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ARTICLE

Fasting and oral glucose-stimulated levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are highly familial traits

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Abstract
Aims/hypothesis Heritability estimates have shown a varying degree of genetic contribution to traits related to type 2 diabetes. Therefore, the objective of this study was to investigate the familiality of fasting and stimulated measures of plasma glucose, serum insulin, serum C-peptide, plasma glucose-dependent insulinotropic polypeptide (GIP) and plasma glucagon-like peptide-1 (GLP-1) among non-diabetic relatives of Danish type 2 diabetic patients.
Methods Sixty-one families comprising 193 non-diabetic offspring, 29 non-diabetic spouses, 72 non-diabetic relatives (parent, sibling, etc.) and two non-related relatives underwent a 4 h 75 g OGTT with measurements of plasma glucose, serum insulin, serum C-peptide, plasma GIP and plasma GLP-1 levels at 18 time points. Insulin secretion rates (ISR) and beta cell responses to glucose, GIP and GLP-1 were calculated. Familiality was estimated based on OGTT-derived measures.
Results A high level of familiality was observed during the OGTT for plasma levels of GIP and GLP-1, with peak familiality values of 74±16% and 65±15%, respectively (h^2±SE). Familiality values were lower for plasma glucose, serum insulin and serum C-peptide during the OGTT (range 8–48%, 14–44% and 15–61%, respectively). ISR presented

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the highest familiality value at fasting reaching 59±16%. Beta cell responsiveness to glucose, GLP-1 and GIP also revealed a strong genetic influence, with peak familiality estimates of 62±13%, 76±15% and 70±14%, respectively.
Conclusions/interpretation Our results suggest that circulating levels of GIP and GLP-1 as well as beta cell response to these incretins are highly familial compared with more commonly investigated measures of glucose homeostasis such as fasting and stimulated plasma glucose, serum insulin and serum C-peptide.

Keywords Genetic variance · Heritability · Incretins · Quantitative trait loci · Type 2 diabetes

Abbreviations

GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
ISR	Insulin secretion rate

Introduction

Fasting and stimulated levels of circulating glucose and insulin are important markers of an individual's ability to regulate blood glucose and are often used to dissect the underlying pathophysiological mechanisms and genetics of type 2 diabetes [1]. Information concerning the degree of genetic influence of various traits can assist the selection of traits for inclusion in genetic studies.

Heritability is a measure of the proportion of the total variation due to genetics and can be estimated from twin and family studies where the genetic similarity between individuals is known [2]. Previous studies have estimated the heritability of a variety of glucose homeostasis markers [3–9], overall revealing a higher level of heritability for measures of insulin secretion [10–14] compared with insulin action [5, 7, 9, 11, 15–18]. This observation is compliant with the identification of a majority of type 2 diabetes risk variants affecting beta cell function in contrast to insulin action [19]. However, despite the vast number of published type 2 diabetes risk variants as well as gene variants associated with diabetes-related quantitative traits, only a fraction (about 10–30%) of the heritability has been explained by these variants [1, 20]. Thus, multiple additional gene markers likely await discovery.

Such additional markers may influence incretin secretion and/or function. Incretins are important glucose-dependent insulinotropic hormones released from the intestine after meal ingestion. While an absent or grossly impaired incretin effect characterises type 2 diabetes [21–23], the degree to which genetics regulate measures of the incretin system has been previously investigated in only one study, showing that genes accounted for 53% of the beta cell response to exogenous glucagon-like peptide-1 (GLP-1) among 100 twins and 25 siblings (hyperglycaemic clamp) [24].

Thus, the overall aim of the present study was to further investigate the degree of genetic influence on measures of glucose homeostasis following oral glucose ingestion to assist the selection of traits for inclusion in future genetic studies. To accomplish this, we estimated the familiality of circulating levels of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) as well as glucose, C-peptide and insulin in the fasting state and at multiple time points after an oral glucose load. We then calculated the insulin secretion rate (ISR) and beta cell response to glucose, GLP-1 and GIP, and finally estimated the familiality of these indices of beta cell function.

Methods

Participants Sixty-one patients with verified type 2 diabetes mellitus according to 1999 WHO criteria [25] having four or more offspring and a spouse without known diabetes were identified through the outpatient clinic at Steno Diabetes Center (n=43) or through an ongoing family study at the University of Copenhagen (n=18). All probands had diabetes onset after 40 years of age and had no known family history of type 1 diabetes. All family members (probands, spouses and offspring) were asked to participate in the study. When available, affected parents or affected siblings and their offspring were included in the study. In total, 329 individuals underwent a 4 h OGTT, of whom 265 were glucose-tolerant and 31 had impaired glucose tolerance. Tables 1 and 2 and electronic supplementary material (ESM) Table 1 show the size, structure and physiological description of participating families. Due to secondary effects of hyperglycaemia and/or pharmacological treatment of the disease on the examined traits, we calculated familiality in non-diabetic (including non-proband) individuals only.

Informed consent was obtained from all participants prior to participation in the study. The study was approved by the Ethics Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki II.

Anthropometric measurements Height and weight were measured in light indoor clothing without shoes, and BMI was calculated as weight in kilograms divided by height in metres squared.

Biochemical measurements After a 12 h overnight fast, venous blood samples were drawn in the morning for analysis of plasma concentrations of glucose, GIP and GLP-1, and serum levels of insulin and C-peptide. The plasma glucose concentration was analysed by a glucose oxidase

Table 1 Size and structure of the examined Danish families and total number of individuals in the families

Families	Total family size												
	4	5	6	7	8	9	10	11	12	13	15	19	38
Nuclear families (n=43)	4	9	18	6	1	3		1		1			
Nuclear families+1 half-sib (n=5)			2	2	1								
Nuclear families+1–2 avuncular (n=7)						2	3	2					
Three-generation family (n=1)					1								
Complex families (n=5)								1	1		1	1	1
Total individuals	16	45	120	56	24	45	30	44	12	13	15	19	38

method (Granutest; Merck, Darmstadt, Germany). Serum insulin was determined by ELISA with a narrow specificity excluding des(31,32)- and intact proinsulin [26]. The serum concentration of C-peptide was determined by RIA employing the polyclonal antibody M1230 [27–29]. All blood samples for GIP and GLP-1 analysis were kept on ice and the protease inhibitor aprotinin (Novo Nordisk, Bagsværd, Denmark) was added in a concentration of 0.08 mg/ml blood. GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (vol./vol., final concentration). For the GIP radioimmunoassay [30] we used the C-terminally directed antiserum R 65, which cross-reacts fully with human GIP, but not with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion is uncertain. Human GIP and ^{125}I-labelled human GIP (70 MBq/nmol) were used for standards and tracer. The detection limit was 5 pmol/l. Plasma concentrations of GLP-1 were measured [31] against standards of synthetic GLP-$1_{(7–36)}$ amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore does not react with GLP-1-containing peptides from the pancreas. The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-$1_{(9–36)}$ amide, into which GLP-1 is rapidly converted [32]. For both assays, sensitivity was below 1 pmol/l, intra-assay coefficient of variation was below 0.06 at 20 pmol/l, and recovery of standard, added to plasma before extraction, was about 100% when corrected for losses inherent in the plasma extraction procedure.

OGTT After 12 h of fasting, venous blood samples were drawn in triplicate with 5 min intervals and immediately cooled on ice until performance of cooled centrifugation. All non-diabetic participants underwent a standard 75 g OGTT with frequent blood sampling (at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 140, 160, 180, 210 and 240 min). Plasma glucose, serum insulin and serum C-peptide levels were analysed in duplicates at all time points. Plasma GIP and plasma GLP-1 were analysed in duplicate from samples obtained in the fasting state and from time points 10, 20, 30, 40, 60, 90, 120, 180 and 240 during the OGTT.

Table 2 Descriptive characteristics of non-diabetic family members who were included in calculating familiality estimates in Fig. 1

Characteristic	Non-diabetic individuals			
	Spouses	Offspring	Other relatives	Other non-related
n (men/women)	29 (8/21)	193 (87/106)	72 (32/40)	2 (1/1)
Age (years)	66 (6)	38 (8)	46 (12)	53 (15)
BMI (kg/m^2)	28 (5)	26 (5)	28 (5)	29 (2)
Fasting plasma glucose (mmol/l)	5.4 (0.6)	5.1 (0.5)	5.2 (0.5)	5.4 (0.03)
Fasting serum insulin (pmol/l)	53 (47)	40 (27)	47 (35)	48 (30)
Fasting plasma GLP-1 (pmol/l)	5.0 (3.6)	5.1 (3.2)	6.9 (4.1)	3.5 (0)
Fasting plasma GIP (pmol/l)	7.7 (6.5)	9.3 (12.3)	7.2 (6.3)	7.3 (8.1)
Plasma glucose$_{120\ min}$ (mmol/l)	7.1 (1.3)	5.9 (1.4)	6.4 (1.5)	6.2 (1.9)
Serum insulin$_{120\ min}$ (pmol/l)	417 (368)	224 (224)	278 (188)	244 (158)
Serum GLP-1$_{120\ min}$ (pmol/l)	14.1 (10.2)	12.2 (6.3)	15.7 (7.6)	12 (7.0)
Serum GIP$_{120\ min}$ (pmol/l)	50.0 (20.3)	53 (39)	50 (19)	44 (13)

Values are mean (SD)

Spouses, spouses to probands; offspring, offspring to probands; other relatives, relatives such as parents, grandchildren and siblings to probands; other non-related, spouses to siblings, spouses to children, etc.

Statistical analysis Familiality was estimated from a polygenic model as the proportion of the additive genetic variation to the total variation, which is also the formula for the (narrow sense) heritability. However, based on the available phenotypes, we were unable to estimate heritability, as we have little information regarding shared environment. Thus, we use the term familiality instead of heritability to emphasise that the resulting estimate not only provides information about genetic similarity. Familiality was estimated adjusting for sex, age and BMI using the program SOLAR (http://txbiomed.org/departments/genetics/genetics-detail?P=37). Since adiposity is a known risk factor for diabetes, all traits were also analysed without BMI as a covariate, but the exclusion of BMI in the model only changed the familiality slightly. Ascertainment correction was not included in analyses as OGTT was not performed among probands. However, fasting values for plasma glucose, serum insulin, serum C-peptide, plasma GIP and plasma GLP-1 were available in probands, and ascertainment-corrected analyses were conducted for these traits. This correction decreased familiality by approximately 10% and reduced the confidence intervals by approximately ±5% compared with estimates calculated in non-diabetic participants only (ESM Table 2); yet, the order of magnitude of the familiality estimates was unaltered subsequent to ascertainment correction. Traits not satisfying the assumption of normality were either log- or cubic-root transformed prior to analysis. When traits remained to show some degree of kurtosis, the tdist function was applied by which t distribution modelling was used rather than a normal distribution, which restores the accuracy of the results.

The familiality estimates for fasting glucose, insulin and C-peptide were calculated from the mean of three fasting values, and the familiality estimates for fasting GIP and GLP-1 were calculated from the mean of two observations. The familiality estimates of various traits measured after glucose ingestion are calculated from the measured value at each time point after the oral glucose load minus the fasting value (the incremental value). The AUC was calculated using the trapezoidal method. ISR (pmol kg^{-1} min^{-1}) were estimated from measured serum C-peptide concentrations using deconvolution [33]. This mathematical operation calculates the secretion rate based on predefined C-peptide kinetic parameters from each individual's weight, height, age, sex and clinical status (glucose tolerance and obesity status) determined in a population-based study [33, 34]. ISEC software, which implements all these factors [35], was applied for the present study (ESM). ISR were plotted against plasma levels of glucose, GLP-1 or GIP to establish the beta cell responsiveness to glucose or incretin hormones for each individual. The relationship was assumed to be linear in all participants and the slope of the line was used as an index of beta cell response to glucose or incretins (ESM) [36].

Results

The phenotypic characteristics, treatment and age of diabetes onset of the probands did not differ from 500 unrelated, consecutively sampled diabetic patients from the outpatient clinic of the Steno Diabetes Center (data not shown). The quantitative traits obtained during the OGTT were examined in a total of 296 non-diabetic individuals (Table 2, ESM Table 1). These individuals include first-degree relatives (556 relationships), second-degree relatives (86 relationships), third-degree relatives (106 relationships), fourth-degree relatives (96 relationships) and unrelated individuals (25 individuals). This combination of relationships enabled us to exclude some degree of shared environment.

None of the examined traits exhibited a significant dominating genetic effect (i.e. $\sigma_d^2 = 0$), and the familiality results shown are all estimated without this variation component. Also, familiality estimates have been performed with and without inclusion of individuals with impaired glucose tolerance. The estimates were slightly higher when calculations were performed among glucose-tolerant individuals only compared with calculations among individuals having either impaired glucose tolerance or normal glucose tolerance (ESM Table 1). We have also calculated the familiality estimates at all time points using the actual measured trait values or the relative incremental values (measured value divided by fasting value) and these estimates were in the same range as the presented familiality estimates using incremental trait values (data not shown).

Overall, familiality estimates ranged from 8% to 76%. The familiality estimate for plasma glucose was 43±13% (h^2±SE) in the fasted state and between 8±13% (90 min) and 48±14% (240 min) after glucose ingestion (Fig. 1). The familiality estimates calculated from the incremental $AUC_{0–240\ min}$ for glucose were within the same range as the familiality for the individual time points, with approximately 30% of the variation resulting from genetic resemblance between relatives (Table 3). Familiality estimates for serum insulin were 40±14% during fasting and between 14±12% (20 min) and 44±18% (180 min) in the stimulated state (Fig. 1). $AUC_{0–240\ min}$ for insulin showed a low level of familiality of 24±14% (Table 3). Familiality estimates for both fasting and incremental values of serum C-peptide

Fig. 1 Observed means and SD among non-diabetic participants for ▶ plasma glucose (**a**), serum insulin (**c**), serum C-peptide (**e**), plasma GLP-1 (**g**), plasma GIP (**i**) levels and ISR (**k**) at different time points during an OGTT. For the same time points, the corresponding familiality estimates (h^2±2SE) for incremental values of plasma glucose (**b**), serum insulin (**d**), serum C-peptide (**f**), plasma GLP-1 (**h**), plasma GIP (**j**) and ISR (**k**), respectively, after correcting for age, sex and BMI are shown. *Insignificant familiality estimate using a threshold of 0.05

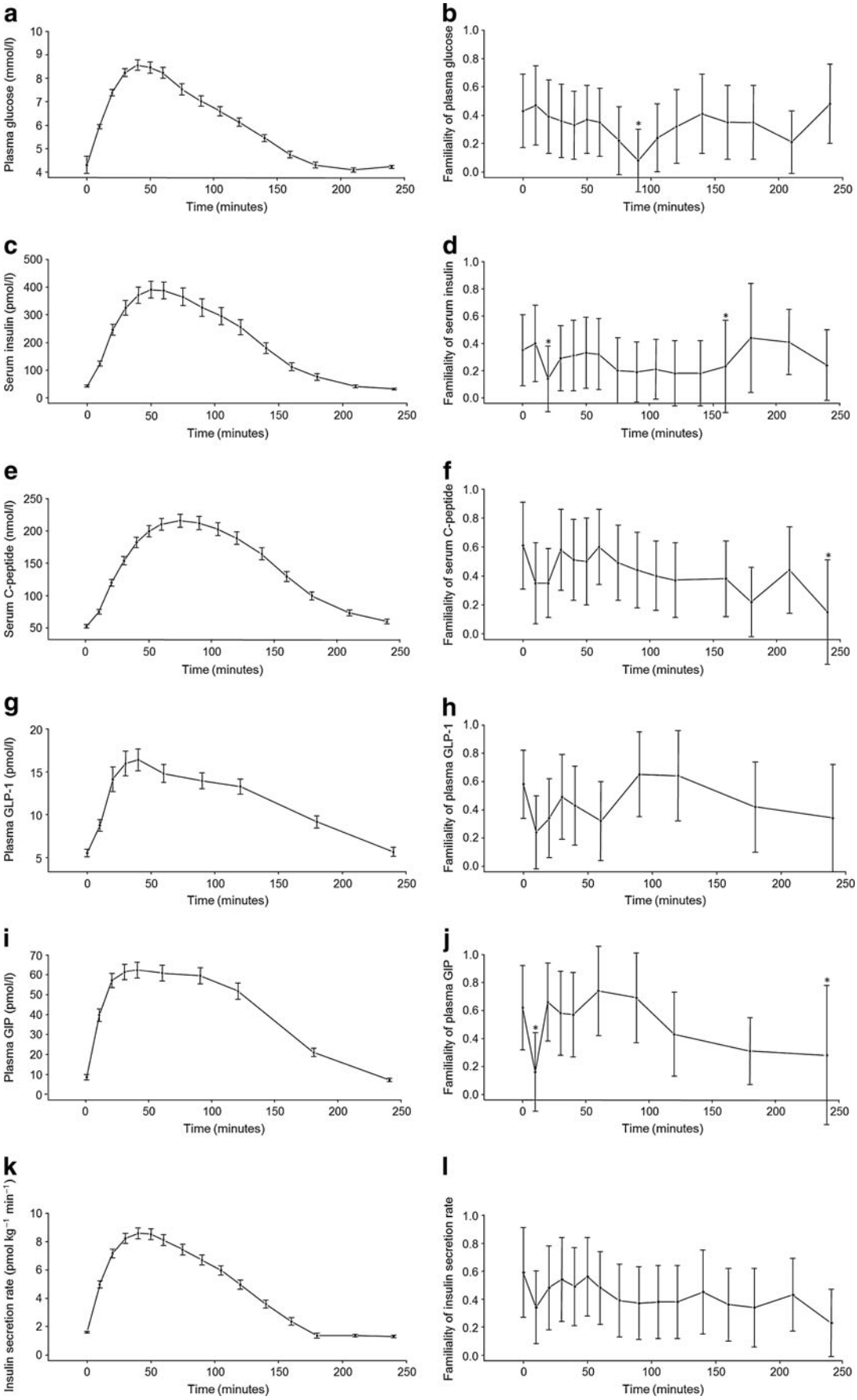
a
Plasma glucose (mmol/l)
Time (minutes)
b
Familiality of plasma glucose
Time (minutes)
c
Serum insulin (pmol/l)
Time (minutes)
d
Familiality of serum insulin
Time (minutes)
e
Serum C-peptide (nmol/l)
Time (minutes)
f
Familiality of serum C-peptide
Time (minutes)
g
Plasma GLP-1 (pmol/l)
Time (minutes)
h
Familiality of plasma GLP-1
Time (minutes)
i
Plasma GIP (pmol/l)
Time (minutes)
j
Familiality of plasma GIP
Time (minutes)
k
Insulin secretion rate (pmol kg−1 min−1)
Time (minutes)
l
Familiality of insulin secretion rate
Time (minutes)

Table 3 Estimates of familiality for OGTT-derived traits (h^2±SE) in non-diabetic relatives of type 2 diabetic patients

Trait	h^2±SE
Plasma glucose $IAUC_{120\ min}$	0.34±0.12***
Plasma glucose $IAUC_{240\ min}$	0.29±0.12*
Serum insulin $IAUC_{120\ min}$	0.28±0.14*
Serum insulin $IAUC_{240\ min}$	0.24±0.14*
Serum C-peptide $IAUC_{120\ min}$	0.60±0.15***
Serum C-peptide $IAUC_{240\ min}$	0.60±0.15***
Plasma GIP $IAUC_{120\ min}$	0.60±0.16***
Plasma GIP $IAUC_{240\ min}$	0.69±0.15***
Plasma GLP-1 $IAUC_{120\ min}$	0.60±0.14***
Plasma GLP-1 $IAUC_{240\ min}$	0.66±0.15***
ISR (pmol/kg) $IAUC_{120\ min}$	0.40±0.13***
ISR (pmol/kg) $IAUC_{240\ min}$	0.53±0.14***

Values are familiality ± SE

*p<0.05, ***p<0.001; p-values represent the significance of the familiality

IAUC, incremental AUC

were higher than for serum insulin and peaked 60 min after glucose ingestion, reaching a familiality of 60±15% (Fig. 1). Also, $AUC_{0–240\ min}$ for C-peptide showed that 60±15% of the variation could be attributed to genetic similarity between family members (Table 3). Based on measured serum C-peptide concentrations, we calculated ISR, which is a more accurate measure of prehepatic insulin secretion. Familiality for ISR ranged between 23% and 56%, with the highest genetic influence before glucose ingestion (Fig. 1).

Familiality estimates for both fasting plasma GIP (62±15%) and fasting plasma GLP-1 (58±8%) were high (Fig. 1); however, estimates of plasma GLP-1 and more particularly plasma GIP levels lowered 10 min after glucose ingestion. Both GLP-1 and GIP levels returned to a high level for the remaining duration of the OGTT (Fig. 1). Throughout the time course of the OGTT, both GIP and GLP-1 displayed an overall high estimate of $AUC_{0–240\ min}$ of 69±15% and 66±15%, respectively (Table 3).

The ability of the beta cell to respond to stimulus from insulinotropic substances can be estimated by using the linear relation between ISR and levels of glucose, GLP-1 or GIP. This was estimated for the first hour as well as during the full OGTT (Table 4). The familiality of the beta cell responsiveness to GIP was similar between the two time intervals (1 h: 71±14%; 4 h: 70±14%), whereas the responsiveness to glucose and GLP-1 appeared under stronger genetic influence considering the full duration of the test (62±13% and 76±15%, respectively) compared with the first hour (47±14% and 54±1%, respectively).

Table 4 Estimates of familiality (h^2±SE) in non-diabetic relatives of type 2 diabetic patients of the beta cell responsiveness to glucose, GLP-1 and GIP within the first hour of an OGTT and the full duration of the glucose challenge

Trait	h^2±SE
Beta cell responsiveness to oral $glucose_{0–60\ min}$ (mmol kg^{-1} min^{-1})	0.47±0.14***
Beta cell responsiveness to oral $glucose_{0–240\ min}$ (mmol kg^{-1} min^{-1})	0.62±0.13***
Beta cell responsiveness to $GLP\text{-}1_{0–60\ min}$ (pmol kg^{-1} min^{-1})	0.54±0.15***
Beta cell responsiveness to $GLP\text{-}1_{0–240\ min}$ (pmol kg^{-1} min^{-1})	0.76±0.15***
Beta cell responsiveness to $GIP_{0–60\ min}$ (pmol kg^{-1} min^{-1})	0.71±0.14***
Beta cell responsiveness to $GIP_{0–240\ min}$ (pmol kg^{-1} min^{-1})	0.70±0.14***

Data are familiality ± SE

***p<0.001; p-values represent the significance of the familiality

Discussion

In this study of Danish type 2 diabetes families, we used measurements of plasma glucose, serum insulin, serum C-peptide, plasma GIP and plasma GLP-1, as well as estimations of prehepatic ISR obtained from a 4 h OGTT, to estimate familiality of various prediabetic quantitative traits. We demonstrated high levels of familiality for fasting and OGTT-stimulated values of both plasma GIP and GLP-1 levels.

Generally, we found that fasting plasma glucose and serum insulin were traits with modest but significant familiality estimates of 43% and 35%, respectively, which is in line with previous studies in pedigrees and twins [8, 11, 12, 16, 37, 38]. Fasting serum C-peptide showed a stronger familiality (61%) than fasting serum insulin; yet, considering the standard errors, the familiality of the two traits are not substantially different. The only study previously investigating the heritability of fasting serum C-peptide reported a lower genetic impact compared with fasting serum insulin among 811 non-diabetic relatives [39].

We also undertook a detailed time course study during the OGTT of the familiality of plasma glucose, serum C-peptide and serum insulin to identify possible peaks with a high degree of familiality, but observed only minor fluctuations in familiality when considering the observed confidence intervals. However, a short-term decrease in familiality estimates of incretins, C-peptide and insulin was observed 10 min after glucose ingestion, which may be related to an increased phenotypic variation in gastric emptying, the number of GIP- and GLP-1-secreting cells in the upper intestinal tract, the amount of hormone stored in these cells and/or the amount of dipeptidyl peptidase-4 locally affecting the hormone concentrations at this time point. By contrast, no

common time point or time interval was identified with a consistently high degree of familiality for all traits. This may imply that different genes influence oral glucose-stimulated levels of plasma glucose, serum C-peptide and serum insulin levels.

A heritability study based on a meal challenge among 149 twins and 34 siblings also included measures of ISR and reported a fasting heritability of 43%, incremental $AUC_{0–30\ min}$ of 47% and $AUC_{30–120\ min}$ of 42%, with the remaining variance of ISR attributed to the unique environment [13]. The familialities of ISR in the present study are of the same level as the heritabilities reported previously, supporting the earlier observation that ISR variations indeed are explained by an additive genes/unique environment model.

The heritability of beta cell responsiveness to glucose has previous been reported to be 50% [13], which is concordant with the outcome of the present study. However, in the present study, beta cell responsiveness to glucose as well as to GLP-1 was subjected to a stronger genetic influence when measured over a period of 4 h compared with the first hour. Therefore, the immediate insulin response appears to be less influenced by genetics compared with the stabilisation of plasma glucose several hours after glucose ingestion. By contrast, both the acute and long-term beta cell response to GIP appears equally highly regulated by genetics. Thus, the response of the beta cell to GLP-1 (especially the slower response) and to GIP could be of great interest to further investigate in relation to the missing heritability of type 2 diabetes.

Overall, familiality estimates among glucose-tolerant individuals were slightly higher than familiality estimates in non-diabetic individuals. This was not surprising as glucose-tolerant individuals have less overall phenotypic variation, leaving a higher proportion of the total phenotypic variation accredited to genetics. Inclusion of probands (mean age 65 years) lowered the familiality estimates. This is not surprising considering that the highest heritability for type 2 diabetes is present in individuals aged between 35 and 60 years [40].

In conclusion, using a variance component approach, we have estimated the familiality of type 2 diabetes-related quantitative traits in non-diabetic relatives of type 2 diabetic patients. The traits were derived from values obtained in the fasting state and at several time points during an extended OGTT. A high degree of familiality was found in particular for circulating GIP and GLP-1 levels, for beta cell response to GIP and GLP-1 and a more modest degree of familiality was found for the fasting and oral glucose-stimulated levels of plasma glucose, serum insulin and serum C-peptide.

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Duality of interest T. Hansen and O. Pedersen hold personal shares in Novo Nordisk. The remaining authors declare that there is no duality of interest associated with this manuscript.

Contribution statement CTE, HE, SAU, JJH, OP and TH contributed to the conception and design of the study. APG, CTE, JJH, OP and TH contributed to the analysis and interpretation of data. APG, CTE, OP and TH contributed to drafting the article. All authors have revised manuscript critically for important intellectual content and approved the final version of the paper.

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