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Clinical genetics of Type 1 Diabetes Genetic correlates of early growth and disease progression

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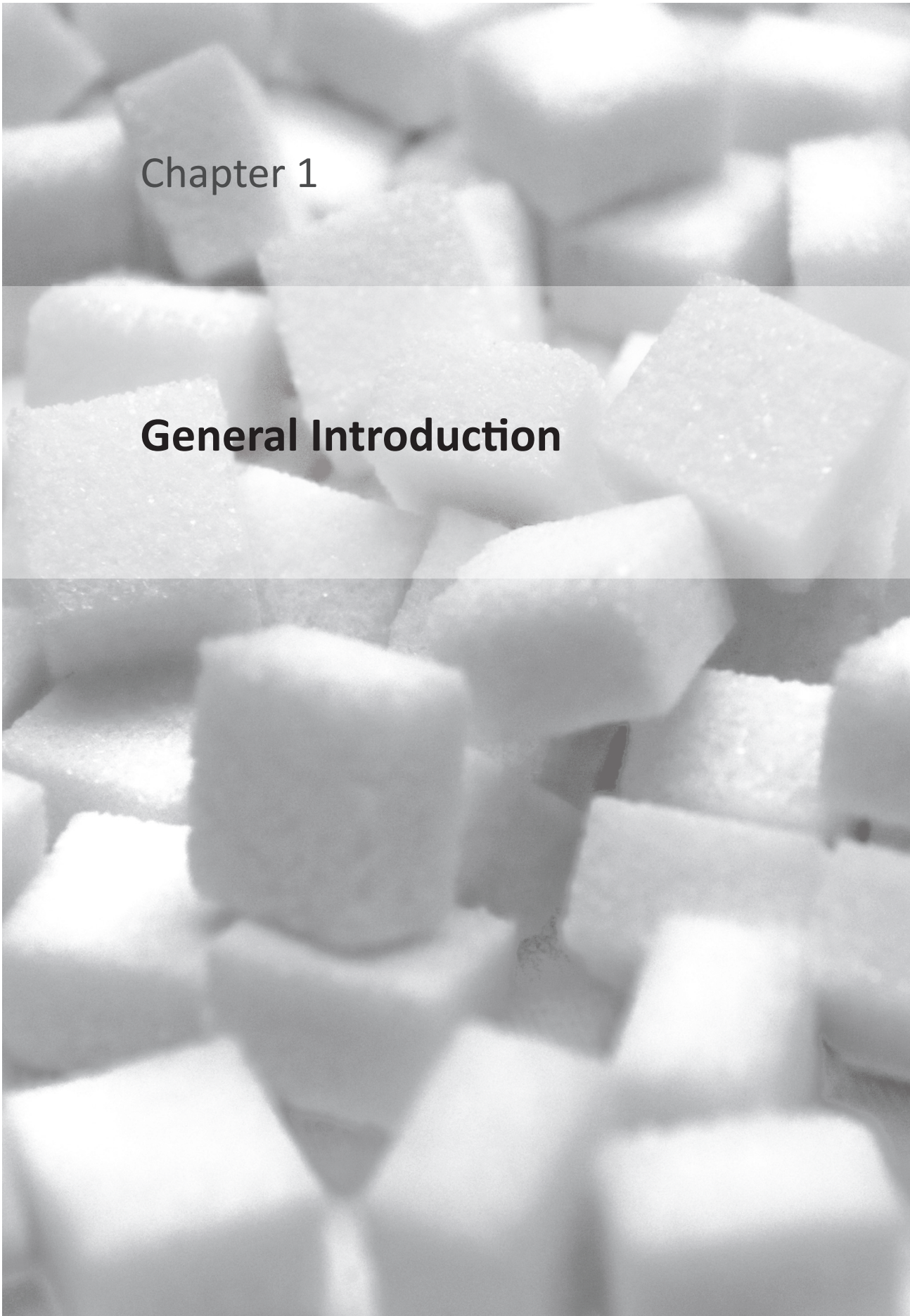

Chapter 1

General Introduction

Between 400 and 500 BC the Hindu physicians Charaka and Susruta for the first time recognized and described the sweet taste of diabetic urine. They correlated an increased bodyweight with this typical disease and inferred that it was a disease mostly contracted by the rich, consuming excessive amounts of rice, starch and sugar. At the same time they observed the existence of a lean more serious phenotype, whilst recognizing that this disease could be passed down from generation to generation (Frank 1957; Pickup and Williams 2003). In the 2nd century AD, Aretaeus was the first to use the term "diabetes" [Greek meaning siphon to signify the extensive fluid loss], while John Rollo [1809] added the adjective mellitus [honey]. Later pioneers: Adolf Kussmaul [1822-1902] described the typical breathing pattern of a patient with severe acidosis; Oskar Minkowski [1851-1931] reported pancreatectomy of a dog leading to diabetes; Paul Langerhans [1847-1888] who in his early twenties was the first to describe the clusters of clear cells [islands] throughout the gland without speculating about their function. All are still honoured today in the diabetes community (Frank 1957; Pickup and Williams 2003)

Diabetes is a chronic metabolic disease due to a relative or absolute deficiency of insulin. This deficiency leads to high blood glucose levels and eventually gives rise to organ damage. Worldwide there are approximately 220 million people diagnosed with diabetes. The diagnosis of diabetes is made on clinical and laboratory findings as published in the summary of revisions from the 2009 Clinical Practice Recommendations (2009). The disease is often classified into two types: type I and type 2 diabetes. The majority of patients have the latter form, although it is recognised that the distinction between the two types does not fully reflect the clinical spectrum [see **table 1**]. Therefore the classification of the several forms of diabetes based on aetiology is regularly revised, and remains a continuous debate (Gale 2006; Leslie, Cain et al. 2008; 2009).

Table 1 |

Classification of DM	
I	Type 1, a/b: auto-immune/ non auto-immune
II	Type 2
III	Other specific types of diabetes
IV	Gestational diabetes

Type 1 diabetes is the second most common chronic childhood disease in the western world with a variable geographic incidence of 3 to 40 per 100 000 per year. However, the majority of newly manifested T1D is diagnosed in adults. Since many of these patients are misdiagnosed as T2D, the true prevalence of T1D in the Netherlands is unknown, but exceeds 0.3% of the Dutch population.

An increase in incidence has been observed worldwide in the last decades (2006; Patterson, Dahlquist et al. 2009). These epidemiological trends also show (Evertsen, Alemzadeh et al. 2009) an increase of childhood onset type 1 diabetes in younger age groups with a predicted doubling in this group by the year 2020. The prevalence of childhood [<16 years of age] onset T1D in the Netherlands is approx 1 per 1000 children (van Wouwe, Mattiazzo et al. 2004); males and females equally are affected. The cause of this increase is unknown, but conceivably involves the interaction between genetic predisposition and environment. This thesis will address that interaction.

Clinical Manifestation and Therapy

Clinical manifestations of type 1 diabetes appear when a substantial part of the beta cells have been destroyed [~80%]. At this point, residual functioning beta cells still exist but are insufficient to maintain glucose tolerance [figure 1]. Prior to this phase is a preclinical phase in which insulin secretory function is impaired.

The events that initiate the transition from glucose intolerance to frank diabetes are often associated with increased insulin requirements, as occurs during infections or increases in growth [for example in puberty]. Following initial insulin treatment of diabetes patients, a "honeymoon" phase, also known as clinical remission, may occur during which time glycaemic control is achieved with modest doses of insulin or, rarely, no insulin at all. Disease remission develops in approximately 50% of patients, with a variable duration of weeks to months.

Although this phenomenon is long known by diabetologists, it is still an ill-defined phase in pathophysiological context. The assumed explanation, i.e. beta cell recovery as a response to exogenous insulin treatment, seems to be incomplete. Important features postulated in this honeymoon phase are the enhanced beta cell insulin secretion and decreased insulin resistance (Block, Rosenfield et al. 1973; Yki-Jarvinen and Koivisto 1984; Agner, Damm et al. 1987; Lombardo, Valenzise et al. 2002). Presently, beta-cell mass cannot be determined, and can only be estimated through beta-cell function (insulin release in response to hyperglycaemia) as a surrogate measure. This maintains the gap in knowledge on disease progression or temporary clinical remission. Even though beta-cell function is an imperfect correlate of beta-cell mass, it serves its duties when progression of beta-cell loss or possibly recovery, repair or regeneration in case of clinical remissions needs to be determined. In this thesis, beta-cell function will be used as correlate of beta-cell mass, to define mechanisms of disease progression and remission.

The consequences of diabetes are potentially devastating: especially subjects with type 1 diabetes require immediate insulin therapy, bringing along the risk of hypoglycaemia in addition to the

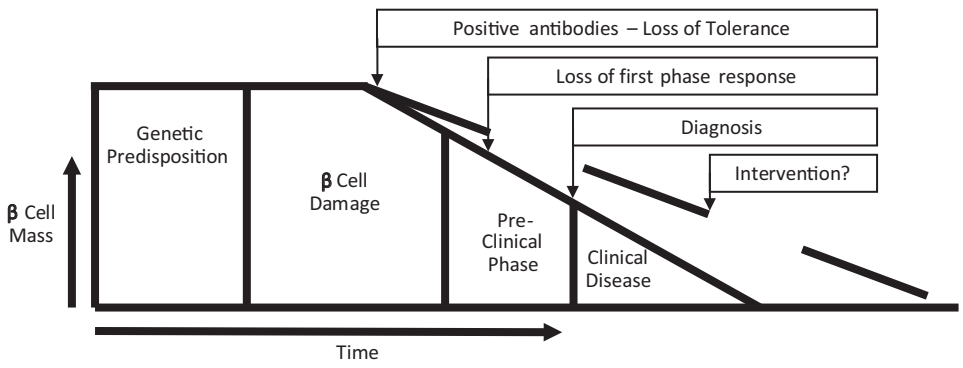

Figure 1 | Schematic view of type 1 diabetes patho-genesis *(adapted from Eisenbarth 1986)*.

immediate psychosocial impact on both the individual and his family. In the long term, complications include micro- and macro-vascular disease including retinopathy, nephro-pathy, neuropathy and cardiovascular disease. These may eventually lead to amputation, blindness, end-stage renal disease and cardio- and cerebro-vascular events. The consequences of morbidity and mortality are enormous with a huge impact on health care costs. Indeed, until 1922 type 1 diabetes was a fatal disease. Since then great progress has been made in improving patient treatment, although only as damage control rather than disease prevention. Despite significant progress over time, most patients still rely on insulin as the mainstay of therapy while awaiting promising therapeutic, and hopefully curative, interventions that are beyond the scope of this thesis.

Immunopathogenesis

Type 1 diabetes is defined as an organ specific auto-immune disease occurring in genetically predisposed individuals leading to hypoinsulinemia due to beta-cell destruction (Eisenbarth 1986). The early initiating events of T1D are of unknown origin and lead to either misreading of inflammatory signals *or* defective resolution of inflammation, causing auto-immunity. The inflammatory response within the islets is named insulitis and is characterised by immune cell infiltration of the pancreas as well as the appearance of auto-antibodies to beta-cell antigens. Disease onset of T1D is considered to start long [months to years] before clinical symptoms become apparent. Auto-antibodies are found to precede clinical onset of T1D by many years (Pihoker, Gilliam et al. 2005). Both cytokines and islet antigen reactive T cells play an important role in this process (Roep 2007; Rabinovitch and Suarez-Pinzon 2003).

Box 1 | *Auto-immune destruction of the beta cells*

It is currently believed that beta cell derived antigens are being released and presented on the surface of the beta cells. Antigen presenting cells (APC) pick up the antigens and present them on their surface. The antigens are then being recognized by specialized white blood cells: T-helper-1-cells (Th-1) and regulating T cells (Tregs); this recognition causes activation of the T cells. The Tregs would normally inhibit the activated Th-1 cells blocking a potential auto-reaction. In T1D the Tregs fail to block the auto-immune response. The activated Th-1 cell excretes cytokines, activating cytotoxic T-cells (CTLs) and B cells. The B cells produce antigen specific antibodies, recognizing the antigens on the beta cells. The activated CTL recognizes the antigens on the beta cells. Recognition of the beta cells by both the CTL's and B cells, leads to destruction of the beta cells.

Immune cells that recognise the body's own tissue, i.e. self antigens, are eliminated by a process of negative selection. Tolerance is the state in which the immune system no longer responds to a given "self" antigen. In the last decade it has become apparent that both antigen-presenting cells such as dendritic cells and a special subset of T-cells called regulatory cells play an important role in the induction of tolerance. However, we still do not know why or how this loss of tolerance, an essential step in auto-immune disease development, is initiated.

Further unsolved and obvious questions remain: why do few contract T1D, even within families [as in identical twins], whilst most do not?; why has the disease incidence risen, especially in the very young, in a relatively short time frame?; why is there such enormous geographic variation in this incidence?

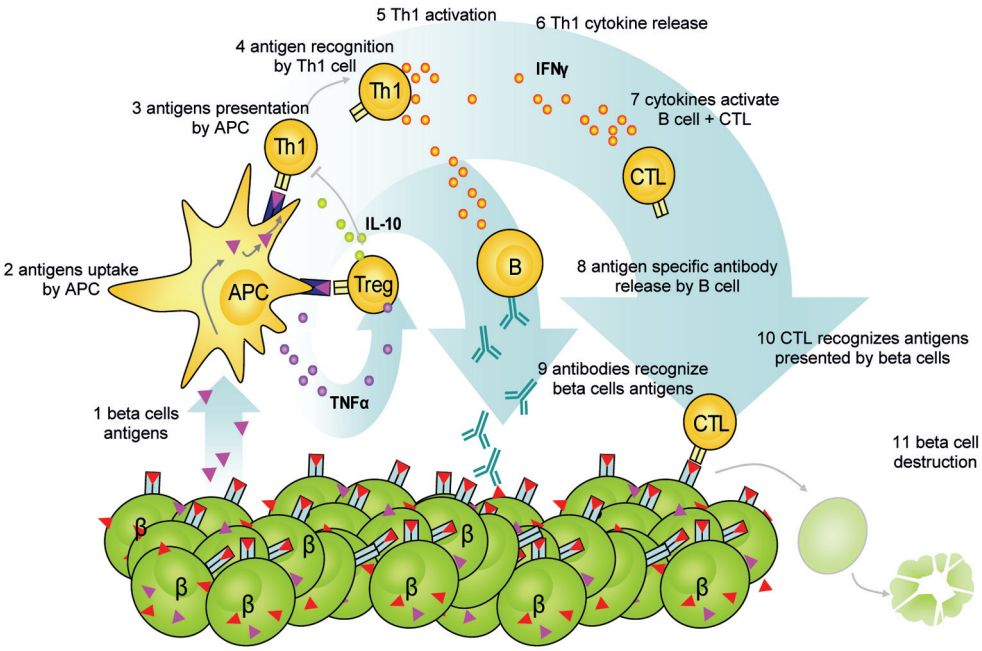

Figure 2 | **Sequence of molecular events leading to Type 1 diabetes.** Beta cells of the pancreas express and shed antigens. These antigens are picked up by antigen presenting cells (APC). These antigens are subsequently being presented on their surface and are recognized by specialized T helper 1 cells (Th1) and regulating T cells (Treg). These Tregs are impaired in T1D and therefore do not inhibit the Th-1 cells as they do under normal circumstances preventing an immune response against the "self". The activated Th-1 cells excrete cytokines thereby activating B-cells and cytotoxic T-cells (CTLs). The activated B cells produce antigen specific antibodies recognizing the beta cells. The activated CTLs recognize the self-antigens on the beta cells. The antibodies and CTL response against the beta cells ultimately lead to their destruction. APC: Antigen Presenting Cells, Th1: T helper 1 cells, B: B cells, β: pancreatic beta cells, ▲: beta cell antigens, CTL: Cytotoxic T lymphocytes, IFN-γ: Interferon gamma, TNFα Tumour Necrosis Factor alpha.

Genetic considerations

An important role in the (immuno-)pathogenesis of T1D is played by genetics. Although this genetic component is evident, simple Mendelian laws of inheritance do not apply. Having an affected family member variably increases the risk on type 1 diabetes with 1-16% whilst in 85% of those affected, a family history of T1D is absent.

Comparable to the vast majority of auto-immune diseases, the most important susceptibility locus is formed by the human leukocyte antigen [HLA] complex, accountable for around 50% of disease susceptibility (Sheehy, Scharf et al. 1989). The HLA region is formed by a cluster of genes located on chromosome 6. These HLA genes encode the glycoproteins called major histocompatibility complex (MHC). The glycoproteins are found on most cells and assist the immune system in distinguishing between self (i.e. its own cells, e.g., beta cells of the pancreas) and non-self (e.g., bacteria, viruses). Of the 3 classes of HLA genes, class II

alleles have the strongest association with type 1 diabetes. This class of MHC encodes for molecules participating in antigen presentation. Certain HLA variants depend on the amino acid composition of their antigen-binding sites to confer either predisposition or protection relating to T1D. Contrary to celiac disease in which the immunogenic epitope presented to CD4 T cells allows a good molecular understanding of HLA association, the epitope in T1D is still unknown, although serious efforts are underway (Di Lorenzo, Peakman et al. 2007). Functional genetic mechanisms have been proposed to "explain" the risk effect of the HLA system on type 1 diabetes (Nepom and Kwok 1998).

Since insulin, an islet cell specific beta cell marker, or its precursors may act as auto-antigens in T1D, it is not surprising that the second most important genetic marker of T1D is found in the region of the insulin gene. A polymorphism in the variable number of tandem repeats of the insulin gene, the INS-VNTR (also known as the IDDM2 locus) is the most widely studied polymorphism and is involved in the regulation of insulin expression and "self and non-self" education (Bell, Horita et al. 1984), a process evolving early in life. The risk alleles of the INS-VNTR of the promoter region, account for approximately 10% of familial clustering of T1D (Davies, Kawaguchi et al. 1994; Bennett, Wilson et al. 1996; Pugliese, Zeller et al. 1997).

Other genetic risk factors

Since the identification of the HLA system and INS-VNTR, several genes have been identified to be associated with T1D (Kharagjitsingh and Roep 2005;Alizadeh and Koeleman 2008). Initially these 'candidate' genes were selected based on biological reasoning, i.e. gene products were chosen based on their involvement in the pathogenesis of the disease. Together with family-based linkage studies, these two forms of genetic research formed the basis to identify candidate gene loci for T1D.

Since the relatively low number of T1D patients renders power issues in the quest of additional susceptibility loci, several international initiatives have been set up (Concannon, Erlich et al. 2005). These initiatives were welcomed some years ago, since technology enabled, by means of genome wide scans [GWAS], association studies of complex polygenetic diseases like T1D, to further identify susceptibility loci (Kharagjitsingh and Roep 2005; Rich, Concannon et al. 2006).

At present more than 20 susceptibility loci have been identified albeit forming only a modest impact on disease risk as compared to the HLA alleles. Although their exact role in the pathophysiology is only slowly being unravelled, preliminary data suggests that the vast majority of the genes in these loci, like PTPN22 and CTLA4, exert their effects on the immune system [immunogenetics], thus implicating important pathways (Concannon, Rich et al. 2009). So, while genetic association studies mainly focus on metabolic genes and genes involved in beta-cell impairment in type 2 diabetes, in T1D candidate genes predominantly seem to exert their effects on the immune system.

Since T1D is a metabolic disease, one may pose the question whether "metabolic genes" or genes involved in growth, may also be implicated in its pathogenesis. The most obvious candidate would be the aforementioned insulin gene, especially considering insulin's important role as a growth-promoting hormone (Hill and Hogg 1989; Laron 2004). Other examples of candidate genes in

this context are the vitamin D receptor gene and the insulin-like growth factor 1 gene. The latter has been identified earlier by our group to be associated with T1D (Eerligh, Roep et al. 2004). However, we were unable to establish the exact mechanism explaining this association.

In addition to genetic risk factors with immunological features, a range of candidate genes can be earmarked as contributing to disease risk and progression through their impact on growth and metabolism. These candidates offer targets for investigation on the role of genes in the context of observed correlations between accelerated growth, birth weight, and risk of development of T1D, which is the topic of this thesis.

Environment

Having discussed two main pillars of T1D pathogenesis, the last but not least important pillar in disease pathogenesis, environmental factors, will now be addressed. The environmental factors [**table 2**] potentially play important roles in TID development. These factors were mainly identified in epidemiological studies. Unfortunately, none of them have been conclusively linked to diabetes nor do we know whether they act as disease modifiers or triggers in the pathogenesis of T1D. Furthermore, studying these environmental factors presents a challenge since they may precede clinical manifestation of diabetes by several years.

In addition to the factors reviewed in **table 2**, other factors strongly suggest an environmental influence, such as the observed variation in geographic disease distribution, and the change of disease prevalence as seen in migration studies (Peng and Hagopian 2006; Adeghate, Schattner et al. 2006; Akerblom and Knip 1998).

Table 2 |

Environmental factors associated with T1D
Early feeding pattern/ Infant nutrition and food supplements
Gluten
Breastfeeding
Vitamin D
Birth weight
Postnatal growth
Infectious agents [e.g. viral infections] / Toxins

Strikingly, most compelling environmental risk factors impose their effect soon after birth, or perhaps even *in utero*, despite disease development 10-20 years later in life. This implies that early events may be a major checkpoint in disease development ['perinatal programming'], possibly prohibiting intervention. This phenomenon is increasingly acknowledged in the context of several diseases (Gluckman, Hanson et al. 2008), and will be addressed in the discussion. In this thesis, several of the candidate risk factors are revisited and challenged. The epidemiological observations pointing to risk of type 1 diabetes also emphasize the need for careful selection of the control cohort to define whether the candidate environmental factors are relevant for T1D or other biometric features (growth, height, and weight) that are not necessarily independent risk factors for T1D.

Rather than being a single point starting an irrevocable domino cascade towards apparent T1D, it appears that there exists a more complex process, involving cross-talk between the beta cell and the immune system. This dynamic dialogue is influenced by environmental factors, set against a predisposed genetic background, leading to clinical diabetes. Examples of genetic predisposi-

tion include: losing T cell tolerance e.g. via the HLA system through immunogenic epitopes (Di Lorenzo, Peakman et al. 2007) or via cytokines expression manifested by cross talk with the beta cell (Nepom and Kwok 1998; Concannon, Rich et al. 2009). This cross talk may then be affected by environmental factors which impact beta cell function (Dahlquist 2006; Ludvigsson 2006).

Examples of this cross-talk between the beta cell and the immune system, mediated by environmental factors, are the following:

1. Several viruses have been implicated in the aetiopathogenesis of T1D (Richer, Straka et al. 2008). Besides direct beta-cell damage, post-viral beta-cell damage can induce activation of a response leading to the production of auto-reactive T lymphocytes against the beta cells, in which molecular mimicry between viral proteins and beta cell antigens cause cross-activation (Atkinson, Bowman et al. 1994; Horwitz, Ilic et al. 2002; Hyoty and Taylor 2002; Roep, Hiemstra et al. 2002).
2. Exposure to certain food constituents like gluten (Norris, Barriga et al. 2005), cow milk proteins (Virtanen, Saukkonen et al. 1994), toxins (Parslow, McKinney et al. 1997) and vitamin D (1999) have all been associated with T1D risk. Based on clinical and experimental work, Varaala hypothesized that in a “fertile” enteral environment normal oral tolerance may be impaired through increased permeability of the mucosal barrier leading to abnormal handling of antigens by the gut (Vaarala 2008). The hygiene hypothesis purports that early in life decreased exposure to microbes shifts the immune system towards a state more susceptible to auto-immune disease such as diabetes (Strachan 1989; Bach 2002).

Given the possibility that viral infection, food constituents or toxins, may contribute to disease development, when studying these environmental influences, the choice of nested (family) controls is indispensible. Differences in these (viral and other) exposures may indeed contribute to the marked discrepancies between different studies and cohorts.

Early growth and T1D

As mentioned [**table 2**], an additional environmental factor found to be associated with T1D is increased growth prior to clinical disease onset. Several large studies found a high birth weight to be positively associated with increased T1D risk (Dahlquist, Patterson et al. 1999; Stene, Magnus et al. 2001; Cardwell, Carson et al. 2005; Dahlquist, Pundziute-Lycka et al. 2005). Various other studies, including ours, have shown early increased growth prior to clinical diabetes onset, to be positively associated with T1D.

The first group which described increased growth in pre-diabetic children (Baum, Ounsted et al. 1975) already suggested overfeeding or relative hyperinsulinism as possible explanations for this phenomenon. Increased growth appears to be associated with insulin resistance, likely due to counter-regulatory hormones (Amiel, Caprio et al. 1991; Ong, Petry et al. 2004).
Whether these phases of insulin resistance may place the beta cell in a more vulnerable state for inflammatory and autoimmune stimuli, remains speculative. The challenge set when researching growth preceding clinical onset of T1D, is to characterize in greater detail this phenomenon of accelerated growth prior to clinical onset of childhood T1D.

Insulin-like growth factor I [IGF-I] and insulin are both important hormones of the growth axis, and in interaction with environmental factors, both are responsible for the complex process of early growth (Oliver, Harding et al. 1996; Gluckman and Pinal 2003; Laron 2004)

IGF-I is a pleiotropic polypeptide hormone (Froesch, Hussain et al. 1996) which has an important role in somatic growth (Bach 2004). Circulating IGF-I level has a marked genetic component (Juul 2003). It was shown that a polymorphism of the IGF1 gene, located on chromosome 12 near the promoter region, is associated with birth weight (Vaessen, Janssen et al. 2002), body height, type 2 DM and myocardial infarction (Vaessen, Heutink et al. 2001). As mentioned above, we have previously shown an IGF1 variant to be associated with T1D (Eerligh, Roep et al. 2004). The pathophysiological relevance of this association is yet to be elucidated.

Whereas insulin is also important in growth, insulin or its precursors may in T1D also act as auto-antigens. The most extensively studied genetic variant in the insulin gene region, located on chromosome 11, is a Variable Number of Tandem Repeats (VNTR) polymorphism in the insulin gene promoter region, the IDDM-2 locus, also known as the INS-VNTR. This polymorphism is involved in the regulation of insulin expression (Bell, Horita et al. 1984), and is variably reported to be associated with birth size and growth (Frayling, Hattersley et al. 2002; Dunger, Ong et al. 1998; Mitchell, Hattersley et al. 2004; Mook-Kanamori, Miranda Geelhoed et al. 2007; Bouatia-Naji, De Graeve et al. 2008). The risk alleles of the INS-VNTR of the promoter region account for approximately 10% of familial clustering of T1D (Davies, Kawaguchi et al. 1994; Bennett and Todd 1996; Pugliese, Zeller et al. 1997).

Our group previously showed that early accelerated growth, i.e. an increased gain in weight in the first years of life and length [and height] in the following second and third year, occurs in prediabetic children (Bruining 2000). In this study it was not found that an increased birth weight was associated with later T1D, in contrast to results obtained by others (Dahlquist, Patterson et al. 1999; Stene, Magnus et al. 2001; Cardwell, Carson et al. 2005; Dahlquist, Pundziute-Lycka et al. 2005). Neither did we find length in the first year of life, of later T1D patients, to be different from their siblings. It was not established whether growth differences were restricted to the first year of life or extended beyond this first year. Possible genetic correlates of growth could have influenced our observation, though these were not included (Bruining 2000).

The findings of this study (Bruining 2000) are in line with several studies confirming increased growth to precede later onset childhood diabetes. However, these case-control studies found different time windows for increased growth, either defined as weight, height or BMI gain. Most of them analysed the growth data via t-tests, using population [non familial] controls, thereby possibly adding confounders [such as early food patterns or infectious exposures] known to influence diabetes risk. Furthermore, high birth weight was not consistently found to be associated with increased T1D risk. Additionally, none of these studies linked the increased growth prior to T1D onset to potential genetic correlates of this early growth.

Study populations

We have recognised the need for large, qualified cohorts to study the relation between genes, environment and risk for T1D. Indeed, several reports were flawed by inappropriate ascertainment of reference cohorts. One of the key objectives of this thesis was to collect a new large family cohort with nested controls, to validate the intriguing findings in our first Dutch family cohort. In addition, we had access to several large, multicenter and international paediatric cohorts permitting us to replicate our findings, or to assess whether regional factors affected risk development between centres and cohorts.

Dutch Diabetic Growth cohort I

From 91 children with clinical onset of type1 diabetes at 4-15 years of age and their 125 healthy siblings, changes in length, weight, and body mass index (BMI) in infancy and early childhood was assessed. This cohort was part of the Kolibrie Diabetes Register and the Sophia Children's Hospital Register (95% ascertainment), consisting of 181 newly diagnosed Children with diabetes, identified between 1995 and 1998 in the south-western part of the Netherlands. Permission from parents of children with diabetes and healthy siblings of non-immigrant origin was obtained. The average age at clinical onset in the diabetic child was 8.3 years. The parents retrieved lengths and weights from the files in their local child-health clinics.

Diab Marker cohort

This cohort is nested within the European Multicentre Remission (REM) Trial aimed at unravelling the determinants of partial clinical remission in T1D. Patients were diagnosed with T1D according to World Health Organization (WHO) and the American Diabetes Association criteria and were included within 3-7 days after diagnosis and initiation of insulin therapy. At the time of patient enrolment an age- and gender-matched unrelated volunteer from the same residential area was recruited. Participants were Caucasian without a relevant medical history. A full clinical examination took place at the time of diagnosis and on completion of a follow-up period of 3 months by the same study physician, using a standardized questionnaire and checklist. Blood samples were obtained for cytokine measurement and genotyping. This cohort allows us to study markers of disease progression and [partial] clinical remission. The international multicentre case control design permits us to assess the impact of the difference in environmental and genetic influence/background.

The Hvidøre cohort

This cohort was part of the framework of the Hvidøre Study Group on childhood diabetes: a multi-centre longitudinal investigation consisting of 275 children and adolescents (144 females) under the age of 16 with new onset T1D. The patients were enrolled between August 1999 and December 2000 from 18 paediatric departments in 14 countries throughout Europe. Exclusion criteria were other types of diabetes, decline of enrolment into the study by the patients or their parents, or patients initially treated outside the participating departments for more than 5 days. T1D was diagnosed according to the World Health Organisation's (WHO) criteria. From the study base 227 children and adolescents, who had complete stimulated C-peptide data, were grouped in one of the four progression pattern groups. Median age at diagnosis was 9.1 years (range 0.2-16.8). Of these 227 patients (116 females*)* who had sufficient C-peptide data to be allocated to a progression pattern groups, 85% were white

Caucasians with a median age at diagnosis of 9.6 years (range 0.2-16.3). Blood samples were obtained for cytokine measurements and genotyping, while a mixed meal stimulated C –peptide test (MMST) was performed at two time points. This cohort allows us to study remission from a genetic and immunological perspective, whilst the MMST will enable us to evaluate beta cell function over time. Given its multicentre pan-European design, the impact of differences in environmental and genetic background can be assessed from this cohort.

Project On Preterm and Small-for-gestational-age infants a.k.a. POPS cohort

This cohort was retrieved from the POPS study. The POPS cohort comprises 94% of all live-born infants in the Netherlands between the period of January 1 and December 31 1983, after a pregnancy of less than 32 completed weeks and/or with a birth weight of less than 1,500 gram. Only subjects with a gestational age below 32 weeks were included in the study. Subjects with congenital malformations leading to changes in body proportions and body composition like focomely, chromosomal abnormalities, and inborn errors of metabolism were not eligible for inclusion. This cohort could allow assessment of catch-up growth, comparable to the increased growth preceding childhood onset T1D. Moreover, we could review whether this growth pattern – in a non-diabetic population- shows similar associations with either of the two selected gene variants (IGF1 and INS-VNTR).

Aims and scope of the thesis

In spite of the huge gain in knowledge of the epidemiology, pathogenesis and treatment of type 1 diabetes as outlined in this introduction, there are still major inconsistencies and critical gaps in knowledge regarding the role of environmental factors in disease pathogenesis and the factors influencing disease progression upon clinical disease onset. These form the basis of this thesis. Existing and debated issues considered in this thesis are the putative association of birth weight with T1D, duration of the period of increased early growth prior to clinical T1D manifestation and factors involved in disease progression. In researching these issues, the important role of the choice of the control population, of paramount importance and often underestimated, is also taken into account.

Several models have been postulated to include any or all of these open ends [**table 3**]. While these models are not mutually exclusive, they have different emphases (as will be addressed in the discussion). Yet, the mere existence of models (rather than facts), leading to multiple hypotheses, all of which still await compelling evidence, provide fertile ground for this thesis.

Table 3 |

Hypotheses aiming to capture relevant factors in T1D aetiopathogenesis
Accelerator hypothesis (Wilkin 2001)
Overload hypothesis (Dahlquist 2006)
Beta cell stress hypothesis (Ludvigsson 2006)
Hygiene Hypothesis (Bach 2002)

Given our ambition, a need for an independent, new cohort from the same background population with appropriate control subjects to validate our earlier findings, was imperative. This posed a major challenge, as those, who have ever collected a cohort for whatever reason, will acknowledge.

Several pediatricians in the Rotterdam area were approached to participate in this project. Research nurses, students and parents were all recruited to reach the goal of collecting a new cohort. All of these individuals proved invaluable in collecting data and material from patients and their non-affected sibling. We decided to extract DNA from sputum. This non-invasive method of DNA collection allowed us to include younger children in this cohort. We called this cohort the Dutch Diabetic Growth cohort II.

Dutch Diabetic Growth cohort II

Families with diabetic children with ages at disease onset 1-15 (inclusive) years were recruited from five childhood diabetes clinics in the Rotterdam area. Weight and height data were collected from Caucasian non-immigrant families. We selected families with two or more children: one diabetic patient and at least one non-affected healthy sibling. The subjects and controls were comparable in terms of age. In cases of more than one healthy sibling, the one whose date of birth was closest to the diabetic child was included in the study. Growth data (height and weight) during infancy and early childhood (0-4 years of age) were systematically collected from all participating children. Patients and healthy siblings were genotyped for the INS-VNTR (IDDM2) and for a microsatellite in the IGF1 gene. We excluded diabetic mothers, birth outside gestational ages 37-42 weeks, and diagnosis of diabetes of the prediabetic child <1 year old or any condition or medication possibly influencing growth.

With this new clinical cohort we wished to validate our earlier claims (Bruining 2000) suggesting an increased gain in weight in the first years of life, and length and height in the following second and third year, occurring in prediabetic children who did not show increased birth weight. Furthermore, this cohort would permit us to assess genetic correlates of the clinical association of growth and disease predisposition, while comparing these findings with other cohorts assessing associations between growth, birth weight, immunological parameters, genetics, environment, family history and/or metabolic parameters with type 1 diabetes.

This thesis aims to scrutinize infant growth, genetic correlates thereof and disease progression in type 1 diabetes from a clinical perspective.

In **chapter 2** we expand our previous work on early accelerated growth prior to disease onset in T1D families and address the importance of very early differences in growth pattern between those children with childhood onset DM compared to their unaffected siblings.

In **chapter 3** we examine the role of two important [growth] gene variants in the established growth patterns of chapter 2.

In **chapter 4** we describe these gene variants in the context of growth in a unique population of [very] premature children, known to show catch-up growth per se. This was to test the hypothesis that accelerated growth for other reasons than acceleration towards childhood type 1 diabetes may indeed show different associations with the studied gene polymorphisms.

In **chapter 5 and 6** we evaluate genetic correlates for remission in pro/anti-inflammatory interleukins in an attempt to establish whether these were associated with [partial] remission in newly diagnosed T1D patients in a multicentre European case control study.

In **chapter 7** we test the hypothesis that correlates of blood group genotypes linked with intestinal immunity associate with T1D in a case control study.

In **chapter 8 and 9** all presented data and their findings are summarized and discussed.

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