The genetics of type 2 diabetes

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Chapter 1

Introduction

Glucose homeostasis

Glucose levels are tightly regulated in the human body. Even after food-intake or fasting, glucose levels remain relatively stable. The major organs involved in regulating glucose levels are the pancreas, liver, skeletal muscle and adipose tissue. In the pancreas beta-cells are present, which are part of the Islets of Langerhans. Triggered by increased glucose levels these beta-cells will secrete insulin. The main target tissues of insulin action are liver, skeletal muscle and adipose tissue. Insulin promotes uptake of glucose from the periphery in adipoytes and muscle. The liver is involved in gluconeogenesis in which glucose is synthesized from C-3 compounds such as lactate, glycerol and pyruvate, derived from amino acids and by glycolysis. The rate limiting step of gluconeogenesis is the activity of the enzyme phosphoenolpyruvate carboxykinase (PEPCK). In the presence of high insulin levels, PEPCK expression is suppressed, yielding a decrease in gluconeogenesis. In addition, in the liver and muscle insulin regulates the balance between glycogenesis, which forms glycogen from glucose and glycogenolysis, yielding glucose from glycogen breakdown. Glycogen is a polysaccharide chain, which is an efficient form of glucose storage. In the presence of high insulin and low glucagon levels (the latter produced by pancreatic alpha-cells within the islets of Langerhans), glucose is converted into glycogen, whereas in situations with low insulin and high glucagon concentrations, glycogen is degraded into glucose. Furthermore, insulin increases glucose uptake predominantly by skeletal muscle and adipose tissue resulting in a drop of blood glucose levels. After uptake, excess of glucose is either stored as glycogen (muscle) or converted into triglyceride (adipocytes). In addition it may be degraded into lactate via a process called glycolysis and further into CO_2 via the tricarboxylic acid cycle and oxidative phosphorylation when metabolic energy is needed. These degradations are coupled to ATP synthesis as described in more detail later in this introduction. Moreover, insulin inhibits lipolysis and increases triglyceride synthesis in the adipose tissue. These processes are reviewed in references 1-3.
Taken together, increased glucose levels will result in insulin secretion by beta-cells leading to decreased gluconeogenesis by the liver and increased glucose uptake by skeletal muscle and adipose tissue. In addition glycogenesis is

increased while glycogenolysis is decreased. Together, this will result in a drop in glucose levels and subsequently decreased insulin secretion preserving the physiological glucose levels around 5 mmol/L. This is important because high levels of glucose (hyperglycaemia) cause damage to blood vessels and nerves by chemical modification of proteins, while low levels of glucose (hypoglycaemia) can results in dysfunction of the brain leading to coma and death (1-3).

Diabetes

Diabetes Mellitus is a common disease and its prevalence is rapidly increasing. It is expected that the global burden of diabetes mellitus is 300 million people in 2025, while this was 150 million in 2000 (4). The disease is divided in several subtypes; type 1 diabetes and type 2 diabetes being the most common forms. Both subtypes are characterized by chronic hyperglycaemia defined as having fasting plasma glucose (FPG) concentrations above 7.0 mmol/L (4-6). The prevalence of especially type 2 diabetes exhibits an explosive growth in societies with a western life style.

Long term hyperglycaemia can lead to several complications like micro-vascular complications in the eye and kidney and neurologic complications. In addition, type 2 diabetes is strongly associated with an increased risk for cardiovascular disease due to macro-vascular complications. The latter is predominantly responsible for the increased mortality (6-9). Next to type 1 diabetes and type 2 diabetes several other specific subgroups of diabetes exist like Maturity Onset Diabetes of the Young (MODY) and Maternally Inherited Diabetes and Deafness (MIDD). Both are high penetrance, monogenic forms of the disease, caused by single nucleotide polymorphisms (SNP). Another diabetes subtype is Gestational Diabetes Mellitus (GDM), which occurs in pregnant women. Women who suffered from GDM are more likely to develop type 2 diabetes later in life (6). Latent Autoimmune Diabetes in Adults (LADA) is yet another subtype of diabetes mellitus. This syndrome associates with autoimmunity against glutamic acid decarboxylase. The disease is often diagnosed as type 2 diabetes because of its late onset (10).

Type 1 diabetes and type 2 diabetes

Type 1 diabetes is characterized by an absolute insulin deficiency, most commonly caused by auto-immune destruction of insulin producing beta-cells in the pancreas (6). The disease has an early onset with a peak at 12 years of age. Patients are fully dependent on exogenous insulin, administered by injections (11). There are genetic risk factors, determining the vulnerability to develop type 1 diabetes. HLA haplotypes account for ~50% of the familial clustering of type 1 diabetes. Genome wide association studies (described in more detail later in this introduction) have now increased this number to 12 loci (12;13).

Type 2 diabetes is a disease with a gradual onset, mostly later in life. In most cases the disease manifests above 40 years of age with a peak at 60 years. Type 2 diabetes patients show a relative rather than an absolute insulin deficiency in combination with an insulin resistant state in which target tissues have an impaired reaction on insulin. When the beta-cells cannot produce sufficient amounts of insulin to correct for the increased demand, hyperglycaemia will develop.

Patients are initially treated by diet and life style interventions, aimed at reducing the level of insulin resistance by weight loss and enhanced muscle glucose uptake, independent of insulin, by physical exercise. When this approach fails pharmaceutical treatment is initiated. Commonly used drugs include metformin, which suppresses hepatic glucose production and sulfonylurea which triggers additional insulin secretion by pancreatic beta-cells independent of glucose. Gradually, the severity of the disease progresses which can lead to insulin dependency (6;14).

Several risk factors for type 2 diabetes are known, like ageing, overweight, low levels of physical activity and genetic variation. The increased prevalence of type 2 diabetes is most likely caused by western life style, which means overfeeding and too little exercise. Which genes are involved in the onset of type 2 diabetes, is not completely clear (14;15). Because type 2 diabetes is a very heterogeneous disease it is difficult to make a good estimation of the heritability, but a Danish twin study has resulted in an estimation up to 72%, depending on the applied model (16). In theory, genetic risk factors may directly affect the risk for type 2 diabetes, e.g. by modulating the capacity of the beta cells to secrete insulin or by inducing insulin

resistance. Genetic variation may also indirectly affect the risk for type 2 diabetes, e.g. by affecting the satiety feeling after food intake as excessive food intake enhances the risk for diabetes. This distinction is important when interpreting data from genetic studies

Genetics of type 2 diabetes

In the last decades efforts were made to identify type 2 diabetes genes, using the candidate gene approach and linkage studies in which the transmittance of an allele or locus is examined in families with multiple cases of type 2 diabetes. When applying candidate gene studies, the genes to be examined are selected based on data on their direct or indirect involvement in glucose homeostasis. Genetic variation, most commonly single nucleotide polymorphisms (SNPs), in these genes is measured in a sample of subjects with and without type 2 diabetes. Such sample could be a population based study, which is a random selection of subjects from a population. The allele frequency of a SNP is than compared between healthy participants and type 2 diabetes patients in the selected population. More commonly case-control studies are used, which are selected groups of control and type 2 diabetes subjects. Matching can be performed e.g. for age and BMI as these are major additional determinants for type 2 diabetes susceptibility. The allele frequency of the SNP in the type 2 diabetes group is compared to the control group. If the allele frequency is enriched in the type 2 diabetes group, the allele is assumed to predispose for type 2 diabetes susceptibility. The benefit of a case-control study compared to a population based study is that it is a better controlled experiment. This approach is well powered for common SNPs with a Minor Allele Frequency (MAF) above 5% even if the penetrance is low, as long as the selected population is of suitable size. A different approach is the linkage study. In linkage studies SNPs or loci are measured in family pedigrees and the transmittance of a risk allele or risk loci to type 2 diabetes family members is analyzed. The latter approach is more applicable for rare variants with high penetrance (minor allele frequency (MAF) below 1%), but is underpowered for SNPs with a low penetrance. Both approaches require replication of novel findings in additional populations. It is important that a replication study is of suitable size in order to obtain sufficient

statistical power. Differences in for instance ethnicity of participants in the studies could result in a false negative replication. Therefore, a replication study should be well designed.

The linkage studies were quite successful in identifying the genes for the MODY subtypes and for MIDD, which were found to be monogenetic diseases, caused by SNPs with a high penetrance. These approaches had, however, very little success in identifying the genes for common type 2 diabetes. Only three type 2 diabetes genes were identified via case-control studies: Peroxisome Proliferator-activated Receptor Gamma (*PPARG*), Calpain10 (*CAPN10*) and Potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*). The effect of these genes on the risk for type 2 diabetes in the general population was found to be small (17-21). The only gene which was associated with type 2 diabetes with a higher impact was Transcription Factor 7-like2 (*TFC7L2*) (OR ~1.35), which was found in an Icelandic population and widely replicated in various ethnic populations. This gene was identified by extensive analysis of a genomic region which showed evidence for linkage with type 2 diabetes in previous studies and therefore its identification was not the result from a classical candidate gene study (21;22).

More progression in identifying type 2 diabetes susceptibility genes was made when genome wide association studies (GWAS) were developed. Using dense arrays with up to 500,000 SNPs a large proportion of genetic variation in the human genome was covered. Because the GWAS approach covers up to 500,000 SNPs randomly spread through the human genome, the outcome is not biased by candidate gene hypothesis. However, the statistical drawback of testing 500,000 SNPs is that the p-value has to be corrected for 500,000 tests. Using Linkage Disequilibrium (LD) even more SNPs can be assessed. If there is no recombination hotspot between SNPs, they will not be separated by recombination during meiosis and therefore be in high LD and inherited together. So, a group of SNPs which are in between two recombination hotspots will be in high LD. Such group of SNPs is called an LD-block. Using this knowledge one SNP can be predicted by genotyping of several others. Therefore, association of a SNP can be tested without directly genotyping the SNP. This strategy is called imputation. Data of linkage between SNPs is available on the HapMap database (www.HAPMAP.org) (23-25). Using

this strategy it is currently possible to test the association of up to 2,000,000 SNPs with type 2 diabetes from GWAS data. The p-value has to be corrected for 2,000,000 tests when this strategy is applied and only a p-value smaller then $2.5x10^{-8}$ can be considered as statistical significant. In order to reach such small p-values very large study samples have to be used consisting of up to 50,000 participants and even more.

GWAS allows the identification of common SNPs that have a relatively low effect on type 2 diabetes susceptibility. However, it does not allow the detecting of rare and low frequency SNPs (MAF < 5%) with a modest effect on type 2 diabetes susceptibility. These studies are underpowered to detect such associations. Moreover, the coverage of low frequency and rare variation on GWAS genotyping chips is relatively low and much variation will therefore be missed.

After several GWAS and a meta-analysis of three of these, the list of known type 2 diabetes loci was expanded to 20, all with marginal effect sizes (OR 1.05 – 1.35) (21;26-32). Most of the newly identified SNPs are not located within the introns or exons of a gene and the pathogenic mechanism of associated SNPs is often unknown. If there is a suggested mechanism, it is mostly involved in decreased insulin secretion via an impaired beta-cell function. Remarkably, only one gene was identified as an insulin resistance gene (*PPARG*). This gene controls fatty acid metabolism and by that, indirectly insulin resistance. Insulin resistance was considered a major candidate pathway to be involved in the pathogenesis of type 2 diabetes (table 1).

The development of GWAS, during the preparation of this thesis, has dramatically changed the approach of genetic association studies. Therefore, my PhD-project starts with ‘old-fashioned’ candidate gene studies and is expanded with GWAS data, which came available after completion of our association studies.

Table 1. Confirmed type 2 diabetes susceptibility loci

Gene (nearest)	Strategy	Mechanism	Location
PPARG	Candidate gene	Insulin sensitivity/ lipid metabolism	Exon
CAPN10	Linkage	Glucose transport	Intron
KCNJ11	Candidate gene	beta-cell function	Exon
TCF7L2	Candidate region	beta-cell function	Intron
CDKAL1	GWAS	beta-cell function	Intron
CDKN2A/B	GWAS	beta-cell function	Intergenic
HHEX/IDE	GWAS	beta-cell function	Intergenic
SLC30A8	GWAS	beta-cell function	Exon
IGF2BP2	GWAS	beta-cell function	Intron
WFS1	Candidate gene	Unknown	Intron
TCF2	GWAS	Unknown	Intron
FTO	GWAS	Obesity	Intron
NOTCH2	GWAS	Unknown	Intron
ADAMTS9	GWAS	Unknown	Intergenic
THADA	GWAS	Unknown	Exon
TSPAN8/LGR5	GWAS	Unknown	Intergenic
CDC123/CAMK1D	GWAS	Unknown	Intergenic
JAZF1	GWAS	Unknown	Intron
KCNQ1	GWAS	beta-cell function	Intron
MTNR1B	GWAS	beta-cell function	Intron

Confirmed type 2 diabetes loci. OR ranging between 1.05 and 1.35.

Adapted from Ridderstrale M et al, Mol.Cell Endocrinol (2009) (21).

Scope of the thesis

The aim of this thesis was to elucidate the role of genetic variation in type 2 diabetes susceptibility and related traits, in particular in genes involved in mitochondrial function. The experimental part of this thesis is divided in three parts.

1. **Nuclear encoded mitochondrial proteins**: In the first part (chapters 2 and 3) we have examined the relation between SNPs in nuclear encoded mitochondrial candidate genes and the risk for type 2 diabetes. We selected these mitochondrial targeted genes because we hypothesized that mitochondrial function is associated with type 2 diabetes, as described in this section.
2. **Mitochondrial DNA content and type 2 diabetes:** In this part (chapter 4) we have investigated whether mitochondrial DNA content is associated with the risk for type 2 diabetes.
3. **Genes regulating fasting plasma glucose concentrations**: In this part (chapter 5) we have analyzed the association of four combined fasting plasma glucose genes with glucose levels and type 2 diabetes susceptibility.

The main topic is genetics of type 2 diabetes. The first two parts describe the role of mitochondria in type 2 diabetes; with a distinct focus on candidate genes (part 1) and mitochondrial DNA content (part 2). The third part does not focus on mitochondria, but describes cytosolic targeted genes influencing FPG levels.

Finally the results obtained during this PhD-project are summarized and discussed in chapter 6.

Part1: Nuclear encoded mitochondrial proteins

Mitochondria and energy homeostasis

Mitochondria are the organelles in cells which are responsible for most of the adenosine triphosphate (ATP) production. Furthermore, they play a role in oxidative removal of fatty acids and of metabolites from amino acids. They are also involved in apoptosis. Because mitochondria have a crucial role in ATP production, cells requiring high levels of ATP like cardiac and skeletal muscle, neurons and beta-cells show a high mitochondrial density. Mitochondria have their own genome, which encodes for only a small fraction of mitochondrial components. It is estimated that a mitochondrion contains ~1500 different proteins. Only 13 of these are encoded by the mitochondrial genome, the remaining are nuclear encoded. In addition, the mitochondrial genome encodes for 2 rRNAs and 20 tRNAs (33;34). The mitochondrial genome is described in more detail in part 2 of this introduction.

Oxidative Phosphorylation

Cells take up glucose by different glucose transporters. Only muscle and adipocytes contain Glut4 transporters which are translocated to the cell membrane in response to insulin. In the cytosol glucose is metabolized into pyruvate via glycolysis. Pyruvate is transported into the mitochondria where it is further converted into CO_2 by the Tricaboxylic acid cycle (TCA cycle). This cycle yields hydrogens in form of Nicotinamide Adenine Dinucleotide (oxidized form) (NADH) and Flavin Adenine Dinucleotide reduced ($FADH_2$). These hydrogens are oxidized to H_2O and the energy that is released by this oxidation is converted into chemical energy in form of ATP. This conversion is performed by the respiratory chain, which consists of 5 enzyme complexes, embedded in the mitochondrial inner membrane. The electrons flowing from H to O during the oxidation of NADH and $FADH_2$ enter the respiratory chain at complex 1 in case of NADH and at complex 2 in case of $FADH_2$ oxidation. At complex 4 the electrons are transferred onto oxygen. The flow of electrons through the respiratory chain is coupled to a proton pumping outwards the mitochondrial matrix by complex I, III and IV. This results in formation of a proton gradient with excess of protons at the outside of the inner

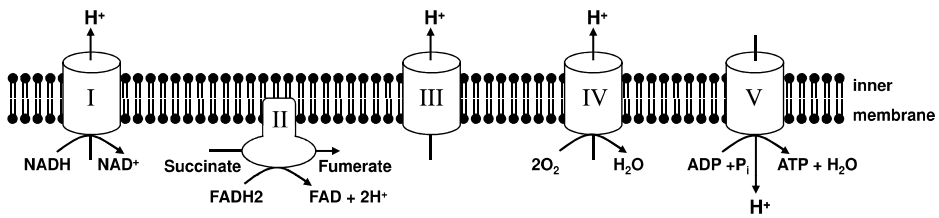
mitochondrial membrane. The back flow of protons through complex 5 drives the synthesis of ATP from adenosine diphosphate (ADP) (35-39). This process is called oxidative phosphorylation and is shown in figure 1. Normally, the respiratory chain is not active, even when NADH, FADH2 and oxygen are present. Only when ADP is generated by catabolic activity, the increased ADP level activates the respiratory chain. Thus, cells can only oxidize glucose, fatty acids and amino acids when ADP is generated by metabolic activity. Activation of the respiratory chain is coupled to conversion of ADP into ATP. In addition, mitochondria can exist in an uncoupled state. In this situation, protons flow back into the mitochondrial matrix, independent of ATP synthesis, with generation of heat as result. By that, uncoupled mitochondria can oxidize large amounts of fuel. Uncoupling proteins and chemical agents like dinitrophenol and high concentrations of fatty acids induce mitochondrial uncoupling.

The complexes from the respiratory chain consist of ~90 subunits encoded by both the nuclear and mitochondrial genome. Since the mitochondrial genome encodes for only 13 of these subunits the majority of subunits are encoded by the nuclear genome (37).

Insulin secretion and mitochondria

In the pancreatic beta-cell, mitochondria play a key role in insulin secretion. Human beta-cells express high Km Glut2 transporters, which are present at the cell membrane and transport glucose into the cytosol. Glucose is phosphorylated into glucose-6-phosphate by a beta cell specific hexokinase, called glucokinase. This is also a high Km enzyme (40-42). Subsequently, glucose-6-phosphate is further

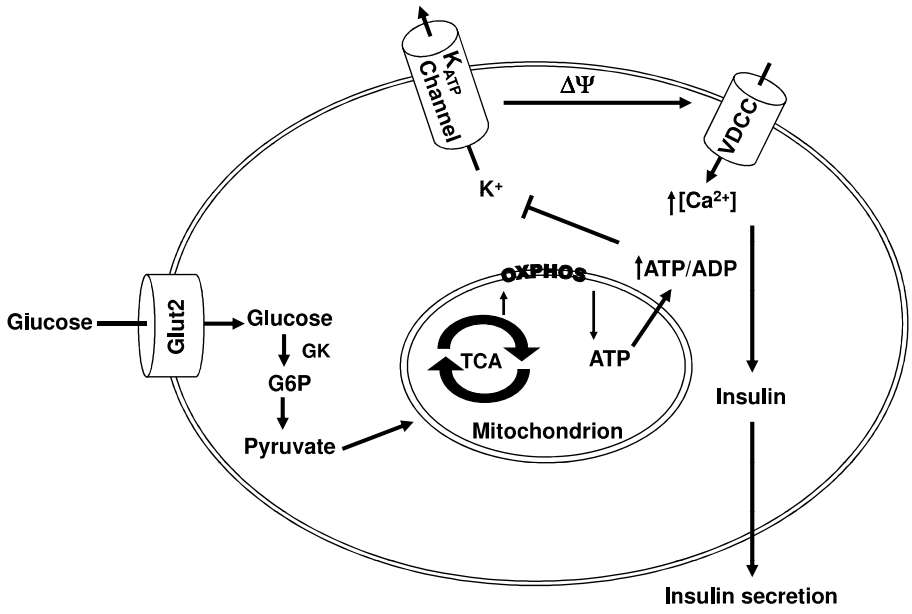
Figure 1. Oxidative phosphorylation by the respiratory chain



Complexes I – V are shown, embedded in the inner mitochondrial membrane. These complexes generate a proton flow directed outwards the mitochondrion, resulting in ATP synthesis by complex V.

metabolized resulting in the synthesis of ATP via processes described in the previous section. The presence of two high Km enzymes at the entrance of this glycolytic pathway makes that the flux through this pathway is very sensitive to variations in glucose concentrations around the physiological concentration of 5mmol/L (40;41). Therefore, variations in the ATP/ADP ratio in the cytoplasm depends on variations in the glycemic state. An increase in plasma glucose levels will increase the ATP/ADP ratio. An increased ATP/ADP ratio inhibits the ATP sensitive potassium channel, resulting in depolarization of the cell membrane. This activates the voltage-dependent calcium channel (VDCC) leading to an increased Ca^{2+} concentration in the cytoplasm, which is the main trigger for insulin secretion by fusion of insulin granules with the cell membrane (38;42). This process is shown in figure 2. Mitochondrial dysfunction could lead to an impaired glucose induced insulin secretion and subsequently type 2 diabetes.

Figure 2. Glucose stimulated insulin secretion



Glucose is transported into the beta-cell and processed into pyrovate. This enters the mitochondrion and is further processed into ATP, which is the main trigger for insulin secretion

Insulin resistance and mitochondria

Mitochondria are also involved in the oxidation of fatty acids. This process is called beta-oxidation and degrades fatty acids into acetyl-CoA, which is subsequently oxidized through the TCA cycle. In physically active muscle, in which large amounts of ADP are generated, fatty acids are the main fuel for ATP-synthesis. Fatty acids normally induce insulin resistance in muscle cells when insulin-stimulated glucose uptake is considered. This physiological adaptation process ensures that during fasting, when carbohydrate is scarce and fatty acids are generated by lipolysis in adipocytes, the physically active muscle uses fatty acids for energy supply, so that sufficient glucose remains available to provide the brain with energy since the brain uses predominantly glucose as fuel.
An inherited defect in mitochondria is often associated with triglyceride deposits in muscle, suggesting impaired removal of fatty acids. A similar situation is observed in HIV-patients treated with Highly Active Anti Retroviral Therapy containing nucleoside analogues, in which mitochondrial DNA content is decrease (43;44). This excess of fatty acids contributes to insulin resistance of muscle and liver, a situation also seen in type 2 diabetes. By this mechanism a mitochondrial dysfunction may contribute to the development of insulin resistance and type 2 diabetes (45-47). Furthermore, fatty acids are toxic to pancreatic beta-cells leading to a decline in insulin secretory capacity.

Aim of Part 1

Evidence is accumulating that mitochondrial dysfunction is a risk factor for the onset of type 2 diabetes. It has been shown that mitochondrial encoded enzymes of the oxidative phosphorylation are down regulated in type 2 diabetes patient muscle and these muscles have an impaired bioenergetic capacity (48;49). As described above, proper mitochondrial function is involved in both insulin secretion and insulin sensitivity. Alterations in these processes are the hallmarks of type 2 diabetes. Relatively rare mutations in the mitochondrial DNA are shown to be associated with MIDD in part through a decreased insulin secretion (50;51). Furthermore, it has been shown that insulin resistant offspring of type 2 diabetes patients have an impaired mitochondrial activity. However, common SNPs in the

mitochondrial genome are not found to be associated with type 2 diabetes (52;53). Since the majority of mitochondrial proteins are encoded by the nuclear genome and translocated to the mitochondria, we hypothesized that defects in nuclear encoded mitochondrial proteins may be associated with type 2 diabetes. The aim of this part is:

Analyzing the association of nuclear encoded mitochondrial targeted genes with type 2 diabetes susceptibility.

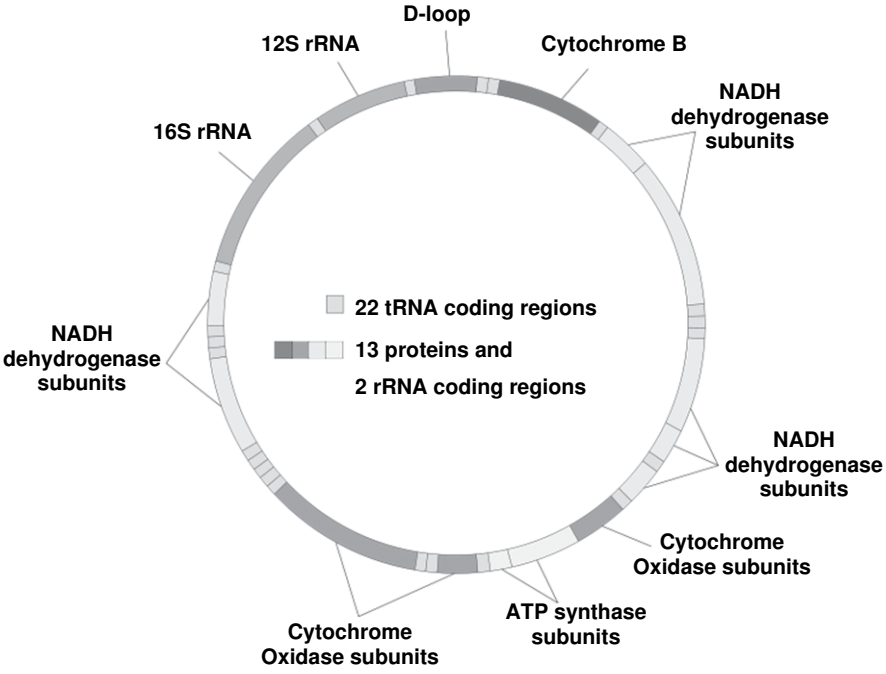
In chapter 2 I describe our search for an association of various SNPs in the *LARS2* gene with type 2 diabetes. The protein encoded by this gene is involved in mitochondrial protein synthesis. It encodes the charging enzyme for the mitochondrial leucyl-tRNA(UUR). A mutation in the mitochondrial leucyl-tRNA(UUR) gene can result in MIDD, but also mitochondrial myopathy, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) and chronic progressive external ophthalmoplegia (CPEO) (50;54). A particular polymorphism in the *LARS2* gene was previously found to be associated with type 2 diabetes (55). I investigated potential associations between several SNPs in *LARS2* with type 2 diabetes in various cohorts from the Netherlands, UK, Denmark Sweden and Finland.
In chapter 3 we have analyzed a selection of 13 candidate genes, all encoding mitochondrial proteins, for association with type 2 diabetes in the Netherlands and putative associations are replicated in cohorts from the Netherlands and Denmark. These candidate genes were selected from four clusters regarded essential for correct mitochondrial protein synthesis and biogenesis: aminoacyl tRNA synthetases, translation initiation factors, tRNA modifying enzymes and mitochondrial DNA transcription and replication.

Part 2: mitochondrial DNA content and type 2 diabetes

Mitochondrial DNA

As described in part 1 of this introduction, mitochondria have their own genome. This circular genome is approximately 16.6 kb in length and encodes for 13 genes of the oxidative phosphorylation, 2 rRNAs and 20 tRNAs (figure 3). Mitochondrial DNA (mtDNA) is predominantly maternally inherited. The quantity of mtDNA, the so-called mtDNA content, varies between different cell types. Cells contain approximately 1000 – 10,000 mtDNA copies (37;56). Mitochondrial activity in human fibroblasts declines upon aging, probably by accumulating somatic mutations in their DNA (57;58).

Figure 3. Map of mitochondrial DNA



Mitochondria have their own DNA of ~16.6 kb, encoding for all tRNAs, 2 rRNAs and 13 subunits for the oxidative phosphorylation.

Replication of mitochondrial DNA

Replication of mtDNA is not dependent on cell division, but there is continuous mtDNA turn-over. Mitochondrial DNA is synthesized by DNA polymerase gamma (POLγ), a RNA-dependent DNA polymerase. This enzyme consists of 2 subunits, POLγA and POLγB. The proteins replicative mitochondrial helicase (TWINKLE) and mitochondrial Single-Stranded DNA-Binding protein (mtSSB) are responsible for unwinding and stabilizing of the mtDNA respectively (56;59-62). Mice expressing a proof reading defective POLγA, are characterized by accumulation of point mutations and deletions in the mtDNA, resulting in decreased life span and aging phenotypes like weight loss, hear loss and osteoporosis (63).

Transcription of mitochondrial DNA

Transcription of mtDNA is directed from two promotor sites, the Heavy Strand Promotor and Light Strand Promotor. Transcription is performed by the Mitochondrial RNA Polymerase (POLRMT). POLRMT forms a complex with the Mitochondrial Transcription Factor A (TFAM) and one of the two other transcription factors; Mitochondrial Transcription Factor B1 (TFB1M) or Mitochondrial Transcription factor B2 (TFB2M), which play important roles in DNA binding, unwinding, and prevention of RNA/DNA hybrid formation. After transcription, the mRNA is processed into proteins by the mitochondrial protein synthesis machinery consisting of nuclear encoded proteins and two mtDNA encoded rRNAs (56;64-66).

Aim of Part 2

As described in part1, mitochondrial function is altered in type 2 diabetes patients. Whether this is a cause or consequence is largely unknown. It has been shown that patients treated with highly active antiretroviral therapy have 30-50% decreased mtDNA content and are at increased risk for developing diabetes and the metabolic syndrome. Furthermore, a reduction of peripheral fat and development of central obesity is observed (44;67). This indicates that a reduction in mtDNA content is rather a cause than consequence of type 2 diabetes development. It has also been shown that mitochondrial content declines during aging in pancreatic islets and decreased mitochondrial content in beta-cells is

associated with decreased insulin secretion (68;69). Furthermore, a small study showed evidence that low mtDNA content in blood precedes the development of type 2 diabetes, further suggesting that mtDNA content may contribute to the development of type 2 diabetes (70). In a twin study it has been observed that mtDNA content is higher correlated in monozygotic twin pairs, compared to dizygotic twin pairs in blood, indicating that regulation of mtDNA content has a genetic component (71). Another study showed that this heritability might be linked with a genomic region on chromosome 10q (72). Taken together variation in the mtDNA content is a plausible candidate to modulate the risk for type 2 diabetes. The aim of this part is:

Analysis of the heritability of mitochondrial DNA content in different tissues in relation to the risk for type 2 diabetes.

In *chapter 4* the heritability of mtDNA content is examined, using monozygotic and dizygotic twins, derived from the Dutch Twin Register. Mitochondrial DNA content is analyzed in samples obtained by buccal swabs. These cells represent a more homogenous cell sample compared to whole blood which was used in other studies. Furthermore, the association between mtDNA content and the onset of type 2 diabetes is examined in a case control study in the Netherlands and in two prospective studies from the Netherlands and Sweden.

Part3: Genes regulating fasting plasma glucose concentrations

Plasma glucose levels are tightly regulated. Despite fluctuations in food intake and physical exercise, the variation in plasma glucose is limited. Deregulation of the glucose homeostasis may lead to hyperglycemia, which is the major hallmark of type 2 diabetes. Variation of FPG levels within healthy limits (FPG < 7 mmol/L) is clinical important as it has been found that FPG levels in the higher region of the healthy range result in an elevated risk for heart disease and type 2 diabetes later in life (8;73-76). Furthermore, FPG levels in pregnant women are an important predictor of the offspring birth weight, which is associated with the development of type 2 diabetes later in life of the offspring (77;78). FPG levels are approximately 50% genetically determined (79). Therefore, a genetic predisposition for increased FPG levels may also represent an elevated risk for type 2 diabetes. A main component of the glucose sensing system, controlling FPG, consist of the pancreatic Glut2-Glucokinase system and its downstream pathway (80).

The aim of this part of my thesis is:

To analyze the effect of known FPG genes on FPG levels and subsequent risk for type 2 diabetes.

The results of this part are described in *chapter 5*. Using a population based study in the Netherlands we examined the effects of known FPG genes on several clinical variables like FPG and HbA_{1C}. Next we used a case-control study from the same region in the Netherlands to investigate the combined effect of these established FPG genes on type 2 diabetes risk.

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