Chapter 8

Summary and conclusions

Summary

This thesis describes an experimental study in healthy MZ and same-sex DZ twins and siblings registered at the Netherlands Twin Register. The main aim was to estimate the heritability of different aspects of the β-cell function and to identify part of the genes causing this heritability. A total of 77 twin families were successfully included in the study. In well over 3 years (2004-2007), 190 screening OGTTs were performed at home and 189 mixed meal tests at the clinical research unit. Moreover, euglycaemic-hyperinsulinaemic clamps and modified extended hyperglycaemic clamps with three different stimuli (glucose, GLP-1 and arginine) were performed in 130 twins and siblings that were willing to participate in all three investigations. The first part of the thesis describes the procedures and analyses that were performed to obtain a heritability estimation of glycaemia and β-cell function parameters derived from above mentioned tests. By using the multivariate extension of the twin design it could be tested to what extent different glycaemia and β-cell function parameters are influenced by the same genetic factors, and to what extent β-cell genetic factors are independent of the genetic factors influencing body composition and insulin sensitivity. The second part describes two studies of the association between genetic variants that increase the risk for type 2 diabetes mellitus and some of the β-cell function parameters. This chapter provides a summary of the results, the main conclusions and suggestions for further research.

HbA1c and fasting blood glucose

HbA1c and fasting blood glucose are both used as diagnostic parameters for type 2 diabetes mellitus. Chapter 3 estimated the heritability of these traits and examined their phenotypic and genetic correlation structure. Heritability of HbA1c was estimated at 75%. Fasting blood glucose was measured in three different settings (pre-OGTT at home, pre-meal test and pre-clamp test in the clinic). The heritability of fasting blood glucose was different across these settings (range 38% to 66%). However, the genetic correlation between them was high ($0.53 < r < 0.95$) and I concluded that gene finding efforts may safely pool FBG samples from different settings. The most remarkable finding was the small and non-significant correlation between fasting blood glucose (FBG) and HbA1c, and that FBG assessed in the three different settings appeared to have no overlapping genetic influences

with HbA1c. I concluded that these two glycaemic parameters cannot be used interchangeably in diagnostic procedures or in studies attempting to find genes for diabetes. Both contribute unique (genetic) information.

The insulin response of the β-cell to a mixed meal

Chapter 4 used the mixed meal test to assess the heritability of classical and mathematical model derived β-cell function parameters during a real physiologic challenge. The heritabilities of waist circumference and insulin sensitivity, with the formula of Oral Glucose Insulin Sensitivity (OGIS), were also estimated because these variables are known to be associated with β-cell function. The results showed significant heritabilities of most of the classical but only some of the model derived β-cell function parameters. The insulinogenic index, an important parameter of early insulin response and an independent predictor of worsening glucose tolerance, had the highest heritability (63%) of the post prandial parameters, but one third of this heritability was shared with the genetic influences on waist and OGIS. The model derived β-cell glucose sensitivity, which quantifies the ability of the β-cell to respond to changes in glucose concentration and is a significant independent predictor of glucose intolerance, had a high heritability (50%) with a negligible overlap with waist and OGIS. Fasting insulin level and fasting insulin secretion rate (ISR) had comparable heritability estimates (38% and 43% respectively) but the fasting insulin secretion rate may be a better measure of the activity of the β-cell than fasting insulin level, because insulin level is strongly co-determined by insulin clearance. The incremental ISR during the first two of the four postprandial hours showed a significant heritability in the first 30 minutes (47%) as well the next one and a half hour (42%). However, the genetic influences on ISR in the first 30 minutes had only a negligible overlap with waist and OGIS, while one third of the heritability of the ISR during the next one and a half hour was shared with waist circumference and OGIS. My conclusion was that the mixed meal test provides multiple heritable aspects of the β-cell function that can help us examine the biology underlying the wealth of genetic variants produced by genome wide association studies. Most promising parameters are the model derived β-cell glucose sensitivity and the insulin secretion rate in the first 30 minutes, because they are relatively independent of body composition and insulin sensitivity.

The insulin response of the β-cell to different secretagogues

Chapter 5 explored the heritability of the insulin response of the beta cell during a modified version of the hyperglycaemic clamp test used by Fritsche and colleagues. The heritability of the first phase (52%) and second phase (77%) glucose stimulated insulin response were estimated, as well as the heritability of the insulin response to additional GLP-1 (53%) and GLP-1 + arginine (80%). From this, I concluded that genetic factors explain most of the individual differences in insulin response after administration of glucose and glucose combined with GLP-1 or GLP-1 + arginine in healthy adults.

The heritabilities of BMI and insulin sensitivity (ISI) were assessed on the same day (74% and 60% respectively), the latter by the euglycaemic-hyperinsulinaemic clamp. We found that the genetic variance unique to β-cell function, i.e. independent of the genetic factors influencing BMI and ISI, contributed less strongly to individual differences in the first-phase response (only 14%) than in the second-phase response (30%) or in the responses to additional GLP-1 (36%) and GLP-1 + arginine (37%). Hence, I concluded that the often used first-phase response may give an incomplete picture of the genes that are specific to beta cell function. I further concluded that the genetic factors influencing the β-cell function are partly the same as the factors that influence BMI and ISI, and that in genetic designs 'correction' for BMI and ISI may not always be desirable.

Association between type 2 diabetes mellitus related gene variants and β-cell function parameters

Chapter 6 and 7 describe two studies on the association between established (chapter 6) and new (chapter 7) type 2 diabetes mellitus related gene variants and β-cell function. Up till now mainly OGTT data were used in genetic association studies on β-cell function. The novelty of our studies was the use of hyperglycaemic clamps, including the clamp that combined three different stimuli (glucose, GLP-1 and arginine). Because of multiple hypothesis testing results were regarded significant at $P \leq 0.008$ (six tests).

In the first study the combined risk allele score based on eight proven beta cell loci (*TCF7L2*, *KCNJ11*, *HHEX/IDE*, *CDKAL1*, *IGF2BP2*, *SLC30A8, CDKN2A/B* and *MTNR1B*) was used. Data came from three independent studies in the Netherlands (Hoorn, Utrecht and NTR/VUmc Amsterdam) and one study from Tübingen, Germany. Only the rapid first phase glucose stimulated insulin secretion (GSIS) and the disposition index (DI =

first phase GSIS x ISI) were significantly inverse associated with this combined risk allele score. In contrast, the slower second phase GSIS, GLP-1 and arginine stimulated insulin secretion and insulin sensitivity were not associated. Furthermore we observed a strong correlation between our combined risk allele score and the absence of a first phase insulin peak in our subjects with IGT which is a strong predictor of future development of type 2 diabetes mellitus. We concluded that these eight β-cell loci seem to act mainly via detrimental effects on processes involved in the early, rapid recruitment and exocytosis of insulin granules after glucose stimulation.

The aim of the second association study, described in chapter 7, was to assess separately the association of each of the 12 genetic risk alleles in recently detected type 2 diabetes mellitus loci with β-cell function parameters. Data came from the above mentioned three independent Dutch clamp studies. The only association with insulin sensitivity was found in carriers of the T risk allele in *THADA,* who showed a significant lower ISI. An increased first phase GSIS was associated with the C risk allele of the *ADAMTS9* gene, while carriers of the TT risk genotype of the *BCL11A* locus had a lower first phase GSIS. Risk variants in the *CDC123/CAMK1D* gene and the T risk allele in *THADA* were associated with a significantly decreased second phase GSIS. GLP-1 and arginine induced insulin secretion were reduced in the homozygous *THADA* TT risk genotype, although not always statistically significant, suggesting lower beta cell mass as a possible pathogenic mechanism.

Remarkably, carriers of the risk allele of the *MTNR1B* gene had, although not statistically significant, increased responses to GLP-1 (+30%, p=0.03) and arginine stimulation (+19%, p=0.037). If replicated, these results indicate that carriers of the G risk allele may well benefit from treatment with GLP-1 agonists or dipeptidyl-IV inhibitors, which may offer new therapeutic possibilities. The *ADAMTS9* risk allele was associated with a higher disposition index in contrast to the risk alleles for *BCL11A* and *MTNR1B* that were significantly associated with a decreased disposition index. Seven of the 12 risk alleles in the recently discovered type 2 diabetes mellitus loci showed no association with any β-cell function parameter. We concluded that type 2 diabetes mellitus risk alleles in *CDC123/CAMK1D, THADA, ADAMTS9, BCL11A* and *MTNR1B* are associated with specific aspects of β-cell function. These findings point to a clear diversity in the impact that these different gene variants may have on (dys)function of pancreatic β-cells.

Main conclusions

- FBG and HbA1c are heritable traits but cannot be used interchangeably because both contribute unique (genetic) information.
- FBG samples from different settings may safely be pooled in gene finding efforts.
- Classical and model derived β-cell function parameters, used in a mixed meal test, show a significant heritability and represent different aspects of β-cell function.
- The β-cell glucose sensitivity and the insulin secretion rate during the first 30 post prandial minutes provide the most specific genetic information of the β-cell function after an oral challenge.
- In the hyperglycaemic GLP-1/arginine challenge test genetic factors explain most of the individual differences in insulin response after intra venous administration of the three different secretagogues.
- The responses to glucose combined with GLP-1 and GLP-1 + arginine are the best indicators of β-cell function, while the often used first phase GSIS may give an incomplete picture of the genes that are specific to β-cell function.
- The combined score of type 2 diabetes mellitus risk alleles in *TCF7L2*, *KCNJ11*, *CDKAL1*, *IGF2BP2*, *HHEX/IDE*, *CDKN2A/B, SLC30A8* and *MTNR1B* is mainly associated with a decreased first phase glucose induced insulin secretion and a lower disposition index.
- Type 2 diabetes mellitus risk alleles in *CDC123/CAMK1D, THADA, ADAMTS9, BCL11A* and *MTNR1B* are each associated with specific aspects of β cell function.
- There is not one single test that can give comprehensive genetic information about the function of the β-cell. Instead, the mixed meal test and the hyperglycaemic GLP-1/arginine challenge test each contribute unique genetic information about the function of the β-cell.

Suggestions for further research

In this twin-family study a tremendous amount of data was obtained, of which only a part could be analysed and presented in this thesis. It is important that these valuable data (including serum specimens in the freezer, autonomic nervous system and blood pressure measurements, food consumption questionnaires) are used in further research. Such future studies may reveal the genetic and environmental contribution to individual variation in hormone secretion during a meal (e.g. proinsulin, incretins and glucagon). In addition it could be investigated whether there is a relation between the autonomic nervous system responses (e.g. measured by blood pressure, heart rate variability or pre-ejection period responses) and β-cell function during meal and clamp tests, and whether genetic factors are involved in this relation. The questionnaires about food consumption give the possibility to investigate the relation between the consumption of different nutrients and β-cell function. The additional use of the research materials gathered during my studies will hopefully contribute to a better understanding of the development of type 2 diabetes mellitus. In view of the growing impact of this disease on daily life of millions of people, there is much to be gained if we can improve primary prevention and further optimize our treatment strategies.