In total 305 patients with type 2 diabetes participated in a study investigating the effects of the above mentioned gene variants on response to DPP-4 inhibitor treatment (vildagliptin n=11/sitagliptin n=280/other n=14). Data are n and estimated means ± SE corrected for pre-treatment HbA1c, stopped other baseline medication, time between measures and BMI.
**Supplemental Table 7.** Phenotypes of patients treated with DPP4-inhibitors carrying different genotypes of rs7202877 near *CTRB1/2.*

| Genotype | DCS West-Friesland | GoDARTS |
|----------|--------------------|---------|
|          | TT (n=39)          | TG + GG (n=10) | TT (n=246) | TG + GG (n=58) |
| Age (yrs) | 64.7 ± 11.3 | 66.2 ± 9.3 | 65.1 ± 9.5 | 63.7 ± 10.6 |
| Gender (M/F) | 16/23 | 3/7 | 134/112 | 24/34 |
| BMI (kg/m²) | 31.6 ± 5.1 | 35.3 ± 11.5 | 33.0 ± 5.8 | 34.5 ± 6.1 |
| Diabetes duration (yrs) | 6.6 ± 3.8 | 8.2 ± 4.3 | 9.1 ± 6.1 | 8.0 ± 6.8 |
| eGFR (ml/min/1.73m²) | 80 ± 18 | 89 ± 27 | 82 ± 23 | 85 ± 23 |
| Vildagliptin/Sitagliptin/other (n) | 21/17/1 | 7/3/0 | 8/225/13 | 3/51/4 |
| Time period (days) | 773 ± 216 | 771 ± 219 | 204 ± 121 | 198 ± 111 |
| Stopped other medication (% stopped) | 26% | 20% | 14% | 16% |
| Pre-treatment HbA1c (%; mmol/mol) | 7.2 ± 1.1; 55 ± 12 | 7.4 ± 0.9; 57 ± 10 | 8.8 ± 1.1; 73 ± 12 | 8.9 ± 1.4; 74 ± 15 |
| Treatment HbA1c (%; mmol/mol) | 6.8 ± 0.9; 51 ± 10 | 7.8 ± 1.6; 62 ± 18 * | 7.9 ± 1.2; 63 ± 13 | 8.4 ± 1.8; 68 ± 20 * |
| Delta HbA1c (%; mmol/mol) | -0.43 ± 1.22; -4.7 ± 13.3 | +0.36 ± 1.29; +3.9 ± 14.1 | -0.92 ± 1.38; -10.1 ± 15.1 | -0.49 ± 1.54; -5.4 ± 16.8 * |
| Delta BMI (kg/m²) | -1.0 ± 1.5 | -0.6 ± 0.6 | -0.4 ± 1.7 | -0.3 ± 2.6 |

Data are means ± SD or n. eGFR estimated glomerular filtration rate. Time period is defined as the number of days between baseline and lowest on treatment HbA1c. Stopped other medication reflects the percentage of participants that stopped other glucose lowering medication at or after the introduction of GLP-1 RA or DPP-4 inhibitor. * p<0.05 for TG+GG vs TT using ANOVA.
### Supplemental Table 8. Clinical characteristics and results of incretin secretion during OGTT in healthy non-diabetic participants.

|                      | Dutch Hoorn meal study | Danish study | German study |
|----------------------|------------------------|--------------|--------------|
|                      | TT (n=126)             | TG+GG (n=19) | TT (n=153)   | TG+GG (n=28) | TT (n=102) | TG+GG (n=29) |
| Age (yrs)            | 53.5 ± 6.5             | 51.9 ± 7.4   | 41.0 ± 11.4  | 41.6 ± 10.7  | 43.1 ± 12.3 | 45.6 ± 10.0  |
| Gender (M/F)         | 60/66                  | 10/9         | 69/84        | 12/16        | 37/65       | 6/23         |
| BMI (kg/m²)          | 26.6 ± 3.6             | 26.3 ± 2.2   | 25.8 ± 4.6   | 25.9 ± 4.4   | 31.7 ± 7.5  | 29.3 ± 5.9   |
| Fasting plasma glucose (mmol/l) | 5.3 ± 0.3   | 5.3 ± 0.4   | 5.1 ± 0.5    | 5.1 ± 0.4    | 5.2 ± 0.4   | 5.2 ± 0.4    |
| Fasting plasma insulin (pmol/l) | 36 (25-55) | 40 (32-74)  | 34 (24-48)   | 30 (19-50)   | 52 (36-88)  | 39 (29-71)   |
| Fasting GLP-1 (pmol/l) | 10.0 (8.0-13.0) | 9.0 (5.0-14.0) | 4.5 (3.0-7.0) | 5.3 (3.5-6.4) | 17.0 (13.0-23.0) | 17.0 (10.5-21.0) |
| Fasting GIP (pmol/l) | 6.5 (1.0-10.0)        | 7.0 (2.0-15.0) | 7.0 (3.0-10.5) | 7.5 (4.5-12.0) | 14.0 (8.0-19.0) | 16.0 (9.0-22.0) |
| AUC GLP-1 (pmol/l/min) | 16.5 (12.9-23.3) | 13.5 * (11.1-15.6) | 12.2 (8.2-17.8) | 11.9 (8.6-15.1) | 30.9 (21.8-37.2) | 25.4 (21.3-42.0) |
| AUC GIP (pmol/l/min) | 36.9 (29.1-56.8)      | 36.0 (26.4-51.4) | 46.4 (34.7-58.7) | 44.9 (33.0-71.0) | 68.6 (52.5-86.0) | 75.5 (63.9-103.0) |

The Dutch Hoorn meal study population represents a random sample of the general population aged 40-65 years (n=208) from the middle-sized town Hoorn in the Netherlands who all underwent an OGTT (1). From this cohort we selected 145 normal glucose tolerant subjects for which incretin measurements and DNA were available. Secondly we used data from a Danish family study representing 61 families (2). From this study we included 181 non-diabetic participants. Finally we used data from a German cohort which includes 131 normal glucose tolerant subjects (3). All subjects participating in this study underwent a standard 2 hour 75g OGTT after overnight fasting and total GLP1 and GIP levels were measured at various time points according standard procedures as described previously (1-3). Areas under the curves (AUC) were calculated using a trapezoidal method. Data are unadjusted means with SD or median (inter quartile range). * P≤0.05 for TG+GG versus TT genotypes after correction for age, gender, BMI and fasting value.
Supplemental figure 1. Quantile-Quantile plot for GLP-1 stimulated insulin secretion during hyperglycemic clamps of SNPs in loci previously associated with QT interval.

Quantile-Quantile plot for GLP-1 stimulated insulin secretion during hyperglycemic clamps of SNPs in loci previously associated with QT interval (4;5). The genomic inflation factor was 1.014. Grey areas indicate the 95% confidence interval.
Supplemental figure 2. Regional plots of the loci associated with GLP-1 stimulated insulin secretion using the additive model.
Supplemental Figure 3. LD plot of the chromosome 16q23.1 region.

Linkage disequilibrium plot showing D’ prime in the region surrounding rs7202877 in CEU+TSI participants using HAPMAP phase III (release 2) data. Bright red indicates D’=1 while, lighter shades of red indicate D’< 1 but > 0, white indicates D’=0. The arrow indicates the position of rs7202877
References

1. Rijkelijhuizen, JM, Girman, CJ, Mari, A, Alssema, M, Rhodes, T, Nijpels, G, Kostense, PJ, Stein, PP, Eekhoff, EM, Heine, RJ, Dekker, JM: Classical and model-based estimates of beta-cell function during a mixed meal vs. an OGTT in a population-based cohort. *Diabetes Res Clin Pract* 83:280-288, 2009

2. Gjesing, AP, Ekstrom, CT, Eiberg, H, Urhammer, SA, Holst, JJ, Pedersen, O, Hansen, T: Fasting and oral glucose-stimulated levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are highly familial traits. *Diabetologia* 55:1338-1345, 2012

3. Schafer, SA, Tschritter, O, Machicao, F, Thamer, C, Stefan, N, Gallwitz, B, Holst, JJ, Dekker, JM, 't Hart, LM, Nijpels, G, Van Haeften, TW, Haring, HU, Fritsche, A: Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. *Diabetologia* 50:2443-2450, 2007

4. Morris, AP, Voight, BF, Teslovich, TM, Ferreira, TA, Segre, AV, Steinthorsdottir, V, Strawbridge, RJ, Khan, H, Grallert, H, Mahajan, A, Prokopenko, I, Kang, HM, Dina, C, Esko, T, Fraser, RM, Kanoni, S, Kumar, A, Lagou, V, Langenberg, C, Luan, J, Lindgren, CM, Muller-Nurasyid, M, Pechlivanis, S, Rayner, NW, Scott, LJ, Wiltshire, S, Yengo, L, Kinnunen, L, Rossin, EJ, Raychaudhuri, S, Johnson, AD, Dimas, AS, Loos, RJ, Vedantam, S, Chen, H, Florez, JC, Fox, C, Liu, CT, Rybin, D, Couper, DJ, Kao, WH, Li, M, Cornelis, MC, Kraft, P, Sun, Q, van Dam, RM, Stringham, HM, Chines, PS, Fischer, K, Fontanillas, P, Holmen, OL, Hunt, SE, Jackson, AU, Kong, A, Lawrence, R, Meyer, J, Perry, JR, Platou, CG, Potter, S, Rehnberg, E, Robertson, N, Sivapalaratnam, S, Stancakova, A, Stirrups, K, Thorleifsson, G, Tikkanen, E, Wood, AR, Almgren, P, Atalay, M, Benediktsson, R, Bonnycastle, LL, Burtt, N, Carey, J, Charpentier, G, Crenshaw, AT, Doney, AS, Dorkhan, M, Edkins, S, Emilsson, V, Eury, E, Forsen, T, Gertow, K, Gigante, B, Grant, GB, Groves, CJ, Guiducci, C, Herder, C, Hreidarsson, AB, Hui, J, James, A, Jonsson, A, Rathmann, W, Klopp, N, Kravic, J, Krjutskov, K, Langford, C, Leander, K, Lindholm, E, Lobbs, S, Mannisto, S, Mirza, G, Muhleisen, TW, Musk, B, Parkin, M, Ralidis, L, Saramies, J, Sennblad, B, Shah, S, Sigurdsson, G, Silveira, A, Steinbach, G, Thorand, B, Trakalo, J, Veglia, F, Wenneu, R, Winckler, W, Zabaneh, D, Campbell, H, Van, DC, Utterlinden, AG, Hofman, A, Sijbrands, E, Abecasis, GR, Owen, KR, Zeggini, E, Trip, MD, Forouhi, NG, Syvanen, AC, Eriksson, JG, Peltonen, L, Nothen, MM, Balkau, B, Palmer, CN, Lyssenko, V, Tuomi, T, Isomaa, B, Hunter, DJ, Qi, L, Shuldiner, AR, Roden, M, Barroso, I, Wilskaard, T, Beilby, J, Hovingh, K, Price, JF, Wilson, JF, Rauramaa, R, Lakka, TA, Lind, L, Dedoussis, G, Njolstad, I, Pedersen, NL, Khaw, KT, Wareham, NJ, Keinanen-Kiukaanniemi, SM, Saaristo, TE, Korpi-Hyovalti, E, Saltevo, J, Laakso, M, Kuusisto, J, Metspalu, A, Collins, FS, Mohlke, KL, Bergman, RN, Tuomilehto, J, Boehm, BO, Gieger, C, Hveem, K, Cauchi, S, Froguel, P, Baldassarre, D, Tremoli, E, Humphries, SE, Saleheen, D, Danesh, J, Ingelsson, E, Ripatti, S, Salomaa, V, Erbel, R, Jockel, KH, Moebus, S, Peters, A, Illig, T, de, FU, Hamsten, A, Morris, AD, Donnelly, PJ, Frayling, TM, Hattersley, AT, Boerwinkle, E, Melander, O, Kathiresan, S, Nilsson, PM, Deloukas, P, Thorsteinsdottir, U, Groop, LC, Stefansson, K, Hu, F, Pankow, JS, Dupuis, J, Meigs, JB, Altshuler, D, Boehnke, M, McCarthy, MI: Large-scale
association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet* 44:981-990, 2012

5. Voight,BF, Kang, HM, Ding, J, Palmer, CD, Sidore, C, Chines, PS, Burtt, NP, Fuchsberger, C, Li, Y, Erdmann, J, Frayling, TM, Heid, IM, Jackson, AU, Johnson, T, Kilpelainen, TO, Lindgren, CM, Morris, AP, Prokopenko, I, Randall, JC, Saxena, R, Soranzo, N, Speliotes, EK, Teslovich, TM, Wheeler, E, Maguire, J, Parkin, M, Potter, S, Rayner, NW, Robertson, N, Stirrups, K, Winckler, W, Sanna, S, Mulas, A, Nagaraja, R, Cucca, F, Barroso, I, Deloukas, P, Loos, RJ, Kathiresan, S, Munroe, PB, Newton-Cheh, C, Pfeuffer, A, Samani, NJ, Schunkert, H, Hirschhorn, JN, Altshuler, D, McCarthy, MI, Abecasis, GR, Boehnke, M: The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS Genet* 8:e1002793, 2012