Elin Pettersen Sørgjerd

Markers of autoimmunity in Latent Autoimmune Diabetes in Adults (LADA) and non-diabetic adults: Impact in phenotype and genetic predisposition

Results from the Nord-Trøndelag health study

Thesis for the degree of Philosophiae Doctor

Levanger, April 2013

Norwegian University of Science and Technology
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Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the degree of Philosophiae Doctor

Faculty of Medicine
Department of Cancer Research and Molecular Medicine

ISBN 978-82-471-4326-1 (printed ver.)
ISBN 978-82-471-4327-8 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2013:113

Printed by NTNU-trykk

Markører for autimmunitet hos pasienter med LADA og en ikke-diabetisk voksen befolkning: påvirkning av fenotype og genetisk predisposisjon.
-Resultater fra Helseundersøkelsen i Nord-Trøndelag.

Diabetes er ikke en enhetlig sykdom, men finnes i flere former. Diabetes er hovedsakelig klassifiert i to hovedgrupper; type 1 og type 2 diabetes. Type 1 diabetes er en autoimmun sykdom der kroppens immunforsvar angriper og ødelegger beta-cellene som produserer insulin. Pasienter med type 2 diabetes har fremdeles bevart en del insulin produksjon, men en viss grad reduksjon sammen med dårlig insulin virkning på cellene i kroppen fører til økende blodsukker og diabetes. I 1986 kom den første rapporten om en gruppe pasienter som avvek fra den klassiske type 2 diabetes diagnosen. De hadde tegn til autoimmunitet i form av påvisbart antistoff (hovedsakelig antiGAD) mot de insulinproduserende beta-cellene i samme grad som type 1 diabetikere, men pasientene hadde likevel fremdeles god beta-celle funksjon. Pasientene kunne i starten kost- og tablett-behandles som type 2 diabetikere før de senere ble insulin avhengige, men ofte på et tidligere tidspunkt en type 2 diabetikere. Denne pasientgruppen ble etter hvert kalt «Latent Autoimmune Diabetes in Adult» (LADA). I likhet med type 2 diabetes er LADA pasientene voksne og ofte overvektige og karakterisert ved såkaldt metabolsk syndrom. Likevel har LADA pasientene høy risiko for progresjon mot insulin-avhengighet. Dette tyder på at LADA kan være en mellomting mellom type 1 og type 2 diabetes.

LADA har en minst like høy prevalens blant befolkingen som type 1 diabetes. Men sykdomsbildet til LADA er i mye mindre grad forklart enn hos type 1 og type 2 diabetes. Målet med denne studien var å kartlegge både den genetiske og fenotypiske bakgrunnen til LADA. Vi ville også se på hvordan tilstedeværelsen av antiGAD positivitet påvirket en generell voksen og ikke-diabetisk befolkning. Studien ble basert på data fra den andre (1995-1997) og tredje (2006-2008) helseundersøkelsen i Nord-Trøndelag.

Artikkel 1: Målet var å kartlegge de genetiske risikofaktorene som påvirker utviklingen av LADA. Dette ble gjort ved å se på allerede kjente risiko gener for både type 1 og type 2 diabetes og deres kobling til LADA pasientene som hadde deltatt i HUNT2. I tillegg ble grad av autoimmunitet hos LADA pasientene bestemt ut fra antiGAD titer fra serumprøver. Det ble funnet genetiske likheter med både type 1 og type 2 diabetes hos LADA pasientene. Type 1 diabetes genene var assosiert med LADA pasienter som hadde høy antiGAD titer, mens type 2 diabetes genene var assosiert med LADA pasienter som hadde lav antiGAD titer. Samlet indikerer dataene at LADA pasienter med høy autoimmunitet er genetisk mer type 1 diabetes lik, mens LADA pasienter med lav autoimmunitet er genetisk mer type 2 diabetes lik.

Artikkel 2: Målet var å studere den autoimmune prossesen hos LADA pasientene både før og etter de hadde fått sin diagnose. Dette ble gjort ved å måle ulike antistoffer som man visste var relatert til autoimmunitet hos type 1 diabetes pasienter (antiGAD, antiIA-2 og antiZnT8) i LADA pasienter som hadde deltatt i både HUNT2 og HUNT3. Blant disse LADA pasientene hadde over 50 % ikke lenger de målte antistoffene i blodet (antistoff negativ) etter 10 års perioden. LADA pasientene som ble antistoff negative var mer type 2 diabetes like - de var bl.a. tykkere og hadde høyere alder når de fikk sin sykdom enn de som beholdt sin positivitet. Men, de antistoff negative LADA pasienten hadde betydelig lavere C-peptidverdier (et mål på egen

insulinprosduksjon) enn type 2 diabetikere. Dette tyder på at selv en kort periode med antistoff positivitet er av klinisk betydning ved at slike LADA pasienter får dårligere beta-celle funksjon. Det ble også funnet at mange av dem som utviklet LADA i tiden etter HUNT2 hadde påvisbart antistoff (antiGAD) i blodet allerede ved HUNT2 – altså før de fikk sykdommen. En del LADA pasienter har derfor en lang periode med "prediabetes" i form av en pågående autoimmun prosess. LADA pasienter med tidlig positivitet for antistoff var mer type 1 diabetes lik sammenlignet med de som var antistoff negative ved HUNT2. Disse funnene viser at antistoff mønsteret hos LADA pasientene er assosiert med både sykdomsutvikling og fenotype.
Artikkel 3: Tilstedeværelsen og kliniske implikasjoner av antiGAD positivitet i ikke-diabetiske populasjoner er dårlig belyst. Disse aspektene ble undersøkt prospektivt i et utplukk av voksne ikke-diabetikere (n=4496) som hadde deltatt i både HUNT2 og HUNT3. AntiGAD positivitet ble funnet i 1,7 % av denne gruppen. Positivitet var ikke assosiert med kjønn, første grad familiehistorie med diabetes (FHD), røyking, glukose eller BMI. Men HLA-DQA1/DQB1, en risiko-haplotype for autoimmun diabetes ble forbundet med antiGAD positivitet. Det samme ble positivitet for antiTPO, et antistoff funnet ved hypotyreose med autoimmun årsak. Ca. 50 % av pasientene som var antiGAD positive ved HUNT2 var senere antiGAD negative (HUNT3). AntiGAD positivitet i vedvarende ikke-diabetiske individer er delvis konsistent, er ikke forbundet med kliniske parametre relatert til diabetes, men forbundet med HLA risiko og autoimmunitet i skjoldbruskkjertelen.

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Ovennevnte avhandling er funnet verdig til å forsvares offentlig
for graden PhD i Molekylærmedisin
Disputas finner sted i Auditoriet BS31,Bevegelsessenteret.
Mandag 29. April 2013, kl. 12:15.

Table of contents

Acknowledgments **5**

List of publications **7**

Abbreviations **9**

Summary **11**

1 Introduction
1.1 World-wide scope of diabetes and classification 15
1.2 Type 1 diabetes 16
In general 16
The epidemiology and etiology 16
Genetics 17
Molecular pathogenesis 18
1.2 Type 2 diabetes 19
In general 19
The epidemiology and etiology 19
Genetics 19
Pathogenesis and treatment 20
1.3 Latent autoimmune diabetes in adult 21
In general 21
The epidemiology and etiology 21
Genetics of LADA 22
Pathophysiology of LADA 23
1.5 Antibodies in autoimmune diabetes 23
In general 23
AntiGAD 24
AntiIA-2 25
AntiZnT8 25
1.6 AntiGAD in the general non-diabetic population 25
2 Aims **27**
3 Methods **29**
3.1 Study population 29
The HUNT Study 29
Data collection 30
Classification of diabetes 31
Classification of diabetic cases with missing data on insulin treatment (paper I) 31
Final study population 32
3.2 Biochemical analysis 36
C-peptide measurements 36
Antibody measurements 36
3.3 Genetic analysis 38

DNA extraction 38
Selection of SNPs 39
Single SNP genotyping analysis 39
HLA-haplotyping 43
3.4 Statistical methods 44
Paper I 44
Paper II 44
Paper III 45

4 Summary of results
4.1 Paper I 47
4.2 Paper II 49
Cross-sectional 49
Prospectively 49
4.3 Paper III 51

5 Discussion
5.1 Methodological considerations 53
The HUNT study 53
Antibody measurements (information bias) 54
Storage time of serum samples 55
Classification criteria of LADA 56
Candidate gene studies 57
5.2 Genetic and autoimmune markers associated with risk of developing LADA 57
5.3 The significance of autoimmunity in a general adult non-diabetic population 58

6 Conclusions 61

7 Future perspectives 63

8 References 65

Appendix I: Q1 HUNT2

Appendix II: Q1 HUNT3

Appendix III: Diabetes questionnaire HUNT2

Appendix IV: Diabetes questionnaire HUNT3

Acknowledgments

The present PhD thesis was carried out during the years 2007-2012 at the Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology.

First of all I am deeply grateful for having the opportunity for having professor Valdemar Grill as my main supervisor. Thank you, Valdemar, for introducing me to de field of science and for shearing your never-ending knowledge in the etiology and physiology of diabetes. You have always been there for discussion and assistance.

I also want to thanks my two co-supervisors professor Frank Skorpen and Dr. Kristi Kvaløy. Thank you both for introducing me to the fields of human genetics. Special thanks to you Kirsti for our many good discussions were I think we both always learned something new.

In addition I want to thanks all my colleagues at the Faculty of Medicine, specially the HUNT gang, who has created a fantastic working environment.

A thanks goes also to Dr. Peter A Torjesen and Kari Julien at Oslo University Hospital, Aker Hospital for always answering my questions about the antibody measurements. Torill Flatvad and Anne Heidi Skogholt at NTNU for their appreciated assistance with the SNPlex analysis and Oddrun Storrø, Marit Aarhaug and Anne Kristin Lysakerrud at St Olavs Hospital, for their technical assistance with the HLA haplotyping.

I want to acknowledge all the participants in the Nord-Trøndelag Health Study, and the people who started and accomplished the HUNT study. Thanks to Kristian Midthjell who made the diabetes part in the three HUNT surveys possible and for me to complete this work.

Finally, a special thanks to my husband and soul mate Arve and to the rest of my family and friends, for endlessly love and support.

I could never have done this work without you all. Thanks.

Our project was supported by the Liaison Committee between the Central Norway Regional Health Authority and NTNU and the Liaison Committee between St.Olav's Hospital Trust and the Faculty of Medicine, NTNU.

List of publications

This thesis is based on the following three papers

Paper I
Elin Pettersen, Frank Skorpen, Kirsti Kvaløy, Kristian Midthjell, Valdemar Grill. **Genetic heterogeneity in latent autoimmune diabetes (LADA) is linked to a varying degree of autoimmune activity. Results from the Nord-Trøndelag Health Study.** *Diabetes* 2010;59(1):302-310

Paper II
Elin Pettersen Sørgjerd, Frank Skorpen, Kirsti Kvaløy, Kristian Midthjell, Valdemar Grill. **Time dynamics of auto-antibodies are coupled to phenotypes and add to the heterogeneity of autoimmune diabetes in adults: the HUNT Study, Norway.** *Diabetologia* 2012;55(5):1310-1318

Paper III
Elin Pettersen Sørgjerd, Frank Skorpen, Kirsti Kvaløy, Valdemar Grill. **Presence of antiGAD; its clinical influence in a non-diabetic population of adults. Results from the HUNT study.** *Manuscript*

Abbreviations

ADA	American Diabetes Association
ai	antibody index
antiGAD	Antibody for Glutamic Acid Decarboxylase
antiIA-2	Antibody for tyrosine phosphatase-like protein Insulinoma Antigen-2
antiTPO	Antibody for Thyroid Peroxide
antiZnT8	Antibody for Zinc Transporter 8
APC	Antigen-Presenting Cell
ASO	Allele Specific Oligonucleotides
BMI	Body Mass Index
CI	Confidence Intervals
cpm	counts per minute
CV	Coefficient of Variation
DASP	Diabetes Autoantibody Standardization Program
ELISA	Enzyme-Linked Immunosorbent Assays
FHD	First-degree family History of Diabetes
GABA	Gamma-Aminobutyric Acid
GAD	Glutamic Acid Decarboxylase
GAD67	67kDalton Glutamic Acid Decarboxylase
GWAS	Genome Wide Association Studies
HLA	Human Leukocyte Antigen
HUNT	The Nord-Trøndelag Health Study
IA-2	tyrosine phosphatase-like protein Insulinoma Antigen-2
ICA	Islet cell Cytoplasmic Antibody
IDF	the International Diabetes Federation
LADA	Latent Autoimmune Diabetes in Adult
LSO	Locus Specific Oligonucleotide
LYP	Lymphoid protein tyrosine Phosphatase

MAF	Minor Allele Frequency
MHC	Major Histocompatibility Complex
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PE	R-Phycoerytrin-bound
Q1	Questionnaire 1
Q2	Questionnaire 2
RIA	Radiobinding Assays
SNP	Single Nucleotide Polymorphisms
SSO	Sequence-Specific Oligonucleotide
TCR	T-Cell Receptor
T1D	Type 1 diabetes
WHO	World Health Organization
ZnT8	Zinc Transporter 8

Summary

Diabetes is mainly classified: type 1 and type 2 diabetes. Type 1 diabetes is an autoimmune disease in which the body's immune system attacks and destroys the beta cells that produce insulin. Patients with type 2 diabetes have somewhat reduced insulin production which coupled to poor insulin efficiency leads to increased levels of blood glucose.

In 1986 a group of patients who deviated from the classical type 2 diabetes diagnosis was reported. These patients showed signs of autoimmunity in form of detectable antibodies (mainly antiGAD) against the insulin-producing beta cells, antibodies which are commonly found in type 1 diabetes. The patients had still considerable good beta-cell function and were initially diet and/or orally treated like type 2 diabetes. However, as a group they developed insulin dependency faster than type 2 diabetes. This patient group was later called Latent Autoimmune Diabetes in Adult (LADA). As with type 2 diabetes the LADA patients are older at diagnosis and often overweight. Nevertheless, the LADA patients display a high risk for progression to insulin dependency. This suggests that the etiology of LADA is a mix of type 1 and type 2 diabetes.

The prevalence of LADA is similar to that of type 1 diabetes; however the etiology and phenotype of LADA is less characterized than type 1 and type 2 diabetes. The aim of this study was therefore primarily to investigate the genetic and phenotypic background of LADA. We also looked at the presence and clinical implications of antiGAD positivity in a general adult non-diabetic population. The study was based on data from the second (HUNT2: 1995-1997) and third (HUNT3:2006-2008) Nord-Trøndelag health surveys.

Paper I: The aim was to identify genetic risk factors that could affect the development of LADA. This was done by looking at known risk genes for both type 1 and type 2 diabetes and their link to LADA. Genetic similarities were found with both type 1 and type 2 diabetes. Further, the type 1 diabetes genes were associated with LADA with higher degree of autoimmunity (high titres of antiGAD), while type 2 diabetes genes

were associated with LADA with lower autoimmunity. Overall, the data suggest that LADA patients with high autoimmunity are genetically more similar to type 1 diabetes, and LADA patients with low autoimmunity are genetically more similar to type 2 diabetes.

Paper II: The aim was to study the autoimmune process in LADA patients, both before and after diagnosis of diabetes. We followed the LADA patients who had participated in both HUNT2 and HUNT3 by measuring antibodies that are known to be related to autoimmunity in patients with type 1 diabetes (anti-GAD, anti-IA-2 and anti-ZnT8). Over 50% of the LADA patients, who had participated in both HUNT2 and HUNT3, were antibody negative after the 10-year period between HUNT2 and HUNT3. LADA patients who were antibody negative were more type 2 diabetes like; i.e. they were more obese and older when they developed diabetes, than those who kept their positivity. However, the antibody negative LADA patients had significantly lower C-peptide values than patients with type 2 diabetes. This suggests that even a short period of antibody positivity is of clinical importance. Samples analysed for antiGAD also showed that many of the LADA patients who developed LADA after HUNT2 had detectable antibody in the blood at HUNT2, i.e. before the onset of the disease. Thus, for some LADA patients there is a long period of pre-diabetes in the form of an on-going autoimmune process. LADA patients with positivity for antibodies at HUNT2 were more type 1 diabetes like compared with those who were antibody negative. These findings show that the antibody patterns in LADA patients affect the LADA patients' disease progression and phenotype.

Paper III: The presence and clinical implications of antiGAD positivity in non-diabetic populations are poorly elucidated. We examined these aspects prospectively in a cohort of adult non-diabetic patients (n = 4496) who had participated in both HUNT2 and HUNT3. AntiGAD positivity was found in 1.7% of the group. Positivity was not associated with gender, first-degree family history of diabetes (FHD), smoking, glucose or BMI. However, the HLA-DQA1/DQB1 haplotype, a known risk haplotype for type 1 diabetes was associated with antiGAD positivity. Association was also found with positivity for antiTPO, an antibody found in hypothyroidism. Approximately 50% of the

patients who were positive by antiGAD at HUNT2 had turned antiGAD negative at HUNT3. We conclude that antiGAD positivity in persistently non-diabetic individuals is partly consistent, is not associated with clinical parameters related to diabetes, but is associated with high risk HLA haplotypes and autoimmunity in the thyroid gland.

1 Introduction

1.1 World-wide scope of diabetes and classification

Diabetes mellitus is a chronic metabolic disorder characterized by increased plasma glucose occurring when insulin is not acting as it should (insulin resistance) and/or the insulin production from pancreas is poor (insulin deficiency). According to the International Diabetes Federation (IDF), there are today estimated more than 350 million people worldwide with diabetes and the incidence every year is still rising[1] (http://www.idf.org/diabetesatlas). Diabetes has become a serious global health problem. Despite the effort of many researchers across the world, the etiology of the disease is still not fully elucidated. However, investigations support the fact that diabetes is a heterogeneous disease.

The World Health Organization (WHO) has since 1965 given advice on definitions, diagnosis and classifications of diabetes based on published epidemiological studies regarding etiology and pathogenesis of diabetes. Before 1999 the major forms of diabetes were classified by type of treatment: e.g. insulin dependence or non-insulin dependence at diagnosis. In the late 1990ies, an international expert committee, sponsored by the American Diabetes Association (ADA), and WHO recommended a change in the classification system from a treatment-based one to one more based on etiology[2,3]. Diabetes primarily caused by beta-cell destruction and prone to ketoacidosis should then be classified as type 1 diabetes. Type 1 diabetes includes two subgroups; A) the major subgroup, autoimmune diabetes, with beta-cell destruction due to an autoimmune process and B) a minor subgroup, idiopathic diabetes, where beta-cell destruction is evident but (as of today) no evidence of autoimmunity has been found. Diabetic patients who do not have signs of autoimmunity, who are insulin resistant and who have to some degree insulin deficiency are classified as type 2 diabetes. Other types of diabetes including gestational diabetes, genetic syndromes and monogenic disorders including maturity-onset diabetes of the young are not the main focus of this study.

1.2 Type 1 diabetes

In general

Type 1 diabetes manifests itself in all age groups and accounts for about 10% of all diabetic cases. The disease is mainly caused by an immune-mediated destruction of the insulin producing beta cells in the pancreas. Diabetes becomes overt when the beta cells are no longer able to meet the body's requirement of insulin[4]. This leads to reliance on insulin treatment.

The epidemiology and etiology

The disease is found in all ethnic groups; however, it is more prevalent in European populations, especially in the Northern countries, with Finland showing the highest incidence rate. Most studies show a worldwide rapid increase in incidence of childhood type 1 diabetes[5-7]. The epidemiological studies are mainly performed in children and therefore little is known about the trends in incidence rates in adults.

Type 1 diabetes is a multifactorial disease where both gene predispositions and environmental factors interact. There is a high familial aggregation of type 1 diabetes, with up to 15-fold higher risk of developing the disease in siblings compared to the general population[8]. As outlined below, a predisposing genetic background is indeed a strong factor. However, 90% of the type 1 diabetic patients do not have a first degree relative with type 1 diabetes. This indicates also a strong influence by environmental factors[4]. Potential environmental factors include diet (e.g. breast vs. bottle feeding and D-vitamin intake), environmental toxins (e.g. nitrosamines) and viral infections both intrauterine and in childhood (e.g. enteroviruses and congenital rubella), however the evidence for the importance of such factors is conflicting[9-14]. The "hygiene hypothesis" proposes that improved hygiene and living conditions in the 20th century have decreased the frequency of childhood infections. This situation may then modulate the immune system and increase the risk for type 1 diabetes and also other autoimmune diseases[15].

Genetics

The strongest susceptibility genes for type 1 diabetes are found in the human leukocyte antigen (HLA) class II genes which account for almost 50% of the genetic risk[16]. These genes are located in the Major Histocompatibility Complex (MHC), on the short arm of chromosome 6. The HLA haplotypes *DQA1*03:01-DQB1*03:02* (DQ8) and *DQA1*05:01-DQB1*02:01* (DQ2) are the two high risk haplotypes known to be associated most strongly with type 1 diabetes[17-20]. About 90% of type 1 diabetic children have at least one or both of these high risk haplotypes. On the other hand *DQA1*01:02-DQB1*06:02* is a strongly protective HLA haplotype with a frequency of about 20% in the general population and <1% in individuals with type 1 diabetes[17]. This protection is not absolute since some patients with type 1 diabetes are found to harbor the protective *DQB1*06:02* allele[21]. The mechanism behind why DQ2 and DQ8 are important risk factors for type 1 diabetes remains to be fully elucidated. A leading hypothesis relates to the three-dimensional configuration of different haplotypes for the groove that harbors a presenting antigen (see further below).

Before the advent of genome wide association studies (GWAS) only a few non-HLA loci had been found to be associated with type 1 diabetes, such as the insulin gene (*INS*)[22], protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*)[23,24], cytotoxic T-lymphocyte-associated protein-4 (*CTLA4*)[25] and interleukin-2 receptor-alpha (*IL2RA*)[26,27]. In the last five-six years the numbers of susceptibility genes associated with type 1 diabetes have increased due to GWAS. More than 40 genetic markers with an underlying risk of developing type 1 diabetes have been identified[28-32]. Many of these genes are suggested to influence immune function or beta-cell function and their discovery may be important for the identification of different disease pathways[33]. The impact of these genes on the development of type 1 diabetes is however limited compared to certain of the HLA haplotypes.

The risk allele of the *INS* gene (class I allele) associates with decreased insulin levels in both the pancreas and in the thymus. Lower expression of insulin in the thymus is suggested to affect the specialized antigen presenting cells in the thymus and in the

elimination of autoreactive T-cells something that could influence the development of autoimmunity[33,34]. The *PTPN22* gene codes for the lymphoid protein tyrosine phosphatase (LYP) that, together with Csk kinase, suppresses T-cell activation[24]. The risk allele of *PTPN22* (arginine to tryptophan) is found to disrupt the interaction between LYP and Csk, resulting in weakened suppression of autoreactive T-cells.

Molecular pathogenesis

The severe reduction or abolishment of insulin production in type 1 diabetes is thought to occur due to an irreversible T-cell mediated autoimmune destruction of the insulin-producing pancreatic beta-cells[12]. One of the hypotheses to explain this T-cell response includes the so-called trimolecular complex[35]. This complex consists of the T-cell receptor (TCR), an antigenic peptide, and a HLA molecule on antigen-presenting cells (APCs). The APCs present the peptide, which is bound to the HLA molecules on the surface of an APC, to the TCR. The TCR is then able to recognize it and with varying affinity bind to the peptide. The TCR is crucial for T-cell selection in the thymus. If the TCR recognition of a certain self-peptide is modest (due to weak binding) thymus may fail to "kill" autoreactive T-cells which can then react with self-antigens in the periphery and trigger an immune response that may end in tissue destruction.

T-cell activation is regarded as the major cause of autoimmunity in type 1 diabetes. However, there are also signs of humoral autoimmunity in form of antibodies against islet proteins[36-39]. Well-documented antibodies that are of clinical interest are glutamic acid decarboxylase (antiGAD), tyrosine phosphatase-like protein insulinoma antigen-2 (antiIA-2), insulin (antiIA), zinc transporter 8 (antiZnT8) and islet cell cytoplasmic antibody (ICA). These antibodies are described in more detail below.

1.2 Type 2 diabetes

In general

Type 2 diabetes is the most common type of diabetes and comprises more than 80% of the diabetic population world-wide. Patients with type 2 diabetes are characterized by being insulin resistant and/or having inadequate insulin secretion with the disease typically developing in adulthood and old age. Type 2 diabetes is usually, but not always, accompanied by obesity.

The epidemiology and etiology

The incidence of type 2 diabetes has been rising in all age groups even in children, although the risk of type 2 diabetes increases with age. The increase may be due to more people getting obese. Regions with the highest prevalence of diabetes in adults are the Middle East and North Africa followed by North America and the Caribbean (data from 2011)[1].

Type 2 diabetes, like type 1 diabetes, is a heterogeneous disorder; however both the predisposing genes and environmental factors involved are different from the ones implicated in type 1 diabetes. Behavioral risk factors like overweight, smoking, diet and lack of physical activity are strongly associated with type 2 diabetes, with overweight as the most important one[40-42]. It has been estimated that approximately 80% of all new type 2 diabetes cases are due to overweight[43] and both physical activity and a healthy diet significantly reduce the risk of type 2 diabetes[41,44]. Also low birth weight, which is an indicator of fetal malnutrition, is a risk factor for developing type 2 diabetes later in life[45-47].

Genetics

The heritability of type 2 diabetes is as high as in type 1 diabetes[40]. However, the risk genes so far documented for type 2 diabetes only explain a small part of the risk deduced from family history. This is in contrast to the situation in type 1 diabetes. A

"simplified" conclusion would be that environmental factors are well documented in type 2 diabetes, genetic factors less so, whereas the opposite is true for type 1 diabetes.

The genes coding for calpain 10 (*CAPN10*), transcription factor-7-like 2 (*TCF7L2*), the pancreatic beta cell K_{ATP} channel subunit Kir6.2 (*KCNJ11*), peroxisome proliferator-activated receptor gamma (*PPARG*) and wolframin (*WFS1*) were the first genes to be associated with type 2 diabetes through linkage and candidate gene studies[48-52]. Many more risk loci have later been identified through GWAS and meta-analysis[53-55]. The majority of the genes found are considered to be important for reduced insulin secretion through reduced beta-cell mass and beta cell dysfunction. This pertains to the *TCF7L2*, *HHEX*, *KCNJ11*, *WFS1*, *HNF1B*, *SLC30A8*, *CDKAL1*, *IGF2BP2*, *CDKN2A*, *CDKN2B*, *THADA*, *TSPAN8* and *KCNQ1* genes[56-58]. Only a few genes, such as *PPARγ, IRS1, ADAMTS9* and *FTO*, affect insulin sensitivity[57,58]. The *FTO* gene is also strongly associated with obesity[59,60]. The clinical pay-off of genetic studies in type 2 diabetes has however been minor. Hence, the associations found have modest effect sizes and the associated genes have limited predictive ability, and only 5-10% of the genetic susceptibility is currently explained[57,61].

Pathogenesis and treatment

Hyperglycemia, which leads to development of type 2 diabetes, occurs because of a combinations of A) insulin resistance in different tissues in the body most importantly skeletal muscles, adipose tissue and liver and B) beta-cell defects and/or reduced beta-cell mass leading to impaired insulin secretion. During pre-diabetes the beta-cell is still able to compensate for the insulin resistance and produces enough insulin to maintain normal glucose levels. At onset of type 2 diabetes one finds disparity between insulin and glucose levels. Thus insulin levels are "normal" despite high glucose levels which should have resulted in elevated insulin levels. This indicates that the insulin secretion is no longer able to compensate for the insulin resistance[62]. It is still unclear whether a reduction in beta-cell mass or cellular signal secretion defects is the most important factor behind insufficient insulin secretion.

Obesity, especially with abdominal fat distribution, (a feature which is more strongly associated with type 2 diabetes than body mass index, BMI), lowers insulin sensitivity[63]. The degree to which the beta cells are able to compensate for the insulin resistance determines whether type 2 diabetes develops or not.

At diagnosis patients with type 2 diabetes are still able to produce much insulin and the disease can usually be treated with diet and oral antidiabetic drugs. As the disease progresses many patients gradually lose their ability to produce insulin and will therefore eventually benefit from insulin treatment.

1.3 Latent autoimmune diabetes in adult

In general

Adult patients with signs of autoimmunity may masquerade as type 2 diabetes[64,65]. These patients are termed slow-onset type 1 diabetes or more commonly latent autoimmune diabetes in adult (LADA). There is still an ongoing discussion whether LADA is a subgroup of type 1 diabetes, a mixture of type 1 and type 2 diabetes or an entity of its own. However, by WHO definitions, LADA is classified as type 1 diabetes or autoimmune diabetes[3].

The epidemiology and etiology

The frequency of LADA among diabetic patients varies between 4-10% in different populations indicating that the prevalence of LADA is as high as type 1 diabetes[66,67]. There are few reports on the incidence of LADA. Available information indicates about 10 per 100,000 people per year[68,69].

Clinical features of LADA share similarities with both type 1 and type 2 diabetes. LADA patients, like type 1 diabetes, have (by definition) autoantibodies indicating an autoimmune disease, but are more likely to be positive for only one antibody than being multiple antibody positive[70]. Similar to type 2 diabetic patients, LADA patients develop

diabetes as adults, and are often, but not always, overweight. Compared to type 1 diabetic patient LADA patients have higher C-peptide levels and do not need insulin treatment at diagnosis[66,71,72]. LADA patients are, however, more prone to progress earlier to insulin dependence than patients with type 2 diabetes.

Age, antibody positivity and initiation time of insulin treatment are common criteria used to classify LADA. However, definition of these criteria varies between studies. Some studies use no age limit[73,74], others use cut-offs like age >30[68,72] years or >35 years[75]. LADA patients should be antibody positive for at least one antibody, however which antibody is not defined. AntiGAD is the most commonly used and also shown to be the most prevalent one in LADA[70]. LADA patients should be non-insulin dependent at diagnosis, but for how long after diagnosis is unclear. Some studies use three months[74], others six months[68] and some use up to 12 months[73].

Genetics of LADA

The genetic risk factors of LADA have not been elucidated to the same extent as for type 1 and type 2 diabetes. Some evidence suggest that the genetic risk of LADA is a mixture of type 1 and type 2 diabetes associated genes as described below.

Like in type 1 diabetes, the high risk *HLA-DQB1*03:02* and *DQB1*02:01* alleles are associated with a higher risk of developing LADA[66,74,76]. However, compared to type 1 diabetes, the frequency of the high risk HLA alleles *DQB1*03:02/*02:01* is reported to be lower and the highly protective allele *DQB1*06:02* is higher in LADA[66]. Studies have also reported that increased frequency of *HLA-DQB1*02:01* may be the most prevalent risk HLA allele in LADA[67,74]. Regarding susceptibility genes in addition to HLA the *INS* gene, as well as the *PTPN22* and the *CTLA4* genes, are reported to be associated with higher risk in LADA[76-78].

When this study started in the fall 2007, there had been few studies looking at the association between susceptibility genes for type 2 diabetes and LADA. A few studies reported that the *TCF7L2* gene, highly associated with type 2 diabetes, was also

associated with LADA[76,79-81]. There were no reports on genes associated with LADA only.

Pathophysiology of LADA

Available results on LADA indicate an interaction of both autoimmunity (shown by presence of antibodies) and insulin resistance. On one hand studies have shown that LADA patients require insulin after a much shorter time subsequent to diagnosis compared to type 2 diabetic patients[73,82]. Hence, an autoimmune attack against the beta-cells in LADA patients is bound to have an impact over time, although at a slower rate than in type 1 diabetic patients. In line with an impact of autoimmunity on insulin producing cells, the levels of antibodies like antiGAD correlate with a need of insulin treatment among LADA patients[73]. On the other hand, many patients with LADA are obese, with obesity being a marker of insulin resistance, and studies have shown insulin resistance in LADA patients to the same degree as in type 2 diabetic patients[71,83].

1.5 Antibodies in autoimmune diabetes

In general

The first autoantibody found to be associated with type 1 diabetes was the ICA[84,85]. Later several other autoantibodies have been defined. These includes antibodies against GAD[36], insulin[39], IA-2[38] and most recently ZnT8[37].

ICA is detected by indirect immunofluorescence on cryocut sections of human pancreas. This antibody is difficult to measure since the method is labor-intensive and requires human pancreas. AntiGAD, antiIA-2, antiIA and antiZnT8 are usually analyzed by immunoprecipitation (radiobinding) assays (RIA) with ^{3}H- or ^{35}S-methionine as labeled reagent. However, also enzyme-linked immunosorbent assays (ELISA) are used. Several international workshops, in particular the Diabetes Autoantibody Standardization Program (DASP) which was established in 2000, have resulted in standardization of the antibody assays. This has over the years led to improvement of

both the sensitivity and specificity of the assays[86-88]. Presently, measurements of autoantibody markers are the most reliable diagnostic tool to identify and predict type 1 diabetes[88].

There is still no evidence that these antibodies have an active role in development of type 1 diabetes. Rather, they appear to reflect an ongoing autoimmune process. Thus development of autoimmune diabetes is strongly associated with the presence of autoantibody markers. About 90-95% of newly diagnosed type 1 diabetic patients are positive for at least one antibody[89,90]. It is also well known that antibodies can be present months and up to several years before clinical diagnosis of type 1 diabetes, indicating a long pre-diabetic phase with autoimmune activity[91-93]. Further, the appearance of multiple antibodies is highly predictable of the development of type 1 diabetes[94,95].

AntiGAD

Glutamic acid decarboxylase (GAD) is an enzyme which catalyzes the conversion of glutamic acid to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). GAD was by Baekkeskov *et al* in 1990[36] discovered to be the 64kDa beta-cell antigen which earlier was found to be a target of antibodies in type 1 diabetes. GAD is found to be highly expressed in the nervous system, but is also found in other tissues such as pancreas[96]. The function of GAD in the pancreas is not clear, however the presence of both GAD and GABA and of GABA receptors on the islet beta cells suggests that GABA is involved in paracrine signaling within these cells[97]. Other isoforms of GAD like the 67kDa GAD (GAD67) have been discovered[98], but have added little to the detection of type 1 diabetes compared to antibodies against the 64kDa GAD[99].

AntiGAD is present in up to 80% of new-onset type 1 diabetic patients[100]. AntiGAD does not seem to be influenced by age to the same extent as antiIA and ICA[101,102].

AntiIA-2

The tyrosine phosphatase-like protein insulinoma antigen-2 (IA-2) is an enzymatically inactive member of the tyrosine phosphatase family. It is a transmembrane glycoprotein located in islet secretory granules and may be involved in insulin secretion[103]. AntiIA-2 was identified when the 64kDa immunoprecipitate was trypsin treated and revealed three different fragments of 37kDa, 40kDa and 50kDa. The 50kDa fragment was identified by GAD antibodies; however the 37kDa and 40kDa fragments seemed to be derived from a different autoantigen which was found to be IA-2[38].

AntiIA-2 is found in about 80% of newly diagnosed type 1 diabetic patients. This antibody is found to have higher prevalence in younger age groups compared to adults[104].

AntiZnT8

In 2004 Chimienti *et al.* identified and cloned a beta-cell specific zinc transporter 8 (ZnT8) which is a product of the gene *SLC30A*[105]. The zinc transporter was found to be localized together with insulin in the insulin secretory granules. Since zinc is an important part of insulin storage and secretion, ZnT8 is believed to play an important role for maintaining zinc in the beta-cells, something which is necessary for insulin maturation and storage[106]. Antibodies against ZnT8 are the fourth major and the most recently identified antibody marker in autoimmune diabetes[37]. AntiZnT8 has been found in about 60-80% of newly diagnosed type 1 diabetic cases[90,107,108] and 10-20% of LADA patients[70,109,110]. Some studies have also shown that AntiZnT8 is present in about 1-7% of patients originally diagnosed with type 2 diabetes and presumably antibody negative[70,111].

1.6 AntiGAD in the general non-diabetic population

Previous studies in adults and school children have shown that antibodies, in particular antiGAD, are present in a minority of non-diabetic subjects who do not have close relatives with autoimmune diabetes[94,112-115]. The frequency (which varies between 1-4%

between studies) and the clinical importance of antiGAD in non-diabetic individuals is however still unclear and debated. It is still not known if the presence of anti-GAD in adult non-diabetic individuals reflects an extremely slow progress of beta-cell destruction or whether it is attributable to other factors, such as aging. It has been argued that positivity under these conditions does not predict the development of diabetes[116,117], that it is unspecific (particularly if it is weak), and should be regarded as falsely positive[118]. However, it is also reported that the high risk type 1 diabetes HLA haplotypes are associated with antiGAD positivity and high antiGAD levels in adult non-diabetic individuals from the general population[112,119]. This may indicate that a genetic predisposition can induce antibody positivity, but that other factors drive or are at least necessary for development of autoimmune mediated diabetes.

2 Aims

The specific aims of this study were to investigate;

1) A: the association of type 1 and type 2 diabetes candidate genes in LADA patients compared to non-diabetic controls and B: the variability of the genetic background in LADA patients in relation to a marker of autoimmunity (antiGAD titre) and to a phenotypic risk factor for type 2 diabetes (BMI) (Paper I).

2) A: prospectively the pre-diabetic appearance of antiGAD, antiIA-2 and antiZnT8, B: the persistence of these antibodies in LADA patients after a 10-13 years follow-up and C: cross-sectionally the presence of the same antibodies in LADA and adult-onset type 1 diabetes in relation to diabetes onset and other phenotypic characteristics (Paper II)

3) The prevalence, persistence and the potential clinical impact of antiGAD positivity in a persistently non-diabetic population of adults (Paper III).

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3 Methods

3.1 Study population

The HUNT Study

The Nord-Trøndelag health Study (HUNT) consist of three health surveys performed in 1984-1986 (HUNT1), 1995-1997 (HUNT2) and 2006-2008 (HUNT3). In all three surveys the entire adult population (aged ≥20 years) in the Nord-Trøndelag county located in the central part of Norway (Figure 3.1), were invited to participate (n=87,259 in HUNT1, n=93,898 in HUNT2 and n=93,860 in HUNT3). The participants who formed the basis of our study were collected from the HUNT2 and HUNT3 surveys.

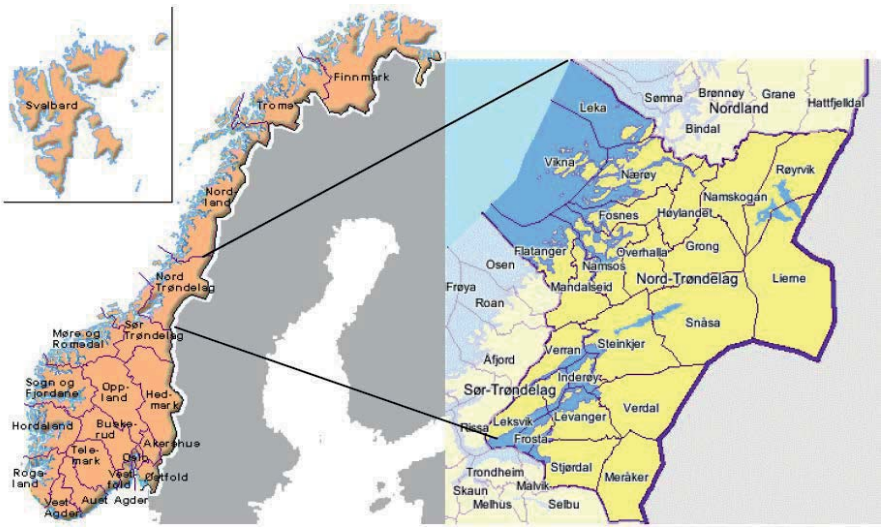

Figure 3.1: Norway and the location of Nord-Trøndelag County

The HUNT2 survey had an overall response rate of 69.5% (n=65,237). The survey included a clinical examination (including blood pressure and anthropometric measurements), non-fasting blood sampling and two basic questionnaires (Q1: Appendix I and Q2) which included more than 200 health-related items. The HUNT2

survey has been described in detail elsewhere[120]. The HUNT3 survey had an overall response rate of 54.1% (n=50,807). Fifty-seven percent of the participants in HUNT2 also participated in HUNT3 (n=37,059). The HUNT3 survey had a similar design as HUNT2 and thus included clinical examination, blood-sampling and two basic questionnaires (Q1: Appendix II and Q2) as described in detail[121]. Biological samples collected from HUNT2 and HUNT3 were stored at HUNT Biobank (Levanger, Norway) prior to analysis, serum being stored at minus 70^{o}C and DNA at minus 20^{o}C.

Data collection

Papers I, II and III

Individuals with diabetes were identified from a self-reported answer of "Yes" to the question "Do you have or have you had diabetes?" in the Q1 questionnaire from HUNT2 and HUNT3. In HUNT2, 1,972 individuals and in HUNT3 2,189 answered affirmative. At both HUNT2 and HUNT3 participants declaring diabetes were invited to a diabetes-oriented follow-up investigation. They completed a more detailed questionnaire concerning diabetes (HUNT2: Appendix III and HUNT3: Appendix IV) and underwent an interview by a nurse to ensure year of diagnosis and details on type and start of treatment. They furthermore provided a fasting blood sample for measurements of blood glucose, serum C-peptide, and antiGAD. In HUNT3 antiIA-2 was also measured. A total of 1,630 and 1,824 participants filled out the diabetes-oriented questionnaire and a total of 1,455 and 1,168 participants rendered a fasting blood sample at the follow-up respectively in HUNT2 and HUNT3.

Subsequent to the surveys we additionally analyzed antiGAD in participants who had declared diabetes but had not provided a blood sample at the follow-up, but had serum available from the baseline blood sampling (n=432 in HUNT2 and n=984 in HUNT3). Analysis of antiGAD in the HUNT2 samples was performed in the spring of 2008 (average 12 years after sampling) and the HUNT3 samples were analyzed in late 2009 (average 3 years after sampling). This gave us the opportunity to classify all cases who had answered affirmative to the question on diabetes.

Participants who answered “no” to the question of having diabetes served as controls. They were frequency-matched by sex and by 10 year of age category to the diabetic patients.

Paper III

Equal numbers of men and women who had stated that they did not have diabetes both at the HUNT2 and HUNT3 surveys were randomly selected from different age groups: 500 individuals from the age group 20-29 years, 500 from the age group 30-34 years etc. up to the last age group of 65 years and above. Altogether a total of 4,500 non-diabetic individuals were sampled to represent the general adult population.

Classification of diabetes

Diabetic cases were classified as having type 1 diabetes if they had started insulin treatment within 12 months of diagnosis and were 1) antibody positive, or 2) antibody negative but in addition had fasting C-peptide levels <150 pmol/l.

Cases were classified as having LADA if they were antibody positive and had not been treated with insulin within 12 months of diagnosis. No age limit was set for LADA.

Cases were classified as having type 2 diabetes if they were antibody negative and had not been treated with insulin within 12 months of diagnosis.

Classification of diabetic cases with missing data on insulin treatment (paper I)

For the identified diabetic cases in HUNT2 who did not attend the follow-up investigation we lacked data on insulin treatment. These non-attendees were therefore classified by less stringent criteria; i.e. as type 1 diabetes if antiGAD positive and age at diagnosis ≤35 years old, LADA if antiGAD positive and age at diagnosis >35 years old and as type 2 diabetes if antiGAD negative and age of diagnosis >35 years old.

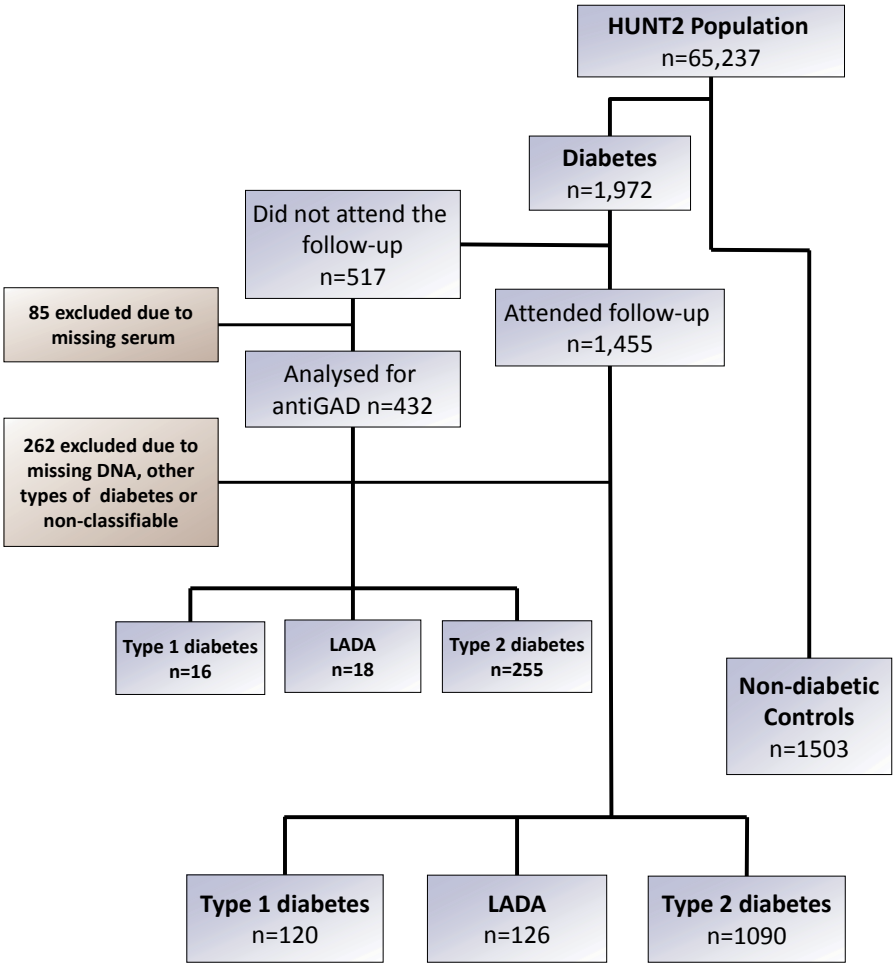

Figure 3.2: Study population in paper I.

Final study population

Paper I

This was a case-control study nested within the HUNT2 cohort (Figure 3.2). All diabetic cases identified at baseline who had DNA available (n=1,642) and 1,503 age and gender matched healthy non-diabetic controls were included in the study. The

diabetic cases classified by the criteria that included insulin treatment, comprised of 120 type 1 diabetic patients, 126 LADA patients and 1,090 type 2 diabetic patients. Sixteen type 1 diabetic, 18 LADA and 255 type 2 diabetic patients were classified by the less stringent criteria. Cases that we were not able to classify (n=17) were excluded.

Paper II

This was both a prospective and a cross-sectional study. Serum samples were collected from the diabetic subjects classified as type 1 diabetes and LADA from both the HUNT2 and HUNT3 surveys. Diabetic cases were analyzed for antiIA-2 (if not done already in HUNT3) and for antiZnT8. Also serum samples from HUNT2 were used to analyze antiGAD, antiIA-2 and antiZnT8 in incident cases in HUNT3 (i.e. in those not having a diagnosis of diabetes in HUNT2). All of these antibody measurements were done in late 2009.

For LADA and type 1 diabetic cases we included for analysis those with complete data on all three antibody assays. These cases comprised 120 type 1 diabetic and 120 LADA cases from HUNT2 and 147 type 1 diabetic and 85 LADA cases from HUNT3 (Figure 3.3). Type 2 diabetic cases who had participated in both HUNT2 and HUNT3 surveys (n=302) were also included for comparison.

Prospective data were obtained (i.e. from cases that had participated in both HUNT2 and HUNT3; providing 10-13 years of follow-up) on 44 LADA, 59 type 1 diabetic and 302 type 2 diabetic cases from HUNT2 who were followed to HUNT. In addition we analyzed data from 31 LADA and 24 type 1 incident cases of diabetes from HUNT3 who also participated and were non-diabetic in HUNT2.

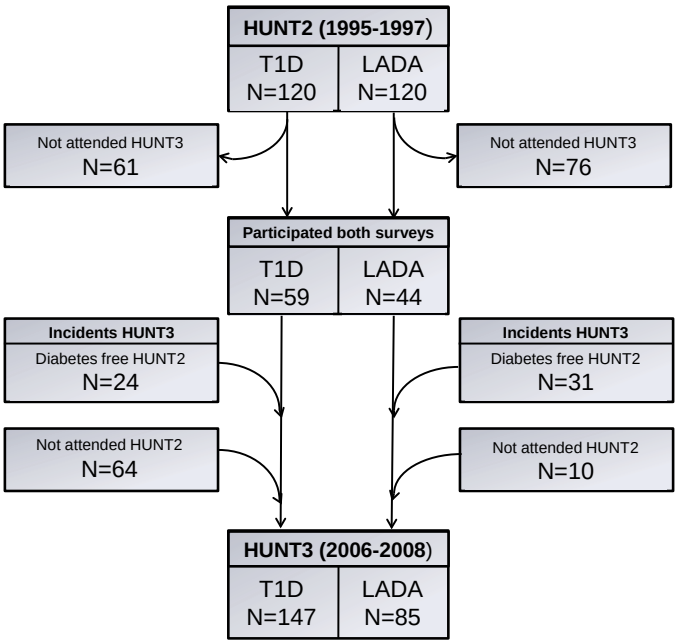

Figure 3.3: Study population in paper II. T1D = type 1 diabetes

Paper III

This was a prospective study. Serum samples from HUNT2 to be used for antiGAD measurements were available from 4,496 of the 4,500 selected individuals (Figure 3.4). All individuals who were antiGAD positive in HUNT2 were analysed for positivity in HUNT3. For these individuals antiGAD was measured in the fall of 2011.

Additionally, 55 incident diabetic cases (Figure 3.4) who developed autoimmune diabetes between HUNT2 and HUNT3 (i.e. reported not having diabetes in HUNT2 but reported having diabetes in HUNT3, n=24 type 1 diabetes and n=31 LADA) were included for analysis. Thirty-three of these cases were antiGAD positive already at HUNT2, i.e. several years before diagnosis (n=13 type 1 diabetes and n=21 LADA). Data from these patients who over time developed diabetes, were compared with data from the antiGAD positive persistently non-diabetic population.

Persistently non-diabetic individuals who were antiGAD positive at HUNT2 as well as a control group of antiGAD negative non-diabetic individuals were typed for *HLA-DQA1* and *HLA- DQB1*. A control group was age and gender matched to the antiGAD positive, non-diabetic group. Two controls were selected per antiGAD positive individual. The same HLA genotypes were also analysed in individuals who developed autoimmune diabetes during the interval between HUNT2 and HUNT3.

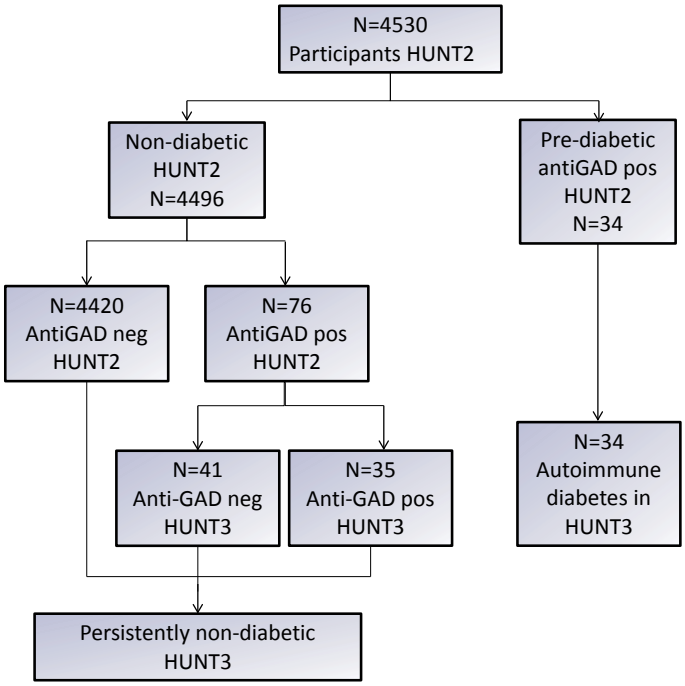

Figure 3.4: Study population paper III

3.2 Biochemical analysis

C-peptide measurements

The most common way to assess insulin secretion is by measurements of C-peptide. C-peptide is a cleavage product from pro-insulin and is released together with insulin. C-peptide is therefore a measure of insulin release.

Serum levels of C-peptide were analysed at the Hormone Laboratory of Aker Hormone laboratory, Oslo University hospital (Oslo, Norway) by radioimmunoassay (Diagnostic system Laboratories, USA).

Antibody measurements

All antibody measurements were carried out at the Aker Hormone laboratory, Oslo University hospital (Oslo, Norway).

AntiGAD

AntiGAD was measured by immuno-precipitation using translation labeled ^{3}H-GAD65 as labeled reagent (Novo Nordisk, Denmark). Separation of bound antiGAD and free labeled antigen was done by protein A coupled to Sepharose (procedure developed at the Hormone laboratory). Antibody levels were expressed as an antibody index (ai) relative to a standard serum given by the formula [(counts per minute (cpm) in the patients sample – cpm from negative reference sample) / (cpm of a positive reference sample – cpm from negative reference sample)]. The lower limit of detection was 0.01ai, whereas no upper limit was defined. Intra-assay variation coefficient (CV) was 14% in the lowest (0.11ai), 8% in the middle (0.22ai) and 17% in the highest (2.0ai) range of measurements. Total assay CV was 19% in the lower (0.21ai) and 23% in the higher (0.66ai) measurement range.

In paper I and II an antibody index of 0.08ai or greater was considered positive. This cut-off level of positivity was the one used by the Hormone laboratory based on participation in DASP. Cut-off was set to achieve the highest possible specificity with

an acceptable corresponding sensitivity. Based on participation in DASP this corresponds to a 68% workshop-sensitivity and 100% workshop-specificity.

In paper III subjects above the 98.5th percentile of the antiGAD levels in the total cohort were considered to be antiGAD "positive". This corresponded to a value >0.05ai. Based on Aker Hormone Laboratory's participation in DASP this corresponded to an 82% workshop-sensitivity and a 99% workshop-specificity.

AntiIA-2

Antibody to IA-2 was measured by immuno-precipitation using translation labeled ^{3}H-IA-2_{ic} as a labeled reagent. Separation of bound antiIA-2 and free labeled antigen was done by protein A coupled to Sepharose, using a procedure developed at the Hormone laboratory. Antibody levels were expressed as an index value relative to a standard serum. A value of 0.11ai or greater was considered positive (method range: 0.01-3.00ai). The level of cut-off was based on the same considerations as for antiGAD. As calculated from DASP 2003 this assay has 70% workshop-sensitivity and 99% workshop-specificity. Intra-assay CV was 17% in the lowest (0.10 ai), 10% in the middle (0.48 ai) and 7% in the highest (1.96 ai) range of measurements. Total assay CV was 22% in the lower (0.14 ai) and 11% in the higher (3.60 ai) range of measurements.

AntiZnT8

AntiZnT8 was measured by immuno-precipitation using a translation labeled ^{3}H-ZnT8 C-terminal Arg325 variant fused to C-terminal Trp325 variant as a labeled reagent (based on a plasmid pJH5.2 SP6, a Dimer human ZnT8 C-terminal Arg325 variant fused to human ZnT8 C-terminal Trp variant from Dr. Hutton, University of Colorado, Denver, CO, USA). Separation of bound antiZnT8 and freely labeled antigen was achieved by protein A plus protein C coupled to Sepharose using a procedure developed at the Hormone laboratory. Antibody levels were expressed as an index value relative to a standard serum. A value greater of 0.08ai was considered positive (method range: >0.01 ai). The level of cut-off was based on the same considerations as for antiGAD. As calculated from DASP 2010 the antiZnT8 assay has 46% workshop-sensitivity and 100% workshop-specificity. Intra-assay CV was 7% in the lowest (0.18 ai) and 6% in

the highest (0.88 ai) range measurements. Total assay CV was 20% in the lower (0.18 ai) and 16% in the higher (0.85 ai) range of measurements.

3.3 Genetic analysis

DNA extraction

DNA samples were mainly collected from the HUNT2 survey. DNA from HUNT2 was extracted from peripheral blood leukocytes from EDTA whole blood or blood clots using the Gentra Purgene blood kit (QIAGEN Science, Maryland, USA). EDTA blood samples were kept frozen at -70^{o}C, whereas clots were stored at -20^{o}C. The blood samples were removed from the freezer and thawed in a 37^{o}C water bath immediately before DNA extraction and transferred to 50 ml tubes (Sarstedt). The clots were liquidized using an OMNI TH homogenizer with disposable OMNI Tip generator probes, using one cycle of 20 sec.

DNA from liquidized clots (5-10ml) and EDTA blood (1-5ml) were isolated on an Autopure LS instrument according to protocols designed by Gentra, or manually, using the same reagents and protocols. In brief, RBC Lysis Solution and Cell Lysis Solution were added to lysate the red and white blood cells. Protein Precipitation Solution and Proteinase K (only for blood clots) were added to precipitate the proteins in the solution. Then the free DNA was precipitated in 100% isopropanol added Gentra Glycogen Solution (only for EDTA blood) and finally the DNA pellet was washed in 70% ethanol. The DNA was rehydrated in DNA Hydration Solution (Tris-EDTA-buffer).

DNA from low volumes (EDTA blood <400 µl) was isolated on a GenoVision BioRobot GenoM-48 (QIAGEN Science, Maryland, USA) according to protocols designed by GenoVision.

In paper III a few DNA samples were collected from the HUNT3 survey when DNA was not available from HUNT2. DNA from HUNT3 was extracted from buffy-coat which was fractionated from 10 ml EDTA whole blood at sampling. The buffy-coat was

stored at -70^{o}C at HUNT Biobank prior to DNA extraction. The DNA extraction protocol was in general the same as that used for the HUNT2 samples.

Selection of SNPs

The selected single nucleotide polymorphisms (SNPs) were based on publicly available results (mainly retrieved from searches on the PubMed database) from studies focusing on genetic association analysis in type 1 (Table 3.1) and type 2 diabetes (Table 3.2).

Single SNP genotyping analysis

The genotyping technologies used for SNP analysis in this study were Taq-Man Discrimination analysis and SNPlex assay (both from Applied Biosystems, Foster City, CA, USA).

TaqMan Discrimination analysis

SNPs genotyped by applying TaqMan SNP allelic discrimination using ABI 7900HT Fast Real-Time PCR System (Applied Bioasystems, Foster City, CA, USA) are indicated in the table 3.1 and 3.2.

The TaqMan allelic discrimination assay is an endpoint analysis in which the presence of two primer and probe pairs in each reaction allows you to differentiate between two possible variations in a single SNP. Each probe is color labeled with its own reporter at the 5'-end. The reporter is a specific fluorescent (typically VIC and FAM) which helps to distinguish between the two alleles. In addition a non-fluorescent quencher which suppresses the fluorescence signal of the reporter is bound at the 3'-end of the probe. During the amplification the probes hybridize specifically to each complementary target sequence (wild-type and mutant) between the primer sites. The DNA polymerase enzyme then cleaves the reporter from the probe and quencher, resulting in increased fluorescence signal from the reporter. The polymerase can only cleave probes hybridized to the target sequence. The fluorescence signal generated during the amplification is therefore an indicator of the alleles present in the sample. After amplification an endpoint reading of the fluorescence signal is done using the Sequence

Detection System (SDS) software. This software plots the signals from each sample in a scatterplot where each signal indicates which alleles are present in the sample.

SNPlex analysis

The SNPs genotyped by applying SNPlex™ genotyping system (Applied Biosystems, Foster City, CA, USA) are indicated in tables 3.1 and 3.2.

The SNPlex assay is a multiplex assay which at the time of the study allowed us to analyse up to 48 SNPs simultaneously. The assay is a migration specific assay designed to discriminate alleles by the application of three SNP specific probes. Two of the probes are allele specific oligonucleotides (ASO) designed to discriminate the two alleles at each SNP. The third probe is a locus specific oligonucleotide (LSO). All probes have a universal PCR priming site; however, the ASO probes have a unique ZipCode identifier that hybridizes to the added complementary ZipChute probe. The ZipChute probe allows for the discrimination between the SNPs in the assay by providing a unique migration pattern for each SNP. Fluorescent signals from each SNP are interpreted by using Applied Biosystem GeneMapper Software (Applied Biosystems, Foster City, CA, USA).

Genotyping performance:

Cases and controls were equally distributed with four or more negative controls per 384-plate. Criteria to pass the assay were 1) call rates >90%, 2) minor allele frequency (MAF) >1% in the genotyped population and 3) agreement with Hardy-Weinberg equilibrium in the whole population (if p-value <0.001 the assay did not pass). The SNP assays that did not pass quality control were excluded from further analysis.

Table 3.1: Selected SNPs associated with higher risk of type 1 diabetes

Chromo-some	SNP	Gene	Full gene name	Alleles*	MAF†	Reference	Analyse method
1p13	rs2476601 rs2488457	*PTPN22*	Protein tyrosine phosphatase, non-receptor type 22	A/G C/G	0.12 0.25	[23,24]	TaqMan TaqMan
2q33.2	rs231775 rs3087243	*CTLA4*	Cytotoxic T-lymphocyte-associated protein-4	G/A A/G	0.43 0.42	[25,30,122]	TaqMan TaqMan
2q24	rs1990760	*IFIH1*	Interferon-induced with helicase C domain 1	C/T	0.36	[25,29]	SNPlex
5p13.2	rs1445898	*CAPSL*	Calcyphosine-like	T/C	0.42	[25]	TaqMan
6p21	rs2296336	*ITPR3*	Inositol 1,4,5-trisphosphate receptor, type 3	C/G	0.32	[123]	TaqMan
10p15.1	rs3118470 rs706778 rs9663421	*IL2RA*	Interleukin-2 receptor-alpha	C/T T/C T/C	0.40 0.45 0.22	[26,124]	TaqMan TaqMan SNPlex
11p15.5	rs689 rs3842753	*INS*	Insulin	A/T A/C	0.26 0.27	[22,122]	TaqMan
16p13.13	rs2903692	KIAA0350	C-type lectin domain family 16, member A	A/G	0.33	[31]	SNPlex

*Minor allele is listed first

†MAF= Minor allele frequency

Table 3.2: Selected SNPs associated with risk of type 2 diabetes

Chromo-some	SNP	Gene	Full gene name	Allels*	MAF†	Reference	Analyse method
1p12	rs10923931	NOTCH2	Prospero-related homeobox-1	T/G	0.12	[55]	TaqMan
1p12	rs2641348	ADAM30	ADAM metallopeptidase domain 30	G/A	0.13	[55]	TaqMan
1q32.1	rs1342387	ADIPOR1	Adiponectin receptor 1	A/G	0.46	[122,123]	SNPlex
2p16.1	rs10490072	BCL11A	B-cell cell/lymphoma 11A	C/T	0.25	[55]	TaqMan
2p21	rs7578597	THADA	Thyroid adenoma associated	C/T	0.08	[55]	TaqMan
3q25.2	rs1801282	PPARG	Peroxisome proliferator-activated receptor-gamma	G/C	0.14	[51,54]	TaqMan
3p27.2	rs4402960 rs7633675	IGF2BP2	Insulin-like growth factor 2 mRNA-binding protein-2	T/G G/T	0.30 0.30	[54,124]	TaqMan TaqMan
3p25	rs17036101	SYN2/PPARG	Peroxisome proliferator-activated receptor gamma	A/G	0.07	[55]	TaqMan
3p14.1	rs4607103	ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9	T/G	0.24	[55]	TaqMan
4p16.1	rs10010131	WFS1	Wolfram syndrome 1 (wolframin)	A/G	0.41	[52]	TaqMan
6p22.3	rs7754840	CKAL1	CDK5 regulatory sub-unit-associated protein-1-like 1	C/G	0.31	[54,124]	TaqMan
6p12	rs9472138	VEGFA	Vascular endothelial growth factor A	T/C	0.32	[55]	TaqMan
6p22-q23	rs1044498	ENPP1	Ectonucleotide pyrophosphatase/ phosphodiesterase 1	C/A	0.16	[125,126]	SNPlex
7p15.2	rs864745	JAZF1	Juxtaposed with another zinc finger gene-1	C/T	0.47	[55]	TaqMan
8q24.11	rs13266634	SLC30A8	Solute carrier family 30 (zinc transporter), member 8	T/C	0.29	[53,54]	SNPlex
9p21.3	rs10811661	CDKN2A/B	Cyclin-dependent kinase inhibitor 2A/B	C/T	0.15	[54,124]	TaqMan
10p13	rs12779790	CDC123/ CAMK1D	Cell division cycle 123 Homologue/calcium/calmodulin-dependent protein kinase ID	G/A	0.17	[55]	TaqMan
10q25.2	rs7903146	TCF7L2	Transcription factor-7 like 2	T/C	0.29	[49,53,54]	TaqMan
10q23.33	rs1111875	HHEX	Homeobox hematopoietically expressed	A/G	0.43	[53,54]	SNPlex
11p12	rs9300039		Intragenic region	A/C	0.13	[54]	TaqMan
11p15.1	rs5219	KCNJ11	ATP-sensitive inward rectifier potassium channel	T/C	0.43	[54,124]	TaqMan
12q21.1	rs7961581	TSPAN8/ LGR5	Tetraspanin 8/leucine-rich repeat-containing G protein-coupled reseptor-5	C/T	0.25	[55]	TaqMan
12q13.1	rs1153188	DCD	Dermcidin	T/A	0.25	[55]	TaqMan
12p13.31	rs767870 rs2286384	ADIPOR2	Adiponectin receptor 2	C/T G/C	0.17 0.48	[123]	SNPlex SNPlex
16q12.2	rs8050136	FTO	Fat mass and obesity associated	A/C	0.44	[54,124]	TaqMan
18p11.31	rs3745012	LPIN2	Lipin 2	T/C	0.24	[127]	SNPlex

*Minor allele is listed first

†MAF= Minor allele frequency

HLA-haplotyping

Paper I and II:

HLA-haplotyping was performed as described by de Bakker *et al*[128]. They captured nearby single tag SNPs or haplotypes of combination of up to three SNPs as a predictor of known HLA-alleles. The recommended tag SNPs or haplotypes given by deBakker *et al.* for the HLA-risk alleles for type 1 diabetes are shown in Table 3.3. We genotyped these tag SNPs by using the SNPlex genotyping system as described above.

Table 3.3: Genotyped tagSNPs from known risk or protective HLA alleles for type 1 diabetes selected from supplementary table 3 in deBakker *et al.*[128]

HLA allele	Frequency	Tag SNPs
HLA-DQA*0101	0.1333	rs6457614,rs1794265
HLA-DQA*0102	0.2389	rs14004,rs6457594
HLA-DQA*0201	0.1333	rs7745002,rs2858333
HLA-DQA*0301	0.2278	rs3129883,rs7745002,rs3844313
HLA-DQA*0401	0.0333	rs7743506
HLA-DQA*0501	0.1778	rs10485326,rs2040406
HLA-DQB*0201	0.1556	rs4988889
HLA-DQB*0301	0.1667	rs7744001,rs2856691
HLA-DQB*0302	0.1444	rs7454108,rs2071876
HLA-DQB*0303	0.0611	rs3834857,rs2395533
HLA-DQB*0402	0.0333	rs7743506
HLA-DQB*0501	0.0944	rs6457614
HLA-DQB*0503	0.0389	rs1794265
HLA-DQB*0602	0.2000	rs3135388
HLA-DRB*0101	0.0889	rs4947332,rs6457614
HLA-DRB*0301	0.0944	rs2040410
HLA-DRB*0401	0.0889	rs6910071,rs3817964,rs660895
HLA-DRB*0402	0.0056	rs3130071,rs416352
HLA-DRB*0403	0.0167	rs7454108,rs206765,rs399604
HLA-DRB*0405	0.0056	rs1057149
HLA-DRB*0701	0.1000	rs7745002,rs2647087,rs241398
HLA-DRB*0801	0.0333	rs7743506
HLA-DRB*0901		Not reported
HLA-DRB*1101	0.0500	rs434841,rs1061172,rs5875439
HLA-DRB*1401	0.0333	rs2763979,rs1794265
HLA-DRB*1404	0.0056	rs805262,rs1794265
HLA-DRB*1501	0.2000	rs3135388

Paper III:

HLA-DQA1 and *HLA-DQB1* genotypes were analysed at the Unit for Immunology, St Olavs Hospital, Trondheim, Norway by sequence-specific oligonucleotide probes (SSO) using LABType^{R} SSO *DQA1/DQB1* Typing Tests (One Lambda Inc., CA, USA) as described by the manufacturer. In brief, the target DNA was amplified by polymerase chain reaction (PCR) using HLA-locus specific target primers. The amplified target DNA was denatured and re-hybridized with complementary DNA probes bound to fluorescently coded microspheres, which allowed detection using R-Phycoerytrin-bound (PE) Strepavidin. The flow analyser Luminex 100 (Luminex Corporation, Texas, USA) was used to measure the fluorescent signal of PE. The HLA fusion 2.0 (One Lambda Inc., CA, USA) software was used to process the results.

3.4 Statistical methods

Paper I

PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/)[129] was used to assess whether genotypes were in Hardy-Weinberg equilibrium and to test differences in genotype distribution between affected and unaffected subjects by logistic regression under additive, dominant and recessive models. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Adjustment for diabetes specific risk factors such as age, sex and BMI was applied when appropriate. Correction for multiple testing was done by max(T) permutation where 1,000 permutations were performed. Phasing HLA-haplotypes and testing for association within cases and controls were also carried out using PLINK.

Paper II

Statistical Package for the Social Sciences, version 14.0 and 18.0 (SPSS Inc, Chicago) was used when comparing categorical data by Chi-square or Fisher's exact test as appropriate. Comparing continuous data between groups was done by a non-parametric analysis of variance test (Mann-Whitney test and Kruskal-Wallis test as appropriate). A

Kaplan-Mayer log-rank test was performed to assess time to insulin requirement in relation to antibody positivity. For comparison of antibody titer change over time (from HUNT2 to HUNT3) a nonparametric Wilcoxon signed Rank test for two related samples was used.

Paper III

Statistical analyses were performed by the PASW Statistics (version 19, SPSS, Inc, Chicago, IL). Chi-square test or Fisher exact test (when appropriate) was used to compare differences in categorical data. Mann Whitney U test was used to test differences in continuous data between two groups. Kruskal-Wallis test was used to test differences in continuous data between more than two groups. Logistic regression models were used to examine whether HLA haplotypes were associated with antiGAD after adjusting for other confounding factors such as age, gender and BMI.

A two-tailed *p*-value of 0.05 was considered to be significant in all three papers.

4 Summary of results

4.1 Paper I

Previous studies have indicated that LADA shares some genetic risk factors with type 1 diabetes. However, data from the HUNT study indicate that LADA phenotypically may be more similar to type 2 diabetes. These findings suggest that LADA may share genetic features both with type1 and type 2 diabetes. This notion was tested by comparing genetic associations in type 1 and type 2 diabetes and LADA. We also tested whether high titres of antiGAD correlated positively with alleles conferring risk of type 1 and negatively with alleles conferring risk of type 2 diabetes.

The type 1 diabetes genes *INS* (rs689 and rs3842753) and *PTPN22* (rs2476601) did not show any association with LADA. However, the most strongly associated HLA haplotypes for type 1 diabetes, were also found to be significantly associated with LADA. One distinct haplotype GCA of *DRB1*0401-DQA1*0301-DQB1*0301* was found to be associated with higher risk only of LADA (p=0.013, Table 4.1).

Table 4.1: Frequency of the two HLA-haplotypes associated only with higher risk in LADA compared to non-diabetic controls.

	LADA Total (n=121)		High antiGAD LADA (n=56)		Low antiGAD LADA (n=65)	
Risk HLA-haplotypes	Frequency (n)	P-value*	Frequency (n)	P-value*	Frequency (n)	P-value*
DRB1*0401 - DQA1*0301 - DQB1*0302 GCTA	0.04 (5)	0.107	0.01 (1)	0.341	0.07 (4)	0.002
DRB1*0401 - DQA1*0301 - DQB1*0301 GCA	0.09 (11)	0.013	0.04 (2)	0.543	0.14 (9)	7.51×10^{-5}

* P-value corrected for age, sex and BMI

For the type 2 diabetes associated genes (Table 4.2), the CC/CT genotypes of rs7961581 upstream of the *TSPAN8* gene and the obesity-linked AA/AC genotypes of the SNP

rs8050126 in the *FTO* gene were associated with LADA. The TT/TC genotypes within the strongly type 2 diabetes associated *TCF7L2* gene (rs7903146) were not obviously associated with LADA.

Table 4.2: Genotypes of known type 2 diabetes associated loci in LADA compared to non-diabetic controls.

Gene name, SNP	Genotype	LADA vs. controls		Low antiGAD LADA vs. controls	
		OR (95% CI)	P-value*	OR (95% CI)	P-value*
TCF7L2, rs7903146	CT/TT vs. CC	1.25 (0.85-1.83)	0.141	1.32 (0.78-2.24)	0.162
TSPAN8/LGR5, rs7961581	CC/CT vs. TT	1.68 (1.15-2.46)	0.01	2.17 (1.28-3.69)	0.006
FTO, rs8050136	AA/AC vs. CC	1.94 (1.22-3.09)	0.005	2.85 (1.39-5.84)	0.003
FTO, rs1861866	CT/TT vs. CC	2.55 (1.45-4.50)	0.003	3.85 (1.52-10.0)	0.004
FTO, rs9931494	CG/GG vs. CC	2.72 (1.63-4.53)	2.43×10^{-4}	3.29 (1.56-6.95)	0.001

* Adjusted p-value from logistic regression for age, sex and BMI.

After dichotomising into high and low antiGAD titre the type 2 diabetes associated genes (Table 4.3) *FTO* (rs8050136, rs1861866 and rs9931491) and *TSPAN8* (rs7961581) were associated only with low-antiGAD titre LADA. The strongly associated type 2 diabetes gene (*TCF7L2*, rs7903146) which was not associated with LADA in general, was neither associated with sub-groups of antiGAD. The HLA haplotypes (Table 4.1) were found to be mainly associated with high antiGAD LADA patients, except the GCA-haplotype for *DRB1*04:01-DQA1*03:01-DQB1*03:01* which was associated with higher risk in low antiGAD LADA patients. Interestingly, yet another distinct haplotype was found, the GCTA-haplotype for *DRB1*04:01-DQA1*03:01-DQB1*03:02*, which was only associated with higher risk in low antiGAD LADA and not with type 1 or type 2 diabetes.

4.2 Paper II

LADA comprises a significant proportion of all diabetic cases. Yet, the etiology of LADA assessed by autoimmune markers is not completely clarified, nor is the distinction from "classical" type 1 diabetes. We investigated cross-sectionally the prevalence and prospectively the pre-diabetic and post-diabetic presence of antiGAD, antiIA-2 and antiZnT8 in LADA and in type 1 diabetes.

Cross-sectional

LADA was positive for only one antibody in 90% of the cases (10% were multiple-antibody-positive). Doubly and triply positive LADA patients vs. singly positive had lower systolic blood pressure ($p=0.005$), higher non-fasting blood glucose ($p=0.011$) and higher antiGAD titer ($p<0.001$). Age at onset was not associated with the number of antibodies.

Comparing LADA with adult onset type 1 diabetes (age >24 years, n=103), LADA patients were less frequently positive for more than one antibody ($p<0.001$). Those with LADA displayed higher levels of C-peptide ($p<0.001$) and antiGAD titer ($p<0.001$) but lower antiIA-2 titer ($p=0.002$) compared to type 1 diabetic subjects.

Prospectively

Fifty-nine percent of antiGAD-positive LADA patients in HUNT2 were no longer positive in HUNT3 (Table 4.3). LADA patients who became negative possessed less frequently risk HLA haplotypes and were phenotypically more akin to those with type 2 diabetes than those who stayed positive. The former patients were also older at onset ($p=0.001$), more obese with both higher BMI and waist circumference ($p=0.013$ and $p=0.009$, respectively) and displayed higher levels of C-peptide ($p=0.003$). Still, those losing positivity differed from those with type 2 diabetes by lower C-peptide levels ($p=0.009$). Persistent antibody positivity was associated with earlier requirement of insulin treatment ($p=0.001$).

Table 4.3: Comparison of clinical characteristics from the HUNT2 survey between LADA cases who participated in both HUNT2 and HUNT3 and either became antibody negative or stayed antibody positive during follow-up (10-13 years between HUNT2 and HUNT3)

	LADA		
Clinical characteristics HUNT2	Ab negative HUNT3	Ab positive HUNT3	P-value*
N	26	18	
Age at onset (yr)	53.5 (42-75)	44.5 (21-60)	0.001
Waist circumference (cm)	96 (78-122)	88.5 (71-103)	0.009
BMI (kg/m^2)	27.9 (24.6-44.8)	25.7 (21.9-36.9)	0.013
HDL cholesterol (mmol/l)	1.1 (0.6-2.4)	1.75 (0.7-3.3)	0.017
Triacylglycerol (mmol/l)	1.6 (0.86-4.42)	1.12 (0.44-4.48)	0.002
C-peptide (pmol/l)	492 (30-1,384)	118.5 (30-588)	0.003
Glucose, fasting (mmol/l)	7.55 (2.9-11.5)	9.0 (3.9-16.9)	0.111
GADA titer (ai)	0.11 (0.08-0.46)	0.51 (0.07-2.43)	<0.001
Numbers of abs positivity			0.023
1 Ab	65% (26)	37% (14)	
2-3 Abs	-	100% (4)	

Data presented as percentages (n) or median (min-max-value)
*Unadjusted p-value.

Of incident LADA cases in HUNT3, 21 out of 31 (68%) were already antibody-positive 10-13 years earlier at HUNT2, i.e. before diabetes diagnosis (Table 4.4). Incident LADA cases who displayed antibody positivity before diagnosis were phenotypically more akin to type 1 diabetes than those who were antibody negative in HUNT2. Antibody positive patients at HUNT2 were diagnosed at younger age (p=0.001) and were associated with higher antiGAD-titer (p=0.006) and higher fasting blood glucose (p=0.013) at HUNT3 than antibody negative patients.

Thirteen out of 24 incident type 1 diabetic cases (54%) were antibody positive already at HUNT2. There were no distinctive differences between antibody positive and antibody negative type 1 diabetic patients either at pre-diabetes (HUNT2) or at overt diabetes (HUNT3).

Table 4.4: Comparison of clinical characteristics from HUNT3 survey between antibody negative and antibody positive cases at HUNT2 who were diagnosed with LADA in HUNT3. Antibodies were measured at HUNT2 before diabetes diagnosis.

	LADA		
Clinical characteristics HUNT3	Ab negative HUNT2	Ab positive HUNT2	p-value*
N	10	21	
Age at onset (year)	70 (57-80)	55 (31-79)	0.001
Glucose, non-fasting (mmol/l)	6.8 (5.7-12.2)	11.1 (5.0-22.4)	0.033
GADA titer (ai)	0.12 (0.08-1.09)	1.17 (0.1-2.09)	0.002
Glucose, fasting (mmol/l)	5.65 (5.2-6.0)	8.0 (5.5-19.6)	0.013

Data presented as percentages (n) or median (min-max-value)
*Unadjusted p-value.

4.3 Paper III

Development of autoimmune diabetes is strongly associated with autoantibody markers with high affinity towards the islet of Langerhans, such as antiGAD. Studies on the presence and clinical implications of antiGAD positivity in the general non-diabetic population are however few, especially in adults. We aimed to investigate these aspects prospectively in a large sample of non-diabetic adults from the HUNT2 and HUNT3 surveys.

In persistently non-diabetic individuals the prevalence of antiGAD positivity in HUNT2 was 1.69% (n=76). Prevalence was highest in the 30-34 years age group (3.2 %, n=16). AntiGAD positivity was not associated with sex, first degree family history of diabetes, smoking, non-fasting glucose or BMI. There was association with the presence of thyroid peroxide antibody (antiTPO) positivity ($p<0.025$).

AntiGAD positivity persisted at follow-up (HUNT3) in 35 out of the 76 persistently non-diabetic individuals (46%). Persistently non-diabetic subjects who were antiGAD positive at HUNT2 were compared with cases who were antiGAD positive and non-diabetic at HUNT2 but had diabetes at HUNT3 (n=34, Table 4.5). The latter cases had higher frequency of first degree family history of diabetes ($p=0.01$), had higher antiGAD titre ($p<0.001$) and non-fasting glucose in HUNT2 ($p=0.001$) compared to those who did not develop diabetes.

Table 4.5: Clinical characteristics of antiGAD positive and initially non-diabetic individuals at HUNT2, divided in persistently non-diabetic and pre-diabetic individuals.

	Persistently non-diabetic* **(n=76)**	**Pre-diabetic†** **(n=34)**	***p*-value‡**
Sex (male)	40 (52.6%)	18 (52.9%)	1.00
Waist circumference (cm)	84 (64-109)	92 (65-109)	0.03
BMI (kg/m^2)	26 (19-35)	27 (18-40)	0.07
Systolic blood pressure (mmHg)	131 (102-189)	136 (107-190)	0.06
Diastolic blood pressure (mmHg)	78 (53-120)	85 (58-122)	0.09
Glucose, non-fasting (mmol/l)	5.05 (3.50-7.10)	5.60 (4.10-27.0)	0.001
Triacylglycerol (mmol/l)	1.31 (0.24-4.73)	1.62 (0.58-7.76)	0.07
AntiGAD level (ai)	0.08 (0.06-3.58)	1.43 (0.07-1.98)	<0.001
First degree relative	21 (27.6%)	18 (52.9%)	0.02

Data are presented as n (*%*) or median (min–max values).

*Persistently non-diabetic; non-diabetic individuals at both HUNT2 and HUNT3.

†Pre-diabetic; individuals reported not having diabetes in HUNT2 and reported having diabetes in HUNT3. Thirteen out of these were classified as classic type 1 diabetes and the rest was classified as LADA (n=21).

‡Unadjusted *p*- value calculated by Mann–Whitney *U* test for continuous data and by Fisher's exact test for categorical data.

5 Discussion

5.1 Methodological considerations

The HUNT study

This study was based on subjects selected from the second and third HUNT surveys in the HUNT study which encompasses the population of the Nord-Trøndelag County. This county consists of mainly rural areas and is thereby sparsely populated; however, the county is fairly demographically representative of Norway with regard to geography, economy, age distribution, morbidity and mortality. The population is stable and ethnically homogenous[120]. The HUNT study is population-based and can provide a long observation time. These features make the HUNT study very suitable for genetic and epidemiological studies on slowly developing diseases such as diabetes. However, there are important concerns regarding the HUNT study that needs to be addressed.

Selection bias

There has been a noticeable decrease in the attendance rate from the HUNT1 survey to the HUNT3 survey raising the question if non-responders could lead to increased selection bias over time. Because of this concern a non-responder study was carried out both in HUNT1 and HUNT3[130,131]. The attendance rate was lowest in the youngest (20-29 years old) and oldest (80+ years old) age groups both in HUNT1 and HUNT3. The main reasons for not attending had not changed between the two surveys. “Had no time / absent” was the most frequently reason given for not attending, especially in the youngest age groups whereas “too ill to attend” was the main reason amongst the oldest people (80+).

In the HUNT1 survey one could not find any differences in health status between participants and non-participants in the youngest age groups, however there was a selection on marital status and social network. Among the elderly the non-participants showed a higher frequency in mortality and morbidity indicating a more “healthy” population among those who participated.

In the HUNT3 survey the prevalence of reported diabetes diagnosis (assessed by questionnaire) was higher among non-participants than among participants. Also the prevalence of diabetes was higher when based on records from the general practitioners compared to reported diabetes diagnosis among the participants. This difference was most marked among those over 60 years old.

This indicates that the prevalence and incidence of diabetic cases in paper I and II may be underestimated. The decreasing prevalence among participants above 60 years old may also explain why the prevalence of LADA in HUNT3 is lower than in HUNT2 (paper II).

Self-reported information (information bias)

As described, the presence of diabetes in this study is based on answering affirmative to the question "Do you have/or have had diabetes?". Also, the use of insulin (or use of any other treatment for diabetes), which was used in the classification of type of diabetes, was based on self-reporting. One may question the reliability in self-reported answers and the possibility of misclassification. Midthjell *et al.* performed a validation study regarding these aspects of the HUNT1 survey[132]. Correct diagnosis diabetes/no diabetes was verified in 96.4% of those reporting having diabetes and in 99.7% in those reporting not having diabetes. Regarding treatment this was verified as correct in 95% of those reporting using insulin and in 100% of those reporting using oral agents. A negative answer regarding treatment was verified in 100% and 99%, respectively. This indicated a very good correlation between the self-reported answers from the questionnaires and the medical records and a minimized possibility of misclassification of diabetic cases.

Antibody measurements (information bias)

By setting a cut-off of antibody positivity, it is possible that we have "recruited" falsely antibody positive cases. However, in paper I and II the cut-off was the same as the cut-off used by the Aker Hormone Laboratory, which was based on data from participation

in DASP. The laboratory wanted highest possible specificity to ensure that the patients who were found to be antibody positive were true positive (correspondence with Peter Torjesen, Aker Hormone Laboratory, Oslo, Norway). The cut-offs in both the antiGAD and antiZnT8 assays had a 100% specificity and the antiIA-2 had 99% specificity according to DASP. All assays had however a somewhat low sensitivity which will rather underestimate than overestimate the prevalence of antibody positive individuals. This may have influenced the classification of LADA and type 2 diabetes, leaving an underestimation of the LADA group. However, having this high cut-off will also lead to a more clearly defined LADA phenotype because we are more certain that these patients are truly antibody positive. Since the LADA group is much smaller than the type 2 diabetic group, it is more important that the LADA cases are identified are true LADA than if a few LADA cases are misdiagnosed as type 2 diabetes.

Storage time of serum samples

Could long-term storage at low temperatures influence the antibody levels in serum? Very few studies have addressed this potential problem. One study reported that long-term storage (>14 years) of serum at -25^{o}C markedly increased the antiTPO level in the samples[133]. These results may indicate that there is a probability of evaporation of water over time from the stored serum samples that could affect the antibody level in samples. If evaporation of water from serum samples occurs, this may lead to increased antibody titer and consequently false positive antibody results. This could affect the prevalence of LADA cases (paper I and II) and the prevalence of antiGAD positivity in the non-diabetic population (paper III) in our study. The serum samples used in this study, which were collected from the HUNT2 survey, have been stored for up to 16 years (paper III), a time span which could potentially lead to evaporation. However, the HUNT serum samples have been stored at -70^{o}C and have been exposed to a limited number of freeze and thaw cycles, which most likely will be better for the quality of the serum samples and minimize the probability of evaporation compared to storage at -25^{o}C.

Classification criteria of LADA

Age, antibody positivity and independence of insulin treatment have been used in the past to diagnose LADA.

The age limit (usually <35 years) is to some extent arbitrary. We agree with the view of Rolandsson and Palmer that there should be no age limit in the classification of LADA[134].

Antibody positivity (usually anti-GAD) is regarded as a sine qua non for diagnosis of LADA. Is it possible to have LADA without being antibody positive? Data, from this study (paper II) do indicate so. About 50% of individuals with diabetes who were found to have LADA at HUNT2 because they were antibody positive, lost anti-GAD positivity when they were re-examined 10-13 years later at HUNT3. Hence, if diabetes had been diagnosed for the first time at HUNT3 these individuals would have been classified as having type 2 diabetes. If one accepts that even fleeting positivity for autoimmune markers in diabetes does have clinical importance, then one must conclude that "mild" autoimmunity plays a role for developing diabetes not only for those individuals that are diagnosed as LADA at a given time point of examination but also for a significant part of those individuals who are currently diagnosed as having type 2 diabetes.

The last criterion for LADA is independence of insulin treatment at diagnosis and for a significant period of time thereafter. Whether an individual with new-onset diabetes is in need of insulin treatment is open to subjective evaluation and to current norms of treatment. Clearly, the insulin independence criterion is the fussiest one when defining LADA. Accordingly, it has been proposed to merge LADA with type 1 diabetes into a single category of autoimmune diabetes in adults[134]. Still, the insulin independence feature separates LADA patients phenotypically and genetically from those with adult onset "classical" type 1 diabetes (papers I and II).

Candidate gene studies

The candidate gene approach was chosen for several reasons. First: GWAS are effective for identification of common variants; however the effect sizes have been small with low odds ratios. Correction for hundreds of thousands of variants (multiple testing) is also essential in GWAS leading to the requirement of large sample sizes. The LADA group was therefore too small to consider GWAS. When this study was started there were already established several well-known loci associated with either type 1 diabetes or type 2 diabetes, identified from both candidate gene analysis and GWAS, and which had not been tested for associations in LADA. Candidate gene studies where therefore at the time of investigation the most suitable approach for testing for the type 1 and type 2 diabetes associated SNPs in the LADA patients.

5.2 Genetic and autoimmune markers associated with risk of developing LADA

Paper I was concerned with two important issues 1) evaluating the genetic risk factors for LADA and 2) assessing if genetic risk factors were dependent on the degree of autoimmunity (assessed by anti-GAD titre) and/or insulin resistance (assessed by BMI).

We did, as others, find that the type 1 diabetes high risk HLA haplotypes were associated with LADA, but still with a lower frequency compared to type 1 diabetes[66,76]. The HLA risk haplotypes were also found to be mainly associated with high antiGAD titer LADA patients. It is well known that associations of risk HLA haplotypes diminish with increasing age of type 1 diabetes[135]. However, this does not explain altogether the difference in strength of association between LADA and type 1 diabetes.

Regarding type 2 diabetes genes we found two novel associations with LADA, the *FTO* and *TSPAN8* genes. Both genes were associated with only low antiGAD LADA, however, the *FTO* gene was associated with obese LADA cases whereas the *TSPAN8* gene was associated with non-obese LADA.

Because of the small sample size and consequently low statistical power, we may have failed to detect previously reported genetic associations like the non-HLA type 1 diabetes genes *INS*, *PTPN22*, *CTLA4* and the type 2 diabetes gene *TCF7L2*[76,77]. Apart from the sample size in our study the discrepancies may also be due to the differences in classification criteria between ours and other studies.

Paper II was mainly concerned with one important issue; the pre- and post-diabetic presence of different beta-cell antibodies in patients with LADA. We found that about 50% of our LADA patients seroconverted to antibody negative during 10-13 years of follow-up (from HUNT2 to HUNT3), a percentage which was higher than previously reported[136,137]. Cases who reverted to negative were found to be phenotypically more similar to cases with type 2 diabetes. However, the patients with LADA displayed lower levels of C-peptide compared to patients with type 2 diabetic which indicates that even a temporary appearance of the GADA marker of autoimmunity is of clinical importance. We have also documented that a large proportion of the LADA patients displayed antiGAD positivity several years before their diagnosis. This is in line with one earlier study that has shown that antiGAD positivity can predict development of "type 2 diabetes" in adults[114].

The existence of LADA as a subgroup of autoimmune diabetes has been debated[118,134,138,139]. In this study we have documented that the genetic risk factors for LADA includes both type 1 and type 2 diabetes associated genes. These differences indicate important etiological differences between LADA and type 1 diabetes.

5.3 The significance of autoimmunity in a general adult non-diabetic population

We found an antiGAD positivity prevalence of 1.7% in our adult persistently non-diabetic population, a prevalence which is in agreement with previous reports[95,113,114,140]. We could not find any clinical parameters conferring risk of diabetes that were associated with antiGAD positivity in the non-diabetic subjects. However,

having high risk *HLA-DQA1/DQB1* haplotypes was associated with the presence of GAD antibodies. This associations is in line with another recent study[119]. Given the etiological importance of these haplotypes it is reasonable to conclude that even minor and fleeting antiGAD positivity is coupled to autoimmunity. A comparison of antiGAD positive persistent non-diabetic individuals with pre-diabetic antiGAD positive individuals revealed higher titers of antiGAD, higher BMI and age in HUNT2 in the latter group. This could indicate higher autoimmune activity in the pre-diabetic group as a cause of precipitating overt diabetes in these individuals. However, also risk factors for type 2 diabetes (such as age and obesity) could have contributed to the development to diabetes.

If antiGAD or also other antibodies are to be used in classification of patients with diabetes as proposed by the World Health Organization and the American Diabetes Association, it is necessary to know the prevalence of these antibodies in the adult non-diabetic population. The presence of antibody positivity, in particular antiGAD has been argued as being non-specific, not only in the non-diabetic population but also in diabetic cases who display low titres of antiGAD[118]. However, our data show that antiGAD positivity in long term (>10 years) non-diabetic individuals associate to risk HLA haplotypes and also to other markers of autoimmunity (antiTPO). Hence, the antiGAD findings in non-diabetic individuals cannot easily be dismissed as false positives.

6 Conclusions

I Genetic predisposition in LADA is a mixture of both type 1 and type 2 diabetes associated genes. The type 1 diabetes genes are associated with LADA with higher degree of autoimmunity (high antiGAD titer) and the type 2 diabetes genes are associated with LADA with lower degree of autoimmunity (low antiGAD titer).

II The dynamic patterns of antibody positivity in patients classified as LADA influence the phenotype of disease.

III AntiGAD positivity in adult persistently non-diabetic individuals from the general population is partly persistent over a long time period and is not associated with clinical parameters related to diabetes, but associated with high risk HLA haplotypes known to be associated with type 1 diabetes.

7 Future perspectives

Since we started this study a new generation of DNA sequencing techniques have developed which makes it possible to identify new variants and loci contributing to disease risk[141]. Next-generation sequencing like exome sequencing and whole genome sequencing are now being used to detect low-frequent (<0.5%) variants. Some causal rare variants may have large effect sizes relative to the typically small effect sizes seen for common variants genotyped by current GWAS, and it is hypothesized that multiple rare variants, each occurring at low frequency but with larger effect size, may contribute to common diseases. The larger effect size of such variants also make re-sequencing approaches more suitable for diseases with low sample sizes like LADA, and in this regard it would have been interesting to carry out exome or whole genome sequencing of the diabetic population from HUNT and particularly of the LADA population. This approach would gather genetic information beyond what can be obtained by conventional GWAS and might contribute to define the genetic architecture of the LADA phenotype.

In this study (paper II and III) antiGAD was analyzed in pre-diabetic state (at HUNT2) only in cases found to have autoimmune diabetes at HUNT3. It would also have been interesting to explore the antiGAD status at a pre-diabetic state in those cases who developed diabetes between HUNT2 and HUNT3 but who were antiGAD negative in HUNT3. Another point that we did not address but which would have been interesting to study further is the appearance of antiGAD positivity in the entire cohort at the visit in HUNT3 and not only in those being antiGAD positive in HUNT2 (paper III). One may expect that some of the antiGAD negative individuals in HUNT2 should turn antiGAD positive during the follow-up. A third point would have been to screen for not only antiGAD but also antiIA-2, antiZnT8 and antiIA in the non-diabetic population. All of these points would add important and very interesting information, and clearly is something we will focus on in the nearest future.

It will also be interesting to follow the as of now persistently non-diabetic individuals with antiGAD for possible development of diabetes in coming years (hopefully in a HUNT4).

8 References

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011;94:311-21.
2. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26 Suppl 1:S5-20.
3. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.
4. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 1994;331:1428-36.
5. Aamodt G, Stene LC, Njolstad PR, Sovik O, Joner G. Spatiotemporal trends and age-period-cohort modeling of the incidence of type 1 diabetes among children aged <15 years in Norway 1973-1982 and 1989-2003. Diabetes Care 2007;30:884-9.
6. Onkamo P, Vaananen S, Karvonen M, Tuomilehto J. Worldwide increase in incidence of Type I diabetes--the analysis of the data on published incidence trends. Diabetologia 1999;42:1395-403.
7. Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 2009;373:2027-33.
8. Steck AK, Rewers MJ. Genetics of type 1 diabetes. Clin Chem 2011;57:176-85.
9. Lempainen J, Tauriainen S, Vaarala O, et al. Interaction of enterovirus infection and cow's milk-based formula nutrition in type 1 diabetes-associated autoimmunity. Diabetes Metab Res Rev 2012;28:177-85.
10. Honeyman M. How robust is the evidence for viruses in the induction of type 1 diabetes? Curr Opin Immunol 2005;17:616-23.
11. Oikarinen M, Tauriainen S, Oikarinen S, et al. Type 1 diabetes is associated with enterovirus infection in gut mucosa. Diabetes 2012;61:687-91.
12. Akerblom HK, Vaarala O, Hyoty H, Ilonen J, Knip M. Environmental factors in the etiology of type 1 diabetes. Am J Med Genet 2002;115:18-29.
13. Stene LC, Oikarinen S, Hyoty H, et al. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). Diabetes 2010;59:3174-80.
14. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001;358:1500-3.
15. Gale EA. A missing link in the hygiene hypothesis? Diabetologia 2002;45:588-94.
16. Noble JA, Valdes AM, Cook M, Klitz W, Thomson G, Erlich HA. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. Am J Hum Genet 1996;59:1134-48.
17. Redondo MJ, Fain PR, Eisenbarth GS. Genetics of type 1A diabetes. Recent Prog Horm Res 2001;56:69-89.
18. Thomson G. HLA disease associations: models for insulin dependent diabetes mellitus and the study of complex human genetic disorders. Annu Rev Genet 1988;22:31-50.

19. Ounissi-Benkalha H, Polychronakos C. The molecular genetics of type 1 diabetes: new genes and emerging mechanisms. Trends Mol Med 2008;14:268-75.
20. Lambert AP, Gillespie KM, Thomson G, et al. Absolute risk of childhood-onset type 1 diabetes defined by human leukocyte antigen class II genotype: a population-based study in the United Kingdom. J Clin Endocrinol Metab 2004;89:4037-43.
21. Pugliese A, Kawasaki E, Zeller M, et al. Sequence analysis of the diabetes-protective human leukocyte antigen-DQB1*0602 allele in unaffected, islet cell antibody-positive first degree relatives and in rare patients with type 1 diabetes. J Clin Endocrinol Metab 1999;84:1722-8.
22. Barratt BJ, Payne F, Lowe CE, et al. Remapping the insulin gene/IDDM2 locus in type 1 diabetes. Diabetes 2004;53:1884-9.
23. Kawasaki E, Awata T, Ikegami H, et al. Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22): association between a promoter polymorphism and type 1 diabetes in Asian populations. Am J Med Genet A 2006;140:586-93.
24. Bottini N, Musumeci L, Alonso A, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 2004;36:337-8.
25. Ueda H, Howson JM, Esposito L, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003;423:506-11.
26. Vella A, Cooper JD, Lowe CE, et al. Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. Am J Hum Genet 2005;76:773-9.
27. Lowe CE, Cooper JD, Brusko T, et al. Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. Nat Genet 2007;39:1074-82.
28. Barrett JC, Clayton DG, Concannon P, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009;41:703-7.
29. Smyth DJ, Cooper JD, Bailey R, et al. A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nat Genet 2006;38:617-9.
30. Todd JA, Walker NM, Cooper JD, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007;39:857-64.
31. Hakonarson H, Grant SF, Bradfield JP, et al. A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. Nature 2007;448:591-4.
32. Cooper JD, Smyth DJ, Smiles AM, et al. Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. Nat Genet 2008;40:1399-401.
33. Pociot F, Akolkar B, Concannon P, et al. Genetics of type 1 diabetes: what's next? Diabetes 2010;59:1561-71.
34. Pugliese A. The insulin gene in type 1 diabetes. IUBMB Life 2005;57:463-8.
35. Baker PR, Eisenbarth GS. Type 1 Diabetes—Pathogenesis, Prediction, and Prevention. US Endocrinology 2009;5:79-84.
36. Baekkeskov S, Aanstoot HJ, Christgau S, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 1990;347:151-6.

37. Wenzlau JM, Juhl K, Yu L, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A 2007;104:17040-5.
38. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. J Immunol 1995;155:5419-26.
39. Palmer JP, Asplin CM, Clemons P, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 1983;222:1337-9.
40. Lyssenko V, Almgren P, Anevski D, et al. Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. Diabetes 2005;54:166-74.
41. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343-50.
42. van Dam RM. The epidemiology of lifestyle and risk for type 2 diabetes. Eur J Epidemiol 2003;18:1115-25.
43. Wild SH, Byrne CD. ABC of obesity. Risk factors for diabetes and coronary heart disease. BMJ 2006;333:1009-11.
44. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403.
45. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 1991;303:1019-22.
46. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull 2001;60:5-20.
47. Frayling TM, Hattersley AT. The role of genetic susceptibility in the association of low birth weight with type 2 diabetes. Br Med Bull 2001;60:89-101.
48. Horikawa Y, Oda N, Cox NJ, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 2000;26:163-75.
49. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 2006;38:320-3.
50. Gloyn AL, Weedon MN, Owen KR, et al. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes 2003;52:568-72.
51. Altshuler D, Hirschhorn JN, Klannemark M, et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 2000;26:76-80.
52. Sandhu MS, Weedon MN, Fawcett KA, et al. Common variants in WFS1 confer risk of type 2 diabetes. Nat Genet 2007;39:951-3.
53. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445:881-5.
54. Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341-5.

55. Zeggini E, Scott LJ, Saxena R, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008;40:638-45.
56. Perry JR, Frayling TM. New gene variants alter type 2 diabetes risk predominantly through reduced beta-cell function. Curr Opin Clin Nutr Metab Care 2008;11:371-7.
57. McCarthy MI. Genomics, type 2 diabetes, and obesity. N Engl J Med 2010;363:2339-50.
58. Bonnefond A, Froguel P, Vaxillaire M. The emerging genetics of type 2 diabetes. Trends Mol Med 2010;16:407-16.
59. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889-94.
60. Dina C, Meyre D, Gallina S, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 2007;39:724-6.
61. Lango H, Palmer CN, Morris AD, et al. Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. Diabetes 2008;57:3129-35.
62. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev 2005;26:19-39.
63. Macor C, Ruggeri A, Mazzonetto P, Federspil G, Cobelli C, Vettor R. Visceral adipose tissue impairs insulin secretion and insulin sensitivity but not energy expenditure in obesity. Metabolism 1997;46:123-9.
64. Groop LC, Bottazzo GF, Doniach D. Islet cell antibodies identify latent type I diabetes in patients aged 35-75 years at diagnosis. Diabetes 1986;35:237-41.
65. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 1993;42:359-62.
66. Tuomi T, Carlsson A, Li H, et al. Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. Diabetes 1999;48:150-7.
67. Buzzetti R, Di Pietro S, Giaccari A, et al. High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. Diabetes Care 2007;30:932-8.
68. Szepietowska B, Glebocka A, Puch U, Gorska M, Szelachowska M. Latent autoimmune diabetes in adults in a population-based cohort of Polish patients with newly diagnosed diabetes mellitus. Arch Med Sci 2012;8:491-5.
69. Davies H, Brophy S, Fielding A, et al. Latent autoimmune diabetes in adults (LADA) in South Wales: incidence and characterization. Diabet Med 2008;25:1354-7.
70. Lampasona V, Petrone A, Tiberti C, et al. Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes: Non Insulin Requiring Autoimmune Diabetes (NIRAD) 4. Diabetes Care 2010;33:104-8.
71. Carlsson S, Midthjell K, Tesfamarian MY, Grill V. Age, overweight and physical inactivity increase the risk of latent autoimmune diabetes in adults: results from the Nord-Trondelag health study. Diabetologia 2007;50:55-8.
72. Juneja R, Hirsch IB, Naik RG, Brooks-Worrell BM, Greenbaum CJ, Palmer JP. Islet cell antibodies and glutamic acid decarboxylase antibodies, but not the clinical

phenotype, help to identify type 1(1/2) diabetes in patients presenting with type 2 diabetes. Metabolism 2001;50:1008-13.
73. Radtke MA, Midthjell K, Nilsen TI, Grill V. Heterogeneity of patients with latent autoimmune diabetes in adults: linkage to autoimmunity is apparent only in those with perceived need for insulin treatment: results from the Nord-Trondelag Health (HUNT) study. Diabetes Care 2009;32:245-50.
74. Desai M, Zeggini E, Horton VA, et al. An association analysis of the HLA gene region in latent autoimmune diabetes in adults. Diabetologia 2007;50:68-73.
75. Olsson L, Ahlbom A, Grill V, Midthjell K, Carlsson S. Sleep disturbances and low psychological well-being are associated with an increased risk of autoimmune diabetes in adults. Results from the Nord-Trondelag Health Study. Diabetes Res Clin Pract 2012;98:302-11.
76. Cervin C, Lyssenko V, Bakhtadze E, et al. Genetic similarities between latent autoimmune diabetes in adults, type 1 diabetes, and type 2 diabetes. Diabetes 2008;57:1433-7.
77. Haller K, Kisand K, Pisarev H, et al. Insulin gene VNTR, CTLA-4 +49A/G and HLA-DQB1 alleles distinguish latent autoimmune diabetes in adults from type 1 diabetes and from type 2 diabetes group. Tissue Antigens 2007;69:121-7.
78. Desai M, Zeggini E, Horton VA, et al. The variable number of tandem repeats upstream of the insulin gene is a susceptibility locus for latent autoimmune diabetes in adults. Diabetes 2006;55:1890-4.
79. Zampetti S, Spoletini M, Petrone A, et al. Association of TCF7L2 gene variants with low GAD autoantibody titre in LADA subjects (NIRAD Study 5). Diabet Med 2010;27:701-4.
80. Bakhtadze E, Cervin C, Lindholm E, et al. Common variants in the TCF7L2 gene help to differentiate autoimmune from non-autoimmune diabetes in young (15-34 years) but not in middle-aged (40-59 years) diabetic patients. Diabetologia 2008;51:2224-32.
81. Lukacs K, Hosszufalusi N, Dinya E, Bakacs M, Madacsy L, Panczel P. The type 2 diabetes-associated variant in TCF7L2 is associated with latent autoimmune diabetes in adult Europeans and the gene effect is modified by obesity: a meta-analysis and an individual study. Diabetologia 2012;55:689-93.
82. Turner R, Stratton I, Horton V, et al. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. Lancet 1997;350:1288-93.
83. Chiu HK, Tsai EC, Juneja R, et al. Equivalent insulin resistance in latent autoimmune diabetes in adults (LADA) and type 2 diabetic patients. Diabetes Res Clin Pract 2007;77:237-44.
84. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974;2:1279-83.
85. MacCuish AC, Irvine WJ, Barnes EW, Duncan LJ. Antibodies to pancreatic islet cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet 1974;2:1529-31.
86. Torn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia 2008;51:846-52.

87. Lampasona V, Schlosser M, Mueller PW, et al. Diabetes antibody standardization program: first proficiency evaluation of assays for autoantibodies to zinc transporter 8. Clin Chem 2011;57:1693-702.
88. Pihoker C, Gilliam LK, Hampe CS, Lernmark A. Autoantibodies in diabetes. Diabetes 2005;54 Suppl 2:S52-61.
89. Decochez K, Tits J, Coolens JL, et al. High frequency of persisting or increasing islet-specific autoantibody levels after diagnosis of type 1 diabetes presenting before 40 years of age. The Belgian Diabetes Registry. Diabetes Care 2000;23:838-44.
90. Andersson C, Larsson K, Vaziri-Sani F, et al. The three ZNT8 autoantibody variants together improve the diagnostic sensitivity of childhood and adolescent type 1 diabetes. Autoimmunity 2011;44:394-405.
91. Gorsuch AN, Spencer KM, Lister J, et al. Evidence for a long prediabetic period in type I (insulin-dependent) diabetes mellitus. Lancet 1981;2:1363-5.
92. Tarn AC, Smith CP, Spencer KM, Bottazzo GF, Gale EA. Type I (insulin dependent) diabetes: a disease of slow clinical onset? Br Med J (Clin Res Ed) 1987;294:342-5.
93. Kukko M, Kimpimaki T, Korhonen S, et al. Dynamics of diabetes-associated autoantibodies in young children with human leukocyte antigen-conferred risk of type 1 diabetes recruited from the general population. J Clin Endocrinol Metab 2005;90:2712-7.
94. LaGasse JM, Brantley MS, Leech NJ, et al. Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. Diabetes Care 2002;25:505-11.
95. Knip M, Korhonen S, Kulmala P, et al. Prediction of type 1 diabetes in the general population. Diabetes Care 2010;33:1206-12.
96. Reddy S, Wu D, Poole CA. Glutamic acid decarboxylase 65 and 67 isoforms in fetal, neonatal and adult porcine islets: predominant beta cell co-localization by light and confocal microscopy. J Autoimmun 1996;9:21-7.
97. Leslie RD, Atkinson MA, Notkins AL. Autoantigens IA-2 and GAD in Type I (insulin-dependent) diabetes. Diabetologia 1999;42:3-14.
98. Bu DF, Erlander MG, Hitz BC, et al. Two human glutamate decarboxylases, 65-kDa GAD and 67-kDa GAD, are each encoded by a single gene. Proc Natl Acad Sci U S A 1992;89:2115-9.
99. Hagopian WA, Michelsen B, Karlsen AE, et al. Autoantibodies in IDDM primarily recognize the 65,000-M(r) rather than the 67,000-M(r) isoform of glutamic acid decarboxylase. Diabetes 1993;42:631-6.
100. Tuomilehto J, Zimmet P, Mackay IR, et al. Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease. Lancet 1994;343:1383-5.
101. Hagopian WA, Sanjeevi CB, Kockum I, et al. Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. J Clin Invest 1995;95:1505-11.
102. Vandewalle CL, Falorni A, Svanholm S, Lernmark A, Pipeleers DG, Gorus FK. High diagnostic sensitivity of glutamate decarboxylase autoantibodies in insulin-dependent diabetes mellitus with clinical onset between age 20 and 40 years. The Belgian Diabetes Registry. J Clin Endocrinol Metab 1995;80:846-51.

103. Saeki K, Zhu M, Kubosaki A, Xie J, Lan MS, Notkins AL. Targeted disruption of the protein tyrosine phosphatase-like molecule IA-2 results in alterations in glucose tolerance tests and insulin secretion. Diabetes 2002;51:1842-50.
104. Gorus FK, Goubert P, Semakula C, et al. IA-2-autoantibodies complement GAD65-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. The Belgian Diabetes Registry. Diabetologia 1997;40:95-9.
105. Chimienti F, Devergnas S, Favier A, Seve M. Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. Diabetes 2004;53:2330-7.
106. Chimienti F, Favier A, Seve M. ZnT-8, a pancreatic beta-cell-specific zinc transporter. Biometals 2005;18:313-7.
107. Nielsen LB, Vaziri-Sani F, Porksen S, et al. Relationship between ZnT8Ab, the SLC30A8 gene and disease progression in children with newly diagnosed type 1 diabetes. Autoimmunity 2011;44:616-23.
108. Wenzlau JM, Liu Y, Yu L, et al. A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes 2008;57:2693-7.
109. Kawasaki E, Nakamura K, Kuriya G, et al. Differences in the humoral autoreactivity to zinc transporter 8 between childhood- and adult-onset type 1 diabetes in Japanese patients. Clin Immunol 2011;138:146-53.
110. Trabucchi A, Faccinetti NI, Guerra LL, et al. Detection and characterization of ZnT8 autoantibodies could help to screen latent autoimmune diabetes in adult-onset patients with type 2 phenotype. Autoimmunity 2012;45:137-42.
111. Trabucchi A, Faccinetti NI, Guerra LL, et al. Detection and characterization of ZnT8 autoantibodies could help to screen latent autoimmune diabetes in adult-onset patients with type 2 phenotype. Autoimmunity 2011.
112. Rolandsson O, Hagg E, Janer M, et al. High GAD65 autoantibody levels in nondiabetic adults are associated with HLA but not with CTLA-4 or INS VNTR. J Intern Med 2003;253:447-53.
113. Ruige JB, Batstra MR, Aanstoot HJ, et al. Low prevalence of antibodies to GAD65 in a 50- to 74-year-old general Dutch population. The Hoorn Study. Diabetes Care 1997;20:1108-10.
114. Lundgren VM, Isomaa B, Lyssenko V, et al. GAD antibody positivity predicts type 2 diabetes in an adult population. Diabetes 2010;59:416-22.
115. Schlosser M, Strebelow M, Rjasanowski I, Kerner W, Wassmuth R, Ziegler M. Prevalence of diabetes-associated autoantibodies in schoolchildren: the Karlsburg Type 1 Diabetes Risk Study. Ann N Y Acad Sci 2004;1037:114-7.
116. Rolandsson O, Hagg E, Nilsson M, Hallmans G, Mincheva-Nilsson L, Lernmark A. Prediction of diabetes with body mass index, oral glucose tolerance test and islet cell autoantibodies in a regional population. J Intern Med 2001;249:279-88.
117. Bosi EP, Garancini MP, Poggiali F, Bonifacio E, Gallus G. Low prevalence of islet autoimmunity in adult diabetes and low predictive value of islet autoantibodies in the general adult population of northern Italy. Diabetologia 1999;42:840-4.
118. Gale EA. Latent autoimmune diabetes in adults: a guide for the perplexed. Diabetologia 2005;48:2195-9.
119. Andersen MK, Lundgren V, Isomaa B, Groop L, Tuomi T. Association of variants in HLA-DQA1-DQB1, PTPN22, INS, and CTLA4 with GAD autoantibodies

and insulin secretion in nondiabetic adults of the Botnia Prospective Study. Eur J Endocrinol 2012;167:27-33.
120. Holmen J, Midthjell,K, Krüger,Ø, Langhammer,A, Holmen,TL, Bratberg,GH, Vatten,L, Lund-Larsen,PG. The Nord-Trøndelag Health Study 1995–97 (HUNT2): objectives, contents, methods and participation. Norsk Epidemiologi 2003;13:19-32.
121. Krokstad S, Langhammer A, Hveem K, et al. Cohort Profile: The HUNT Study, Norway. Int J Epidemiol 2012.
122. Damcott CM, Ott SH, Pollin TI, et al. Genetic variation in adiponectin receptor 1 and adiponectin receptor 2 is associated with type 2 diabetes in the Old Order Amish. Diabetes 2005;54:2245-50.
123. Vaxillaire M, Dechaume A, Vasseur-Delannoy V, et al. Genetic analysis of ADIPOR1 and ADIPOR2 candidate polymorphisms for type 2 diabetes in the Caucasian population. Diabetes 2006;55:856-61.
124. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336-41.
125. Meyre D, Bouatia-Naji N, Tounian A, et al. Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. Nat Genet 2005;37:863-7.
126. Grarup N, Urhammer SA, Ek J, et al. Studies of the relationship between the ENPP1 K121Q polymorphism and type 2 diabetes, insulin resistance and obesity in 7,333 Danish white subjects. Diabetologia 2006;49:2097-104.
127. Aulchenko YS, Pullen J, Kloosterman WP, et al. LPIN2 is associated with type 2 diabetes, glucose metabolism, and body composition. Diabetes 2007;56:3020-6.
128. de Bakker PI, McVean G, Sabeti PC, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. Nat Genet 2006;38:1166-72.
129. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-75.
130. Holmen J, Midthjell K, Forsén L, Skjerve K, Gorseth M, Oseland A. [Attendance and comparative analysis of attendants and non-attendants]. Tidsskr Nor Laegeforen 1990;110:1973-7.
131. Langhammer A, Krokstad S, Romundstad P, Heggland J, Holmen J. The HUNT study: participation is associated with survival and depends on socioeconomic status, diseases and symptoms. BMC Med Res Methodol 2012;12:143.
132. Midthjell K, Holmen J, Bjorndal A, Lund-Larsen G. Is questionnaire information valid in the study of a chronic disease such as diabetes? The Nord-Trondelag diabetes study. J Epidemiol Community Health 1992;46:537-42.
133. Mannisto T, Surcel HM, Bloigu A, et al. The effect of freezing, thawing, and short- and long-term storage on serum thyrotropin, thyroid hormones, and thyroid autoantibodies: implications for analyzing samples stored in serum banks. Clin Chem 2007;53:1986-7.
134. Rolandsson O, Palmer JP. Latent autoimmune diabetes in adults (LADA) is dead: long live autoimmune diabetes! Diabetologia 2010;53:1250-3.
135. Okruszko A, Szepietowska B, Wawrusiewicz-Kurylonek N, Gorska M, Kretowski A, Szelachowska M. HLA-DR, HLA-DQB1 and PTPN22 gene

polymorphism: association with age at onset for autoimmune diabetes. Arch Med Sci 2012;8:874-8.
136. Desai M, Cull CA, Horton VA, et al. GAD autoantibodies and epitope reactivities persist after diagnosis in latent autoimmune diabetes in adults but do not predict disease progression: UKPDS 77. Diabetologia 2007;50:2052-60.
137. Borg H, Gottsater A, Fernlund P, Sundkvist G. A 12-year prospective study of the relationship between islet antibodies and beta-cell function at and after the diagnosis in patients with adult-onset diabetes. Diabetes 2002;51:1754-62.
138. Fourlanos S, Dotta F, Greenbaum CJ, et al. Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia 2005;48:2206-12.
139. Groop L, Tuomi T, Rowley M, Zimmet P, Mackay IR. Latent autoimmune diabetes in adults (LADA)--more than a name. Diabetologia 2006;49:1996-8.
140. Siljander HT, Veijola R, Reunanen A, Virtanen SM, Akerblom HK, Knip M. Prediction of type 1 diabetes among siblings of affected children and in the general population. Diabetologia 2007;50:2272-5.
141. Mohlke KL, Scott LJ. What will diabetes genomes tell us? Curr Diab Rep 2012;12:643-50.

Paper I

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Paper II

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Paper III

Presence of antiGAD; its clinical influence in a non-diabetic population of adults. Results from the HUNT study.

Running head:

AntiGAD in a non-diabetic population

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Word Count:

Manuscript: 3043 words (without ref.)

Abstract: 200

Tables: 3

Figures: 2

Supplement: 3

Abstract

The presence and clinical implications of antiGAD positivity in non-diabetic populations are poorly elucidated. We investigated these aspects prospectively in an all-population based cohort from the Nord-Trøndelag health study (HUNT). We selected 4496 individuals (randomly from different age groups, 50% men/women) who were non-diabetic in two consecutive surveys, HUNT2 (1995-97) and HUNT3 (2006-08). AntiGAD positive subjects at HUNT2 were followed up at HUNT3. HLA-DQA1/DQB1 was genotyped in antiGAD positive individuals and in matched antiGAD negative controls. In persisting non-diabetic individuals, prevalence of antiGAD positivity at HUNT2 was 1.69% (n=76). Positivity was not associated with gender, first degree family history of diabetes (FHD), smoking, glucose or BMI. However, HLA-DQA1/DQB1, a risk-haplotype for autoimmune diabetes was associated with antiGAD positivity as was thyroid peroxidase antibody positivity. AntiGAD positivity was lost at follow-up (HUNT3) in 41 out of 76 individuals (54%). Results in the non-diabetic cohort were compared with pre-diabetic subjects (autoimmune diabetes at HUNT3 but non-diabetic and antiGAD positive at HUNT2, n=34). Pre-diabetic subjects had higher frequency of FHD, higher antiGAD levels and glucose levels. AntiGAD positivity in persistently non-diabetic individuals is partly consistent, is not associated with clinical parameters related to diabetes, but associated with HLA risk and thyroid autoimmunity.

Introduction

Development of autoimmune diabetes is strongly associated with the presence of autoantibody markers. The commonly used antibody for diagnostic purpose is against Glutamic Acid Decarboxylase (antiGAD). This antibody is present in over 80% of the young-onset type 1 diabetic patients (1).

AntiGAD and other antibodies to pancreatic antigens can be detected many years before the clinical onset of autoimmune mediated diabetes, indicating a long pre-diabetic phase of autoimmune activity (2, 3). Studies which identify subjects at risk for autoimmune diabetes by antibodies are however mainly based on relatives of patients with type 1 diabetes (4, 5) who comprises only about 10% of all type 1 diabetic cases (6). There are few data available on antibody profiling in the remaining 90% of the subjects from the general population. Previous studies based on adults (7-9) and school children (10, 11) have shown that antibodies, in particular antiGAD, are present in a proportion of non-diabetic subjects who do not have close relatives with autoimmune diabetes. The frequency and clinical importance of antiGAD in non-diabetic individuals is however still unclear and debated. It has been argued that positivity under these conditions do not predict developing of diabetes (12, 13), that it is unspecific (particularly if it is weak), and should be regarded as falsely positive (14). More large and prospective epidemiological studies which are population-based are needed to clarify this issue.

The human leukocyte antigene (HLA) genes are strongly associated with risk of developing type 1 diabetes (15). HLA *DQA1*0301-DQB1*0302* (DQ8) and *DQA1*0501-DQB1*0201* (DQ2) are two high risk haplotypes possessed by about 90% of type 1 diabetic children (16). It is still not fully clarified whether these haplotypes are associated or not with antiGAD

positivity in adult non-diabetic individuals from the general population. To our knowledge only one study has investigated an association in persistently non-diabetic subjects and found that antiGAD positivity was associated with the type 1 diabetic HLA DQA-DQB high risk haplotype (17).

The primary aim of this study was to investigate the prevalence, persistence and the potential clinical impact of antiGAD positivity together with HLA genotypes in a non-diabetic population of adults. To this end we have analysed relevant data prospectively in a large non-diabetic adult population based cohort collected from the Norwegian Health Study in Nord-Trøndelag (HUNT). Our analysis also included tests for association between antiGAD positivity and other autoimmune diseases.

Material and methods

Study population

The study is based on the second and third health surveys (HUNT2 and HUNT3) in Nord-Trøndelag county located in Norway. HUNT2 was performed in 1995-1997 and HUNT3 in 2006-2008 Details about the HUNT study have been published (18). For our analysis we collected a random sample from the 37,059 adults ≥ 20 years of age who took part in both HUNT2 and HUNT3 surveys, comprising 40% of all adults in the county.

Equal numbers of men and women who were non-diabetic at both surveys (in the following termed persistently non-diabetic individuals), were randomly selected from different age groups: 500 individuals aged 20-29, 500 aged 30-34 etc. up to the last age group with 500 individuals aged from 65 years and above. Altogether we sampled a total of 4,500 persistently non-diabetic individuals to represent the general adult population in Norway (Figure 1).

Serum specimens to be used for antiGAD measurements were available for 4,496 of the 4,500 selected individuals from both HUNT2 and HUNT3 surveys. All individuals who were found to be antiGAD positive in HUNT2 were analysed for positivity in HUNT3.

We also included 55 cases with autoimmune diabetes (including both type 1 diabetes and LADA, Figure 1) who developed diabetes between HUNT2 and HUNT3 (i.e. reported not having diabetes in HUNT2 but reported having diabetes in HUNT3). Thirty-four of these cases were antiGAD positive already at HUNT2, i.e. several years before diagnosis. Some results on these cases have been published earlier (3). Diabetic cases were classified as having type 1 diabetes (n=13) if they started insulin treatment within 12 months of diagnosis and were antibody positive, or antibody negative but in addition had fasting C-peptide levels <150 pmol/l. Cases were classified as having Latent Autoimmune Diabetes in the Adult (LADA, n=21) if they were antibody positive and had not been treated with insulin within 12 months of diagnosis. No age limit was set for LADA. Data from the patients who with time developed diabetes and were antiGAD positive prior to diabetes diagnosis (here called pre-diabetic cases, n=34) were compared with data from the population that was antiGAD positive and persistently non-diabetic.

Assays of antiGAD

The serum samples to be used were stored at the HUNT Biobank, Levanger, Norway, at minus 80^{o}C until analysed. AntiGAD was measured at the Aker Hormone Laboratory, Oslo University Hospital, Oslo, Norway in 2011. AntiGAD was measured by immune precipitation using ^{3}H leucine translation labelled GAD65 as reagent (Novo Nordisk, Bagsværd, Denmark). Separation of bound antiGAD and free labelled GAD65 was done by protein A coupled to

Sepharose. Antibody levels were expressed as an antibody index (ai) relative to a standard serum given by the formula [(counts per minute (cpm) in the patients sample – cpm from negative reference sample) / (cpm of a positive reference sample – cpm from negative reference sample)]. Subjects above the 98.5th percentile of the antiGAD levels in the total cohort were considered antiGAD "positive". This corresponded to a value >0.05ai. Based on Aker Hormone Laboratory's participation in the Diabetes Autoantibody Standardization Program (DASP) 2010, this corresponded with an 82% DASP-sensitivity and a 99% DASP-specificity. The intra assay coefficient variation (CV) was 14% in the lowest (0.11ai), 8% in the middle (0.22ai) and 17% in the highest (2.0ai) range of measurements. The total CV was 19% in the lowest (0.21ai) and 23% in the highest (0.66ai) measurements range.

HLA-typing

Persistently non-diabetic individuals who were antiGAD positive at HUNT2 as well as a control group of antiGAD negative non-diabetic individuals were typed for HLA- DQA1 and HLA- DQB1. The control group was age and gender matched to the antiGAD positive, non-diabetic group. Two controls were selected per antiGAD positive individual. The same HLA genotypes were also analysed in individuals who developed autoimmune diabetes during the interval between HUNT2 and HUNT3.

HLA-DQA1 and HLA-DQB1 genotypes were analysed at the Unit for Immunology, St Olavs Hospital, Trondheim, Norway by sequence-specific oligonucleotide probes (SSO) using LABType^{R} SSO DQA1/DQB1 Typing Tests (One Lambda Inc., CA, USA) as described by the manufacturer. In brief, the target DNA was amplified by polymerase chain reaction (PCR) using HLA-locus specific target primers. The amplified target DNA was denatured and re-hybridized with complementary DNA probes bound to fluorescently coded microspheres,

which allowed detection using R-Phycoerytrin-bound (PE) Strepavidin. The flow analyser Luminex 100 (Luminex Corporation, Texas, USA) was used to measure the fluorescent signal of PE and the HLA fusion 2.0 (One Lambda Inc., CA, USA) software was used to process the results.

The HLA *DQA1-DQB1* haplotypes were divided into five groups based on known risk for type 1 diabetes: 1) very high risk, having both *DQA1*0301-DQB1*0302* and *DQA1*0501-DQB1*0201*; 2) high risk, having one of the *DQA1*0301-DQB1*0302*, *DQA1*X-DQB1*0302* or *DQA1*0501-DQB1*0201* haplotypes (homozygosity not excluded); 3) moderate risk, having *DQA1*X-DQB1*0201*, *DQA1*0101-DQB1*0501* and *DQA1*0401-DQB1*0402*; 4) neutral/low-risk HLA having *DQA1*X-DQB1*X*; 5) very-low-risk, having *DQA1*X-DQB1*0602 and DQA1*X-DQB1*0603*. For group 2-5, X indicates a non-defined allele and homozygosity was not excluded.

Statistical analysis

Data are given as numbers and percentage for categorical data and as median (min-max value) for continuous data. All statistical analyses were performed by the PASW Statistics (SPSS, Inc, Chicago, IL). χ^2 test or Fisher exact test (when appropriate) was used to compare differences in categorical data. Mann Whitney U test was used to test differences in continuous data between two groups. Kruskal-Wallis test was used to test differences in continuous data between more than two groups. Logistic regression models were used to examine whether HLA haplotypes were associated with antiGAD after adjusting for other confounding factors such as age, gender and BMI. A two-tailed *p*-value of 0.05 was considered to be significant.

Ethics

All participants gave their written consent. The study was approved by the Regional Committee for Ethics in Medical Research.

Results

Prevalence of antiGAD in persistently non-diabetic individuals

Seventy-six out of 4496 persistently non-diabetic participants (1.69%) tested positive for antiGAD in HUNT2 (median level 0.08ai with min-max value of 0.06-3.58ai). The age group with highest prevalence was 30-34 years (16 out of 499, 3.2%). The age group with the lowest prevalence was 45-49 years (2 out of 499, 0.4%, Figure 2).

AntiGAD positivity in relation to clinical data

Positivity for antiGAD was not associated with first degree family history of diabetes (overall), smoking, non-fasting glucose or BMI (Table 1). When splitting first degree family history of diabetes into parents, children and siblings, there tended to be a higher risk of being antiGAD positive in those who had siblings with diabetes (OR [95% CI]: 1.96 [0.83-4.51], p=0.14, 8.2% (6 of 73 individuals) vs. 4.4% (190 of 4292 individuals)].

AntiGAD conversion amongst persistently non-diabetic participants

A follow-up serum sample was obtained 9-13 years later (HUNT3) from the persistently non-diabetic participants who initially (at HUNT2) were positive for antiGAD. Of the 76 initially antiGAD positive adults 41 (54%) converted to negative in the follow-up sample.

There were no phenotypic differences between antiGAD positive persisters compared to converters (Supplementary table 1). However, the initial antiGAD levels in HUNT2 was

higher in those who stayed positive compared to those who converted to negative, with a median level (min-max value) of 0.27ai (0.06-3.58 ai) vs. 0.06ai (0.06-1.34 ai) ($p<0.001$ for difference).

AntiGAD positive non-diabetic persisters compared to antiGAD positive pre-diabetic cases

Persistently non-diabetic subjects who were antiGAD positive at HUNT2 (n=76) were compared with pre-diabetic cases who were antiGAD positive and non-diabetic at HUNT2 but had developed diabetes at HUNT3 (n=34). The pre-diabetic cases (i.e. those that developed diabetes) had higher frequency of first degree family history of diabetes compared to those who did not develop diabetes (53% (n=18) vs. 28% (n=21), p=0.02, Table 2). This difference was significant also after adjusting (by logistic regression) for HLA genotypes, sex, age and BMI (OR 2.86 [1.12-7.34], p=0.03). The individuals who turned diabetic also had higher antiGAD levels at HUNT2 (1.43ai [0.07-1.98 ai] vs. 0.08ai [0.06-3.58 ai], $p<0.001$) and higher non-fasting glucose (5.60mmol/l [4.10-27.0 mmol/l] vs. 5.05mmol/l [3.50-7.10 mmol/l], p=0.001) compared to the persistently non-diabetic individuals. Removing the non-diabetic non-persistent antiGAD positive cases (i.e. in those who became antiGAD negative in HUNT3, n=41) from the analysis, did not affect the results (data not shown).

HLA risk haplotypes and antiGAD

Seventy-five antiGAD positive and 151 antiGAD negative persistently non-diabetic participants as well as 34 antiGAD positive individuals (who were pre-diabetic at HUNT2 and diabetic at HUNT3 were successfully genotyped for HLA DQA1-DQB1. Comparing antiGAD positive persistently non-diabetic individuals with antiGAD negative ones, we found that having the very high risk genotype *DQA1*0301-DQB1*0302*/*DQA1*0501-DQB1*0201* (OR [95% CI]: 9.6 [2.2-42], p=0.003) or having one of the *DQA1*0301-DQB1*0302*,

*DQA1*X-DQB1*0302* or *DQA1*0501-DQB1*0201* high risk haplotypes (OR 2.2 [1.0-4.8], p=0.04) was associated with being antiGAD positive (Table 3). Removing the non-persistent antiGAD positive cases (i.e. those who became antiGAD negative in HUNT3, n=41) from the analysis, did not considerably affect the results (data not shown).

Comparing antiGAD positive persistently non-diabetic individuals with antiGAD positive pre-diabetic individuals, we found only a tendency for higher frequency of HLA *DQA1*0301-DQB1*0302*/*DQA1*0501-DQB1*0201* genotype in antiGAD positive pre-diabetic individuals (23.5% (n=8) vs. 10.7% (n=8), OR 2.76 [0.72-11], p=0.14).

When combining both persistently non-diabetic and pre-diabetic antiGAD positive individuals in the analysis, the antiGAD level was significant higher in the high risk HLA genotypes compared to the weak protective HLA genotypes (p=0.001, Supplementary table 2).

AntiGAD positivity in relation to other autoimmune diseases

We tested for associations between antiGAD positivity and other self-reported immune mediated diseases that were asked for in the HUNT questionnaires. AntiGAD positivity measured in HUNT2 compared to antiGAD negativity was associated with reported hyperthyroidism reported in HUNT3 (7.1% (n=5) vs. 2.9% (n=120) respectively, p=0.05, Supplementary table 3). There was also an association with Thyroid Peroxidase antibody (antiTPO) positivity in both HUNT2 and HUNT3. In HUNT2, we had data on antiTPO from 164 out of 4496 individuals. Four out of these 164 were antiGAD positive and also anti-TPO positive (100%), whereas 62 out of 160 (39%) antiGAD negative were antiTPO positive (p=0.03 for difference, Supplementary table 3). In HUNT3, we had data on antiTPO from 383 out of 4496 individuals. Eight out of 11 (73%) antiGAD positive individuals were also

antiTPO positive compared to 134 out of 238 (36%) antiGAD negative ones (p=0.02 for difference, Supplementary table 3).

Discussion

We investigated the prevalence, evolution and clinical impact of antiGAD in a non-diabetic population recruited from all adults in a cohort from Norway. We also evaluated the associations of high risk type 1 diabetes associated HLA genotypes to the presence of antiGAD positivity in this non-diabetic population.

In our adult persistently non-diabetic population we find an antiGAD positivity prevalence of 1.7% which increases to 2.0% when including also pre-diabetic individuals. This is in fair agreement with previous reports in which the prevalence ranges from 1-4% in both children and adults from the general population (5, 8, 9, 19). However, in previous reports a distinction was usually not made as to whether diabetes developed over time or not, nor to phenotypic characteristics described here.

We observed that antiGAD in the non-diabetic population frequently changed from positivity to negativity over a 9-13 years period, giving a conversion rate of 54%. Seroconversion from antibody positive to antibody negative was associated with lower levels of antiGAD. Such evolution may lead one to question the clinical importance of low and fleeting antibody positivity. However, as elaborated below, the HLA associations constitute tangible evidence of importance. Further, fleeting antiGAD positivity in LADA patients was previously shown to impact on insulin secretion, as assessed from fasting levels of C-peptide (3).

In the persistently non-diabetic individuals we could not find any clinical parameters conferring risk of diabetes that were associated with antiGAD positivity. Neither did the presence or absence of stability of antiGAD positivity (positivity or not both in HUNT2 and HUNT3) affect the clinical parameters. However, having high risk HLA DQA1-DQB1 haplotypes were associated with presence of GAD antibodies. This is in line whit a recent study mainly based one adult relatives of type 2 diabetic cases which found that antiGAD was associated with HLA high risk haplotypes regardless of family history of type 1 diabetes and evolution of diabetes (17).
As mentioned above, given the etiological importance of these haplotypes it is reasonable to conclude that even minor and fleeting antiGAD positivity is coupled to autoimmunity.

This notion is reinforced by finding associations between antiGAD positivity and hyperthyroidism (in this population bound to be Graves' disease) as well as presence of antibody towards thyroid peroxidase. An association between type 1 diabetes and thyroid autoimmune disease is well documented (20-22); however, an association with presence of antiGAD in persistently non-diabetic individuals has, to our knowledge, not been described.

A comparison of antiGAD positive persistent non-diabetic individuals with pre-diabetic individuals (i.e. antiGAD positive but non-diabetic at HUNT 2, diabetic at HUNT3) revealed higher titers of antiGAD in HUNT2 in the latter. This could signify higher autoimmune activity which in turn could precipitate overt diabetes in these individuals. However, also risk factors for type 2 diabetes, such as BMI and age were higher and could participate in evolution to diabetes. In this context it will be of great interest to follow those as of now persistent non-diabetic individuals with antiGAD for possible evolution of diabetes in coming years.

The antiGAD level (in only antiGAD positive individuals pooled with those who also progressed to diabetes) was correlated with genetic risk as assessed by HLA haplotypes. This finding agrees with that of the Botnia Prospective Study (17). The mentioned correlation highlights the potential clinical importance of the risk HLA haplotypes for autoimmunity in adults.

We did not find an association of antiGAD positivity and overall first degree family history of diabetes in the non-diabetic subjects. In contrast such an association was found in the Botnia study (17). This difference between studies may be explained by our questionnaire data not being able to distinguish between type 1 and type 2 family histories of diabetes. It has been shown in earlier studies that risk of developing type 1 diabetes and LADA is higher in siblings of diabetic patients compared to having parents with diabetes (23, 24). We did see a tendency for higher risk of being antiGAD positive in those having siblings with diabetes.

A limitation of this study is the sample size of the antiGAD positive individuals (both non-diabetic and pre-diabetic) and should be considered when interpreting these results. One may also argue one should confirm the positivity by performing competition assays. However, this type of analysis has been done before and did not detect unspecific influence between anyone of the three different antiGAD assays tested (25). A further limitation concerns the small subset of the studied subjects on which the anti-TPO results are based. Therefor studies in other populations should be performed to confirm the present results on autoimmune thyroid diseases.

We conclude that antiGAD positivity in adult non-diabetic individuals from the general population is 1) partly persistent over a long time period 2) not associated with clinical parameters related to diabetes, but associated with increased frequency of high risk HLA haplotypes known to be associated with type 1 diabetes and 3) associated with hyperthyroidism and thyroid autoimmunity.

Acknowledgment

The HUNT study was performed as collaboration between the HUNT Research Centre (Faculty of Medicine, Norwegian University of Science and Technology (NTNU)), Nord-Trøndelag County Council, Central Norway Health Authority and the Norwegian Institute of Public Health. Our study was funded by the liaison committee of the Central Norway Regional Health Authority and the NTNU, and the liaison committee of St Olav's Hospital Trust and the Faculty of Medicine, NTNU. The authors would also thank Ms Oddrun Storrø, Ms Marit Aarhaug and Ms Anne Kristin Lysakerrud at Unit for Immunology at St Olavs Hospital, Trondheim, Norway for their appreciated technical assistance.

E.P.S. did the statistical analyses and wrote the manuscript. K.K. and F.S. participated in the interpretation of data and reviewed/edited the manuscript. V.G. designed the study, participated in the interpretation of data, contributed to the discussion and reviewed/edited the manuscript.

References

1. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 1990;347:151-156

2. Tuomilehto J, Zimmet P, Mackay IR, Koskela P, Vidgren G, Toivanen L, Tuomilehto-Wolf E, Kohtamaki K, Stengard J, Rowley MJ. Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease. Lancet 1994;343:1383-1385

3. Sorgjerd EP, Skorpen F, Kvaloy K, Midthjell K, Grill V. Time dynamics of autoantibodies are coupled to phenotypes and add to the heterogeneity of autoimmune diabetes in adults: the HUNT study, Norway. Diabetologia 2012;
4. Vermeulen I, Weets I, Costa O, Asanghanwa M, Verhaeghen K, Decochez K, Ruige J, Casteels K, Wenzlau J, Hutton JC, Pipeleers DG, Gorus FK. An important minority of prediabetic first-degree relatives of type 1 diabetic patients derives from seroconversion to persistent autoantibody positivity after 10 years of age. Diabetologia 2012;55:413-420
5. Siljander HT, Veijola R, Reunanen A, Virtanen SM, Akerblom HK, Knip M. Prediction of type 1 diabetes among siblings of affected children and in the general population. Diabetologia 2007;50:2272-2275
6. Veijola R, Reijonen H, Vahasalo P, Sabbah E, Kulmala P, Ilonen J, Akerblom HK, Knip M. HLA-DQB1-defined genetic susceptibility, beta cell autoimmunity, and metabolic characteristics in familial and nonfamilial insulin-dependent diabetes mellitus. Childhood Diabetes in Finland (DiMe) Study Group. J Clin Invest 1996;98:2489-2495
7. Rolandsson O, Hagg E, Janer M, Rutledge E, Gaur LK, Nilsson M, Hallmans G, Lernmark A. High GAD65 autoantibody levels in nondiabetic adults are associated with HLA but not with CTLA-4 or INS VNTR. J Intern Med 2003;253:447-453
8. Ruige JB, Batstra MR, Aanstoot HJ, Bouter LM, Bruining GJ, De Neeling JN, Heine RJ. Low prevalence of antibodies to GAD65 in a 50- to 74-year-old general Dutch population. The Hoorn Study. Diabetes Care 1997;20:1108-1110
9. Lundgren VM, Isomaa B, Lyssenko V, Laurila E, Korhonen P, Groop LC, Tuomi T. GAD antibody positivity predicts type 2 diabetes in an adult population. Diabetes 2010;59:416-422
10. LaGasse JM, Brantley MS, Leech NJ, Rowe RE, Monks S, Palmer JP, Nepom GT, McCulloch DK, Hagopian WA. Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. Diabetes Care 2002;25:505-511
11. Schlosser M, Strebelow M, Rjasanowski I, Kerner W, Wassmuth R, Ziegler M. Prevalence of diabetes-associated autoantibodies in schoolchildren: the Karlsburg Type 1 Diabetes Risk Study. Ann N Y Acad Sci 2004;1037:114-117
12. Rolandsson O, Hagg E, Nilsson M, Hallmans G, Mincheva-Nilsson L, Lernmark A. Prediction of diabetes with body mass index, oral glucose tolerance test and islet cell autoantibodies in a regional population. J Intern Med 2001;249:279-288
13. Bosi EP, Garancini MP, Poggiali F, Bonifacio E, Gallus G. Low prevalence of islet autoimmunity in adult diabetes and low predictive value of islet autoantibodies in the general adult population of northern Italy. Diabetologia 1999;42:840-844
14. Gale EA. Latent autoimmune diabetes in adults: a guide for the perplexed. Diabetologia 2005;48:2195-2199
15. Howson JM, Stevens H, Smyth DJ, Walker NM, Chandler KA, Bingley PJ, Todd JA. Evidence that HLA class I and II associations with type 1 diabetes, autoantibodies to GAD and autoantibodies to IA-2, are distinct. Diabetes 2011;60:2635-2644
16. Lambert AP, Gillespie KM, Thomson G, Cordell HJ, Todd JA, Gale EA, Bingley PJ. Absolute risk of childhood-onset type 1 diabetes defined by human leukocyte antigen class II genotype: a population-based study in the United Kingdom. J Clin Endocrinol Metab 2004;89:4037-4043
17. Andersen MK, Lundgren V, Isomaa B, Groop L, Tuomi T. Association of variants in HLA-DQA1-DQB1, PTPN22, INS, and CTLA4 with GAD autoantibodies and insulin secretion in nondiabetic adults of the Botnia Prospective Study. Eur J Endocrinol 2012;167:27-33

18. Holmen J, Midthjell,K, Krüger,Ø, Langhammer,A, Holmen,TL, Bratberg,GH, Vatten,L, Lund-Larsen,PG. The Nord-Trøndelag Health Study 1995–97 (HUNT2): objectives, contents, methods and participation. Norsk Epidemiologi 2003;13:19-32
19. Knip M, Korhonen S, Kulmala P, Veijola R, Reunanen A, Raitakari OT, Viikari J, Akerblom HK. Prediction of type 1 diabetes in the general population. Diabetes Care 2010;33:1206-1212
20. Hansen D, Bennedbaek FN, Hansen LK, Hoier-Madsen M, Jacobsen BB, Hegedus L. Thyroid function, morphology and autoimmunity in young patients with insulin-dependent diabetes mellitus. Eur J Endocrinol 1999;140:512-518
21. Barova H, Perusicova J, Hill M, Sterzl I, Vondra K, Masek Z. Anti-GAD-positive patients with type 1 diabetes mellitus have higher prevalence of autoimmune thyroiditis than anti-GAD-negative patients with type 1 and type 2 diabetes mellitus. Physiol Res 2004;53:279-286
22. Kordonouri O, Charpentier N, Hartmann R. GADA positivity at onset of type 1 diabetes is a risk factor for the development of autoimmune thyroiditis. Pediatr Diabetes 2011;12:31-33
23. Carlsson S, Midthjell K, Grill V. Influence of family history of diabetes on incidence and prevalence of latent autoimmune diabetes of the adult: results from the Nord-Trondelag Health Study. Diabetes Care 2007;30:3040-3045
24. Steck AK, Rewers MJ. Genetics of type 1 diabetes. Clin Chem 2011;57:176-185
25. Daka B, Svensson MK, Lernmark K, Mincheva-Nilsson L, Hallmans G, Rolandsson O. Low agreement between radio binding assays in analyzing glutamic acid decarboxylase (GAD65Ab) autoantibodies in patients classified with type 2 diabetes. Autoimmunity 2009;42:507-514

Table 1: Clinical characteristics of persistently non-diabetic adult individuals stratified by being antiGAD negative and antiGAD positive in HUNT2.

	AntiGAD negative	AntiGAD positive	*p*-value
N	4420	76 (1.7%)	
Sex (male)	2208 (50%)	40 (52.6%)	0.64
Age at attendance	47.6 (19.4-85.1)	41.9 (21.3-77.1)	0.22
Waist circumference (cm)	86 (55-131)	84 (64-109)	0.69
BMI (kg/m^2)	25.6 (16.2-52.8)	26.0 (19.4-34.8)	0.70
Systolic blood pressure (mmHg)	133 (82-225)	132 (102-189)	0.82
Diastolic blood pressure (mmHg)	79 (44-136)	78 (52-120)	0.85
Glucose, non-fasting (mmol/l)	5.1 (0-14.4)	5.1 (3.5-7.5)	0.89
Cholesterol (mmol/l)	5.8 (1.9-13.2)	5.7 (3.4-8.2)	0.35
HDL cholesterol (mmol/l)	1.4 (0.5-3.8)	1.3 (0.6-2.4)	0.62
Triacylglycerol [b] (mmol/l)	1.42 (0.31-18.6)	1.31 (0.24-4.73)	0.30
Family history of diabetes			
No	3229	55	
Yes	1181 (26.8%)	21 (27.6%)	0.86
Smoking			
No	3224	59	
Yes	1052 (24.9%)	17 (22.4%)	0.65

Data is given as numbers (%) and median (min-max value).
Unadjusted *p*- value calculated by Mann–Whitney *U* test for continuous data and by χ^2 test for categorical data.

Table 2: Clinical characteristics of antiGAD positive and initially non-diabetic individuals at HUNT2, divided in persistently non-diabetic and pre-diabetic individuals.

	Persistently non-diabetic[*] (n=76)	**Pre-diabetic[†] (n=34)**	***p*-value[‡]**
Sex (male)	40 (52.6%)	18 (52.9%)	1.00
Age at attendance	42 (21-77)	50 (26-76)	0.22
Waist circumference (cm)	84 (64-109)	92 (65-109)	0.03
BMI (kg/m^2)	26 (19-35)	27 (18-40)	0.07
Systolic blood pressure (mmHg)	131 (102-189)	136 (107-190)	0.06
Diastolic blood pressure (mmHg)	78 (53-120)	85 (58-122)	0.09
Glucose, non-fasting (mmol/l)	5.05 (3.50-7.10)	5.60 (4.10-27.0)	0.001
Cholesterol (mmol/l)	5.65 (3.40-8.20)	6.00 (3.40-7.60)	0.20
HDL cholesterol (mmol/l)	1.30 (0.60-2.40)	1.30 (0.70-2.40)	0.17
Triacylglycerol (mmol/l)	1.31 (0.24-4.73)	1.62 (0.58-7.76)	0.07
AntiGAD level (ai)	0.08 (0.06-3.58)	1.43 (0.07-1.98)	<0.001
First degree relative	21 (27.6%)	18 (52.9%)	0.02
Smoking (Currently)	17 (22.4%)	12 (35.3%)	0.17

Data are presented as n (**%**) or median (min–max values).

[*]Persistently non-diabetic; non-diabetic individuals at both HUNT2 and HUNT3.

[†]Pre-diabetic; individuals reported not having diabetes in HUNT2 and reported having diabetes in HUNT3. Thirteen out of these were classified as classic type 1 diabetes and the rest was classified as LADA (n=21).

[‡]Unadjusted *p*- value calculated by Mann–Whitney *U* test for continuous data and by Fisher's exact test for categorical data.

Table 3: Association of HLA DQA1-DQB1 haplotypes between antiGAD negative and antiGAD positive persistently non-diabetic individuals.

	Haplotype 1	Haplotype 2	AntiGAD			
	HLA DQA1-DQB1	HLA DQA1-DQB1	Negative N=151	Positive N=75	OR (95% CI)	*p*-value*
Very high risk	0301-0302	0501-0201	3 (2.0)	8 (10.7)	9.55 (2.16-42.2)	0.003
High risk	0301-0302/ X†-0302 0501-0201	Z‡ Z	61 (40.4)	37 (49.3)	2.22 (1.03-4.80)	0.043
Moderate risk	X-0201 0401-0402 0101-0501	0401-0402/ 0101-0501/ X-X 0101-0501/ X-X X-X	14 (9.3)	8 (10.7)	2.20 (0.73-6.63)	0.159
Weak protective	X-X	X-X	31 (20.5)	10 (13.3)	1.15 (0.44-3.01)	0.776
Strong protective	X-0602 X-0603	X-0603/ 0401-0402/ 0101-0501/ X-0201 X-X X-0602/ 0401-0402/ 0101-0501/ X-0201 X-X	42 (27.8)	12 (16.0)	1.00	

Data are given as numbers (%).

**p*- value calculated from logistic regression, corrected for age and BMI at HUNT2, sex and first degree family history of diabetes.

†X means non-defined allele, but does not exclude potential homozygosity.

‡Z means any haplotype.

Legends to the figures

Figure 1: Flow sheet over included cases followed from HUNT2 until HUNT3

Figure 2: Prevalence of antiGAD positivity amongst the adult population in the HUNT2 survey distributed across different age categories.

FIGURE 1

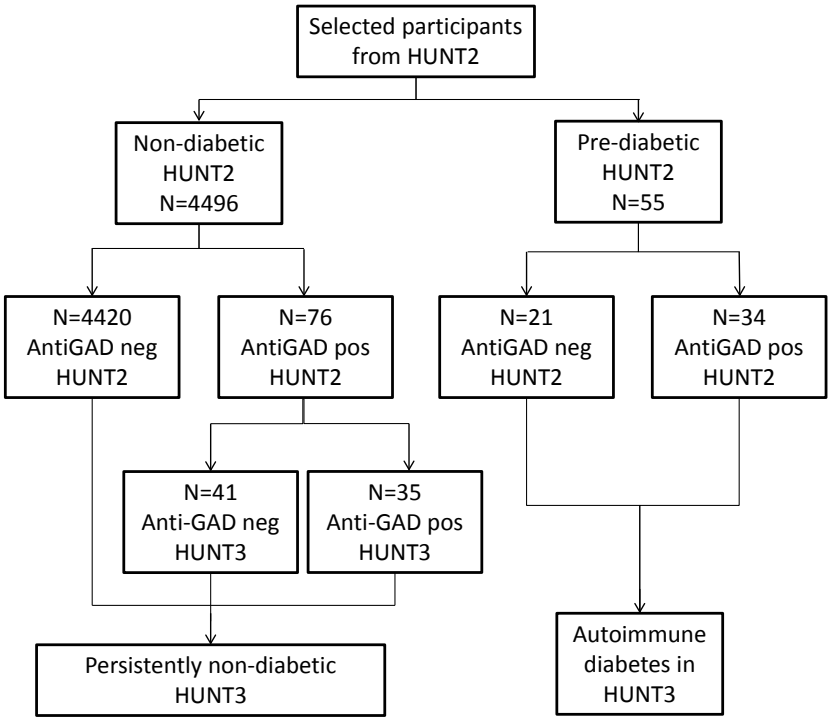
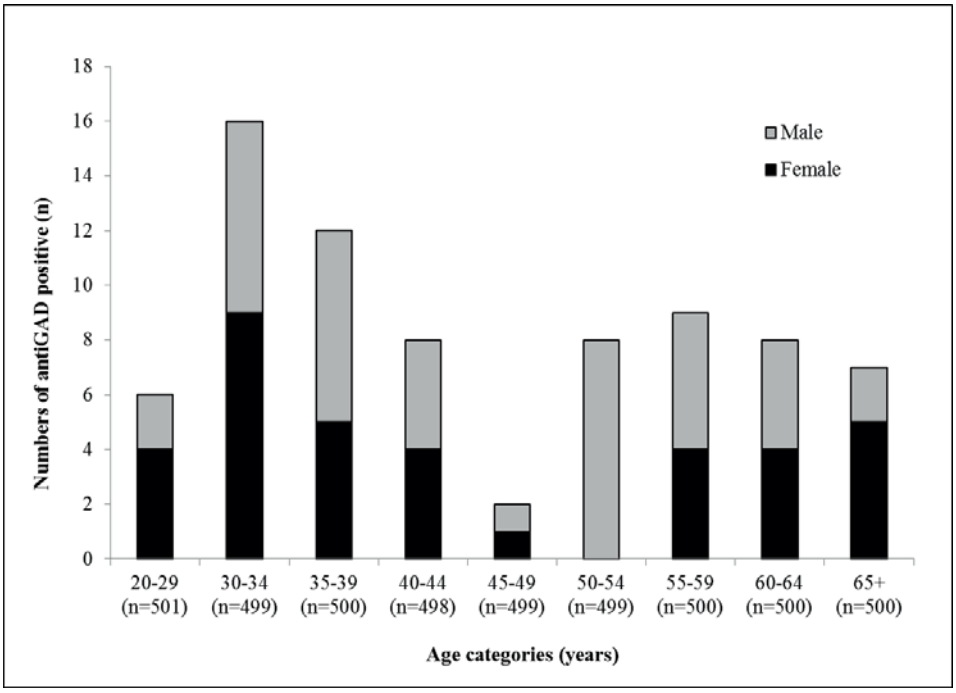

Selected participants from HUNT2
Non-diabetic HUNT2 N=4496
Pre-diabetic HUNT2 N=55
N=4420 AntiGAD neg HUNT2
N=76 AntiGAD pos HUNT2
N=21 AntiGAD neg HUNT2
N=34 AntiGAD pos HUNT2
N=41 Anti-GAD neg HUNT3
N=35 Anti-GAD pos HUNT3
Persistently non-diabetic HUNT3
Autoimmune diabetes in HUNT3

FIGURE 2


Numbers of antiGAD positive (n)
18
16
14
12
10
8
6
4
2
0
Male
Female
20-29 (n=501)
30-34 (n=499)
35-39 (n=500)
40-44 (n=498)
45-49 (n=499)
50-54 (n=499)
55-59 (n=500)
60-64 (n=500)
65+ (n=500)
Age categories (years)

Supplementary table 1: Clinical characteristics from HUNT2 between antiGAD positivity persisters and converters in persistently non-diabetic participants.

	AntiGAD negative	**AntiGAD positive**	***p*-value**
N=79	41	35	
Sex (male)	19 (46%)	21 (60%)	0.24
Age at attendance	44.3 (21.7-77.1)	39.7 (21.3-65.9)	0.24
Waist circumference (cm)	82 (64-120)	85 (64-106)	0.24
BMI (kg/m^2)	25.6 (19.4-34.2)	26.3 (19.4-34.8)	0.32
Systolic blood pressure (mmHg)	131 (102-188)	132 (104-189)	0.89
Diastolic blood pressure (mmHg)	78 (60-120)	79 (53-105)	0.81
Glucose, non-fasting (mmol/l)	5.2 (3.5-7.5)	5.0 (3.7-7.1)	0.90
Cholesterol (mmol/l)	5.5 (3.4-8.2)	5.8 (4.1-7.8)	0.74
HDL cholesterol (mmol/l)	1.3 (0.7-2.4)	1.4 (0.6-2.2)	0.84
Triacylglycerol (mmol/l)	1.3 (0.2-4.0)	1.3 (0.6-4.7)	0.73
AntiGAD titre (ai)	0.06 (0.06-1.34)	0.27 (0.06-3.58)	<0.001
Family history of diabetes			
No	29	26	
Yes	12 (29.3%)	9 (25.7%)	0.80
Smoking (Yes)			
No	30	29	
Yes	11 (26.8%)	6 (17.1%)	0.41

Data presented as numbers (%) and median (min-max) value.
Unadjusted *p*- value calculated by Mann–Whitney *U* test for continuous data and by Fisher's exact test for categorical data.

Supplementary table 2: AntiGAD titre in initially antiGAD positive participants in HUNT2 stratified by HLA genotype risk and in persistently non-diabetic individuals in both HUNT2 and HUNT3 and pre-diabetic individuals who developed diabetes during follow-up (between HUNT2 and HUNT3).

	Haplotype 1	Haplotype 2	AntiGAD titre*	
	HLA DQA1-DQB1	HLA DQA1-DQB1	Non-diabetic N=75	Pre-diabetic N=34
Very high risk	0301-0302	0501-0201	1.11 (0.06-3.58)	1.49 (0.7-1.74)
High risk	0301-0302/ X†-0302	Z‡	0.08 (0.06-1.82)	1.46 (0.34-1.98)
	0501-0201	Z		
Moderate risk	X-0201	0401-0402/ 0101-0501/ X-X	0.08 (0.06-2.05)	0.74 (0.25-1.36)
	0401-0402	0101-0501/ X-X		
	0101-0501	X-X		
Weak protective	X-X	X-X	0.09 (0.06-049)	0.82 (0.07-1.79)
Strong protective	X-0602	X-0603/ 0401-0402/ 0101-0501/ X-0201	0.065 (0.06-1.57	No subjects
		X-X		
	X-0603	X-0602/ 0401-0402/ 0101-0501/ X-0201		
		X-X		

Data are given as median (min-max value).

*AntiGAD titre was significant higher in the vhigh risk HLA genotypes compared to the weak protective HLA genotypes (p=0.001). Data analysed by Kruskal-Wallis Test and the non-diabetic and pre-diabetic combined.

†X means non-defined allele, but does not exclude potential homozygosity.

‡Z means any haplotype.

Supplementary table 3: Comparing antiGAD positivity with other self-reported immune mediated diseases in HUNT2 and HUNT3.

	AntiGAD negative		AntiGAD positive		*p*- value
	No	Yes	No	Yes	
HUNT2					
Anti-TPO positivity	98 (61.3%)	62 (38.8%)	0	4 (100%)	0.03
Asthma	4099 (92.8%)	319 (7.2%)	72 (94.7%)	4 (5.3%)	0.66
Hyperthyroidism	4340 (98.3%)	76 (1.7%)	73 (96.1%)	3 (3.9%)	0.15
Hypothyroidism	4293 (97.2%)	124 (2.8%)	74 (97.4%)	2 (2.6%)	1.00
Other disease in the thyroid gland*	4347 (99.9%)	24 (0.5%)	7 (97.4%)	2 (2.6%)	0.07
Arthritis	4269 (97.9%)	93 (2.1%)	73 (96.1%)	3 (3.9%)	0.23
Bechterews	4311 (98.2%)	77 (1.8%)	74 (97.4%)	2 (2.6%)	0.39
Allergy	3581 (82.6%)	752 (17.4%)	58 (77.3%)	17 (22.7%)	0.22
Myocrdial infarction	4351 (98.4%)	69 (1.6%)	75 (97.7%)	1 (1.3%)	1.00
HUNT3					
Anti-TPO positivity	238 (64.0%)	134 (36.0%)	3 (27.3%)	8 (72.7%)	0.02
Asthma	3955 (89.5%)	465 (10.5%)	72 (94.7%)	4 (5.3%)	0.18
Hyperthyroidism	4070 (97.1%)	120 (2.9%)	65 (92.9%)	5 (7.1%)	0.05
Hypothyroidism	3954 (92.8%)	308 (7.2%)	63 (86.3%)	10 (3.1%)	0.06
Arthritis	4047 (94.7%)	226 (5.3%)	69 (93.2%)	5 (6.8%)	0.60
Bechterews	4188 (98.0%)	87 (2.0%)	72 (97.3%)	2 (2.7%)	0.66
Allergy	3000 (71.3%)	1205 (28.7%)	51 (68.9%)	23 (31.1%)	0.70
Myocrdial infarction	4233 (95.2%)	187 (4.2%)	73 (96.1%)	3 (3.9%)	1.00

Data given as absolute numbers (%)

p- value calculated by Fisher exact test because of numbers less than 5.

*Only included in the questionnaire from HUNT2

Appendix I

Q1-HUNT2

HELSEUNDERSØKELSEN
I NORD-TRØNDELAG

Personlig innbydelse

Spørreskjemaet er en viktig del av Helseundersøkelsen. Her finner du spørsmål om tidligere sykdom og om andre forhold som har betydning for helsa. Vennligst fyll ut skjemaet på forhånd og ta det med til Helseundersøkelsen. Dersom enkelte spørsmål er uklare, lar du dem bare stå ubesvarte til du møter fram, og drøfter dem med personalet som gjennomfører undersøkelsen. Alle svar vil bli behandlet strengt fortrolig.

Flere steder i skjemaet ber vi deg oppgi din alder da eventuell sykdom inntrådte. Hvis du ikke husker nøyaktig hvor gammel du var, skriver du et tall som er nærmest det du antar er korrekt.

Når resultatene fra undersøkelsen foreligger, vil det være enkelte som trenger ny undersøkelse hos egen lege. Dette vil du få beskjed om i det brevet som vi sender deg om dine resultater. Samtidig sender vi melding om resultatene dine til legen din. Det er derfor om å gjøre at du i rubrikken helt til slutt i skjemaet oppgir navnet på den allmennpraktiserende lege, kommunelege eller det helsesenter som du ønsker skal ta hånd om eventuell etterundersøkelse, og som vi skal sende resultatene til.

Med vennlig hilsen

Helsetjenesten i Nord-Trøndelag • Statens helseundersøkelser • Statens Institutt for Folkehelse

DET HANDLER OM HELSA DI

Hvordan er helsa di nå?

Bare ett kryss

Dårlig	12	☐ 1
Ikke helt god		☐ 2
God		☐ 3
Svært god		☐ 4

LUFTVEGSPLAGER

		JA	NEI
Hoster du daglig i perioder av året?			
Hvis JA:			
Er hosten vanligvis ledsaget av oppspytt?	14		
Har du hatt hoste med oppspytt i minst 3 mnd. sammenhengende i hvert av de to siste åra?			
Har du hatt noe anfall med pipende eller tung pust de siste 12 måneder?	16		

		JA	NEI	Alder første gang
Har du eller har du hatt astma?	17			år

		JA	NEI
Har du brukt eller bruker du astmamedisiner?	20		

HJERTE-KARSYKDOMMER, DIABETES

Har du, eller har du hatt:		JA	NEI	Alder første gang
Hjerteinfarkt	21			år
Angina pectoris (hjertekrampe)	24			år
Hjerneslag/hjerneblødning	27			år
Diabetes (sukkersyke)	30			år

Hva ble resultatet siste gang du målte blodtrykket ditt?

Bare ett kryss

Begynne med/fortsette med blodtrykksmedisin	33	☐ 1
Komme til kontroll, men ikke ta blodtrykksmedisin		☐ 2
Ingen kontroll og ingen medisin nødvendig		☐ 3
Har aldri fått målt blodtrykket		☐ 4

Bruker du medisin mot høyt blodtrykk?

Bare ett kryss

Nå	34	☐ 1
Før, men ikke nå		☐ 2
Aldri brukt		☐ 3

	JA	NEI	VET IKKE
Har en eller flere av foreldre eller søsken hatt hjerteinfarkt (sår på hjertet) eller angina pectoris (hjertekrampe)?			

STOFFSKIFTE

Har du noen gang fått påvist:		JA	NEI	Alder første gang
for høyt stoffskifte	36			år
for lavt stoffskifte	39			år
struma	42			år
annen sykdom i skjoldbruskkjertelen				år
Bruker du eller har du brukt noen av disse medisinene:				
Thyroxin	48			år
Neo-Mercazole	51			år
Er du operert i skjoldbruskkjertelen				år
Har du fått radiojodbehandling	57			år

MUSKEL/SKJELETT-PLAGER

		JA	NEI
Har du i løpet av det siste året vært plaget med smerter og/eller stivhet i muskler og ledd som har vart i minst 3 måneder sammenhengende?	60		

Hvis NEI, gå videre til neste side øverst.
Hvis JA, svar på følgende:

Hvor har du hatt disse plagene?		JA	NEI
Nakke	61		
Skuldre (aksler)			
Albuer			
Håndledd, hender			
Bryst/mage	65		
Øvre del av ryggen			
Korsryggen			
Hofter			
Knær			
Ankler, føtter	70		

Hvis du har hatt plager i flere områder i minst 3 mnd. det siste året, setter du ring rundt det ja-krysset hvor plagene har vart lengst

Hvor lenge har plagene vart sammenhengende?

Svar for det området hvor plagene har vart lengst

		Antall mnd.	Antall år
Hvis under 1 år, oppgi antall mnd.	71		
Hvis 1 år eller mer, oppgi antall år	73		

Har plagene redusert din arbeidsevne det siste året?

Gjelder også hjemmearbeidende. Bare ett kryss

Nei/ubetydelig	I noen grad	I betydelig grad	Vet ikke
☐	☐	☐	☐

		JA	NEI	IKKE I ARBEID
Har du vært sykmeldt pga. disse plagene det siste året?	76			

	JA	NEI
Har plagene ført til redusert aktivitet i fritida?		

Har lege noen gang sagt at du har/har hatt noen av disse sykdommene:

		JA	NEI
Beinskjørhet (osteoporose)	78		
Fibromyalgi (fibrositt/kronisk smertesyndrom)			
Leddgikt (reumatoid artritt)			
Slitasjegikt (artrose)			
Bechterews sykdom	82		
Andre langvarige skjelett- eller muskelsykdommer			

Har du noen gang hatt:

		JA	NEI	Alder *siste* gang
Lårhalsbrudd	84			år
Brudd i håndledd/underarm	87			år
Nakkesleng (whiplash)	90			år
Skade som førte til sykehusinnleggelse				år

ANDRE PLAGER

I hvilken grad har du hatt disse plagene i de siste 12 månedene?

		Ikke plaget	Litt plaget	Mye plaget
Kvalme	96	☐	☐	☐
Brystbrann/sure oppstøt		☐	☐	☐
Diaré		☐	☐	☐
Treg mage		☐	☐	☐
Hjertebank		☐	☐	☐
Åndenød	101	☐	☐	☐

ANDRE SYKDOMMER

Har du eller har du noen gang hatt:

		JA	NEI	Alder første gang
Epilepsi	102			år
Psykiske plager hvor du har søkt hjelp				år
Kreftsykdom	108			år
Annen langvarig sykdom	111			

DAGLIGE FUNKSJONER

Har du noen langvarig sykdom, skade eller lidelse av fysisk eller psykisk art som nedsetter dine funksjoner i ditt daglige liv? ... 112

JA	NEI

Langvarig: minst ett år

Hvis JA:

Hvor mye vil du si at dine funksjoner er nedsatt?

		Litt nedsatt	Middels nedsatt	Mye nedsatt
Er bevegelseshemmet	113	☐	☐	☐
Har nedsatt syn		☐	☐	☐
Har nedsatt hørsel		☐	☐	☐
Hemmet pga. kroppslig sykdom.		☐	☐	☐
Hemmet pga. psykiske plager...	117	☐	☐	☐

MENN fortsetter øverst neste spalte

BESVARES BARE AV KVINNER

Hvor mange barn har du født?..... 118
Sett 0 hvis du ikke har født barn

Antall barn

Hvis du har født barn, besvar:

Alder

Hvor gammel var du da du fødte ditt første barn? ..... 120 — år

Hvor gammel var du da du fødte ditt siste barn? ..... 122 — år
Besvares ikke hvis du har født bare ett barn

Hvor gammel var du da du fikk menstruasjon? ..... 124 — år
Sett 0 hvis du ikke noen gang har hatt menstruasjon

Fortsett neste spalte øverst

RØYKING

Røykte noen av de voksne hjemme da du vokste opp? 126

JA	NEI

Bor du, eller har du bodd, sammen med noen dagligrøykere etter at du fylte 20 år? 127

JA	NEI

Hvor lenge er du vanligvis daglig til stede i røykfylt rom? 128
Sett 0 hvis du ikke oppholder deg i røykfylt rom

Antall timer

Røyker du selv?

		JA	NEI
Sigaretter daglig?	130		
Sigarer/sigarillos daglig?			
Pipe daglig?	132		
Aldri røykt daglig ..... *(Sett kryss)* ☐			

Hvis du har røykt daglig tidligere, hvor lenge er det siden du sluttet?..... 134

Antall år

Hvis du røyker daglig nå eller har røykt tidligere:

Hvor mange sigaretter røyker eller røykte du vanligvis daglig? ..... 136 — Antall sigaretter

Hvor gammel var du da du begynte å røyke daglig?..... 140 — Alder år

Hvor mange år tilsammen har du røykt daglig? ..... 142 — Antall år

KAFFE/TE/ALKOHOL

Hvor mange kopper kaffe/te drikker du daglig?
Sett 0 hvis du ikke drikker kaffe/te daglig

		Antall kopper
Kokekaffe	144	
Annen kaffe	146	
Te	148	

Alkohol:
Er du total avholdsmann/-kvinne? 150

JA	NEI

Hvor mange ganger i måneden drikker du vanligvis alkohol? 151
Regn ikke med lettøl. Sett 0 hvis mindre enn 1 gang i mnd.

Antall ganger

Hvor mange glass øl, vin eller brennevin drikker du vanligvis i løpet av to uker?

Regn ikke med lettøl.
Sett 0 hvis du ikke drikker alkohol 153

Øl	Vin	Brennevin
glass	glass	glass

FYSISK AKTIVITET

I FRITIDA

Hvordan har din fysiske aktivitet i fritida vært det siste året? *Tenk deg et ukentlig gjennomsnitt for året.*

Arbeidsveg regnes som fritid

		Timer pr. uke: Ingen	Under 1	1-2	3 og mer
Lett aktivitet *(ikke svett/andpusten)*	159	☐	☐	☐	☐
Hard fysisk aktivitet *(svett/andpusten)*	160	☐ 1	☐ 2	☐ 3	☐ 4

UNDER ARBEID
Hvis du er i lønnet eller ulønnet arbeid:

Hvorledes vil du beskrive arbeidet ditt?
Bare ett kryss

For det meste stillesittende arbeid
(f.eks. skrivebordsarbeid, montering) ..... 161 ☐ 1

Arbeid som krever at du går mye
(f.eks. ekspeditørarb., lett industriarb., undervisning) ..... ☐ 2

Arbeid hvor du går og løfter mye
(f.eks. postbud, pleier, bygningsarbeid) ..... ☐ 3

Tungt kroppsarbeid
(f.eks. skogsarbeid, tungt jordbruksarb.,tungt bygningsarb.) ☐ 4

Bla om!

HVORLEDES FØLER DU DEG?

Har du de siste to ukene følt deg:

	Nei	Litt	En god del	Svært mye
Trygg og rolig? 162	☐	☐	☐	☐
Glad og optimistisk?	☐	☐	☐	☐
Har du følt deg:				
Nervøs og urolig?	☐	☐	☐	☐
Plaget av angst? 165	☐	☐	☐	☐
Irritabel?	☐	☐	☐	☐
Nedfor/deprimert?	☐	☐	☐	☐
Ensom? 168	☐ 1	☐ 2	☐ 3	☐ 4

Her kommer noen flere spørsmål om hvorledes du føler deg. For hvert spørsmål setter du kryss for ett av de fire svarene som best beskriver dine følelser ***den siste uka.*** *Ikke tenk for lenge på svaret - de spontane svarene er best*

Jeg gleder meg fortsatt over ting slik jeg pleide før 169
Avgjort like mye ..... ☐ 1 Bare lite grann ..... ☐ 3
Ikke fullt så mye ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg har en urofølelse som om noe forferdelig vil skje 170
Ja, og noe svært ille .... ☐ 1 Litt, bekymrer meg lite . ☐ 3
Ja, ikke så veldig ille ... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg kan le og se det morsomme i situasjoner 171
Like mye nå som før .... ☐ 1 Avgjort ikke som før .... ☐ 3
Ikke like mye nå som før ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg har hodet fullt av bekymringer 172
Veldig ofte ..... ☐ 1 Av og til ..... ☐ 3
Ganske ofte ..... ☐ 2 En gang i blant ..... ☐ 4

Jeg er i godt humør 173
Aldri ..... ☐ 1 Ganske ofte ..... ☐ 3
Noen ganger ..... ☐ 2 For det meste ..... ☐ 4

Jeg kan sitte i fred og ro og kjenne meg avslappet 174
Ja, helt klart ..... ☐ 1 Ikke så ofte ..... ☐ 3
Vanligvis ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg føler meg som om alt går langsommere 175
Nesten hele tiden ..... ☐ 1 Fra tid til annen ..... ☐ 3
Svært ofte ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg føler meg urolig som om jeg har sommerfugler i magen 176
Ikke i det hele tatt ..... ☐ 1 Ganske ofte ..... ☐ 3
Fra tid til annen ..... ☐ 2 Svært ofte ..... ☐ 4

Jeg bryr meg ikke lenger om hvordan jeg ser ut 177
Ja, har sluttet å bry meg ☐ 1 Kan hende ikke nok .... ☐ 3
Ikke som jeg burde ..... ☐ 2 Bryr meg som før ..... ☐ 4

Jeg er rastløs som om jeg stadig må være aktiv 178
Uten tvil svært mye ..... ☐ 1 Ikke så veldig mye ..... ☐ 3
Ganske mye..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg ser med glede frem til hendelser og ting 179
Like mye som før ..... ☐ 1 Avgjort mindre enn før . ☐ 3
Heller mindre enn før ... ☐ 2 Nesten ikke i det hele tatt ☐ 4

Jeg kan plutselig få en følelse av panikk 180
Uten tvil svært ofte ..... ☐ 1 Ikke så veldig ofte ..... ☐ 3
Ganske ofte ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg kan glede meg over gode bøker, radio og TV 181
Ofte ..... ☐ 1 Ikke så ofte ..... ☐ 3
Fra tid til annen ..... ☐ 2 Svært sjelden ..... ☐ 4

UTDANNING

Hvilken utdanning er den høyeste du har fullført?

Grunnskole 7-10 år, framhaldsskole, folkehøgskole ..... 182 ☐ 1
Realskole, middelskole, yrkesskole, 1-2 årig videregående skole..... ☐ 2
Artium, øk.gymnas, allmennfaglig retning i videregående skole ..... ☐ 3
Høgskole/universitet, mindre enn 4 år ..... ☐ 4
Høgskole/universitet, 4 år eller mer ..... ☐ 5

ARBEID

Hva slags arbeidssituasjon har du nå?
Ett eller flere kryss

Lønnet arbeid ..... 183 ☐
Selvstendig næringsdrivende..... ☐
Heltids husarbeid ..... ☐
Utdanning, militærtjeneste ..... ☐
Arbeidsledig, permittert..... ☐
Pensjonist/trygdet..... 188 ☐

Hvor mange timer lønnet arbeid har du i uka? ..... 189 Antall timer

	JA	NEI
Har du skiftarbeid, nattarbeid eller går vakt?		

ALT I ALT

Når du tenker på hvordan du har det for tida, er du stort sett fornøyd med tilværelsen eller er du stort sett misfornøyd?
Bare ett kryss

Svært fornøyd ..... 192 ☐ 1
Meget fornøyd ..... ☐ 2
Ganske fornøyd..... ☐ 3
Både/og..... ☐ 4
Nokså misfornøyd ..... ☐ 5
Meget misfornøyd..... ☐ 6
Svært misfornøyd..... ☐ 7

DIN LEGE

Hvis denne helseundersøkelsen viser at du bør undersøkes nærmere, hvilken allmennpraktiserende lege/kommunelege ønsker du skal foreta undersøkelsen?

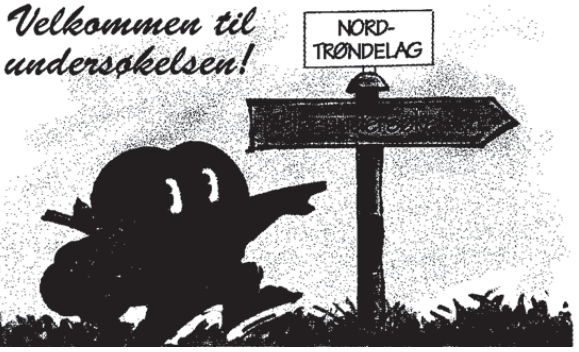
Skriv navnet på legen her: 193

	Ikke skriv her

Takk for utfyllingen!

Nok en gang:

Velkommen til undersøkelsen!



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Appendix II

Q1-HUNT3

Invitasjon til HUNT 3

Viktig
Enkelt
Gratis

Du inviteres herved til å delta i den tredje store Helseundersøkelsen i Nord-Trøndelag (HUNT 3). Ved å delta får du en enkel undersøkelse av din egen helse, og du gir samtidig et viktig bidrag til medisinsk forskning.

Hver deltaker er like viktig, enten du er ung eller gammel, frisk eller syk, er HUNT-veteran eller møter for første gang. Tilsvarende undersøkelse er tidligere gjennomført i 1984-86 (HUNT 1) og 1995-97 (HUNT 2 og Ung-HUNT). For å kunne studere årsaker til sykdom, er det viktig at også de som tidligere har deltatt møter fram.

Vennligst fyll ut spørreskjemaet, og ta det med når du møter til undersøkelse.

Undersøkelsen tar vanligvis ca 1/2 time. Du vil få brev med resultater fra dine prøver etter noen uker. Dersom noen av resultatene er utenom det normale, vil du bli anbefalt undersøkelse hos fastlegen din.

Du kan lese mer om HUNT 3 i den vedlagte brosjyren eller på www.hunt.ntnu.no. Har du spørsmål, kan du også ringe til HUNT forskningssenter, tlf 74075180.

Vel møtt til undersøkelsen!

Vennlig hilsen

Steinar Krokstad
Førsteamanuensis
Prosjektleder HUNT 3

Jostein Holmen
Professor, daglig leder
HUNT forskningssenter

Stig A. Slørdahl
Professor, dekanus
Det medisinske fakultet, NTNU

Tid og sted for oppmøte

Dersom det foreslåtte tidspunktet ikke passer for deg, behøver du ikke bestille ny time. Du kan møte når det passer deg innenfor åpningstiden, men det kan da bli noe ventetid. Du kan også møte i en annen kommune, hvis det skulle passe bedre. Takk for at du deltar!

Åpningstida:

hunt 3
Helseundersøkelsen i Nord-Trøndelag

NTNU
HUNT forskningssenter






En time for bedre folkehelse

VESTVIK REKLAME AS. FOTO: HARALD SÆTEROY OG JOHAN ARNT NESGÅRD.

Slik fyller du ut skjemaet

- Skjemaet vil bli lest maskinelt.
- Det er derfor viktig at du krysser av riktig: **Rett** ☒ **Galt** ☐ ☑
- Krysser du feil sted, retter du ved å fylle boksen slik: ■
- Skriv tydelige tall: 0 1 2 3 4 5 6 7 8 9
- Bruk bare svart eller blå penn. Ikke bruk blyant eller tusj.

HELSE OG DAGLIGLIV

1 Hvordan er helsa di nå?

☐ Dårlig ☐ Ikke helt god ☐ God ☐ Svært god

2 Har du noen langvarig (minst 1 år) sykdom, skade eller lidelse av fysisk eller psykisk art som nedsetter dine funksjoner i ditt daglige liv?

	Ja	Nei
	☐	☐

Hvis ja:
Hvor mye vil du si at dine funksjoner er nedsatt?

	Litt nedsatt	Middels nedsatt	Mye nedsatt
Er bevegelseshemmet	☐	☐	☐
Har nedsatt syn	☐	☐	☐
Har nedsatt hørsel	☐	☐	☐
Hemmet pga. kroppslig sykdom	☐	☐	☐
Hemmet pga. psykisk sykdom	☐	☐	☐

3 Har du kroppslige smerter nå som har vart mer enn 6 måneder?

Ja	Nei
☐	☐

4 Hvor sterke kroppslige smerter har du hatt i løpet av de siste 4 uker?

Ingen	Meget svake	Svake	Mode-rate	Sterke	Meget sterke
☐	☐	☐	☐	☐	☐

5 I hvilken grad har din fysiske helse eller følelses-messige problemer begrenset deg i din vanlige sosiale omgang med familie eller venner i løpet av de siste 4 uker?

Ikke i det hele tatt	En del	Litt	Mye	Kunne ikke ha sosial omgang
☐	☐	☐	☐	☐

HELSETJENESTER

6 Har du i løpet av de siste 12 måneder vært hos:

	Ja	Nei
Fastlege/allmennlege	☐	☐
Annen legespesialist utenfor sykehus	☐	☐
Konsultasjon uten innleggelse		
- ved psykiatrisk poliklinikk	☐	☐
- ved annen poliklinikk i sykehus	☐	☐
Kiropraktor	☐	☐
Homøopat, akupunktør, soneterapeut, håndspålegger eller annen alternativ behandler	☐	☐

7 Har du vært innlagt i sykehus i løpet av de siste 12 måneder?

Ja	Nei
☐	☐

SYKDOMMER OG PLAGER

8 Har du hatt noe anfall med pipende eller tung pust de siste 12 måneder?

Ja	Nei
☐	☐

9 Har du noen gang de siste 5 år brukt medisiner for astma, kronisk bronkitt, emfysem eller KOLS?

Ja	Nei
☐	☐

10 Bruker du, eller har du brukt, medisin mot høyt blodtrykk?

Ja	Nei
☐	☐

11 Har du, eller har du noen gang hatt, noen av disse sykdommene/plagene:
(Sett ett kryss pr. linje)

	Ja	Nei	Hvis ja, hvor gammel var du **første** gang?
Eksempel:			34 år gammel
Hjerteinfarkt	☐	☐	år gammel
Angina pectoris (hjertekrampe)	☐	☐	år gammel
Hjertesvikt	☐	☐	år gammel
Annen hjertesykdom	☐	☐	år gammel
Hjerneslag/hjerneblødning	☐	☐	år gammel
Nyresykdom	☐	☐	år gammel
Astma	☐	☐	år gammel
Kronisk bronkitt, emfysem, KOLS	☐	☐	år gammel
Diabetes (sukkersyke)	☐	☐	år gammel
Psoriasis	☐	☐	år gammel
Eksem på hendene	☐	☐	år gammel
Kreftsykdom	☐	☐	år gammel
Epilepsi	☐	☐	år gammel
Leddgikt (reumatoid artritt)	☐	☐	år gammel
Bechterews sykdom	☐	☐	år gammel
Sarkoidose	☐	☐	år gammel
Beinskjørhet (osteoporose)	☐	☐	år gammel
Fibromyalgi	☐	☐	år gammel
Slitasjegikt (artrose)	☐	☐	år gammel
Psykiske plager som du har søkt hjelp for	☐	☐	år gammel

12 Har du noen gang fått påvist for høyt blodsukker?

Ja	Nei
☐	☐

Hvis ja: I hvilken situasjon første gang?

☐ Ved helseundersøkelse... ☐ Under sykdom ...
☐ Under svangerskap ... ☐ Annet ...

SKADER

13 Har du noen gang hatt:

	Ja	Nei	Hvis ja, hvor gammel var du **første** gang?
Eksempel:			3 4 *år gammel*
Lårhalsbrudd	☐	☐	*år gammel*
Brudd i handledd/underarm	☐	☐	*år gammel*
Brudd/sammenfall av ryggvirvler	☐	☐	*år gammel*
Nakkesleng (whiplash)	☐	☐	*år gammel*

14 Har du foreldre, søsken eller barn som har, eller har hatt, følgende sykdommer?
(Sett ett kryss pr. linje)

	Ja	Nei	Vet ikke
Hjerneslag eller hjerneblødning før 60 års alder	☐	☐	☐
Hjerteinfarkt før 60-års alder	☐	☐	☐
Astma	☐	☐	☐
Allergi/høysnue/neseallergi	☐	☐	☐
Kronisk bronkitt/emfysem/KOLS	☐	☐	☐
Kreftsykdom	☐	☐	☐
Psykiske plager	☐	☐	☐
Beinskjørhet (osteoporose)	☐	☐	☐
Nyresykdom (ikke nyresten, urinveisinfeksjon, urinlekkasje)	☐	☐	☐
Diabetes (sukkersyke)	☐	☐	☐

15 Har noen av dine besteforeldre, dine foreldres søsken eller dine søskenbarn fått diagnosen diabetes (type 1 eller type 2)?

Ja ☐ Nei ☐

HVORDAN FØLER DU DEG?

16 Har du de to siste uker følt deg:
(Sett ett kryss pr. linje)

	Nei	Litt	En god del	Svært mye
Trygg og rolig?	☐	☐	☐	☐
Glad og optimistisk?	☐	☐	☐	☐
Nervøs og urolig?	☐	☐	☐	☐
Plaget av angst?	☐	☐	☐	☐
Irritabel?	☐	☐	☐	☐
Nedfor/deprimert?	☐	☐	☐	☐
Ensom?	☐	☐	☐	☐

17 Har du noen gang i livet opplevd at noen over lengre tid har forsøkt å kue, fornedre eller ydmyke deg?

Ja ☐ Nei ☐

TOBAKK

18 Røykte noen av de voksne innendørs da du vokste opp? Ja ☐ Nei ☐

19 Røykte mora di da du vokste opp? Ja ☐ Nei ☐

20 Røyker du selv?

- **Nei**, jeg har aldri røykt ☐
  Hvis du aldri har røykt, hopp til spørsmål 22.
- **Nei**, jeg har sluttet å røyke ☐
- **Ja**, sigaretter av og til (fest/ferie, ikke daglig) ☐
- **Ja**, sigarer/sigarillos/pipe av og til ☐
- **Ja**, sigaretter daglig ☐
- **Ja**, sigarer/sigarillos/pipe daglig ☐

21 A Svar på dette hvis du nå røyker **daglig** eller tidligere har røykt **daglig**:

Hvor mange sigaretter røyker eller røykte du vanligvis daglig? ☐☐ *sigaretter pr. dag*

Hvor gammel var du da du begynte å røyke daglig? ☐☐ *år gammel*

Hvis du tidligere har røykt daglig, hvor gammel var du da du sluttet? ☐☐ *år gammel*

21 B Svar på dette hvis du røyker eller har røykt **av og til**, men ikke daglig:

Hvor mange sigaretter røyker eller røykte du vanligvis i måneden? ☐☐ *sigaretter pr. mnd*

Hvor gammel var du da du begynte å røyke av og til? ☐☐ *år gammel*

Hvis du tidligere har røykt av og til, hvor gammel var du da du sluttet? ☐☐ *år gammel*

22 Bruker du, eller har du brukt, snus?

Nei, aldri ☐ Ja, av og til ☐
Ja, men jeg har sluttet ☐ Ja, daglig ☐

Hvis du aldri har brukt snus, hopp til spørsmål 23.

Hvis ja:

Hvor gammel var du da du begynte med snus? ☐☐ *år gammel*

Hvor mange esker snus bruker/brukte du pr. måned? ☐☐ *esker snus pr. måned*

Hvis du bruker eller har brukt både sigaretter og snus, hva begynte du med først?

Snus ☐ Sigaretter ☐
Omtrent samtidig ☐ Husker ikke ☐
(innenfor 3 måneder)

Da du begynte å bruke snus, var det for å prøve å slutte å røyke eller for å redusere røykinga?

Nei ☐ Ja, for å redusere røykinga ☐
Ja, for å slutte å røyke ☐

MATVARER

23 Hvor ofte spiser du vanligvis disse matvarene?
(Sett ett kryss pr. linje)

	0-3 ganger pr. mnd	1-3 ganger pr. uke	4-6 ganger pr. uke	1 gang pr. dag	2 ggr el mer pr. dag
Frukt/bær	☐	☐	☐	☐	☐
Grønnsaker	☐	☐	☐	☐	☐
Sjokolade/smågodt	☐	☐	☐	☐	☐
Kokte poteter	☐	☐	☐	☐	☐
Pasta/ris	☐	☐	☐	☐	☐
Pølser/hamburgere	☐	☐	☐	☐	☐
Fet fisk (laks, ørret, sild, makrell, uer som pålegg/middag)	☐	☐	☐	☐	☐

24 Bruker du følgende kosttilskudd?
(Sett ett kryss for hvert kosttilskudd)

	Ja, daglig	Av og til	Nei
Tran	☐	☐	☐
Omega-3-kapsler	☐	☐	☐
Vitamin- og/eller mineraltilskudd	☐	☐	☐

25 Hvor mange glass drikker du vanligvis av følgende?
½ liter = 3 glass *(Sett ett kryss pr. linje)*

	Sjelden eller aldri	1-6 gl. pr uke	1 gl. pr. dag	2-3 gl. pr. dag	4 gl. eller mer pr. dag
Vann, farris o.l	☐	☐	☐	☐	☐
Helmelk (søt/sur)	☐	☐	☐	☐	☐
Annen melk (søt/sur)	☐	☐	☐	☐	☐
Brus/saft med sukker	☐	☐	☐	☐	☐
Brus/saft uten sukker	☐	☐	☐	☐	☐
Juice eller nektar	☐	☐	☐	☐	☐

26 Hvor mange kopper kaffe/te drikker du pr. døgn?
(Sett 0 dersom du ikke drikker kaffe/te daglig)

	Koke-kaffe	Annen kaffe	Te
Antall kopper			

27 Hvor mange kopper kaffe drikker du om kvelden (etter kl 18)?
Antall kopper

ALKOHOLBRUK

28 Omtrent hvor ofte har du i løpet av de siste 12 måneder drukket alkohol? *(Regn ikke med lettøl)*

4-7 ganger pr. uke ☐ Ca 1 gang pr. måned ☐
2-3 ganger pr. uke ☐ Noen få ganger pr. år ☐
ca 1 gang pr. uke ☐ Ingen ganger siste år ☐
2-3 ganger pr. måned ☐ Aldri drukket alkohol ☐

29 Har du drukket alkohol i løpet av de siste 4 uker? Ja ☐ Nei ☐

Hvis ja:
Har du drukket så mye at du har kjent deg sterkt beruset (full)?
Nei ☐
Ja, 1-2 ganger ☐
Ja, 3 ganger eller mer ☐

30 Hvor mange glass øl, vin eller brennevin drikker du vanligvis i løpet av 2 uker? *(Regn ikke med lettøl)*
(Sett 0 hvis du ikke drikker alkohol)

	Øl	Vin	Brenne-vin
Antall glass			

31 Hvor ofte drikker du 5 glass eller mer av øl, vin eller brennevin ved samme anledning?

Aldri ☐ Ukentlig ☐
Månedlig ☐ Daglig ☐

MOSJON/FYSISK AKTIVITET

Med mosjon mener vi at du f.eks går tur, går på ski, svømmer eller driver trening/idrett.

32 Hvor ofte driver du mosjon? *(Ta et gjennomsnitt)*

Aldri ☐
Sjeldnere enn en gang i uka ☐
En gang i uka ☐
2-3 ganger i uka ☐
Omtrent hver dag ☐

33 Dersom du driver slik mosjon, så ofte som en eller flere ganger i uka; hvor hardt mosjonerer du?
(Ta et gjennomsnitt)

Tar det rolig uten å bli andpusten eller svett ☐
Tar det så hardt at jeg blir andpusten og svett ☐
Tar meg nesten helt ut ☐

34 Hvor lenge holder du på hver gang?
(Ta et gjennomsnitt)

Mindre enn 15 minutter ☐ 30 minutter – 1 time ☐
15-29 minutter ☐ Mer enn 1 time ☐

35 Har du vanligvis minst 30 minutter fysisk aktivitet daglig på arbeid og/eller i fritida? Ja ☐ Nei ☐

36 Omtrent hvor mange timer sitter du i ro på en vanlig hverdag? *(Regn med både jobb og fritid)* Antall timer ☐

ARBEID

37 Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du beskrive arbeidet ditt? *(Sett ett kryss)*

For det meste stillesittende arbeid *(f.eks skrivebordsarbeid, montering)* ☐

Arbeid som krever at du går mye *(f.eks ekspeditørarbeid, lett industriarb., undervisning)* ☐

Arbeid hvor du går og løfter mye *(f.eks postbud, pleier, bygningsarbeid)* ☐

Tungt kroppsarbeid *(f.eks skogsarbeid, tungt jordbruksarbeid, tungt bygningsarbeid)* ☐

HØYDE/VEKT

38 Omtrent hva var din høyde da du var 18 år?

☐ cm Husker ikke ☐

39 Omtrent hva var din kroppsvekt da du var 18 år?

☐ kg Husker ikke ☐

40 Er du fornøyd med vekta di nå?

Ja ☐ Nei, for lett ☐ Nei, for tung ☐

41 Har du forsøkt å slanke deg i løpet av de siste 10 år?

Nei ☐ Ja, noen ganger ☐ Ja, mange ganger ☐

42 Er din kroppsvekt minst 2 kg lavere nå enn for 1 år siden? Ja ☐ Nei ☐

Hvis ja:

Hva er grunnen til dette?

Slanking ☐ Sykdom/stress ☐ Vet ikke ☐

ALVORLIGE LIVSHENDELSER SISTE 12 MÅNEDER

43 Har det vært dødsfall i nær familie? *(barn, ektefelle/samboer, søsken eller foreldre)* Ja ☐ Nei ☐

44 Har du vært i overhengende livsfare pga. alvorlig ulykke, katastrofe, voldssituasjon eller krig? Ja ☐ Nei ☐

45 Har du hatt samlivsbrudd i ekteskap eller i lengre samboerforhold? Ja ☐ Nei ☐

46 Hvis du har svart ja på et eller flere av spm 43, 44 eller 45; i hvilken grad har du hatt reaksjoner på dette de siste 7 dager?

Ikke i det hele tatt ☐ I moderat grad ☐
Litt ☐ I høy grad ☐

OPPVEKST - DA DU VAR 0-18 ÅR

47 Hvem vokste du opp sammen med?

Mor ☐ Andre slektninger ☐
Far ☐ Adoptivforeldre ☐
Stemor/stefar ☐ Foster-/pleieforeldre ☐

48 Ble dine foreldre skilt, eller flyttet de fra hverandre, da du var barn?

Nei ☐
Ja, før jeg var 7 år ☐
Ja, da jeg var 7-18 år ☐

49 Døde noen av dine foreldre da du var barn?

Nei ☐
Ja, før jeg var 7 år ☐
Ja, da jeg var 7-18 år ☐

50 Vokste du opp med kjæledyr?

Nei ☐
Ja, katt ☐ Ja, hund ☐
Ja, hest ☐ Ja, annet levende dyr ☐

51 Hvor mye melk eller yoghurt drakk du vanligvis?

Sjelden/ aldri	1-6 gl. pr. uke	1 glass pr. dag	2-3 gl. pr. dag	Mer enn 3 glass pr. dag
☐	☐	☐	☐	☐

52 Vokste du opp på gård med husdyr? Ja ☐ Nei ☐

53 Når du tenker på barndommen/oppveksten din, vil du beskrive den som:

Svært god ☐ Vanskelig ☐
God ☐ Svært vanskelig ☐
Middels ☐

ALT I ALT

54 Når du tenker på hvordan du har det for tida, er du stort sett fornøyd med tilværelsen eller er du stort sett misfornøyd? *(Sett ett kryss)*

Svært fornøyd ☐ Nokså misfornøyd ☐
Meget fornøyd ☐ Meget misfornøyd ☐
Ganske fornøyd ☐ Svært misfornøyd ☐
Både/og ☐

Appendix III

Diabetes questionnaire HUNT2

hunt-diabetes

Helseundersøkelsen i Nord-Trøndelag

Hvis du ikke ønsker å besvare spørreskjemaet, sett kryss her og returner skjemaet. Da slipper du purring.
Jeg ønsker ikke å besvare skjemaet ○

Du har opplyst at du har eller har hatt diabetes. Vi ber deg derfor svare så godt du kan på disse spørsmålene om diabetes. Opplysningene vil bli brukt i det videre arbeidet for å bedre diabetesomsorgen og finne ut hvordan man best kan forebygge problemer knyttet til sykdommen. Les forøvrig brosjyren «hunt-spesial», som du fikk ved Helseundersøkelsen

Alle opplysningene blir behandlet av oss med streng taushetsplikt. *Lykke til !*

UTFYLLING

Dato for utfylling av skjema: ..... ☐ / 199

DIAGNOSE

Hvordan ble din diabetes oppdaget?

	Ja	Nei
Jeg søkte lege pga. symptomer.....	☐	☐
Ble oppdaget uten at jeg hadde symptomer (Ved legeattest, bedriftshelsekontroll, undersøkelse for annen sykdom e.l.)	☐	☐

Hva slags plager hadde du i tilfelle da din diabetes ble oppdaget? *(kryss i minst ei rute)*

Ingen plager	☐	Kvalme	☐
Unormal tørste	☐	Synsplager	☐
Stor vannlating	☐	Smerter i beina	☐
Slapphet, svimmelhet	☐	Underlivskløe ..	☐
Vekttap	☐	Andre plager ...	☐

BEHANDLING

INSULIN

Bruker du insulin (sprøyter, penn) mot din diabetes nå?.....	Ja	Nei
	☐	☐

Hvis «Nei»: Gå til TABLETTER

Hvilket årstall begynte du med insulin? ..... 19☐

Hvordan tar du insulin? *(ett kryss på hver linje)*

	Ja	Nei
Sprøyter jeg fyller selv	☐	☐
Engangs- (ferdigfylt) insulinpenn	☐	☐
Vanlig insulinpenn (penn med ampuller som skiftes når de er tomme)	☐	☐
Insulinpumpe	☐	☐
Jet- (trykk-)injektor	☐	☐

Hvor mange ganger tar du vanligvis insulin hver dag? ..... ☐ ganger

Hvor mange enheter insulin tar du vanligvis tilsammen hver dag?..... ☐ enheter (IE)

TABLETTER **Bruker du tabletter mot din diabetes?.....**	Ja	Nei
	☐	☐

Hvis «NEI»: Gå til EGENKONTROLL

Skriv nedenfor hva diabetestablettene heter, antall mg som står på glasset/pakningen og hvor mange slike tabletter du tar daglig. *(Skriv begge sorter hvis du bruker mer enn en type tabletter mot diabetes)*

skriv navn på tabletten her	mg pr. tablett	antall tabl. pr. dag

EGENKONTROLL

Måler du noen gang heime hvor mye sukker (glukose) du har i blodet (blodsukker)? *(Svar «Ja» også om noen hjelper deg eller gjør det for deg)*	Ja	Nei
	☐	☐

Hvis «Nei»: Gå til LEGEKONTROLL

Omtrentlig hvor mange ganger måler du blodsukker i løpet av ei vanlig uke? ..... ☐ ganger

Hva slags metode bruker du når du måler blodsukker?

Strimmel som leses av mot farge på boksen.....	☐
Apparat som leser av prøven og gir resultatet som tall	☐

Hvis du bruker apparat til avlesing av blodsukker; hva heter apparatet? *(Skriv navnet på linja)*

_____

LEGEKONTROLL

	Ja	Nei
Går du til regelmessig kontroll hos lege for din diabetes?	☐	☐
Hvis nei, går du til kontroll hos sykepleier eller annet helsepersonell?	☐	☐

Hvis du ikke går til kontroll hos lege: Gå til KOSTHOLD

Hva slags lege går du til kontroll hos for din diabetes?

	Ja	Nei
Vanlig lege (kommunelege, allmennpraktiserende lege, bedriftslege osv.).....	☐	☐
Sykehuslege (poliklinikk på sykehus).....	☐	☐
Bor i sykeheim eller annen institusjon og får diabeteskontroll hos lege der.....	☐	☐

Hvor mange forskjellige leger har du hatt de fem siste gangene du har vært til vanlig diabeteskontroll? ..... ☐ leger

Hvor mange ganger i året går du til vanlig diabeteskontroll hos lege? ..... ☐ ganger

Bla om !

KOSTHOLD

Her er noen utsagn om kost og mat. Svar ut fra det kostholdet du har til daglig *(Ett kryss på hver linje)*

	Helt sant	Nesten sant	Nesten usant	Helt usant
Jeg spiser akkurat det samme som de som ikke har diabetes ...	☐	☐	☐	☐
Jeg prøver stadig å gå ned i vekt	☐	☐	☐	☐
Jeg synes det er en plage ikke å kunne spise det jeg vil	☐	☐	☐	☐
De fleste dager prøver jeg bevisst å unngå (mettet) fett	☐	☐	☐	☐
Jeg spiser mye grønnsaker	☐	☐	☐	☐

HVORDAN DU HAR DET

Synes du det er vanskelig å ha diabetes? *(ett kryss)*

Ja, jeg føler det som en plage hver dag ..... ☐
Ja, jeg tenker ofte på det ..... ☐
Ja, av og til ..... ☐
Nei, sjelden ..... ☐
Nei, jeg tenker nesten aldri på det ..... ☐

	Ja	Nei
Har du noen gang hatt for lavt blodsukker? («føling», «insulinsjokk»)	☐	☐

Hvis «Ja», hvor mange ganger har du hatt det den siste uka?..... ☐ ganger

	Ja	Nei
Har du noen gang hatt så lavt blodsukker («sjokk») at du måtte ha hjelp av andre for å komme over det?	☐	☐

Hvor mange ganger har du ligget på sykehus etter at du fikk diabetes?..... ☐ ganger

Hvis du har ligget på sykehus etter at du fikk diabetes, hva har du ligget der for? *(Skriv på linjene nedenfor)*

_____

_____

ANNEN MEDISIN

	Ja	Nei
Bruker du fast (regelmessig) medisin for noe annet enn din diabetes?	☐	☐

Hvis «Ja»: Skriv hva disse medisinene heter. *(Skriv det navnet som står på glasset eller pakningen. Ta med alle du bruker regelmessig.)*

_____ _____
_____ _____
_____ _____
_____ _____
_____ _____

SYN

	Ja	Nei
Har du problemer med synet som lege har sagt skyldes din diabetes?.....	☐	☐

UNDERVISNING - STØTTE

	Ja	Nei
Er du medlem av Norges Diabetesforbund?	☐	☐

Hvis «Ja», omtrent hvor mange år har du vært medlem?..... ☐ år

	Ja	Nei
Har du noen gang deltatt på kurs eller møte om diabetes?.....	☐	☐
Får du grunnstønad gjennom trygdekontoret for diabetes?.....	☐	☐
Får du særfradrag i skattelikninga fordi du har diabetes?.....	☐	☐

FOTPROBLEMER

	Ja	Nei
Er du operert for trange blodårer til beina?	☐	☐

Har du fått amputert (skjært bort) en del av ett eller begge bein svarende til:
(Ett kryss på hver linje, skriv evt. årstall til høyre)

	Ja	Nei	Årstall
tær/forfot?	☐	☐	_____
legg/kne?	☐	☐	_____
lår?	☐	☐	_____

	Ja	Nei
Har du hatt sår på føttene som har brukt over 3 uker på å gro?	☐	☐

Hvis ja, omtrent hvor mange uker tok det før såret grodde? *(Hvis flere ganger, skriv det som varte lengst).* ..... ☐ uker

	Ja	Nei	Husker ikke
Har du noen gang fått undersøkt føttene dine ved vanlig diabeteskontroll hos lege?	☐	☐	☐

Undersøkes føttene dine regelmessig av:	Ja	Nei
Lege	☐	☐
Fotterapeut/fotpleier	☐	☐
Sykepleier/heimesykepleier	☐	☐
Andre	☐	☐
Deg selv	☐	☐

Hvis du får regelmessig undersøkelse av lege/fotterapeut/sykepleier; hvor lenge er det mellom hver gang? ..... ☐ uker

Vennligst legg skjemaet i samme konvolutt som det andre skjemaet du fikk ved Helseundersøkelsen og postlegg den snarest. Porto er betalt.
Tusen takk for hjelpa!

Appendix IV

Diabetes questionnaire HUNT3

Diabetes

Kjære HUNT-deltaker

Takk for at du deltok i den første delen av Helseundersøkelsen. **Du svarte bl.a. at du har eller har hatt diabetes. Ved å fylle ut dette skjemaet vil du gi et viktig bidrag til HUNTs videre arbeid for å bedre diabetesomsorgen og finne ut hvordan man best kan forebygge diabetes og problemer knyttet til sykdommen.**

Dato for utfylling: ____ / ____ 20____
Dag / Måned / År

Slik fyller du ut skjemaet

- Skjemaet vil bli lest maskinelt.
- Det er viktig at du krysser av riktig: Rett ☒ Galt ☐ ✗ ✓
- Krysser du feil sted, retter du ved å fylle boksen slik: ■
- Skriv tydelige tall: 0 1 2 3 4 5 6 7 8 9
- Bruk bare svart eller blå penn. Ikke bruk blyant eller tusj.

Skjemaet returneres i den vedlagte konvolutten, som er ferdig frankert.

DIAGNOSE

1. Hvordan ble din diabetes oppdaget? — Ja / Nei
   - Jeg søkte lege pga. symptomer ☐ ☐
   - Ble oppdaget uten at jeg hadde symptomer *(Ved legeattest, bedriftshelsekontroll, undersøkelse for annen sykdom e.l.)* ☐ ☐
2. Hvilket årstall ble din diabetes oppdaget? *Eks.:* 1 9 9 5 ____

BEHANDLING

INSULIN

3. Bruker du insulin (sprøyter, penn, pumpe) mot din diabetes nå? ☐ ☐
   Hvis nei, gå til spørsmål 8.
4. Hvilket årstall begynte du med insulin? *Eks.:* 1 9 9 5 ____
5. Hvordan tar du insulin? *(Sett ett kryss pr. linje)* — Ja / Nei
   - Insulinpenn ☐ ☐
   - Insulinpumpe ☐ ☐
   - Jet-(trykk-)injektor ☐ ☐
6. Hvor mange ganger tar du vanligvis insulin hver dag? ____ ganger
7. Hvor mange enheter insulin tar du vanligvis tilsammen hver dag? ____ enheter (IE)

TABLETTER

8. Bruker du tabletter mot din diabetes? — Ja ☐ / Nei ☐
9. Hvilket årstall begynte du med tabletter mot diabetes? *Eks.:* 1 9 9 5 ____

EGENKONTROLL

10. Måler du noen gang heime hvor mye sukker (glukose) du har i blodet (blodsukker)? *(Svar «Ja» også om noen hjelper deg eller gjør det for deg)* — Ja ☐ / Nei ☐
11. Omtrent hvor mange ganger måler du blodsukker i løpet av en vanlig dag/uke? *Skriv i den ruta som passer best.*
    - ____ ganger pr. dag
    - ____ ganger pr. uke

LEGEKONTROLL

12. Går du til regelmessig kontroll hos lege for din diabetes? — Ja ☐ / Nei ☐
    Hvis nei:
    Går du til kontroll hos sykepleier eller annet helsepersonell? — Ja ☐ / Nei ☐
    Hvis du ikke går til kontroll hos lege: Gå til spørsmål 16.
13. Hva slags lege går du til kontroll hos for din diabetes? — Ja / Nei
    - Fastlege, allmennpraktiserende lege, bedriftslege osv. ☐ ☐
    - Sykehuslege *(poliklinikk på sykehus)* ☐ ☐
    - Bor i sykeheim eller annen institusjon og får diabeteskontroll hos lege der ☐ ☐
14. Hvor mange forskjellige leger har du hatt de fem siste gangene du har vært til vanlig diabeteskontroll? ____ lege(r)
15. Hvor mange ganger i året går du til vanlig diabeteskontroll hos lege? ____ ganger

UNDERVISNING - STØTTE

16. Er du medlem av Norges Diabetesforbund? — Ja ☐ / Nei ☐
    Hvis ja:
    Omtrent når ble du medlem? *Eks.:* 1 9 9 5 ____
17. Har du noen gang deltatt på kurs eller møte om diabetes? — Ja ☐ / Nei ☐
18. Får du særfradrag i skattelikninga fordi du har diabetes? — Ja ☐ / Nei ☐
19. Hvor har du fått mest informasjon om diabetes?
    Kryss av for de viktigste stedene (inntil 3):
    - Kurs/møter ☐
    - Fastlege, annen lege ☐
    - Sykepleier (evt. diabetessykepleier) ☐
    - Andre som selv har diabetes ☐
    - Bøker/blader/tidsskrifter ☐
    - Internett ☐

90900000019

KOSTHOLD

20 Under er noen utsagn om kost og mat. Svar ut fra det kostholdet du har til daglig.

	Helt sant	Nesten sant	Nesten usant	Helt usant
Jeg spiser akkurat det samme som de som ikke har diabetes	☐	☐	☐	☐
Jeg prøver stadig å gå ned i vekt	☐	☐	☐	☐
Jeg synes det er en plage ikke å kunne spise det jeg vil	☐	☐	☐	☐
De fleste dager prøver jeg bevisst å unngå mettet fett	☐	☐	☐	☐
Jeg spiser mye grønnsaker	☐	☐	☐	☐

21 Hvor ofte spiser du vanligvis:

	Sjelden /aldri	1-2 g pr. uke	3-4 g pr. uke	5-6 g pr. uke	Hver dag
Nøtter?	☐	☐	☐	☐	☐
Erter/bønner/linser?	☐	☐	☐	☐	☐
Havregryn?	☐	☐	☐	☐	☐
Løk?	☐	☐	☐	☐	☐

SYN

22 Har du problemer med synet som lege har sagt skyldes din diabetes? Ja ☐ Nei ☐

23 Går du til regelmessig øyeundersøkelse (av netthinna/ øyenbunnen) på grunn av din diabetes? Ja ☐ Nei ☐

Hvis ja:
Hvor lenge er det vanligvis mellom hver gang du blir undersøkt? ____ måneder

24 Har du fått laserbehandling av øynene pga. øyebunns-forandringer som skyldes diabetes? Ja ☐ Nei ☐

HVORDAN HAR DU DET?

25 Synes du det er vanskelig å ha diabetes?
(Sett ett kryss)

- Ja, jeg føler det som en plage hver dag ☐
- Ja, jeg tenker ofte på det ☐
- Ja, av og til ☐
- Nei, sjelden ☐
- Nei, jeg tenker nesten aldri på det ☐
- Føler meg akkurat som de som ikke har diabetes ☐

26 Hvordan opplever du stort sett at det er å kontrollere blodsukkeret ditt?

- Svært vanskelig ☐
- Vanskelig ☐
- Både/og ☐
- Lett ☐
- Svært lett ☐

27 Har du noen gang hatt for lavt blodsukker? *(«føling», «insulinsjokk»)* Ja ☐ Nei ☐

Hvis ja:
Hvor mange ganger har du hatt det den siste uka? ____ ganger

28 Har du noen gang hatt så lavt blodsukker («sjokk») at du måtte ha hjelp av andre for å komme over det? Ja ☐ Nei ☐

29 Hvor mange ganger har du ligget på sykehus etter at du fikk diabetes? ____ ganger

30 Hvis du har ligget på sykehus etter at du fikk diabetes, hva har du ligget der for?
(Sett ett eller flere kryss)

- Lavt blodsukker/insulinsjokk eller skade pga. dette ☐
- Høyt blodsukker/"sukkerslag" ☐
- Hjerte/karsykdom (hjerteinfarkt, hjertesvikt, slag osv.) ☐
- Nyresykdom ☐
- Annen sykdom ☐

FOTPROBLEMER

31 Er du operert for trange blodårer til beina? Ja ☐ Nei ☐

32 Har du fått amputert (skjært bort) en del av ett eller begge bein svarende til: *(Sett ett kryss pr. linje, skriv ev. årstall til høyre)*

	Ja	Nei	Årstall
tær/fot?	☐	☐	
legg/kne?	☐	☐	
lår?	☐	☐	

33 Har du hatt sår på føttene som har brukt over 3 uker på å gro? Ja ☐ Nei ☐

Hvis ja:
Omtrent hvor mange uker tok det før såret grodde? *(Hvis flere ganger, skriv det som varte lengst)* ____ uker

34 Har du noen gang fått undersøkt føttene dine ved vanlig diabeteskontroll hos lege?
Ja ☐ Nei ☐ Husker ikke ☐

35 Undersøkes føttene dine regelmessig av:
(Sett ett eller flere kryss)

- Lege ☐
- Fotterapeut/fotpleier ☐
- Sykepleier/heimesykepleier ☐
- Andre ☐
- Deg selv ☐

36 Hvis du får regelmessig fotundersøkelse av lege/fotterapeut/sykepleier; omtrent hvor lenge er det mellom hver gang? ____ måneder

NB!
Det utfylte skjemaet returneres i den vedlagte svarkonvolutten. Porto er betalt.

Takk for hjelpa!