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New Insights into the Epidemiology of Type 1 Diabetes in Children in Finland

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ACADEMIC DISSERTATION
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To Lauri, Daniel, and Sara

Abstract

Epidemiological data on type 1 diabetes is important for understanding disease determinants and heterogeneity, which in turn plays a crucial role in uncovering the pathogenesis and advancing opportunities for treatment and prevention. Finland is an excellent setting for epidemiological research on type 1 diabetes, as it has the highest incidence of type 1 diabetes worldwide, nationwide high-quality administrative and health registers, and comprehensive databases on diabetes.

For many decades, the incidence of type 1 diabetes has been rising—both in Finland and globally—and was predicted to continue rising at an alarming pace, particularly in young children. However, more recently, Finland and some other countries have shown signs of plateauing incidence. The autoimmune etiology of type 1 diabetes involves a complex interplay between genetic and environmental factors, and changes in incidence are believed to reflect changes in exposure to environmental exposures influencing the disease process. While many environmental factors have been implicated, the evidence remains inconclusive—likely due to a heterogeneous disease process. This heterogeneity appears to be age-related, and it has been suggested that different etiopathological mechanisms, representing distinct endotypes of type 1 diabetes, may underlie cases diagnosed before age 7 compared to those diagnosed at age 13 or older.

This thesis utilized nationwide register data to examine trends in the incidence of type 1 diabetes among children in Finland between 2003 and 2022, and to explore certain environmental factors possibly influencing changes in incidence, as well as the age-related heterogeneity of type 1 diabetes. At the population-level, we investigated the association between incidence trends and decreased exposure to rotavirus infections following the introduction of the rotavirus vaccine into the Finnish national vaccination program in 2009. We also examined the impact of the first 18 months of the COVID-19 pandemic on the incidence and disease characteristics of type 1 diabetes in Finnish children and evaluated the role of SARS-CoV-2 infection by analyzing SARS-CoV-2 antibodies in children newly diagnosed with type 1 diabetes during the pandemic. Furthermore, to explore the age-related heterogeneity of type 1 diabetes, we compared demographic, clinical, autoimmune, and HLA-genotype characteristics between groups based on age at diagnosis.

We found that the incidence of type 1 diabetes in Finnish children aged under 15 years fluctuated considerably during the 20-year study period, with no clear long-term trend. The fluctuation did not appear to occur by chance alone and tended to be more pronounced in boys aged under 5 years. Compared to the baseline 2003–2006 period, the incidence was 10% lower overall in the 2015–2018 period, with a 23% reduction in children aged under 5 years and a 28% reduction in boys of this age. During the first 18 months of the COVID-19 pandemic, the incidence increased by 16% compared to a preceding reference cohort, with a 34% increase in boys aged under 5 years. Following

this rise, overall incidence appeared to level off and decreased again in boys under 5 years of age.

When comparing birth cohorts born before and after the national implementation of the rotavirus vaccine, we found that in children aged under 5 years, both the incidence of type 1 diabetes and the exposure to rotavirus infections were significantly lower in the post-vaccine birth cohorts than in the pre-vaccine 2001–2005 birth cohorts. At the population level, a one-percentage-point reduction in the proportion of children exposed to rotavirus was associated with an 8% decrease in the incidence of type 1 diabetes in this age group.

In addition to the increased incidence of type 1 diabetes during the first 18 months of the COVID-19 pandemic, we observed a 36% rise in the frequency of ketoacidosis at diagnosis of new-onset type 1 diabetes. However, only 0.9% of the children diagnosed with type 1 diabetes during this period tested positive for infection-induced SARS-CoV-2 antibodies, suggesting that the observed changes were not attributable to direct effects of COVID-19 infection.

Finally, when comparing demographic data, clinical characteristics, autoantibody profiles, and HLA class II–associated disease risk between those aged under 7, 7–12 and 13 years or more at diagnosis of type 1 diabetes, we found significant age-related differences in most of the characteristics analyzed. Differences were particularly pronounced between the youngest and oldest age groups, with the middle age group displaying intermediate values. These findings support the concept that the heterogeneity of type 1 diabetes is related to age and may reflect age-related differences in the disease process representing distinct endotypes of type 1 diabetes.

In conclusion, this thesis describes trends in the incidence of type 1 diabetes in Finland during the last 20 years and contributes to our understanding of age- and sex-related differences as well as the possible role of certain environmental factors. Increasing understanding of the etiopathogenesis of this heterogeneous disease is crucial in the search for disease-modifying therapy.

Tiivistelmä

Tyypin 1 diabeteksen epidemiologinen tutkimus on keskeistä taudin syntyyn vaikuttavien tekijöiden ja sen heterogeenisyyden ymmärtämisessä. Tämä puolestaan on ratkaisevaa taudin patogeneesin selvittämisessä sekä uusien hoito- ja ehkäisykeinojen kehittämisessä. Suomi tarjoaa erinomaiset edellytykset tyypin 1 diabeteksen epidemiologiseen tutkimukseen, sillä maassa on maailman korkein tyypin 1 diabeteksen ilmaantuvuus, korkeatasoiset valtakunnalliset rekisteri ja kattavat diabetesaineistot.

Tyypin 1 diabeteksen ilmaantuvuus on ollut vuosikymmenten ajan nousussa niin Suomessa kuin muuallakin maailmassa, ja erityisesti pienten lasten ilmaantuvuuden ennakoitiin kasvavan huolestuttavaa vauhtia. Viime aikoina ilmaantuvuuden kasvu on kuitenkin näyttänyt tasaantumisen merkkejä Suomessa ja joissakin muissa maissa. Taudin autoimmuunitaustainen etiologia perustuu monimutkaiseen geneettisten ja ympäristötekijöiden vuorovaikutukseen, ja ilmaantuvuuden muutosten ajatellaan kuvastavan muutoksia ympäristötekijöissä, jotka vaikuttavat tautiprosessiin. Useita ympäristötekijöitä on epäilty, mutta näyttö on ollut epäyhtenäistä, mahdollisesti taudin heterogeenisen luonteen vuoksi. Tämä heterogeenisyys vaikuttaa liittyvän ikään, ja on esitetty, että ennen 7 vuoden ikää sairastuneet ja yli 12-vuotiaina sairastuneet edustaisivat taudin eri endotyyppejä johtuen erilaisista taudin syntyyn vaikuttavista mekanismeista.

Tässä väitöskirjatutkimuksessa hyödynnettiin valtakunnallisia rekisteritietoja tyypin 1 diabeteksen ilmaantuvuustrendien tarkasteluun suomalaisilla lapsilla vuosina 2003–2022. Lisäksi tutkittiin suomalaislapsilla eräitä ilmaantuvuuden muutoksiin mahdollisesti vaikuttavia ympäristötekijöitä sekä taudin ikään liittyvää heterogeenisyyttä. Väestötasolla arvioitiin vuonna 2009 kansalliseen rokotusohjelmaan sisällytetyn rotavirusrokotteen myötä vähentyneen rotavirusaltistuksen yhteyttä tyypin 1 diabeteksen ilmaantuvuuden muutoksiin. Tutkimuksessa selvitettiin myös koronapandemian ensimmäisten 18 kuukauden vaikutuksia tyypin 1 diabeteksen ilmaantuvuuteen ja taudin piirteisiin sekä arvioitiin SARS-CoV-2-viruksen mahdollista roolia analysoimalla viruksen vasta-aineita pandemian aikana tyypin 1 diabetekseen sairastuneilta. Tyypin 1 diabeteksen ikään liittyvän heterogeenisyyden tutkimiseksi verrattiin diagnoosi-iän mukaan demografisia tietoja, kliinisiä piirteitä, autovasta-aineprofiileja ja HLA-luokan II geeneihin liittyvää tautiriskiä.

Tutkimuksessa havaittiin, että alle 15-vuotiaiden suomalaislasten tyypin 1 diabeteksen ilmaantuvuus vaihteli huomattavasti 20 vuoden aikana ilman selkeää pitkän aikavälin trendiä. Vaihtelu ei näyttänyt olevan sattumanvaraista ja oli korostuneinta alle 5-vuotiailla pojilla. Vuosien 2003–2006 vertailujaksoon nähden ilmaantuvuus oli vuosina 2015–2018 keskimäärin 10 % alempi, ja lasku oli 23 % alle 5-vuotiailla lapsilla sekä 28 % alle 5-vuotiailla pojilla. Koronapandemian ensimmäisten 18 kuukauden aikana ilmaantuvuus nousi 16 % edeltävään vertailukohorttiin nähden, ja nousu oli 34 % alle 5-

vuotiailla pojilla. Tämän nousun jälkeen kokonaisilmaantuvuus näytti tasaantuvan, mutta laski uudelleen alle 5-vuotiailla pojilla.

Verratessa syntymäkohortteja ennen ja jälkeen rotavirusrokotteen käyttöönottoa havaittiin, että alle 5-vuotiailla lapsilla sekä rotavirusinfektioiden esiintyvyys että tyypin 1 diabeteksen ilmaantuvuus olivat merkittävästi alhaisempia rokotteen käyttöönoton jälkeisissä syntymäkohorteissa verrattuna vuosien 2001–2005 kohortteihin. Väestötasolla yhden prosenttiyksikön väheneminen rotavirukselle altistuneiden lasten osuudessa liittyi 8 %:n laskuun tyypin 1 diabeteksen ilmaantuvuudessa alle 5-vuotiailla.

Koronapandemian aikaisen tyypin 1 diabeteksen ilmaantuvuuden kasvun ohella havaittiin 36 %:n lisäys ketoasidoosin esiintyvyydessä diabeteksen toteamishetkellä. Vain 0,9 %:lla tyypin 1 diabetekseen pandemian aikana sairastuneista lapsista todettiin infektioon viittaavat SARS-CoV-2-vasta-aineet, mikä viittaa siihen, että muutokset eivät johtuneet viruksen suorasta vaikutuksesta.

Lopuksi havaittiin merkittäviä eroja tyypin 1 diabetekseen sairastuneiden demografisissa tiedoissa, kliinisissä ominaisuuksissa, autovasta-aineprofiileissa ja HLA-luokka II-tautiriskissä diagnoosi-iän mukaan (<7, 7–12 ja ≥13 vuotta). Erot korostuivat erityisesti nuorimman ja vanhimman ikäryhmän välillä. Tulokset tukevat näkemystä siitä, että tyypin 1 diabeteksen heterogeenisyys liittyy ikään ja saattaa heijastaa ikään liittyviä eroja tautiprosessissa, jotka voivat edustaa tyypin 1 diabeteksen erilaisia endotyyppejä.

Yhteenvetona väitöskirja kuvaa tyypin 1 diabeteksen ilmaantuvuuden kehitystä Suomessa viimeisten 20 vuoden aikana ja syventää ymmärrystä taudin ikään ja sukupuoleen liittyvästä heterogeenisyydestä sekä tiettyjen ympäristötekijöiden mahdollisesta roolista. Tämän heterogeenisen taudin syntymekanismin ja syntyyn vaikuttavien tekijöiden parempi ymmärtäminen on keskeistä taudinkulkuun vaikuttavien hoitomuotojen löytämiseksi.

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List of abbreviations

BMI	Body mass index
BMI-SDS	BMI standard deviation score, age- and sex-specific BMI
CI	Confidence interval
COVID-19	Coronavirus disease 2019
FinDM	Diabetes in Finland database
FPDR	Finnish Pediatric Diabetes Register
GADA	Antibodies to glutamic acid decarboxylase
HbA1c	Glycated hemoglobin
HLA	Human leucocyte antigen
IA-2A	Antibodies to islet antigen 2
IAA	Insulin autoantibodies
ICA	Islet cell antibodies
IR	Incidence rate
IRR	Incidence rate ratio
JDFU	Juvenile Diabetes Foundation Units
N	Number
NIDR	National Infectious Diseases Register
PY	Person years
Ref	Reference
RR	Risk ratio
RU	Relative units
SARS-CoV-1	Severe acute respiratory syndrome coronavirus 1
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
T1D	Type 1 diabetes
T1DE1	Type 1 diabetes endotype 1
T1DE2	Type 1 diabetes endotype 2
ZnT8A	Zinc transporter 8 autoantibodies

Index

List of original publications

This thesis is based on the following publications:

I Parviainen A, But A, Siljander H, Knip M; Finnish Pediatric Diabetes Register. Decreased Incidence of Type 1 Diabetes in Young Finnish Children. Diabetes Care. 2020;43(12):2953-2958. doi:10.2337/dc20-0604*

II Parviainen A, But A, Sund R, Arffman M, Siljander H, Knip M. Incidence of Type 1 Diabetes in Relation to Exposure to Rotavirus Infections in Pre- and Post-Vaccine Birth Cohorts in Finland. Diabetes Care. 2024;47(1):97-100. doi:10.2337/dc23-1203*

III Knip M, Parviainen A, Turtinen M, But A, Härkönen T, Hepojoki J, Sironen T, Iheozor-Ejiofor R, Ugurlu H, Saksela K, Lempainen J, Ilonen J, Vapalahti O: Finnish Pediatric Diabetes Register. SARS-CoV-2 and type 1 diabetes in children in Finland: an observational study. Lancet Diabetes Endocrinol. 2023;11(4):251-260. doi:10.1016/S2213-8587(23)00041-4

IV Parviainen A, Härkönen T, Ilonen J, But A, Knip M; Finnish Pediatric Diabetes Register. Heterogeneity of Type 1 Diabetes at Diagnosis Supports Existence of Age-Related Endotypes. Diabetes Care. 2022;45(4):871-879. doi:10.2337/dc21-1251*

The publications are referred to in the text by their roman numerals. Previously unpublished material is also presented. The publications have been reprinted with the permission of their respective copyright holders.

1 Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreatic islets, resulting in lifelong dependence on exogenous insulin. Though it can manifest at any age, its incidence peaks in childhood, and it is one of the most common chronic conditions affecting children, imposing a significant burden on both individuals and society. This thesis focuses on type 1 diabetes in children.

In recent decades, the global incidence of type 1 diabetes among children has been rising (1, 2), highlighting the urgent need for finding strategies for treatment and prevention. However, developing such strategies is challenging, as the precise etiopathogenesis of the disease remains elusive due to its complex and heterogeneous nature. This has driven recent research to focus on identifying distinct disease subtypes (endotypes) that could enable precision medicine approaches (3).

The etiology of type 1 diabetes is multifactorial, involving a complex interplay between genetic and environmental factors. Given that genetic changes in populations occur slowly over time, rapid changes in type 1 diabetes incidence are likely driven by environmental factors. Despite numerous environmental factors having been implicated as possible triggers of autoimmunity, the evidence linking specific environmental factors to type 1 diabetes remains ambiguous and inconclusive.

Interestingly, at the onset of this thesis project, data from some countries with high type 1 diabetes incidence, including Finland, indicated that the alarming rise in incidence might be slowing down or plateauing (4-7). This observation sparked interest in exploring trends in incidence in Finland and environmental factors that might be contributing to changes in incidence rates.

This thesis describes trends in the incidence of type 1 diabetes among Finnish children over the past two decades. It investigates certain environmental factors that have undergone changes in exposure during the study period to determine whether there may be an association between these environmental factors and changes in disease incidence. Furthermore, it explores the heterogeneity of type 1 diabetes in relation to age at diagnosis, to provide insights into potential endotypes of the disease. The overall aim of this thesis is to advance the understanding of the epidemiology of type 1 diabetes, with the hope that this knowledge may contribute to the search for preventive and curative therapies.

2 Review of the literature

2.1 The incidence of type 1 diabetes

Epidemiology is the study of the occurrence and determinants of disease. Measuring the frequency of a disease in a population and identifying how the frequency varies over time or among subgroups may point to potential causes of the disease and imply possibilities for prevention. Incidence is a commonly used measure of disease frequency. Incidence rate (IR) represents the number of new cases of a disease occurring within a specified population during a specified time period, and is often reported in terms of person-years, combining the number of people and the amount of time they were followed (8). Incidence is a valuable tool for studying changes in the occurrence of type 1 diabetes.

2.1.1 Globally

Type 1 diabetes was relatively rare until the 1950s, after which incidence began to increase (9). It has been estimated that annually almost 100,000 children develop type 1 diabetes before the age of 15 years (10). There is considerable variation in the incidence of type 1 diabetes across populations (1, 10, 11). Incidence is low in most Asian populations and the highest incidence rates are found in populations in Europe, North America and Oceania (1). The incidence is generally higher in high-income countries (10, 12), though incidence rates do vary substantially also within populations and countries, for example varying between low and very high incidence within Europe, and there is a 3–5-fold incidence in Sardinia compared with the rest of Italy (1, 11).

Incidence rates in children also vary by age groups and sex. The incidence rate generally increases with age, being lowest in children aged less than 5 years and highest in those aged 10–14 years (1, 11). Whilst overall the sex ratio is fairly equal, male excess is observed in high incidence and female excess in low-incidence populations (13, 14). According to a study by Karvonen et al., all populations with an incidence exceeding 23.0 per 100,000 exhibited a higher incidence among males, whereas populations with an incidence below 4.5 per 100,000 showed a higher incidence among females (14). 77% of populations with a male-dominated

incidence were of European origin, whilst populations with a female-dominated incidence were predominantly from low-risk ethnic groups.

The incidence rate of type 1 diabetes in children has been increasing globally at an annual rate of 3–4% for decades (1, 2, 6, 15). Overall, the incidence trends have been similar for both sexes (1), however a European study revealed a higher rate of increase for boys than girls in children aged 10–14 years (6). The rate of increase has been highest in children aged less than 5 years (1, 16, 17), and the incidence in this age group was predicted to double between 2005 and 2020 in Europe (16). However, the rate of increase has since appeared to be slowing down in some high-incidence countries (6), and even a plateau in incidence has been described in certain Nordic countries (4, 7).

2.1.2 Finland

Finland is infamous for having the highest incidence of type 1 diabetes worldwide (1, 11, 17, 18). Incidence trends similar to the global trends described above have been observed in Finland. The incidence has been rising for decades, with an approximate 3% annual increase among children since 1965 (19, 20). The rise in incidence accelerated in the 1990s, reaching an annual increase of 4.1% between 1992 and 2005 (19). Incidence has been lowest in children aged less than 5 years, but the greatest increase in incidence has been observed in this age group, among which the annual increase in incidence was 4.7% between 1980 and 2005 (19, 20). Incidence doubled between 1980 and 2005 and was projected to double again by 2020, with rates in the youngest age group predicted to surpass the incidence in older children (19). However, after a peak incidence of 64.9 per 100,000 children in 2006, a plateau in incidence was observed until 2011, and incidence between 2006 and 2011 was highest in the 5–9 age group (5).

No major difference was observed in the incidence of type 1 diabetes between the sexes between 1980 and 2005, with an overall boy-to-girl ratio in incidence of 1·1 (19). The ratio did, however, change with puberty, reaching a boy-to-girl ratio of 1.7 at 13 years (19). Between 2006 and 2011 the overall incidence in boys was higher than in girls; 68.4 (95% CI 65.1–71.9) per 100,000 in boys and 55.4 (95% CI 52.7–58.4) per 100,000 in girls (ratio 1.2) (5).

2.2 Pathogenesis

Considering the alarming global rise of type 1 diabetes incidence, there is a strong interest in the pathogenesis of this disease.

Type 1 diabetes is an autoimmune disease. Autoimmune diseases are caused by a malfunction of the immune response, in which the immune system attacks the body's own cells (21). Autoimmunity is marked by the presence of autoantibodies

(produced by B lymphocytes) and autoreactive T lymphocytes, which recognize autoantigens. In type 1 diabetes, the autoimmune process attacks the insulin producing β-cells in the pancreatic islets. The precise pathological mechanisms underlying the process remain unclear (22, 23).

2.2.1 Islet autoimmunity

The β-cell destruction causing type 1 diabetes is considered to be a primarily T-cell mediated process (24). β-cell components become recognized as autoantigens and are presented to autoreactive T-cells by human leucocyte antigen (HLA) I and II molecules (25). This leads to autoantibody production, release of cytokines, cytolysis and stimulation of macrophages and other immune cells, resulting in a positive feedback loop of β-cell destruction (25).

A defining pathologic feature of this autoimmune attack against β-cells is insulitis, characterized by lesions of predominantly lymphocytic infiltration in and around the pancreatic islets (26, 27). Insulitis is, however, present in only a modest number of islets at any given time. It is more prevalent in younger patients and closer to diagnosis, yet even then it is present in only about 30% of residual insulin positive islets (28). This suggests that the extent of the impairment of insulin secretion causing the severe hyperglycemia generally observed at diagnosis is partially due to other mechanisms, such as inflammation and β-cell dysfunction (26, 29). Insulitis, as well as residual β-cells and insulin secretion, are also still observed many years after diagnosis, to a highly variable degree, demonstrating that islet autoimmunity is a chronic, evolving and varied process (26).

Another key element marking islet autoimmunity is the appearance of islet autoantibodies. These are considered to reflect disease activity rather than to be major effectors in β-cell damage and are useful for prediction and diagnostic purposes as biomarkers of islet autoimmunity (22). Five major autoantibodies associated with type 1 diabetes are autoantibodies against islet cells (ICA) (30), insulin (IAA), glutamic acid decarboxylase (GADA), islet antigen-2 (IA-2A) and zinc transporter 8 (ZnT8a) (31). ICA represents a mix of various autoantibodies, while the other four are antibodies against specific molecules and therefore defined as biochemical autoantibodies. The first autoantibody to appear is usually IAA or GADA, which possibly represent two different disease pathways, with IAA associated with younger and GADA with older age at diagnosis (32).

2.2.2 The stages of type 1 diabetes

Type 1 diabetes is considered to be a continuum, progressing from presymptomatic β-cell autoimmunity to symptomatic clinical disease. The disease can progress at highly variable rates, ranging from months to more than two decades (33). Islet

autoantibodies may first appear already before the age of six months, and the incidence of seroconversion to autoantibody positivity peaks during the first two years of life (34-36). The risk of progressing to type 1 diabetes is fairly small if only one of the biochemical autoantibodies is detected, but with the presence of two or more, the risk is high (37). Progression from single to multiple autoantibody positivity is most common in children aged less than five years, and typically takes place within two years of the appearance of single autoantibody positivity (38).

Three distinct stages of the disease have been proposed (39) (Figure 1). In the presymptomatic stage 1, the individual tests positive for two or more islet autoantibodies, indicating advanced autoimmunity, yet with normoglycemia. For children at genetic risk (see section 2.3.1), reaching stage 1 signifies a 70% risk of developing clinical type 1 diabetes within 10 years, and the lifetime risk is close to 100% (37). In stage 2, advanced autoimmunity is combined with presymptomatic dysglycemia, as functional β-cell mass has decreased, but is still sufficient to prevent symptoms from appearing. Upon reaching this stage, approximately 75% develop symptomatic disease within 4 years (40). Finally, stage 3 is marked by the onset of symptomatic type 1 diabetes. Classic clinical symptoms include increased urination (polyuria), thirst (polydipsia), fatigue and weight loss. The blood glucose level is elevated and a variable state of metabolic decompensation exists, which can progress to life-threatening diabetic ketoacidosis if treatment is delayed (41).

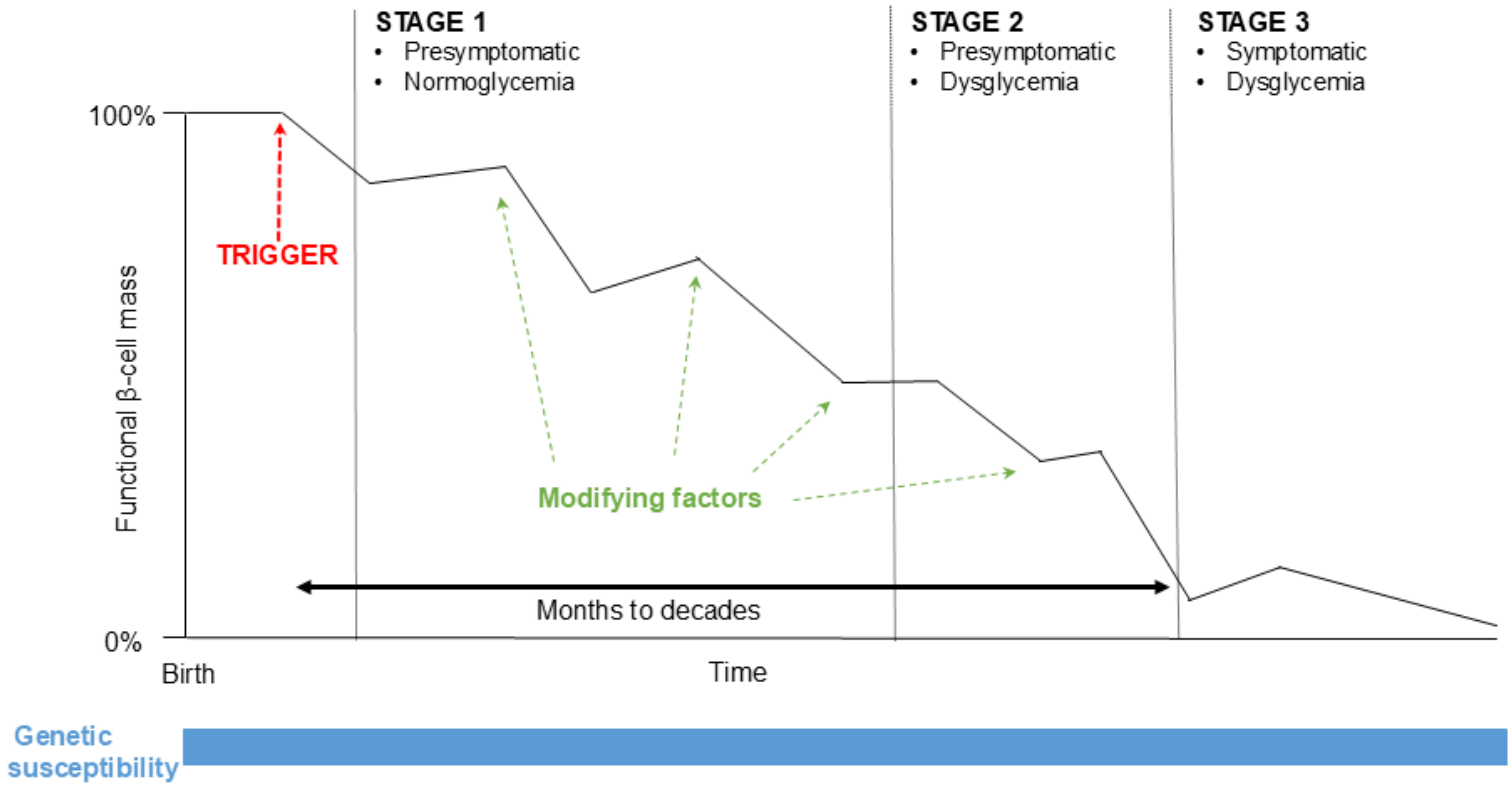

Figure 1 The pathogenesis, etiology, and stages of type 1 diabetes. Modified from (39, 42)

2.3 Etiology of type 1 diabetes

Multiple genetic and environmental factors are considered to be involved in the development of type 1 diabetes.

2.3.1 Genetic factors

It is well established that there is strong genetic predisposition to type 1 diabetes. With long-term follow-up, in geographically or otherwise restricted samples, monozygotic twins have been found to have up to 65% concordance for type 1 diabetes and up to 78% of initially discordant identical twins have been observed to develop autoantibody positivity, type 1 diabetes, or both (43). However, in a Finnish population-based study the concordance for type 1 diabetes was observed to be significantly lower—only 23 % among monozygotic twins and 5% in dizygotic twins (44). Though most cases of type 1 diabetes are sporadic, the risk of developing the disease is manifold in individuals with affected first-degree relatives. Approximately 10–12% of children diagnosed with type 1 diabetes have an affected first-degree relative, and a similar proportion have an affected second-degree relative (45, 46).

2.3.1.1 HLA genotype

Type 1 diabetes is a polygenic disease, but the HLA region on chromosome 6 represents the major determinant of the genetic risk, estimated to explain approximately half of the genetic susceptibility (47). The HLA region is made up of three subregions, class I, class II and class III. Particularly class I (loci A, B and C) and class II (loci DR, DQ and DP) are involved in the immune response, as products of the genes in these loci are cell surface proteins, which bind antigens and present them to T cells (48).

Though HLA class I loci are also involved in type 1 diabetes susceptibility by enhancing progression of islet autoimmunity (49), the major genetic determinant of type 1 diabetes risk is HLA class II, particularly the DR/DQ region, and the HLA class II effect is conferred through initiation of islet autoimmunity (50). The proteins encoded by these genes are found specifically on the surface of antigen presenting cells, and present antigens to T helper cells.

HLA class II mediated disease susceptibility is determined by a combination of two haplotypes, one inherited from each biological parent. The haplotypes consist of several genes, and these genes are highly polymorphic, with many possible variants of each gene (48). This polymorphism affects which antigens the gene products bind, and thus determines the specificity of the immune response, influencing type 1 diabetes risk (48). These genes also exhibit extensive linkage

disequilibrium, meaning that they are often found in certain combinations, and the frequency of certain haplotypic combinations varies in different populations (51).

HLA class II DR/DQ haplotypes can be arranged based on their effect on disease susceptibility from strong susceptibility to strong protection, and HLA class II DR/DQ genotype, formed by two inherited haplotypes, can be used to estimate the risk for type 1 diabetes (Tables 1 and 2) (50). The two major risk haplotypes are the DR3-DQ2 and the DR4-DQ8 haplotypes, and approximately 90% of patients with type 1 diabetes have one of these two haplotypes (47). Whilst the risk effect conferred by two haplotypes is mostly additive, there is a synergistic risk associated with the combination of the DR3-DQ2 and DR4-DQ8 haplotypes (50). This combination is associated with the highest risk of type 1 diabetes and is carried by around 30 % of people with type 1 diabetes, compared with about 2% of the general population (47).

Table 1 Classification of type 1 diabetes risk associated with HLA class II DR/DQ haplotypes found in Finnish children, exhibiting the influence of gene polymorphism on type 1 diabetes susceptibility

S, strong susceptibility; s, weak susceptibility; N, neutral; p, weak protection; P, strong protection

DR3-DQ2 = (DR3)-DQA1*05-DQB1*02

DR4-DQ8 = DRB1*04:01/02/04/05/08-DQA1*03(:01)-DQB1*03:02(/04)

Modified from (50).

Haplotype	Odds Ratio	Haplotype risk classification
DRB1*04:01-DQA1*03-DQB1*03:02	10.11	S
DRB1*04:05-DQA1*03-DQB1*03:02	3.01	S
DRB1*04:04-DQA1*03-DQB1*03:02	2.82	s
(DR3)-DQA1*05-DQB1*02	2.81	s
DRB1*04:02-DQA1*03-DQB1*03:02	1.75	S
(DR13)-DQB1*06:04	1.13	N
(DR9)-DQA1*03-DQB1*03:03	0.97	N
(DR8)-DQB1*04	0.95	N
(DR16)-DQB1*05:02	0.79	N
(DR7)-DQA1*0201-DQB1*02	0.63	N
(DR1/10)-DQB1*05:01	0.58	N
(DR4)-DQA1*03-DQB1*03:01	0.51	N
DRB1*0403-DQA1*03-DQB1*03:02	0.37	p
(DR13)-DQB1*06:09	0.36	N
(DR13)-DQB1*06:03	0.23	p

Haplotype	Odds Ratio	Haplotype risk classification
(DR11/12/13)-DQA1*05-DQB1*0:301	0.23	p
(DR7)-DQA1*02:01-DQB1*03:03	0.08	P
(DR15)-DQB1*06:01	0.07	P
(DR15)-DQB1*06:02	0.03	P
(DR14)-DQB1*05:03	0.03	P

Table 2 Classification of type 1 diabetes risk associated with HLA class II DR/DQ genotype

S, strong susceptibility; s, weak susceptibility; N, neutral; p, weak protection; P, strong protection Modified from (50).

HLA-DR/DQ based risk group	Haplotype risk classification combination	Odds Ratio
High risk	S/s, s/s (if DR3-DQ2/ DR4-DQ8)	13.23
Moderately increased risk	S/s, s/s (except DR3-DQ2/DR4-DQ8), S/S, S/N	7.20
Slightly increased risk	s/N, S/p	1.94
Neutral	N/N, S/P, s/P, s/p	0.38
Slightly decreased risk	p/N	0.10
Strongly decreased risk	P/N, p/p, P/p, P/P	0.02

2.3.1.2 Non-HLA genes

Whilst the HLA-region is recognized as the major determinant of genetic susceptibility to type 1 diabetes, various non-HLA regions are also involved. Currently, there are around 80 non-HLA gene loci confirmed to be associated with type 1 diabetes risk, which together with HLA-related susceptibility are considered to explain approximately 80% of the disease heritability (52, 53). Many of these loci contain genes involved in the immune response (53), and influence either initiation of autoimmunity or progression to clinical disease (54). After the HLA region, the strongest genetic association has been found for polymorphisms of the insulin gene (*INS*), considered to influence the development of immune tolerance for insulin (47). The unexplained portion of heritability may partly be explained by yet unidentified non-HLA genes, but also by, for example, epigenetics (53).

2.3.1.3 Epigenetics

Despite the evident importance of genetic risk in the development of type 1 diabetes, genetics alone do not suffice to explain the etiology; the concordance for the disease in monozygotic twins is not 100%, and only a minority of those with known genetic susceptibility develop the disease (42). It has thus been proposed that epigenetics play an important role in the development of type 1 diabetes (55-57). Epigenetics refers to modifications in gene expression, which affect phenotype, without changes in DNA sequence (53). Environmental factors influence the expression of genes through epigenetic mechanisms, including DNA methylation, modification of histones and activation of microRNA (55, 56), which can, for example, affect the immune response (58). In type 1 diabetes, epigenetic modifications of gene expression caused by environmental exposures are considered to be involved in the interaction between genetic and environmental risk factors and disease pathogenesis (55, 57).

2.3.2 Environmental factors

There are many reasons why environmental factors are considered to play a crucial role in triggering or accelerating islet autoimmunity. First, rapid changes in incidence cannot be explained by genes. Also, incidence varies considerably in populations with similar genetic background but different environmental conditions, for example Finland compared to Russian Karelia (59). Likewise, the incidence increases amongst immigrants from low incidence regions who have migrated to high incidence countries (60-62).

A myriad of environmental risk factors have been implicated, including but not limited to cow's milk (63, 64), gluten (65, 66), vitamin D deficiency (67), obesity (68), stress (69, 70), various viruses (71), decreased environmental biodiversity and changes in gut microbiota (72-74). None have yet been conclusively proven (75). Some of the implicated environmental factors are discussed in more detail below, selected based on the focus of this thesis.

2.3.2.1 The hygiene hypothesis and the biodiversity hypothesis

Microbes are amongst the environmental factors considered to be involved in type 1 diabetes pathogenesis (76). According to the hygiene hypothesis, reduced microbial exposure in early childhood could predispose to type 1 diabetes by steering the immune system away from self-tolerance, towards autoimmunity (77). Similarly, the more recent biodiversity hypothesis suggests that reduced environmental biodiversity, related to the modern urban living style, could increase susceptibility to autoimmune diseases by for example altering the human microbiota, with immunomodulatory effects (72). Evidence supporting these

hypotheses has been seen, for example, in the higher incidence of type 1 diabetes in countries with higher socioeconomic status, associated with lower microbial exposure and environmental biodiversity, as evidenced by the differences between Finland and Russian Karelia (78).

The relationship between microbes and type 1 diabetes is, however, ambiguous (79), as early-life infections have, on the other hand, been reported to increase the risk for developing type 1 diabetes (80-83), and several viruses in particular have been hypothesized to act as triggers of islet autoimmunity.

2.3.2.2 Viruses

Ever since the seasonality of new-onset type 1 diabetes was recognized, viruses have been considered key candidates amongst environmental factors possibly involved in the development of type 1 diabetes (71, 84). They have been hypothesized to either trigger or enhance autoimmune destruction of β-cells in genetically susceptible individuals.

Different mechanisms for viral involvement in β-cell autoimmunity have been proposed (79, 85, 86). The virus could infect and damage β-cells, causing the release of islet cell antigens, targets for the autoimmune response. On the other hand, destruction of the β-cells could be immune mediated. According to the hypothesis of molecular mimicry, similar peptide structures on the virus and the β-cell could cause immune cells to activate in response to the pathogen and to attack β-cells due to cross-reactivity. Another mechanism proposed for viral involvement is bystander activation, in which the general inflammatory environment caused by the infection leads to non-specific activation of autoimmune cells due to loss of self-tolerance.

Amongst viruses which have been hypothesized to be involved in islet autoimmunity are enteroviruses, rotavirus, paramyxovirus (causing mumps), cytomegalovirus, rubella and the newcomer SARS-CoV-2 (85, 87). Currently, the strongest evidence exists for enteroviruses, in particular for the group of Coxsackie B viruses (88-92). Coxsackie B viruses have been implicated to induce a chronic low-grade infection in the β-cells resulting in progressing β-cell damage (93, 94). Vaccines against Coxsackie B viruses have been developed with the hope of providing a strategy for primary prevention of type 1 diabetes (95).

Out of the viral pathogens implicated, the rotavirus and the SARS-CoV-2 virus were studied in this thesis.

Rotavirus

The rotavirus is one of the most common causes of childhood gastroenteritis. Before the introduction of the rotavirus vaccine, it has been estimated that virtually all children globally were affected before the age of 5 years, the majority between the

ages of 6 months and 2 years, with approximately 1 in 5 requiring a clinic visit and 1 in 60 requiring hospitalization (96, 97). The substantial disease burden prompted the development of rotavirus vaccines, resulting in a significant reduction in rotavirus gastroenteritis (98). In Finland, the rotavirus vaccine first became commercially available in 2006 and was introduced into the Finnish national vaccination program in the autumn of 2009, having since been offered in three doses administered at 2, 3 and 5 months of age to children born from July 2009 onwards. During the transition period between 2006 and 2009, it has been estimated that around 1/3 of the birth cohort was vaccinated, and after the introduction of the vaccine into the national vaccination program, vaccination coverage rapidly increased to more than 90% (99). As a result, both hospital outpatient and hospitalized cases decreased by more than 90%, indicating a dramatic decrease in the population-level exposure to circulating rotaviruses (99).

Rotavirus and type 1 diabetes

Rotavirus infections have been hypothesized to be a possible trigger of islet autoimmunity (86, 100-102). Several possible mechanisms have been proposed. Since the rotavirus has peptide sequences very similar to the peptide sequences of islet autoantigens GAD and IA-2, molecular mimicry has been suggested as a possible mechanism for rotavirus involvement (103, 104). It has also been hypothesized that the general inflammation caused by the rotavirus could activate islet autoimmunity through bystander activation. Extra-intestinal spreading of rotavirus and infection of pancreatic tissue could also cause damage to β-cells, resulting in the release of islet autoantigens precipitating autoimmunity. In addition, intestinal inflammation could result in increased intestinal permeability and consequently in increased exposure to several other antigens potentially capable of precipitating islet autoimmunity (86, 105).

Despite extensive research on the topic, evidence for the role of the rotavirus in islet autoimmunity is inconsistent. Rotavirus infection has been demonstrated to affect the risk of type 1 diabetes in mouse-models, but results in human studies have been mixed. For example, an association was demonstrated between the rotavirus infection and the appearance of islet autoantibodies in an Australian study (106), but was unconfirmed in a subsequent Finnish study (107). Nonetheless, current evidence suggests that the rotavirus may be an environmental trigger of type 1 diabetes (101).

Rotavirus vaccine and the incidence of type 1 diabetes

Considering the possible involvement of the rotavirus in the development of type 1 diabetes, there has been an obvious interest in exploring the potential relationship

between the rotavirus vaccine and type 1 diabetes. If rotavirus infections do indeed act as triggers of type 1 diabetes, the vaccine could, in theory, act as either a trigger of islet autoimmunity like the rotavirus due to similar peptide sequences, or have a protective effect through the prevention of rotavirus infections.

Research on the possible association between these two has become more and more feasible with increasing follow-up time since widespread vaccination as part of national vaccination programs around the world. There has been no indication that the rotavirus vaccine would act as a trigger of type 1 diabetes. Rather, some studies have reported an association between the introduction of a nationwide rotavirus vaccination program and a decrease in the incidence rate of type 1 diabetes in young children (108-111). An ecological study conducted in Australia reported a 15% decrease in the incidence of type 1 diabetes in children under the age of 5 years in the 8-year period after the introduction of the rotavirus vaccine into the national vaccination program in 2007, when compared to the preceding 8-year period (109). Likewise, in the United States, the rotavirus vaccine was introduced into the national vaccination program in 2006 and Rogers et al. observed a 3.4% annual decrease in the incidence rate in children aged under 5 years between 2006 and 2017, and a 33% reduction in the risk of type 1 diabetes when comparing 540 317 children who had received the full rotavirus vaccination series to 246 600 unvaccinated peers (111).

On the other hand, there are several studies which have not supported the existence of such a protective association (112-116). Amongst these are two studies conducted in Finland. Vaarala et al. compared 94 437 vaccinated and 27 213 unvaccinated children in a cohort born in 2009–2010 (116). Between 2010 and 2014, there were 345 cases of type 1 diabetes in this cohort, of which 243 in vaccinated children, leading to an adjusted relative risk of 0.91 (95% confidence interval 0.69 to 1.20), thus a non-significant decrease, with the conclusion that the rotavirus vaccine does not significantly alter the risk of type 1 diabetes (116). In the other Finnish study, Hemming-Harlo et al. retrospectively studied participants in the Rotavirus Efficacy and Safety Trial (REST) conducted in 2001–2003, in which more than 23 000 Finnish babies received the RotaTeq vaccine or placebo in a 1:1 ratio (117, 118). In 2015, over 19 000 families that had participated in REST were sent questionnaires asking whether their child had later been diagnosed with any autoimmune disease (115). Of the 5764 (30%) who responded, a similar prevalence of type 1 diabetes was seen in the vaccinated (33/3184, 1.04%) and placebo (25/2580, 0.97%) groups leading to the conclusion that the rotavirus vaccine did not affect the occurrence of type 1 diabetes (115). These individual-level cohort studies were limited by short follow-up time and small cohort sizes relative to the incidence of type 1 diabetes, which may lead to a lack of statistical power to observe small but significant changes. Recently, the aforementioned studies, amongst others, were included in a meta-analysis, which concluded that vaccinated children

under the age of 5 years had a decreased risk of type 1 diabetes (RR=0.84, 95% confidence interval 0.75 to 0.95) (119).

SARS-CoV-2 and the COVID-19 pandemic

Reports of increased incidence of diabetes and increased frequency of diabetic ketoacidosis during the COVID-19 pandemic sparked concern about the possible role of the SARS-CoV-2 virus in the development of diabetes (120-122).

SARS-CoV-2 viruses, like the SARS-CoV-1 viruses which caused the previous SARS pandemic in 2003, use angiotensin-converting enzyme 2 (ACE2) receptors for host cell entry (123). These receptors are expressed in the pancreas, and according to some studies specifically in pancreatic islets and β-cells (124, 125). Reversible β-cell damage and transient insulin-dependent diabetes was documented during the SARS-CoV-1 pandemic (125), and acute pancreatitis was reported during the COVID-19 pandemic (126-130). It has thus been implied that direct damage of pancreatic tissue and pancreatic beta cells by the virus is possible and this, as well as systemic inflammation and multi-organ damage caused by severe COVID-19 infection, could impair insulin production causing symptoms of diabetes and ketoacidosis. Whilst these mechanisms are non-autoimmune, it has also been proposed that the virus could precipitate or accelerate the autoimmune process leading to autoimmune type 1 diabetes (131).

On the other hand, it has been suggested that the increase in incidence could instead be the result of other pandemic related changes in environmental conditions. Lockdown and social distancing led to a reduction in microbial exposure and infections (132, 133), which according to the biodiversity hypothesis could enhance the risk of autoimmune diseases. Lockdown also resulted in decreased physical activity (134) and weight gain (135, 136) in children and adolescents, factors which may also increase the risk of type 1 diabetes (68, 137). Furthermore, the mental health of children and adolescents deteriorated during the pandemic, and psychological stress has been linked to the pathogenesis of type 1 diabetes (69, 138). Increased ketoacidosis at diagnosis could on the other hand be related to delays in seeking or receiving medical care due to pandemic lockdown circumstances, rather than to a direct effect of the virus. Some of the various impacts of the pandemic could thus also explain the changes observed in type 1 diabetes incidence and presentation.

2.4 Heterogeneity and endotypes of type 1 diabetes

As illustrated above, the epidemiology, pathogenesis and etiology of type 1 diabetes are highly variable and multifactorial. Incidence varies by geographic location, ethnicity, sex and age; histologic findings are diverse, and the rate of disease progression is highly variable; genetic susceptibility is polygenic and various environmental factors seem to be involved, likely via unique gene-environment interactions. This complexity is referred to as the heterogeneity of type 1 diabetes

and poses a challenge for type 1 diabetes research. A better understanding of this heterogeneity and the identification of endotypes, disease subtypes with unique etiopathogenesis, would facilitate the search for disease modifying therapy (3).

Heterogeneity in type 1 diabetes has been observed to be related to age (139). Two major patterns of islet autoimmunity have been observed, with IAA as the first appearing autoantibody associated with HLA DR4-DQ8 and younger age, and GADA as the first appearing antibody associated with HLA DR3-DQ2 and older age (22).

More recently two endotypes of type 1 diabetes, type 1 diabetes endotype 1 (T1DE1) and type 1 diabetes endotype 2 (T1DE2) have been proposed (140, 141). The basis of these proposed endotypes was the discovery made from the analysis of pancreatic tissue samples and serum samples, demonstrating distinct immunohistological profiles correlating with age at diagnosis (141-143). The immunohistological findings in individuals diagnosed before the age of 7 years were different from those diagnosed at age 13 or above, and those aged 7 to 12 years at diagnosis exhibited either one or the other of the two profile types.

Based on these findings, T1DE1 is implicated to be predominant in those diagnosed before the age of 7 years and proposed characteristics include genetic association with HLA DR4-DQ8, IAA as the first and persisting islet autoantibody to appear, insulitis lesions with large numbers of CD8+ T cells and CD20+ B cells, few remaining insulin-containing islets, lower circulating C-peptide levels, and higher proinsulin-to–C-peptide ratios (140). In contrast, T1DE2 has been suggested to be predominant in those aged 13 or above at diagnosis and to be marked by association with HLA DR3-DQ2, GADA as the first and persisting autoantibody, low percentage of CD8+ T cells and CD20+ B cells, more residual insulin-containing islets, higher circulating C-peptide levels and lower proinsulin to C-peptide ratio (140).

Due to the scarcity of available pancreatic tissue samples from children and adolescents close to diagnosis of type 1 diabetes, these endotypes have been proposed based on data obtained from relatively few cases. More research is required to confirm whether these do indeed represent distinct underlying mechanisms, rather than reflecting, for example, mere differences in the intensity of the immune attack (140).

2.5 Summary of the literature

In summary, type 1 diabetes is a heterogeneous disease with multifactorial etiology and varied pathogenic pathways. The destruction of β-cells causing type 1 diabetes is perceived to be caused by the actions and interactions of a series of genetic and environmental factors, but the precise etiopathogenesis remains unclear. Particularly as the incidence of type 1 diabetes has long been rising globally, gaining a deeper understanding of factors influencing disease development is urgent. Such insights are essential for advances in precision medicine strategies for the prediction, prevention and treatment of type 1 diabetes.

3 Aims of the study

The aim of this thesis is to contribute to a better understanding of the epidemiological factors influencing type 1 diabetes in children in Finland.

The specific objectives were:

1. To describe trends in the incidence of type 1 diabetes in children in Finland between 2003 and 2022 (I, III and previously unpublished material).

2. To explore changes in the incidence of type 1 diabetes in relation to changes in exposure to rotavirus infections in Finnish children (II).

3. To explore the impact of the first 18 months of the COVID-19 pandemic on the incidence and characteristics of type 1 diabetes in Finnish children, and to evaluate the possible role of the SARS-CoV-2 virus (III).

4. To explore age-related heterogeneity of type 1 diabetes in Finnish children (IV).

4 Materials and methods

4.1 Materials

4.1.1 Registers

The data for this thesis were obtained from several national registers which are described below.

4.1.1.1 The Finnish Pediatric Diabetes Register

The Finnish Pediatric Diabetes Register (FPDR) was established in 2002 and has since collected data from all pediatric units treating children and adolescents with newly diagnosed diabetes. Upon diagnosis, all patients and their families were invited to participate in the register. A study based on a 3-year period from June 2002–May 2005 validated the coverage of the FPDR at 92% when compared to hospital records (144). Data on demographic and clinical characteristics were collected from participating families and pediatric care-units using structured questionnaires. In addition, more than 70% of register subjects provided blood samples, which were analyzed for diabetes-associated autoantibodies and HLA class II DR-DQ haplotypes. Diabetes type was categorized in the register as type 1, type 2, monogenic or other, based on clinical characteristics, autoantibody status and HLA-associated disease risk. The diagnosis of monogenic diabetes was also based on molecular diagnosis (145). Approximately 99% of the register-subjects have type 1 diabetes.

4.1.1.2 The Diabetes in Finland database

The Diabetes in Finland (FinDM) project aims to identify all persons with diabetes in Finland, in order to provide data on the incidence, prevalence, treatment, costs, comorbidities and complications of diabetes in Finland (146). Cases are retrospectively identified by collecting and processing data from several national administrative and healthcare registers. Due to challenges in the classification of diabetes type, classification is based on information from one year following diabetes onset, using the register data in a hierarchical fashion. The registers used

include the Hospital Discharge Register, the Care Register for Health Care, the Register of Primary Health Care Visits, the Medical Birth Register, the Causes of Death Statistics, the Special Reimbursement Register and the Prescription Database. At the time of Publication II, the FinDM database included individuals diagnosed with diabetes between 1964 and 2017, with cases until 2016 ascertained with at least one year of follow-up to confirm the initial diagnosis. Currently data is available until 2023.

4.1.1.3 The Finnish National Infectious Diseases Register

The National Infectious Diseases Register (NIDR) is based on the Communicable Diseases Decree (147) and maintained by the Finnish Institute for Health and Welfare (THL). This register collects data on cases of hazardous and monitored communicable diseases for the purposes of prevention, control and research (148). Data are collected using communicable disease notifications filled out by doctors and laboratories. The rotavirus is one of the infectious diseases considered as a notifiable communicable disease and laboratories are required to notify the NIDR of positive findings. Data on laboratory confirmed rotavirus infections have been gathered since the establishment of the register in 1995.

4.1.1.4 The Population Information System

The Population Information System is a register maintained by Statistics Finland, Finland's national statistical institute, which processes data from many sources to produce a wide selection of up-to-date statistical data on the Finnish population (149). This register includes an open database available through the StatFin service, from which annual age- and sex-specific population data were obtained for use in this thesis to determine the population for incidence and prevalence calculations.

4.1.2 Setting, design and study population

This thesis is a nationwide retrospective register-based cohort study. Publications I and II used group-level aggregated data on counts and person years, Publication III used both group-level and individual-level data, and Publication IV used individual-level data for analyses. The publications are summarized in Table 3.

4.1.2.1 Inclusion criteria

Diagnosis of type 1 diabetes rather than any other form of diabetes was the primary inclusion criteria for study subjects. Children younger than 6 months at diagnosis were generally excluded due to the likelihood of monogenic rather than type 1

diabetes (150). As an exception, in Publication IV, out of those aged less than 1 year at diagnosis, only those with HLA class II associated disease risk and clear positivity for at least one autoantibody were included, which resulted in the inclusion of one child aged less than 6 months at age of diagnosis. In general, the upper limit for age at diagnosis of type 1 diabetes was 14 years (less than 15 years), except in Publication IV, in which there was no upper limit resulting in a maximum age of 17 years. For the purposes of the study, only those cases with data on HLA-associated disease risk and islet autoantibodies were included in Publication IV.

Table 3 Summary of publications

	Aims	Time period(s) / Birth cohorts	Number of cases (data source)	Comparisons	Outcome measures
I	Describe trends in the incidence of T1D in Finland	Time period 1.1.2003–31.12.2018	7871 cases of T1D (FPDR)	Periods 2003–2006 (ref) 2007–2010 2011–2014 2015–2018	Incidence rate of T1D and relative differences in the incidence rate between periods (IR and IRR)
II	Explore changes in the incidence of T1D in relation to changes in exposure to rotavirus infections	Birth cohorts born 1.1.1995–31.12.2015 Follow-up period 1.1.1995–31.12.2016	8674 cases of T1D (FinDM) 18154 cases of rotavirus (NIDR)	Birth cohorts 1995–2000 2001–2005 (ref) 2006–2009 2010–2015	Prevalence of rotavirus infections Incidence rate of T1D and relative differences in the incidence rate between birth cohorts (IR and IRR) Relationship between exposure to rotavirus infections and incidence of T1D (IRR)
III	Explore the impact of the COVID-19 pandemic on the incidence and characteristics of type 1 diabetes	Time periods 1.3.2014–31.8.2015; 1.3.2016–31.8.2017; 1.3.2018–31.8.2019 (pre-pandemic) 1.3.2020–31.8.2021 (pandemic)	2881 cases of T1D (FPDR)	Periods Pre-pandemic (ref) Pandemic	Incidence rate of T1D and relative differences in the incidence rate between pandemic and pre-pandemic periods (IR and IRR) Differences in distribution of characteristics between children diagnosed with T1D during pandemic and pre-pandemic periods
IV	Explore the age-related heterogeneity of T1D	1.1.2003–31.12.2019	6015 cases of T1D (FPDR)	Age groups <7 years 7–12 years ≥13 years	Differences in distribution of characteristics between children diagnosed with T1D in different age groups

4.1.2.2 Publication I

FPDR data from 1.1.2003–31.12.2018 was analyzed to describe trends in the incidence of type 1 diabetes. Data on type 1 diabetes cases included date of birth, date of diagnosis, age at diagnosis and sex. To account for random fluctuation of incidence, data were aggregated into four 4-year time periods (2003–2006, 2007–2010, 2011–2014, and 2015–2018), and incidence rates were compared using the 2003–2006 period as a reference.

4.1.2.3 Publication II

For exploring changes in the incidence of type 1 diabetes in relation to exposure to rotavirus infections, cases of type 1 diabetes were obtained from the FinDM database, as the period of data collection in the FinDM database covers the period included in the NIDR. Birth cohorts 1995–2015 were included in the analyses, with follow-up from 1.1.1995–31.12.2016. Four birth cohort groups were formed based on the availability and coverage of the rotavirus vaccine: the pre-vaccine (1995–2000 and 2001–2005), the partly vaccinated (2006–2009), and the post-vaccine (2010–2015) birth cohorts. The 2001–2005 birth cohort group was used as a reference. Data analyzed were the number of laboratory-confirmed rotavirus infections in children aged under 5 years and the number of individuals diagnosed with type 1 diabetes at the age of 0.5–14.9 years, tabulated by year of birth, sex and age at diagnosis in 1-year age groups.

4.1.2.4 Publication III

To compare type 1 diabetes incidence and characteristics at diagnosis during the COVID-19 pandemic relative to a preceding reference period, the first 18 months of the COVID-19 pandemic (March 1, 2020–August 31, 2021) and three preceding periods of equal length and monthly distribution (March 1, 2014–August 31, 2015; March 1, 2016–August 31, 2017; and March 1, 2018–August 31, 2019), were studied using data from the FPDR. In addition to the data essential for calculating the incidence of type 1 diabetes, including date of birth, date of diagnosis, age at diagnosis and sex, a wide range of data were retrieved on demographic and clinical characteristics, diabetes-related autoantibodies and HLA-II genotype. 583 cases out of the 785 diagnosed during the pandemic period had serum samples taken at a median of 5 days after diagnosis allowing for the analysis of SARS-CoV-2 antibodies.

4.1.2.5 Publication IV

Data on demographic and clinical characteristics, diabetes-related autoantibodies and HLA-II genotype from the FPDR participants between January 1, 2003, and December 31, 2019, were analyzed for exploring age-related differences. Cases were stratified by age at diagnosis into three groups: <7, 7–12 and ≥13 years.

4.1.2.6 Previously unpublished data

Annual and average incidence rates were described overall and for age- and sex-specific groups for FPDR data covering the 20-year period from January 1, 2003, to December 31, 2022.

In addition, to compare data coverage of the FPDR and the FinDM database, the number of cases with type 1 diabetes diagnosed between the ages of 0.5–14.9 years were analyzed by calendar year and age groups between 2003 and 2022.

4.2 Methods

The following section describes the methods used in this thesis.

4.2.1 Definitions

Type 1 diabetes was diagnosed based on the diagnostic criteria of the American Diabetes Association (151). Familial type 1 diabetes was defined as having one or more first-degree family members (parent or sibling) with type 1 diabetes. Season of birth and diagnosis were categorized according to common practice by dividing the calendar year into four seasons: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February).

Body mass index standard deviation scores (BMI SDS) were assessed using the World Health Organization AnthroPlus software (152). The duration of classical symptoms of type 1 diabetes (increased thirst and urination, fatigue and weight loss) prior to diagnosis was divided into 4 categories: no symptoms, less than 1 week, 1–4 weeks or more than 4 weeks. The stage of puberty was categorized as prepubertal (Tanner scale 1) or pubertal/post-pubertal (Tanner scale 2 or more). Impaired consciousness was defined as either impaired consciousness or unconsciousness at diagnosis. Markers of metabolic decompensation, including plasma glucose, glycated hemoglobin (HbA1c), blood pH and β-hydroxybuturate, were analyzed in local hospital laboratories at the time of diagnosis of type 1 diabetes and recorded in the FPDR. Ketoacidosis was defined as a pH <7.30 and severe ketoacidosis as a pH <7.10 (41).

The IAA-dominant profile was defined as being positive for IAA but not GADA, whilst the GADA-dominant profile represented positivity to GADA but not IAA. HLA-DR/DQ genotypes were classified into six risk groups of varying type 1 diabetes risk (Table 2). In Publication IV, these six risk groups were combined to form two risk categories: increased risk (including high risk, moderately increased risk and slightly increased risk) and neutral/protective (including neutral, slightly decreased risk and strongly decreased risk).

4.2.2 Laboratory analyses

4.2.2.1 Analysis of autoantibodies

ICA levels were analyzed in the research laboratory of the Department of Pediatrics at the University of Oulu, using a standardized immunofluorescence staining on pancreatic tissue from blood group O human donors (153). ICA positivity was defined using a detection limit 2.5 Juvenile Diabetes Foundation Units (JDFU).

The biochemical autoantibodies IAA (154), GADA (155), IA-2A (156) and ZnT8A (157) were analyzed using specific radiobinding assays in the PEDIA laboratory at the University of Helsinki. The cutoff limits used to define positivity were 1.57 relative units (RU) for IAA, 5.36 RU for GADA, 0.77 RU for IA-2A and 0.50 RU for ZnT8A based on the 99th percentile in more than 350 healthy Finnish children. The PEDIA laboratory has taken part in the Diabetes Autoantibody Standardization Program (DASP) and the Islet Autoantibody Standardization Program (IASP) between 2010 and 2020, according to which the sensitivities and specificities range from 36–66% and 92–98% for IAA, 64–88% and 94–100% for GADA, 62–72% and 93–100% for IA-2A, and 62–74% and 99–100% for ZnT8A, respectively (158, 159).

Only autoantibody titers of samples taken within 30 days of diagnosis were included in the study, as with a longer delay, positive IAA titers may result from the use of exogenous insulin rather than an autoimmune response (46).

4.2.2.2 Analysis of HLA class II genotypes

Typing of the major HLA class II DR/DQ haplotypes associated with type 1 diabetes risk was performed in the Immunogenetics Laboratory, Institute of Biomedicine, University of Turku, using polymerase chain reaction (PCR) amplification and hybridization with lanthanide labeled probes (160).

4.2.2.3 Analysis of SARS-CoV-2 antibodies

For the purposes of Publication III, serum samples taken soon after diagnosis of type 1 diabetes from those diagnosed during the pandemic were analyzed for SARS-

CoV-2 antibodies. The analyses were performed by first screening the samples with an enzyme-linked immunosorbent assay (ELISA) for antibodies against SARS-CoV-2 Spike protein (161, 162). The samples testing positive were then further analyzed for antibodies against the nucleoprotein (N protein), not induced by the spike-based vaccines used in Finland, as well as for neutralization capacity against the contemporary variants using a pseudovirus neutralization assay (163).

4.2.3 Statistics

We calculated overall, sex-specific and age-specific (0.5–4.9, 5.0–9.9 and 10.0–14.9 years) incidence rates (IR) of type 1 diabetes per 100,000 person years. IR ratios (IRR) were calculated to measure relative differences in incidence rates over time (I and III) and between birth cohorts (II). In addition, IRRs were used to quantify the association between the incidence rate of type 1 diabetes and exposure to rotavirus infections (II). This was performed by calculating the relative change in the incidence rate (IRR) per one percentage point change in the proportion of rotavirus exposed children. Due to the incompletely observed age range, sensitivity analyses were performed using imputed data. Poisson distributed rates were assumed when calculating 95% confidence intervals (CI) for IRs and when applying multiplicative Poisson regression models to quantify IRRs. Poisson regression models were fitted to the number of children diagnosed with diabetes, using the natural logarithm of person years as an offset term. Adjustment for age, sex and period was performed where appropriate. Comparison of the number of cases of type 1 diabetes recorded in the FPDR and the FinDM database was performed by calculating the percentage differences between the two relative to their average. Analyses were performed to describe characteristics of children diagnosed with type 1 diabetes and to compare these characteristics in different groups (III, IV). Categorical data were analyzed using cross-tabulation and reported using frequencies and proportions. Confidence intervals (95% CI) were calculated for frequencies using the interval proportion tool in R (III). Continuous normally distributed data were reported using mean and standard deviation, and continuous skewed data using median and range or interquartile range. For comparisons between unrelated groups, frequencies were compared using Pearson's X2 test, with continuity correction or Fisher exact test when appropriate (III). To compare the means of two or more groups of continuous normally distributed variables, Student's t-test, one way ANOVA and Welch ANOVA were used when appropriate. When these tests were not appropriate, distributions of continuous variables were compared using non-parametric tests, including Mann-Whitney U test /Wilcoxon's rank sum test and Kruskal-Wallis test. When comparisons involving three groups indicated statistically significant differences, pairwise comparisons were performed using the independent samples Student t test or Mann-Whitney U test, as

appropriate. Bonferroni correction or other such techniques for multiple comparisons were not applied because of the risk of overcorrection (164), rather multiplicity issues were taken into account by cautious interpretation of the results. When considered appropriate, adjustment for sex and age at diagnosis were performed using logistic or multinomial regression for categorical variables, linear regression for parametric continuous variables and quantile regression for skewed continuous variables.

Statistical analyses were performed using IBM SPSS Statistics for Windows versions 25 and 27, and R Software for Statistical Computing for Windows, versions 3.4.0 and 3.5.0. For all analyses, a two-tailed p value of <0.05 was considered significant.

4.3 Ethical considerations

The research is register-based, and individuals are not recognizable in the published material. The study protocol for the Finnish Pediatric Diabetes Register has been approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa (Helsinki, Finland). Participation in the register is based on informed consent, obtained from parents and children aged 10 years or more. The FinDM database has been approval by the Research Ethics Workgroup of the Finnish Institute for Health and Welfare. The research was conducted according to principles of the Declaration of Helsinki.

5 Results

5.1 The incidence of type 1 diabetes in Finland

5.1.1 A decrease in incidence between 2003 and 2018 (I)

Between 2003 and 2018, when viewed as four consecutive 4-year periods, using the first period (2003–2006) as a reference, incidence plateaued between 2003 and 2010 and declined thereafter (Table 4). The overall incidence rate decreased by 10%, from 57.9 per 100,000 PY in 2003–2006 to 52.2 per 100,000 PY in 2015–2018. Analyzed by age groups, the overall decline was observed to be due to a decreased incidence in young children; the incidence of type 1 diabetes decreased by 23% and 14% in children aged 0–4 and 5–9 years, respectively, when comparing 2015–2018 to the reference period. In the youngest age group, this decline was statistically significant for both boys and girls, 27% and 18% respectively. In the middle age group, the reduction was only observed in girls, whilst for boys the incidence more or less plateaued between 2007 and 2018. In contrast, in the oldest age group, no changes in the incidence were observed over the study period.

5.1.2 A rise in incidence during the beginning of the COVID-19 pandemic (III)

The overall, age- and sex-specific incidence rates of type 1 diabetes per 100,000 PY in children aged less than 15 years were consistently higher during the first 18 months of the COVID-19 pandemic than during the preceding 54-month reference period (Table 5). Overall, the incidence increased by 16%; by 19% in boys and a nonsignificant 11% in girls. When analyzed by age groups, the change was similar in the two youngest groups, but statistically significant only among children aged 5–9 years, with an 18% increase. In this age group the increase was similar for both sexes, whereas in the youngest group, there was a conspicuous increase of 34% among boys, whilst the incidence in girls remained relatively stable.

Table 4 Overall, age- and sex-specific incidence rates per 100,000 person years and incidence rate ratios of type 1 diabetes in Finnish children between 2003 and 2018, based on data from the Finnish Pediatric Diabetes Register.

Statistically significant results are marked in bold. Modified from Publication I.

		ALL		**BOYS**		**GIRLS**	
Age at diagnosis (years)	Period	IR/100,000 PY (95% CI)	IRR (95% CI)	IR/100,000 PY (95% CI)	IRR (95% CI)	IR/100,000 PY (95% CI)	IRR (95% CI)
0–14	2003–2006	57.9 (55.4–60.4)	1	63.5 (59.9–67.2)	1	52.0 (48.7–55.5)	1
	2007–2010	56.0 (53.6–58.6)	0.97 (0.91–1.03)	61.2 (57.6–64.9)	0.96 (0.89–1.05)	50.7 (47.4–54.1)	0.97 (0.89–1.07)
	2011–2014	53.4 (51.1–55.9)	**0.92 (0.87–0.98)**	58.8 (55.3–62.4)	0.93 (0.85–1.01)	47.9 (44.7–51.2)	0.92 (0.84–1.01)
	2015–2018	52.2 (49.8–54.6)	**0.90 (0.85–0.96)**	57.7 (54.3–61.3)	**0.91 (0.84–0.99)**	46.3 (43.2–49.7)	**0.89 (0.81–0.98)**
0–4	2003–2006	51.1 (47.1–55.5)	1	53.7 (47.9–60.0)	1	48.5 (42.9–54.6)	1
	2007–2010	50.4 (46.5–54.6)	0.99 (0.88–1.1)	53.3 (47.6–59.4)	0.99 (0.85–1.16)	47.4 (42.0–53.3)	0.98 (0.83–1.16)
	2011–2014	43.1 (39.4–46.9)	**0.84 (0.75–0.95)**	44.6 (39.5–50.2)	**0.83 (0.71–0.98)**	41.4 (36.4–46.9)	0.85 (0.72–1.02)
	2015–2018	39.3 (35.7–43.1)	**0.77 (0.68–0.87)**	39.0 (34.1–44.4)	**0.73 (0.61–0.86)**	39.6 (34.5–45.2)	**0.82 (0.68–0.98)**
5–9	2003–2006	68.2 (63.6–73.1)	1	72.7 (66.1–79.8)	1	63.6 (57.2–70.4)	1
	2007–2010	62.3 (57.9–67.1)	0.91 (0.83–1.01)	64.4 (58.1–71.2)	0.89 (0.77–1.02)	60.2 (53.9–66.9)	0.95 (0.82–1.10)
	2011–2014	59.4 (55.1–63.9)	**0.87 (0.79–0.96)**	64.7 (58.5–71.4)	0.89 (0.78–1.02)	53.9 (48.1–60.2)	**0.85 (0.73–0.99)**
	2015–2018	58.6 (54.4–63.0)	**0.86 (0.78–0.95)**	65.1 (58.9–71.7)	0.89 (0.78–1.02)	51.8 (46.2–57.9)	**0.82 (0.70–0.95)**
10–14	2003–2006	54.3 (50.4–58.5)	1	63.6 (57.7–69.9)	1	44.7 (39.7–50.2)	1
	2007–2010	55.6 (51.5–59.9)	1.02 (0.92–1.14)	65.8 (59.6–72.5)	1.03 (0.90–1.18)	45.0 (39.8–50.7)	1.01 (0.85–1.19)
	2011–2014	58.1 (53.8–62.7)	1.07 (0.96–1.19)	67.4 (61.0–74.3)	1.06 (0.92–1.21)	48.4 (42.9–54.5)	1.08 (0.92–1.28)
	2015–2018	57.6 (53.4–62.1)	1.06 (0.96–1.18)	67.7 (61.4–74.6)	1.07 (0.93–1.22)	47.1 (41.7–53.0)	1.05 (0.89–1.24)

Table 5 Overall, age- and sex-specific incidence rates per 100,000 person years and incidence rate ratios of type 1 diabetes in Finnish children during the first 18 months of the COVID-19 pandemic (March 1, 2020–August 31, 2021) and during a preceding 54-month reference period (March 1, 2014–August 31, 2015; March 1, 2016–August 31, 2017; March 1, 2018–August 31, 2019). Based on data from the Finnish Pediatric Diabetes Register.

IRRs are age- and/or sex adjusted, as appropriate. Statistically significant results are marked in bold. Modified from Publication III.

		ALL		**BOYS**		**GIRLS**	
Age at diagnosis (years)	Period	IR/100,000 PY (95% CI)	IRR (95% CI)	IR/100,000 PY (95% CI)	IRR (95% CI)	IR/100,000 PY (95% CI)	IRR (95% CI)
0–14	Reference	52.3 (50.1–54.6)	1	57.8 (54.6–61.2)	1	46.5 (43.5–49.6)	1
	Pandemic	61.0 (56.8–65.4)	**1.16 (1.06–1.25)**	69.6 (63.4–76.3)	**1.19 (1.07–1.32)**	52.0 (46.5–58.0)	1.11 (0.98–1.26)
0–4	Reference	39.6 (36.2–43.2)	1	40.4 (35.6–45.6)	1	38.7 (34.0–44.0)	1
	Pandemic	47.0 (40.2–54.5)	1.19 (1.00–1.41)	53.6 (43.7–65.2)	**1.34 (1.06–1.67)**	40.0 (31.3–50.3)	1.03 (0.79–1.34)
5–9	Reference	59.3 (55.3–63.5)	1	65.6 (59.7–71.8)	1	52.8 (47.5–58.5)	1
	Pandemic	70.0 (62.4–78.2)	**1.18 (1.04–1.34)**	77.4 (66.4–89.6)	1.18 (0.99–1.40)	62.2 (52.2–73.6)	1.18 (0.97–1.44)
10–14	Reference	57.0 (53.1–61.2)	1	66.3 (60.3–72.6)	1	47.3 (42.2–52.9)	1
	Pandemic	63.5 (56.5–71.1)	1.11 (0.97–1.27)	74.8 (64.2–86.5)	1.13 (0.95–1.34)	51.7 (42.8–61.9)	1.09 (0.88–1.35)

5.1.3 Incidence rates of type 1 diabetes between 2003 and 2022 (previously unpublished data)

The total number of children diagnosed with type 1 diabetes before the age of 15 years between January 1, 2003, and December 31, 2022, and recorded in the FPDR was 9757. Of these, 5462 (56.0%) were boys and 4295 (44.0%) were girls. Yearly incidence rates overall and stratified by age groups and sex are shown in Figures 2–5, and average incidence rates over the 20-year period are reported in Table 6. The average incidence rate over the 20-year period was highest in children aged 5–9 years and lowest in children aged 0–4 years. The incidence rate was higher in boys than in girls (IRR 1.22, 95% CI 1.17–1.27). This was the case in all age groups, but the relative difference was highest in the oldest age group; age 0–4 years IRR 1.10 (95% CI 1.02–1.19), age 5–9 years IRR 1.16 (95% CI 1.08–1.23), age 10–14 years IRR 1.40 (95% CI 1.30–1.50).

Table 6 Overall, age- and sex-specific incidence rates of type 1 diabetes per 100,000 person years in Finnish children between 2003 and 2022, based on data from the Finnish Pediatric Diabetes Register.

	ALL	**BOYS**	**GIRLS**
Age at diagnosis (years)	IR/100,000 PY (95% CI)	IR/100,000 PY (95% CI)	IR/100,000 PY (95% CI)
0–14	54.9 (53.8–56.0)	60.2 (58.6–61.8)	49.4 (48.0–50.9)
0–4	46.0 (44.2–47.8)	48.1 (45.6–50.7)	43.8 (41.4–46.3)
5–9	62.8 (60.8–64.8)	67.2 (64.4–70.2)	58.2 (55.4–60.1)
10–14	55.5 (53.7–57.4)	64.5 (61.7–67.4)	46.2 (43.8–48.7)

When observed annually we found considerable fluctuation in the incidence of type 1 diabetes, which appeared most pronounced in young boys (Figure 5). The figures show the decrease in incidence particularly in the youngest age group, followed by the rise observed during the beginning of the COVID-19 pandemic, with an overall peak incidence of 61.0 per 100,000 PY in 2020. The new data show that most recently the incidence has leveled off, and has decreased in the youngest children, more specifically in boys. Compared with 2020 there was a significant decline in 2022 in children aged 0–4 years (IRR 0.68, 95% CI 0.52–0.91), for which the all-time low during the 20-year follow-up period was in 2022 at 37.4 cases per 100,000 PY. The decline was significant for boys (IRR 0.58, 95% CI 0.42–0.78) but not for girls (IRR 0.85, 95% CI 0.61–1.14) (Figure 5).

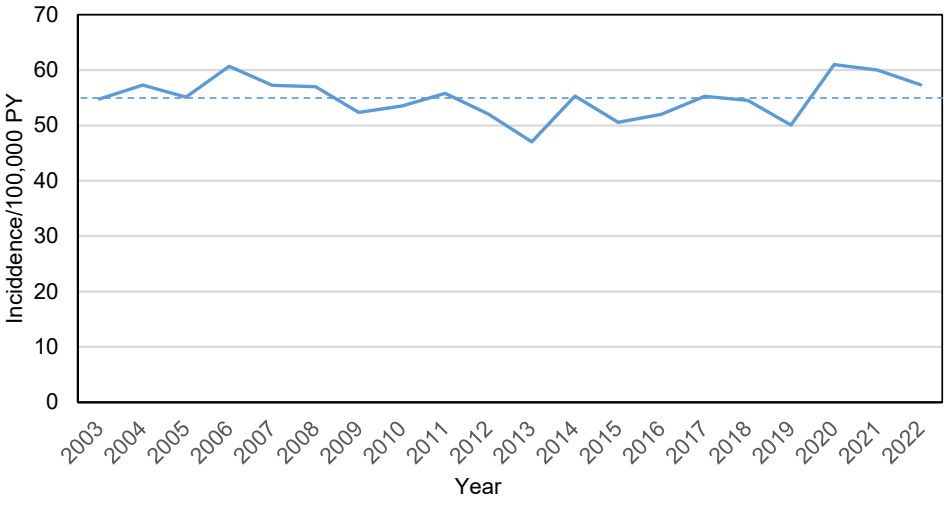

Figure 2 The incidence rate of type 1 diabetes diagnosed before the age of 15 years between 2003 and 2022. Based on data from the Finnish Pediatric Diabetes Register. The dashed line represents the average incidence rate of 54.9 per 100,000 PY.

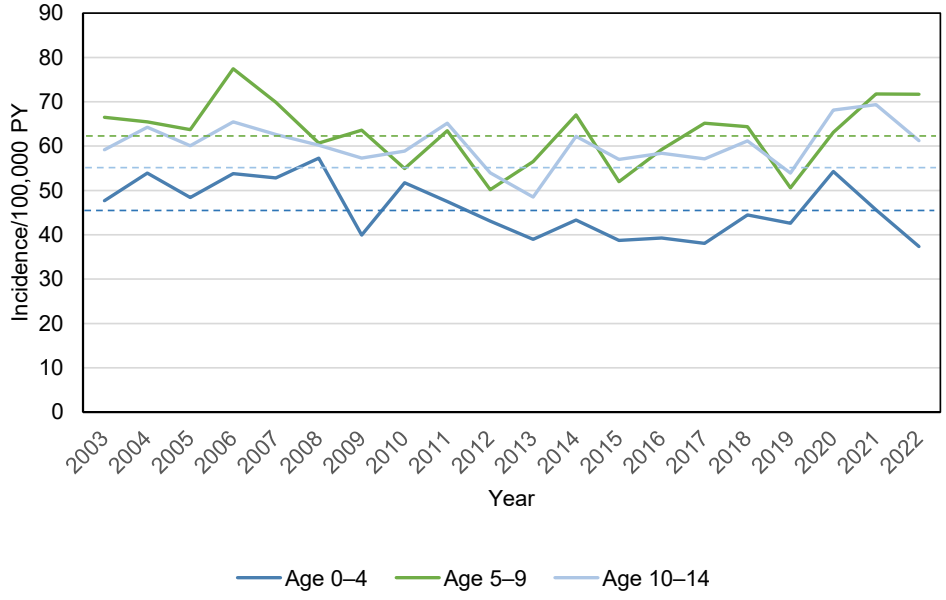

Figure 3 The incidence rate of type 1 diabetes diagnosed in three age groups between 2003 and 2022. Based on data from the Finnish Pediatric Diabetes Register. The dashed lines represent the average incidence rates of 46.0, 62.8 and 55.5 per 100,000 PY in those aged 0–4, 5–9 and 10–14 years, respectively.

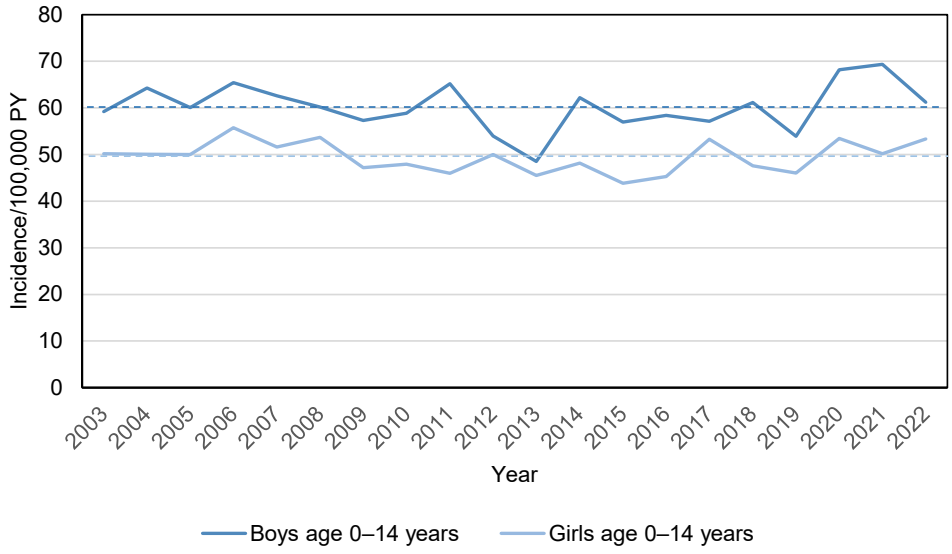

Figure 4 The incidence rates of type 1 diabetes diagnosed in boys and girls before the age of 15 years between 2003 and 2022. Based on data from the Finnish Pediatric Diabetes Register. The dashed lines represent the average incidence rates of 60.2 and 49.4 per 100,000 PY for boys and girls, respectively.

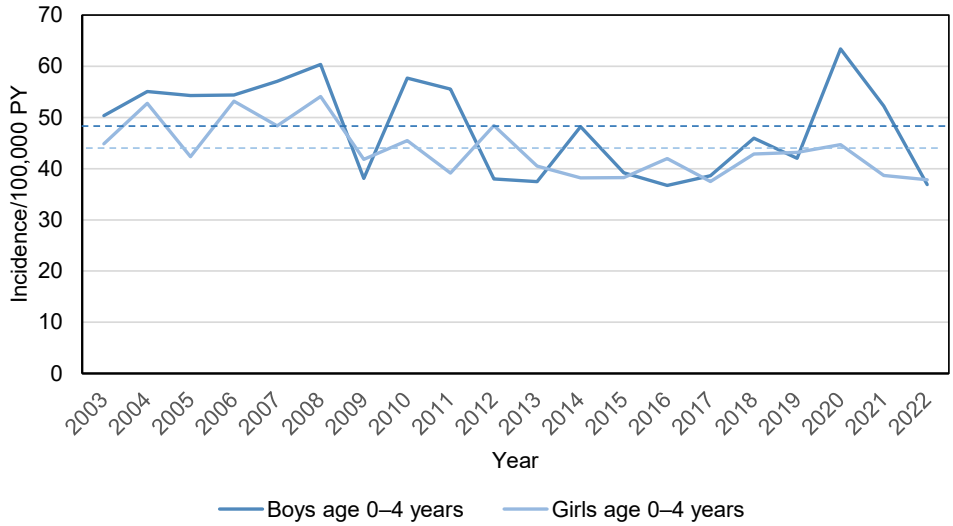

Figure 5 The incidence rates of type 1 diabetes diagnosed in boys and girls aged 0–4 years between 2003 and 2022. Based on data from the Finnish Pediatric Diabetes Register. The dashed lines represent the average incidence rates of 48.1 and 43.8 per 100,000 PY for boys and girls, respectively.

5.1.4 Comparison of the number of cases of type 1 diabetes recorded in the FPDR and the FinDM database (previously unpublished data)

As both the FinDM database and the FPDR were used in this thesis as data sources for retrieving the number of cases of type 1 diabetes, it was of interest to compare the coverage of these two registers (Table 7). The number of cases recorded in the FinDM database was consistently higher. The overall annual difference averaged 19.3%, and was 19.1%, 16.8% and 22.2% in age groups 0–4, 5–10 and 10–14 years respectively. Despite some fluctuation from one year to the other, the trends were similar in the two registers (Figures 6-9).

Table 7 Yearly cases of type 1 diabetes recorded in the Finnish Pediatric Diabetes Register (FPDR) and the Diabetes in Finland (FinDM) database between 2003 and 2016 in children diagnosed before the age of 15 years, and the percentage difference between the two registers

Age group (years)	**0–14**			**0–4**			**5–9**			**10–14**		
	FPDR	FinDM	% diff	FPDR	FinDM	% diff	FPDR	FinDM	% diff	FPDR	FinDM	% diff
2003	504	578	13,7	135	157	15,1	204	216	5,7	165	205	21,6
2004	524	572	8,8	153	162	5,7	196	213	8,3	175	197	11,8
2005	500	623	21,9	138	174	23,1	187	220	16,2	175	229	26,7
2006	547	626	13,5	155	174	11,6	224	243	8,1	168	209	21,8
2007	512	637	21,8	154	182	16,7	200	246	20,6	158	209	27,8
2008	508	629	21,3	169	197	15,3	174	215	21,1	165	217	27,2
2009	465	580	22,0	119	155	26,3	183	217	17,0	163	208	24,3
2010	475	601	23,4	156	181	14,8	159	200	22,8	160	220	31,6
2011	496	608	20,3	144	170	16,6	186	227	19,9	166	211	23,9
2012	464	587	23,4	131	157	18,1	149	197	27,7	184	233	23,5
2013	421	536	24,0	118	155	27,1	170	210	21,1	133	171	25,0
2014	496	631	24,0	130	173	28,4	204	247	19,1	162	211	26,3
2015	453	550	19,3	114	148	26,0	160	192	18,2	179	210	15,9
2016	465	542	15,3	113	129	13,2	183	211	14,2	169	202	17,8
2017	492	582	16,8	106	133	22,6	202	223	9,9	184	226	20,5
2018	481	604	22,7	119	149	22,4	199	244	20,3	163	211	25,7
2019	436	497	13,1	109	129	16,8	155	178	13,8	172	190	9,9
2020	525	604	14,0	134	144	7,2	190	225	16,9	201	235	15,6
2021	511	620	19,3	111	138	21,7	211	250	16,9	189	232	20,4
2022	482	636	27,5	89	138	43,2	205	251	20,2	188	247	27,1
Total	9757	11843	19,3	2597	3145	19,1	3741	4425	16,8	3419	4273	22,2

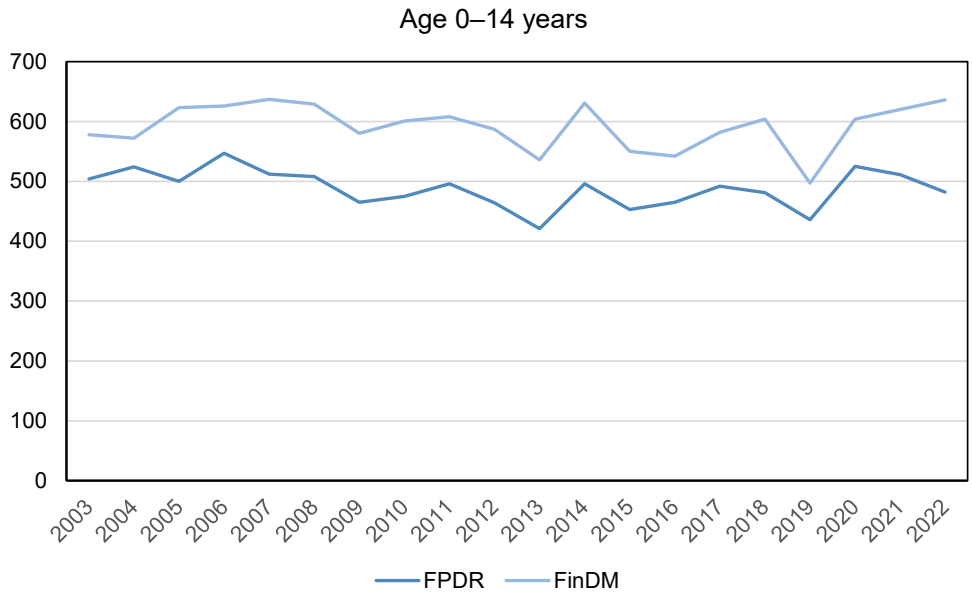

Figure 6 The number of cases of type 1 diabetes diagnosed in children before the age of 15 years between 2003 and 2022, registered in the Finnish Pediatric Diabetes Register (FPDR) and the Diabetes in Finland (FinDM) database

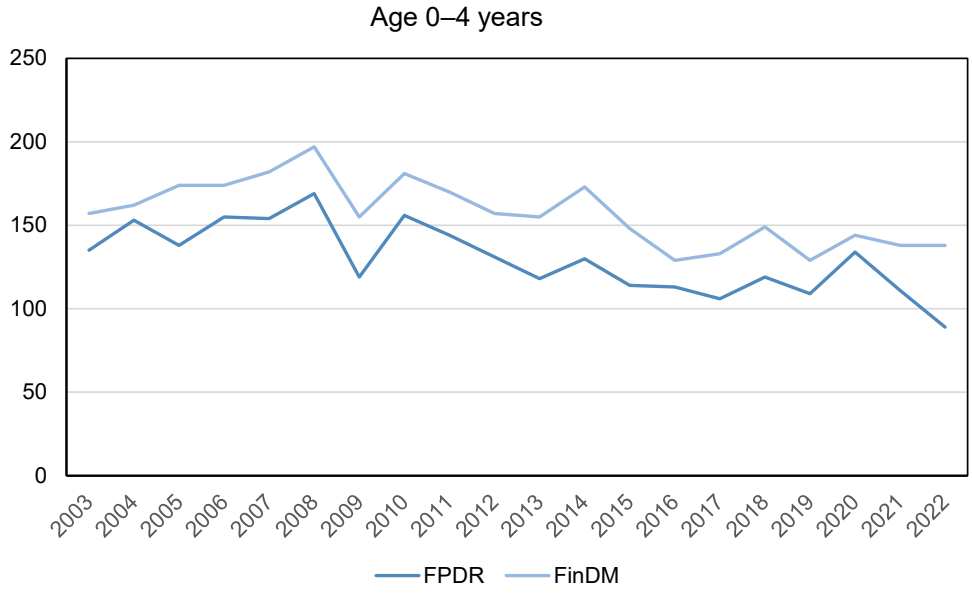

Figure 7 The number of cases of type 1 diabetes diagnosed in children before the age of 5 years between 2003 and 2022, registered in the Finnish Pediatric Diabetes Register (FPDR) and the Diabetes in Finland (FinDM) database

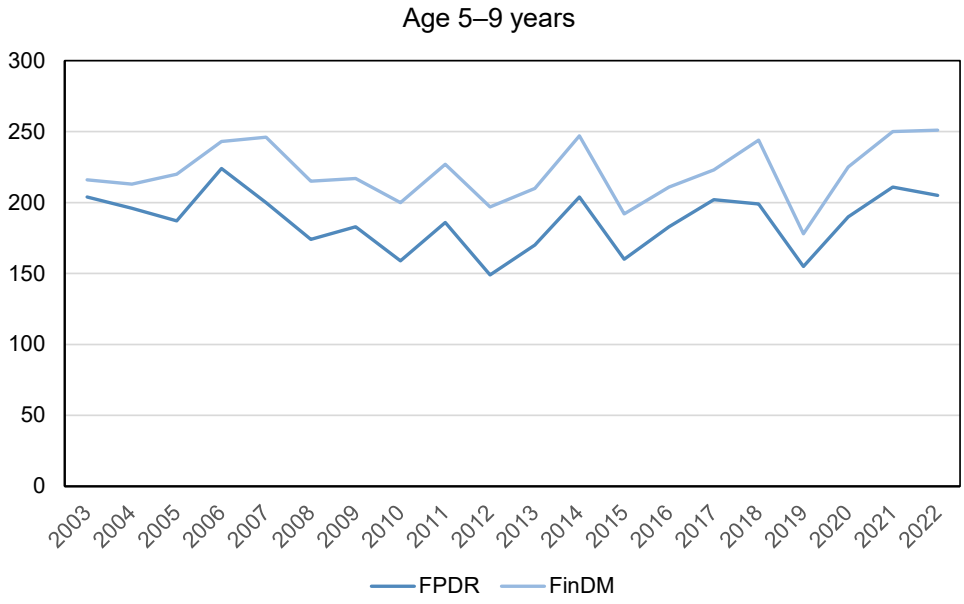

Figure 8 The number of cases of type 1 diabetes diagnosed in children aged 5–9 years between 2003 and 2022, registered in the Finnish Pediatric Diabetes Register (FPDR) and the Diabetes in Finland (FinDM) database

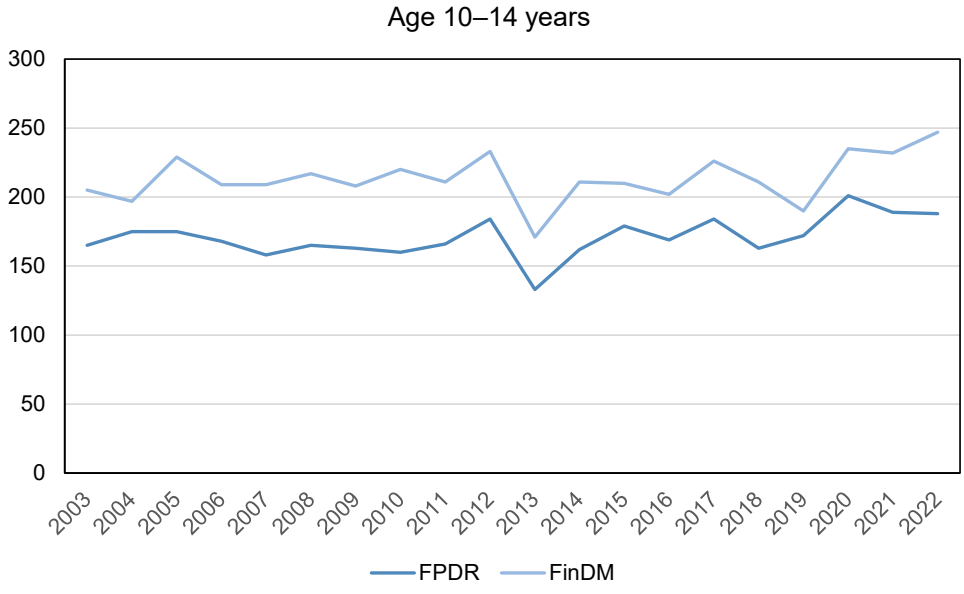

Figure 9 The number of cases of type 1 diabetes diagnosed in children aged 10–14 years between 2003 and 2022, registered in the Finnish Pediatric Diabetes Register (FPDR) and the Diabetes in Finland (FinDM) database

5.2 Environmental exposures and type 1 diabetes

5.2.1 Rotavirus (II)

The total number of laboratory-confirmed rotavirus infections in children aged under 5 years, born between 1995 and 2015 with follow-up until 2016, was 18,154. Of these exposed children, 14 910 (82%) were born during the pre-vaccine era (1995–2005), and across all birth cohorts the majority were males and children under the age of 2 years (Table 8, Figure 10). The number of exposed children peaked in the 2001–2005 birth cohort, decreased during the 2006–2009 birth cohort group coinciding with the commercial availability of the vaccine, and reached a nadir in the post-vaccine 2010–2015 birth cohort group. The number of children exposed to laboratory confirmed rotavirus infection before the age of 5 years per 100 000 children was 2522 in the 2001–2005 birth cohort and 171 in the 2010–2012 birth cohort, resulting in an absolute reduction of 2351 per 100 000 children (95% CI 2291–2411).

Table 8 The number and proportion of children exposed to laboratory confirmed rotavirus infections before the age of 5 years, by birth cohort groups and sex

Follow-up between 1995 and 2016. Due to the incompletely observed age range, birth cohorts 2013–2015 were not included in the analyses. Modified from Publication II.

	Birth cohort group	Exposed (n)	Population (n)	Exposed (%)	Exposed n/100 000 PY (95% CI)
ALL	1995–2000	7649	386 415	2.0	1979 (1936–2024)
	2001–2005	7261	287 851	2.5	2522 (2466–2580)
	2006–2009	2935	230 390	1.3	1274 (1229–1321)
	2010–2012	309	180 481	0.2	171 (153–191)
BOYS	1995–2000	4181	196 961	2.1	2123 (2060–2187)
	2001–2005	4094	147 021	2.8	2785 (2702–2870)
	2006–2009	1635	117 723	1.4	1389 (1324–1457)
	2010–2012	178	92 309	0.2	193 (167–223)
GIRLS	1995–2000	3468	189 454	1.8	1831 (1771–1892)
	2001–2005	3167	140 830	2.3	2249 (2173–2328)
	2006–2009	1300	112 667	1.2	1154 (1093–1218)
	2010–2012	131	88 172	0.2	149 (125–176)

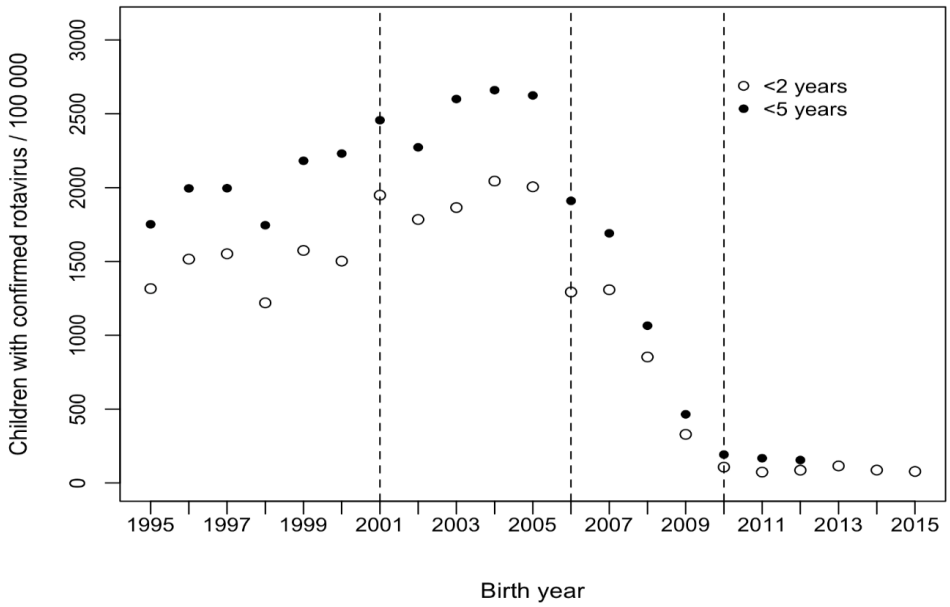

Figure 10 Birth cohort-specific numbers of laboratory confirmed rotavirus infections in children aged less than 2 and less than 5 years per 100,000 children. Dashed lines mark the birth cohort groups (1995–2000, 2001–2005, 2006–2009 and 2010–2015). Reproduced from Publication II with permission of the American Diabetes Association.

A total of 8,674 children and adolescents born between 1995 and 2015 were diagnosed with type 1 diabetes before the age of 15 years. Compared with the 2001–2005 reference birth cohort, the incidence of type 1 diabetes was 11% lower in the 2010–2015 birth cohort (Table 9). In the youngest age group, the incidence followed the same trend as exposure to rotavirus: it peaked in the 2001–2005 birth cohort group, and then declined. In this age group, the same trend was observed for both boys and girls, but the changes were more prominent in boys. Comparing the post-vaccine cohort with the reference pre-vaccine cohort, incidence for children aged less than 5 years was 28% and 20% lower for boys and girls respectively.

A reduction of one percentage point in the proportion of children with laboratory confirmed rotavirus infections was associated with a 5% decrease in the incidence of type 1 diabetes in children aged under 15 years (adjusted IRR 0.95, 95% CI 0.91–0.98). When stratified by age group, the results were significant and consistent in sensitivity analyses only for the youngest age group, for which a reduction of one percentage point in rotavirus exposure was associated with an 8% decrease in the incidence of type 1 diabetes (adjusted IRR 0.92, 95% CI 0.89–0.96). When stratified by sex, results were similar and non-significant in both sexes (boys IRR 0.96, 95% CI 0.92–1.00, girls 0.96 95% CI 0.91–1.01). Stratified for both age-groups and sex the difference was significant for both main and sensitivity analyses only for boys aged 0–4 years (IRR 0.92, 95% CI 0.87–0.97), though results were similar and close to statistical significance for girls in the same age group (0.94, 95% CI 0.87–1.00) (unpublished data).

Table 9 The overall, age- and sex-specific incidence rates and incidence rate ratios of type 1 diabetes in four birth cohorts based on the Diabetes in Finland (FinDM) database

Results statistically significant in both main and sensitivity analyses are marked in bold. IRRs adjusted for age and/or sex when appropriate.

*1 is not included in the CI. Modified from Publication II.

		ALL		**BOYS**		**GIRLS**	
Age at diagnosis (years)	Birth cohort group	IR/100,000 PY (95% CI)	IRR (95% CI)	IR/100,000 PY (95% CI)	IRR (95% CI)	IR/100,000 PY (95% CI)	IRR (95% CI)
0–14	1995–2000	64.6 (62.5–66.7)	**0.95 (0.90–1.00*)**	69.8 (66.7–72.9)	0.94 (0.88–1.01)	59.1 (56.3–62.1)	0.94 (0.88–1.02)
	2001–2005	68.5 (66.0–71.1)	1	74.1 (70.5–77.9)	1	62.7 (59.2–66.3)	1
	2006–2009	71.0 (67.5–74.7)	1.04 (0.98–1.11)	75.8 (70.6–81.2)	1.02 (0.94–1.11)	66.1 (61.2–71.3)	1.05 (0.96–1.16)
	2010–2015	56.8 (53.0–60.9)	0.89 (0.82–0.97)	58.5 (53.1–64.3)	0.79 (0.71–0.88)	55.1 (49.7–60.8)	0.88 (0.78–0.98)
<5	1995–2000	56.4 (52.9–60.1)	**0.79 (0.72–0.86)**	58.1 (53.1–63.4)	**0.76 (0.67–0.86)**	54.7 (49.7–60.0)	**0.83 (0.72–0.94)**
	2001–2005	71.5 (66.9–76.3)	1	76.5 (70.0–83.5)	1	66.2 (60.0–72.9)	1
	2006–2009	67.8 (63.0–73.0)	0.95 (0.86–1.05)	71.7 (64.7–79.2)	0.94 (0.82–1.07)	63.8 (57.1–71.1)	0.96 (0.83–1.11)
	2010–2015	54.4 (50.4–58.6)	**0.79 (0.71–0.87)**	55.8 (50.2–61.8)	**0.73 (0.64–0.83)**	52.9 (47.3–59.0)	**0.80 (0.69–0.92)**
5–9	1995–2000	70.0 (66.3–73.8)	1.03 (0.95–1.11)	74.1 (68.9–79.7)	1.06 (0.95–1.18)	65.7 (60.7–71.0)	0.99 (0.88–1.12)
	2001–2005	68.1 (64.0–72.4)	1	70.0 (64.2–76.1)	1	66.2 (60.5–72.2)	1
	2006–2009	75.7 (70.3–81.3)	**1.11 (1.01–1.22)**	82.7 (74.9–91.1)	1.18 (1.04–1.34)	68.3 (61.1–76.1)	1.03 (0.90–1.19)
	2010–2015	74.3 (62.1–88.1)	1.08 (0.89–1.30)	78.2 (61.1–98.6)	1.12 (0.87–1.43)	70.2 (53.7–90.1)	1.06 (0.81–1.38)
10–14	1995–2000	66.1 (62.5–69.8)	1.03 (0.94–1.12)	75.5 (70.1–81.1)	0.98 (0.88–1.10)	56.4 (51.7–61.4)	1.03 (0.90–1.18)
	2001–2005	66.0 (61.6–70.7)	1	76.8 (70.2–83.9)	1	54.7 (49.0–60.9)	1
	2006–2009	52.2 (35.7–73.7)	0.74 (0.52–1.07)	35.0 (17.5–62.7)	0.46 (0.25–0.83)	70.3 (43.5–107.4)	1.28 (0.83–2.00)

5.2.2 SARS-CoV-2 (III)

As described above (Table 5), the incidence of type 1 diabetes increased by 16% during the first 18 months of the COVID-19 pandemic (IRR 1.16, 95% CI 1.06–1.25) when compared with a previous reference cohort. To examine the possible direct role of the SARS-CoV-2 virus in this increase, SARS-CoV-2 antibodies were analyzed for 583 children diagnosed with type 1 diabetes during the pandemic with available serum samples. Nine children tested positive for SARS-CoV-2 Spike antibodies and were further tested for SARS-CoV-2 N protein antibodies and neutralizing antibodies to contemporary variants. In addition, the parents of the nine children testing positive were interviewed for their child's history of SARS-CoV-2 infection or vaccination before the diagnosis of type 1 diabetes. Based on these results, only 5/583 (0.9%) children were interpreted to have had a SARS-CoV-2 infection before the diagnosis of type 1 diabetes (Table 10).

Data on demographics, clinical characteristics at diagnosis, autoantibodies and HLA-class II genotype were described and compared for the 18-month COVID-19 pandemic cohort and the 54-month preceding reference cohort (Tables 11–13). The average age at diagnosis was 8 years and the majority were boys, with no significant differences in the age and sex distribution between the two cohorts. There was a slight difference reaching statistical significance in the distribution of the seasons of birth, with those diagnosed during the pandemic more often born in the summer and autumn, compared with a larger proportion having been born during the spring in the reference cohort. Spring and summer were the most common seasons of diagnosis in both cohorts. The pandemic cohort had a slightly lower proportion of children with a first-degree family member with type 1 diabetes, though there was a slightly greater proportion with two affected family members, and the only case with four family members with type 1 diabetes.

Children diagnosed with type 1 diabetes during the pandemic had more severe metabolic decompensation at diagnosis. pH was lower and both ketoacidosis (30.8% vs 22.6%) and severe ketoacidosis (8.8% vs 5.6%) were more common in the pandemic cohort. Also, β–hydroxybutyrate levels were higher, and though plasma glucose at diagnosis was slightly lower, HbA1c levels were higher indicating longer history of hyperglycemia. Other clinical characteristics, such as pubertal status, BMI and weight loss, duration of symptoms and proportion with impaired consciousness at diagnosis did not differ significantly between the groups.

Those diagnosed during the pandemic tested positive for a slightly higher number of autoantibodies at diagnosis than those diagnosed during the reference period, and positivity for GAD autoantibodies was more common (73% vs 65%). There were no significant differences in HLA class II conferred risk between the two cohorts.

Table 10 History of acute COVID-19 infection and vaccination, and results of antibody tests in the nine children with newly diagnosed type 1 diabetes who tested positive for SARS-CoV-2 Spike antibodies in samples obtained close to diagnosis of type 1 diabetes

Modified from Publication III.

Cases	**Month and year of T1D diagnosis**	**Interval from T1D diagnosis to sampling**	**History of COVID-19 infection before T1D**	**History of COVID-19 vaccination before T1D**	**Tests for SARS-CoV-2 IgG antibodies**		**Tests for SARS-CoV-2 neutralizing antibody titres**			**Interpretation of history and serological findings**
					Spike	N protein	Alpha	Delta	Omicron	
1. girl 6.9 years	May 2020	4 days	April, 2020	No	Positive	Positive	160	40	<20	Antibodies induced by infection
2. boy 10.5 years	October 2020	6 days	September 2020	No	Positive	Negative	<20	<20	<20	Only Spike antibodies; diagnosis less certain
3. boy 13.1 years	February 2021	40 days	November 2020	No	Positive	Positive	160	80	<20	Antibodies induced by infection
4. girl 13.0 years	August 2021	4 days	July, 2021	No	Positive	Positive	<20	320	<20	Antibodies induced by infection
5. boy 12.7 years	August 2021	46 days	No	Yes	Positive	Positive	80	<20	<20	Antibodies induced probably by both infection and vaccination
6. boy 12.4 years	August 2021	4 days	No	Yes	Positive	Negative	80	40	<20	Antibodies induced by vaccination
7. girl 12.0 years	August 2021	13 days	No	Yes	Positive	Negative	80	20	<20	Antibodies induced by vaccination
8. boy 13.7 years	August 2021	18 days	No	Yes	Positive	Negative	160	40	<20	Antibodies induced by vaccination
9. boy 18 years	August 2021	113 days	No	Yes	Positive	Equivocal	>2560	2560	320	Antibodies induced by vaccination

Table 11 Description and comparison of demographic characteristics of children diagnosed with type 1 diabetes before age 15 years during the COVID-19 pandemic (March 1, 2020–August 31, 2021) and during a preceding reference period (March 1, 2014–August 31, 2015; March 1, 2016–August 31, 2017; March 1, 2018–August 31, 2019)

Modified from Publication III.

	N	**Pandemic cohort, n=785 (27.2 %)**	**Reference cohort, n=2096 (72.8 %)**	***P***	***P* Adjusted for age and sex**
Age at diagnosis (years), mean (SD)	2881	8.41 (3.75)	8.20 (3.86)	0.208	
Sex, male, n (%)	2881			0.406	
Boys		458 (58.3)	1185 (56.5)		
Girls		327 (41.7)	911 (43.5)		
Season of birth, n (%)	2880			**0.005**	**0.004**
Spring		167 (21.3)	565 (27.0)		
Summer		232 (29.6)	524 (25.0)		
Autumn		210 (26.8)	519 (24.8)		
Winter		175 (22.3)	488 (23.3)		
Season of diagnosis, n (%)	2881			0.108	0.101
Spring		242 (30.8)	634 (30.2)		
Summer		282 (35.9)	670 (32.0)		
Autumn		139 (17.7)	413 (19.7)		
Winter		122 (15.5)	379 (18.1)		
Familial, n (%)	2881	89 (11.3)	291 (13.9)	0.083	0.090
Father with T1D, n (%)	2881	45 (5.7)	123 (5.9)	0.961	0.979
Mother with T1D, n (%)	2881	19 (2.4)	64 (3.1)	0.436	0.446
Sibling with T1D, n (%)	2881	36 (4.6)	115 (5.5)	0.383	0.316
N of first-degree relatives with T1D, n (%)	2881			**0.015**	**0.024**
0		696 (88.7)	1805 (86.1)		
1		76 (9.7)	271 (12.9)		
2		12 (1.5)	18 (0.9)		
3		0 (0%)	2 (0.1)		
4		1 (0.1)	0 (0%)		

Table 12 Description and comparison of clinical characteristics of children diagnosed with type 1 diabetes before age 15 years during the COVID-19 pandemic (March 1, 2020–August 31, 2021) and during a preceding reference period (March 1, 2014–August 31, 2015; March 1, 2016–August 31, 2017; March 1, 2018–August 31, 2019)

Modified from Publication III.

	N	Pandemic cohort, n=785 (27.2 %)	Reference cohort, n=2096 (72.8 %)	*P*	*P* Adjusted for age and sex
Pubertal, n (%)	1894	77 (15.7)	211 (15.0)	0.806	0.158
BMI-SDS, median (range)	2493	-0.19 (-10.45–4.23)	-0.24 (-6.43–9.44)	0.830	0.794
Weight loss (%), median (range)	1792	6.5 (0–34.0)	5.5 (0–28.3)	**0.036**	0.220
Duration of symptoms, n (%)	2706			0.195	0.241
No symptoms		37 (5.1)	65 (3.3)		
< 1 week		123 (16.8)	344 (17.4)		
1–4 weeks		425 (58.1)	1173 (59.4)		
> 4 weeks		147 (20.1)	392 (19.9)		
Impaired consciousness, n (%)	2741	56 (7.5)	131 (6.6)	0.419	0.408
pH, median (range)	2723	7.36 (6.82–7.55)	7.37 (6.8–7.55)	**<0.001**	**<0.001**
Ketoacidosis (pH<7.30), n (%)	2723	228 (30.8)	448 (22.6)	**<0.001**	**<0.001**
Severe ketoacidosis (pH<7.10), n (%)	2723	65 (8.8)	116 (5.6)	**0.008**	**0.009**
Plasma glucose (mmol/L), median (range)	2748	22.8 (4.2–71.0)	23.9 (3.2–88.0)	**0.023**	**0.009**
HbA1c (mmol/mol), mean (SD)	2504	97.7 (28.0)	94.2 (27.1)	**0.004**	**0.004**
HbA1c (%), mean (SD)	2504	11.1 (2.6)	10.8 (2.5)	**0.003**	**0.003**
β–hydroxybutyrate (mmol/L), median (range)	2663	2.6 (0–15.0)	2.0 (0–13.8)	**<0.001**	**0.024**

Table 13 Description and comparison of autoantibody status and HLA class II genotype-based risk of children diagnosed with type 1 diabetes before age 15 years during the COVID-19 pandemic (March 1, 2020–August 31, 2021) and during a preceding reference period (March 1, 2014–August 31, 2015; March 1, 2016–August 31, 2017; March 1, 2018–August 31, 2019)

Modified from Publication III.

	N	Pandemic cohort, n=785 (27.2 %)	Reference cohort, n=2096 (72.8 %)	*P*	*P* Adjusted for age and sex
Autoantibodies					
Number of positive autoantibodies, median (mean)	2176	3 (2.78)	3 (2.65)	**0.012**	**0.043**
Autoantibody negative, n (%)	2176	19 (3.3)	61 (3.8)	0.632	0.445
Positivity for multiple (≥2) autoantibodies, n (%)	2176	506 (87.1)	1342 (84.1)	0.102	0.062
IAA positive, n (%)	2176	339 (58.3)	920 (57.7)	0.818	0.341
IAA (RU), median (range)	1259	6.3 (1.7–289.6)	6.4 (1.6–829.8)	0.757	0.845
IA2A positive, n (%)	2176	432 (74.4)	1166 (73.1)	0.596	0.579
IA2A (RU), median (range)	1598	106.9 (0.8–679.2)	100.1 (0.8–266.5)	0.160	0.132
GADA positive, n (%)	2176	424 (73.0)	1036 (65.0)	**<0.001**	**<0.001**
GADA (RU), median (range)	1460	38.5 (5.5–323.4)	33.5 (5.4–473.9)	0.151	0.196
Zn8TA positive, n (%)	2176	420 (72.3)	1104 (69.2)	0.183	0.218
Zn8TA (RU), median (range)	1524	15.0 (0.5–289.5)	11.0 (0.5–263.2)	**0.003**	0.076
HLA-DR/DQ genotype-based risk classification					
Risk group, n (%)	2379			0.495	0.537
Strongly decreased risk		6 (0.9)	11 (0.6)		
Slightly decreased risk		17 (2.6)	44 (2.6)		
Neutral		117 (17.8)	260 (15.1)		
Slightly increased risk		142 (21.6)	420 (24.0)		
Moderately increased risk		247 (37.6)	640 (37.2)		
High risk		128 (19.5)	347 (20.2)		

5.3 Age-related heterogeneity of type 1 diabetes (IV)

Demographic and clinical characteristics, diabetes-related autoantibodies and HLA class II-based disease susceptibility were described and compared in three age groups of children and adolescents diagnosed with type 1 diabetes between 2003 and 2019 (Tables 14–16). The age groups (0–6, 7–12 and 13 or above years) were based on age-related immunohistological findings described above (section 2.4) suggesting different disease endotypes. The maximum age was 17.3 years, and the oldest group had the lowest number of cases (15.8% of the total) due to the pediatric nature of the register.

The analysis of the distribution of seasons of birth and seasons of diagnosis revealed no statistically significant differences between the age groups. Overall, spring was the most common season of birth (26.3%), while winter was the least common (23.3%). In contrast, autumn was the most common season of diagnosis (26.9%), and spring the least common (22.5%). More than 13% had familial type 1 diabetes; 5.7% had a sibling, 5.4% a father, and 3.1% an affected mother. Familial type 1 diabetes was significantly more common in the youngest age group, as was having a mother or a sibling with type 1 diabetes.

Metabolic decompensation at diagnosis was most severe in the oldest age group. Overall, 19.4% had diabetic ketoacidosis at diagnosis and 5.1% had severe ketoacidosis, whilst in the oldest group ketoacidosis was present in 26.4% of the cases and severe ketoacidosis in 8.4%. Older children also experienced a longer duration of symptoms prior to diagnosis, had higher HbA1c and β–hydroxybutyrate levels, and a lower BMI SDS. There was no significant difference in plasma glucose at diagnosis between the age groups.

Autoantibody profiles differed somewhat between the age groups. The most common number of autoantibodies detectable at diagnosis was four in all age groups, and negativity for all five autoantibodies was rare (2.1%). Younger age at diagnosis was related to a higher number of positive autoantibodies. ICA-positivity was most frequent overall (91.1%), and there was a clear difference in the distribution of IAA and GADA positivity, with IAA dominating in the youngest group and GADA in the oldest group.

A majority (81.2%) carried a HLA class II risk genotype. A HLA-conferred risk genotype was most common in the youngest cohort, whilst a neutral or protective HLA genotype was more common in the oldest cohort.

Nearly all the examined characteristics exhibited statistically significant differences in their distribution across the three age groups. Exceptions were ICA titer, plasma glucose at diagnosis, paternal type 1 diabetes and the seasonality of birth and diagnosis. The majority of the characteristics exhibited the most conspicuous differences between the youngest and oldest cohorts, with intermediate values observed in the middle cohort. Here the exceptions were IA-2A

and ZnT8A, and having three or four positive autoantibodies, which were most common/highest in the middle cohort.

Table 14 Description and comparison of demographic characteristics of children and adolescents diagnosed with type 1 diabetes between 2003 and 2019 in three age groups

Modified from Publication IV.

		Age at diagnosis, years			
	Overall	**0–6**	**7–12**	**13–17**	***P***
n (%)	6015	2361 (39.3)	2706 (45.0)	948 (15.8)	
Sex, n (%)					**<0.001**
Boys	3413 (56.7)	1269 (53.7)	1497 (55.3)	647 (68.2)	
Girls	2602 (43.3)	1092 (46.3)	1209 (44.7)	301 (31.8)	
Season of birth, n (%)					0.337
Spring	1581 (26.3)	625 (26.5)	704 (26.0)	252 (26.6)	
Summer	1522 (25.3)	592 (25.1)	675 (24.9)	255 (26.9)	
Autumn	1511 (25.1)	570 (24.1)	717 (26.5)	224 (23.6)	
Winter	1401 (23.3)	574 (24.3)	610 (22.5)	217 (22.9)	
Season of diagnosis, n (%)					0.236
Spring	1353 (22.5)	542 (23.9)	588 (21.7)	223 (23.5)	
Summer	1478 (24.6)	586 (24.8)	674 (24.9)	218 (23.0)	
Autumn	1619 (26.9)	658 (27.9)	709 (26.2)	252 (26.6)	
Winter	1565 (26.0)	575 (24.4)	735 (27.2)	255 (26.9)	
Familial T1D	793 (13.2)	374 (15.8)	325 (12.0)	94 (9.9)	**<0.001**
Father with T1D, n (%)	322 (5.4)	141 (6.0)	137 (5.1)	44 (4.6)	0.204
Mother with T1D, n (%)	185 (3.1)	93 (3.9)	76 (2.8)	16 (1.7)	**0.002**
Sibling with T1D, n (%)	340 (5.7)	160 (6.8)	137 (5.1)	43 (4.5)	**0.008**
N of first-degree relatives with T1D, n (%)					**<0.001**
0	5222(86.8)	1987 (84.2)	2381 (88.0)	854 (90.1)	
1	723 (12.0)	345 (14.6)	294 (10.9)	84 (8.9)	
2	64 (1.1)	26 (1.1)	30 (1.1)	8 (0.8)	
3	5 (0.1)	3 (0.1)	1 (0.0)	1 (0.1)	
4	1 (0.0)	0 (0)	0 (0)	1 (0.1)	

Table 15 Description and comparison of clinical characteristics of children and adolescents diagnosed with type 1 diabetes between 2003 and 2019 in three age groups

Modified from Publication IV.

		Age at diagnosis, years			
	Overall	**0–6**	**7–12**	**13–17**	***P***
BMI-SDS, mean (SD)	-0.22 (1.37)	-0.09 (1.23)	-0.20 (1.43)	-0.62 (1.45)	**<0.001**
Duration of symptoms, n (%)					**<0.001**
No symptoms	99 (1.6)	39 (1.7)	45 (1.7)	15 (1.6)	
<1 week	1184 (19.7)	550 (23.3)	481 (17.8)	153 (16.1)	
1–4 weeks	3140 (52.2)	1340 (56.8)	1352 (50.0)	448 (47.3)	
>4 weeks	1079 (17.9)	250 (10.6)	581 (21.5)	248 (26.2)	
Unknown	513 (8.5)	182 (7.7)	247 (9.1)	84 (8.9)	
pH, median (IQR)	7.38 (0.08)	7.39 (0.06)	7.37 (0.10)	7.36 (0.10)	**<0.001**
Ketoacidosis (pH<7.30), n (%)	1127 (19.4)	318 (14.0)	568 (21.7)	241 (26.4)	**<0.001**
Severe ketoacidosis (pH<7.10), n (%)	298 (5.1)	69 (3.0)	152 (5.8)	77 (8.4)	**<0.001**
Plasma glucose (mmol/L), median (IQR)	23.9 (12.5)	23.8 (12.7)	23.8 (12.5)	24.1 (12.2)	0.106
HbA1c (mmol/mol), mean (SD)	94.6±27.6	82.3±20.6	101.4±27.8	104.7±30.2	**<0.001**
HbA1c (%), mean (SD)	10.8±2.5	9.7±1.9	11.4±2.5	11.7±2.8	**<0.001**
β-hydroxybutyrate (mmol/L), median (IQR)	1.7 (4.4)	1.3 (3.5)	2.1 (4.8)	2.3 (4.9)	**<0.001**

Table 16 Description and comparison of autoantibody status and HLA class II genotype-based risk of children and adolescents diagnosed with type 1 diabetes between 2003 and 2019 in three age groups

Modified from Publication IV.

		Age at diagnosis, years			
	Overall	**0–6**	**7–12**	**13–17**	***P***
Autoantibodies					
Number of positive autoantibodies, n (%)					**<0.001**
0	125 (2.1)	21 (0.9)	65 (2.4)	39 (4.1)	
1	291 (4.8)	100 (4.2)	126 (4.7)	65 (6.9)	
2	671 (11.2)	249 (10.5)	304 (11.2)	118 (12.4)	
3	1444 (24.0)	559 (23.7)	662 (24.5)	223 (23.5)	
4	1984 (33.0)	765 (32.4)	910 (33.6)	309 (32.6)	
5	1500 (24.9)	667 (28.3)	639 (23.6)	194 (20.5)	
IAA positive, n (%)	3282 (54.6)	1741 (73.7)	1209 (44.7)	332 (35.0)	**<0.001**
IAA (RU), median (IQR)	2.0 (7.3)	6.1 (17.96)	1.2 (3.68)	0.8 (2.3)	**<0.001**
GADA positive, n (%)	4002 (66.5)	1467 (62.1)	1850 (68.4)	685 (72.3)	**<0.001**
GADA (RU), median (IQR)	14.5 (56.2)	11.4 (49.5)	16.2 (58.4)	19.7 (72.8)	**<0.001**
IA-2A positive, n (%)	4473 (74.4)	1726 (73.1)	2058 (76.1)	689 (72.7)	**0.024**
IA-2A (RU), median (IQR)	69.0 (120.7)	50.8 (116.0)	81.7 (122.4)	69.5 (124.4)	**<0.001**
ZnT8A positive, n (%)	4167 (69.3)	1526 (64.6)	1987 (73.4)	654 (69.0)	**<0.001**
ZnT8A (RU), median (IQR)	3.5 (24.6)	2.4 (17.2)	5.0 (30.5)	3.4 (26.3)	**<0.001**
ICA positive, n (%)	5477 (91.1)	2210 (93.6)	2451 (90.6)	816 (86.1)	**<0.001**
ICA (JDFU), median (IQR)	49.0 (152.0)	49.0 (143.0)	49.0 (152.0)	49.0 (177.0)	0.097
IAA/GADA-dominant profile, n (%)					**<0.001**
IAA-dominant profile	936 (36.1)	574 (65.7)	302 (24.3)	60 (12.7)	
GADA-dominant profile	1656 (63.9)	300 (34.3)	943 (75.7)	413 (87.3)	
HLA-DR/DQ genotype-based risk classification					
HLA-DR/DQ based risk, n (%)					**<0.001**
Increased risk	4887 (81.2)	1972 (83.5)	2189 (80.9)	726 (76.6)	
Neutral or protective	1128 (18.8)	389 (16.5)	517 (19.1)	222 (23.4)	

6 Discussion

This thesis describes the fluctuation in the incidence of type 1 diabetes in children in Finland over the last two decades. The extent and direction of the fluctuation varied by age at diagnosis and sex and was most prominent in young boys. As a potential explanation for some of the fluctuating incidence of type 1 diabetes, we found, at the population level, an association between exposure to rotavirus and the incidence of type 1 diabetes. The decrease in exposure to rotavirus infection following the national implementation of the rotavirus vaccine with subsequent high coverage was at the population-level associated with a decrease in the incidence of type 1 diabetes diagnosed in children before the age of 5 years. After the decrease in incidence, the incidence of type 1 diabetes increased again during the beginning of the COVID-19 pandemic. Based on sparse SARS-CoV-2 virus positivity in children with new-onset type 1 diabetes diagnosed during the pandemic, the increase in incidence and other observed changes, such as increased ketoacidosis, could not be attributed to a direct effect of the SARS-CoV-2 virus. In addition to fluctuations in incidence being related to age at diagnosis, there was extensive heterogeneity of demographic and clinical characteristics, diabetes-related autoantibodies and HLA class II genotype according to age at diagnosis, suggesting that there may be age-related endotypes of type 1 diabetes.

6.1 The incidence of type 1 diabetes

Based on data on 9757 children with type 1 diabetes registered in the FPDR during the 20-year follow-up between 2003 and 2022, the incidence of type 1 diabetes was highest in children aged 5–9 years, and higher in boys than in girls. Both findings are in agreement with the previous Finnish study reporting incidences between 2006 and 2011 (5). In contrast, most incidence studies from other countries report increasing incidence with age, and thus highest incidence in children aged 10–14 years (1, 11, 165-167). Consistent with findings in Finland, incidence is reported to be higher in boys than in girls in other high incidence countries (13, 14). We observed that the highest relative difference between boys and girls was seen among those aged 10–14 years. Data on sex-related differences in incidence rates in different age groups of children is scarce, but a similar observation has been made

in Germany, and an increase in the male to female ratio has been observed beyond puberty (13, 168, 169).

Incidence in Finland fluctuated over the 20-year period. At the outset of this thesis, research had shown a long-standing global trend of increase in the incidence of type 1 diabetes (1, 2). Yet there had been some indication of a slowing down of the increase and even a plateau in some countries, namely Finland and Sweden (2, 5, 6). This thesis confirmed the plateau period which had been reported by Harjutsalo et al. (5). A novel observation was that, after the plateau period, the incidence rate first declined, but then showed a steep increase during the first 18 months of the COVID-19 pandemic. Our most recent data, however, suggest that after the peak incidence in 2020 incidence again leveled off. The range of the fluctuation of the incidence between 2003 and 2022 was conspicuous (from 49.0 per 100,000 in 2013 to 61.0 per 100,000 in 2020), whilst the overall incidence remained relatively stable; incidence was 54.8 per 100,000 PY in 2003 and 57.4 per 100,000 PY in 2022, with an average of 54.9 per 100,000 over the 20-year period. Some reports suggest a 4- or 5-year cyclical pattern in incidence fluctuation, but we did not test for this in our study (6, 166, 167).

The extent and direction of the fluctuation of the incidence in Finland seemed to vary between age groups and sex. Both the decrease in incidence observed before the COVID-19 pandemic, and the increase in incidence observed during the beginning of the pandemic tended to be most pronounced in boys aged under 5 years. Subsequently, after the peak in incidence in 2020, the incidence again decreased in boys aged under 5. Previously, both in Finland and globally, the increase in incidence had been observed to be greatest in children aged under 5 years (1, 16, 19), whilst the trends have been similar in both sexes (1, 19). There are few reports from other countries documenting a decrease in incidence such as the one we observed before the pandemic. Similar to our findings, incidence was reported to decrease in Australia between 2002 and 2017, both overall (IRR 0.991, 95% CI 0.987–0.994), and in age-specific analyses for children aged 0–4 years (IRR 0.980, 95% CI 0.973–0.988) (167). A study from North East England and North Cumbria on 943 children diagnosed with type 1 diabetes before the age of 15 years reported an overall decrease in incidence per 100,000 from 25.5 (95% CI 20.9–29.9) in 2012 to 16.6 (95% CI 13.0–20.2) in 2020, with no analyses of trends for subgroups of age or sex (170).

The incidence of type 1 diabetes in children was widely reported to increase after the start of the COVID-19 pandemic (121, 171-173). According to a meta-analysis including 17 studies, the incidence rate of type 1 diabetes in children and adolescents (inclusion until age 19 years) was 1.14 times higher during the first year and 1.27 times higher during the second year of the pandemic compared with before the pandemic (172). Most studies on incidence during the COVID-19 pandemic did not stratify children by age or sex, and reports on incidence rates from 2022 onward

are scarce. Interestingly, an increase in the incidence specifically in boys aged less than 5 years during the first 18 months of the COVID-19 pandemic has been observed in data from Sweden (IRR 1.21, 95% CI 1.00–1.41) and Stanford CA, USA (IRR 2.06, 95% CI 1.06–4.02) (S. Tall et al., unpublished data). Recent studies from Scotland and Germany have reported findings similar to ours, with a rise in type 1 diabetes incidence during the beginning of the pandemic followed by a decline in incidence observed in 2022 (174, 175). The study from Germany observed the changes to be most pronounced in boys (175), whilst the report from Scotland stratified children into two age groups (0–5 and 6–14 years), and contrary to our findings, noted the changes only in the older children (174).

6.2 The rotavirus and type 1 diabetes

Exposure to rotavirus decreased greatly in Finland after the introduction of the rotavirus vaccine into the national immunization program. Laboratory confirmed rotavirus infections were more common in boys than in girls in all birth cohorts and most occurred before the age of 2 years. Male excess of rotavirus cases in children has been reported elsewhere, including a study combining data on rotavirus infections from Finland, Germany and Australia, which reported the highest male excess for children aged 0–9 years in Finland (176).

At the population level, the change in exposure to rotavirus infections was associated with changes in the incidence of type 1 diabetes. A reduction of one percentage point in exposure to rotavirus was associated with 5% (IRR 0.95, 95% CI 0.91–0.98) and 8% (IRR 0.92, 95% CI 0.89–0.96) decreases in type 1 diabetes incidence in age groups 0–14 and 0–4 years respectively. Results were similar for both sexes. These findings are applicable only at the population level and no claim can be made based on this study for an association between rotavirus and type 1 diabetes at the individual level.

Our observations are partly in agreement with a meta-analysis published in 2022, which combined data from seven studies investigating the association of the rotavirus vaccine and type 1 diabetes, totaling close to 6 million participants (119). This meta-analysis did not find a significant association between the vaccine and type 1 diabetes incidence in the overall child population studied (RR 0.94, 95% CI 0.82–1.09). However, vaccinated children aged less than 5 years were observed to have a decreased risk of type 1 diabetes (RR 0.84, 95% CI 0.75–0.95). These findings together with our results suggest that there may be an association between rotavirus infection and type 1 diabetes and a possible protective effect of the rotavirus vaccine, specifically in young children.

Interestingly, a recent study from Sweden did not find an association between rotavirus vaccination and the incidence of type 1 diabetes (177). This study had a 10-year follow-up, during which a total of 7893 children aged less than 15 years were

diagnosed with type 1 diabetes. The effects of rotavirus vaccination status on the incidence of type 1 diabetes in children were studied by comparing changes in incidence within three groups with different vaccination status based on regional variations in the introduction of the vaccine. In addition, individual vaccination status was obtained for a subset of 125 children who had developed type 1 diabetes, and no difference was found in vaccine coverage between these children and overall regional vaccine coverage.

It has been suggested that the differing results in studies on the association between the rotavirus vaccine and type 1 diabetes, in addition to being related to for example statistical power, differences in study populations and study methods, may be related to the type of vaccine used (178). In the UK and in Sweden, where association was not observed, the Rotarix vaccine is used, whilst the Rotateq vaccine is widely used in the US, Australia and Israel, and is the only vaccine used in Finland. In line with this theory, in the US study by Rogers et al., a reduction by more than one third in the incidence of type 1 diabetes was observed in those fully vaccinated with the pentavalent Rotateq vaccine compared to unvaccinated children (111). In contrast, no significant difference was found in the incidence of type 1 diabetes when comparing children fully vaccinated with the monovalent Rotarix vaccine and unvaccinated children. However, 83.3% of those having received the full vaccination series had received the pentavalent Rotateq vaccine, so the statistical power was markedly less for the Rotarix group. In another US study by Glanz et al., no difference was observed for either vaccination, nor for fully vaccinated and unvaccinated individuals in general, but in this study the unvaccinated group comprised only 2,8% of the study population (114).

6.3 SARS-CoV2 and type 1 diabetes

As discussed above, the incidence of type 1 diabetes in children was widely reported to increase after the start of the COVID-19 pandemic (121, 172), and in this thesis we observed an increase in the incidence also in Finland during the first 18 months of the pandemic. Considering how few of those diagnosed during the pandemic in our study tested positive for SARS-CoV-2 antibodies (5 cases/0.9%), the increase in incidence we observed cannot be considered to be due to a direct effect of the SARS-CoV-2 virus; if the 16% increase in incidence had been due to the direct effect of SARS-CoV-2 infection, a similar frequency would have been expected for SARS-CoV-2 positivity. Furthermore, the incidence of type 1 diabetes in Finland leveled off after 2021, when the easing of lockdown measures resulted in an increase in exposure to the SARS-CoV-2 virus.

There have been several reports corroborating the findings of this thesis, with no indication of a direct relationship between SARS-CoV-2 infection and an increase in incidence of type 1 diabetes in children (171, 179-182). However, there

are also studies which have found an association between COVID-19 infection and increased risk of new-onset type 1 diabetes (183-186). The meta-analysis by Rahmati et al. found that in children and adolescents, the risk of developing type 1 diabetes >30 days after SARS-CoV-2 infection was 42% higher than in the non-COVID-19 control group, but only in studies from the United States (RR=1.70, 95% CI 1.37–2.11), not from Europe (RR=1.02, 95% CI 0.67–1.55) (183). Another meta-analysis also found COVID-19 infection to be associated with an increased risk of type 1 diabetes (hazard ratio 1.44, 95% CI 1.38–1.55), but in subgroup analyses for region did not specify between type 1 and type 2 diabetes, and age at diagnosis was not specified (186). Detection bias may be a factor in these studies, as increased testing for COVID-19 was likely to occur around the time of diabetes diagnosis.

In this thesis, we observed some demographic, autoimmune and metabolic differences between children diagnosed before and during the pandemic. One observed difference was more severe metabolic decompensation at diagnosis in the pandemic cohort: ketoacidosis increased by 36% and severe ketoacidosis by 57%. Several other studies have reported an increased frequency of ketoacidosis upon diagnosis of type 1 diabetes after the start of the pandemic (121, 122, 187), with a meta-analysis reporting a 25% increase in ketoacidosis and a 19% increase in severe ketoacidosis (121). Due to the sparse SARS-CoV-2 positivity in our study, the observed increase in the frequency of ketoacidosis can hardly be attributed to the direct effect of the virus, and a plausible explanation for the increase is diagnostic delay due to the lockdown. However, some indication of an association between preceding SARS-CoV-2 infection and ketoacidosis at diagnosis of diabetes has been presented (183, 188). For example, the meta-analysis by Rahmati et al. found that the risk for ketoacidosis was increased following COVID-19 infection (RR 2.56, 95% CI 1.07–6.11) (183).

In our study, those diagnosed during the pandemic also had a higher number of autoantibodies, more frequent positivity for GAD autoantibodies, fewer first-degree relatives with type 1 diabetes and a different distribution of seasons of birth compared with those diagnosed before the pandemic. To our knowledge these findings have not been confirmed by other studies, so their significance remains unclear.

As evidence exists both for and against the increased risk of new-onset type 1 diabetes and ketoacidosis after COVID-19 infection, the matter remains ambiguous (189). Damage to β-cells directly by the virus or due to systemic inflammation could cause a lack of insulin production and (possibly reversible) diabetes symptoms, but this would not by definition be autoimmune type 1 diabetes (190). There is a risk of misclassification of diabetes type, and particularly in children such non-autoimmune diabetes could be classified as type 1, affecting the statistics on association. So far there is a scarcity of evidence to indicate that the SARS-CoV-2 virus or COVID-19 infection trigger or accelerate islet autoimmunity. However,

patients with COVID-19 infection have exhibited increased autoantibody reactivity (191), so autoimmune mechanisms could be involved. Lugar et al. reported an association between COVID-19 infection and islet autoantibody appearance in a cohort of 885 infants with increased genetic risk of type 1 diabetes: the incidence rate of islet autoantibodies was 3.5 per 100 PY (95% CI 2.2–5.1) among children without SARS-CoV-2 antibodies, compared with 7.8 per 100 PY (95% CI 5.3–19.0) among children with SARS-CoV-2 antibodies (131). On the other hand, a report based on 4586 children aged 9–15 years participating in The Environmental Determinants of Diabetes in the Young (TEDDY) study did not observe any association between SARS-COV-2 infection and seroconversion to islet autoantibody positivity or progression to clinical type 1 diabetes (182).

Regardless of the ambiguity surrounding the association between SARS-CoV-2 and diabetes, the sparse SARS-CoV-2 positivity observed in this thesis indicates that any differences we observed in the incidence and characteristics of children diagnosed with type 1 diabetes before and during the pandemic were not due to a direct effect of the virus, but rather likely due to other pandemic related factors, such as for example the dramatically reduced microbial load related to the decreased frequency of viral and bacterial infections in children (133, 192, 193). As progression from the triggering of islet autoimmunity to clinical type 1 diabetes is, however, a process which generally takes several years (37), it is likely that the full effects of the COVID-19 pandemic and the SARS-CoV-2 virus on type 1 diabetes pathogenesis and incidence are yet to be determined.

6.4 Age-related heterogeneity and endotypes of type 1 diabetes

The results of this thesis highlight the association between the heterogeneity of type 1 diabetes and age at diagnosis. Almost all the characteristics analyzed in Publication IV demonstrated statistically significant differences in their distribution across the three age groups. Notably, for most characteristics, the differences were particularly pronounced between the youngest and oldest age groups, with the middle age group displaying intermediate values.

We analyzed the data by categorizing individuals into predetermined age groups at diagnosis, based on previously established evidence suggesting age-related disease endotypes (141, 143). We were not able to directly confirm the proposed endotype characteristics described in section 2.4, as we did not have information on C-peptide and proinsulin levels, first appearing autoantibody, islet histology etc. We observed that children diagnosed with type 1 diabetes before the age of 7 years exhibited a greater prevalence of first-degree relatives with type 1 diabetes, stronger HLA-mediated genetic susceptibility, and a higher number of autoantibodies at diagnosis, particularly IAA. In contrast, those diagnosed at 13 years or older

demonstrated a marked male predominance, an increased frequency of GADA, more frequent neutral and protective HLA genotype, a longer duration of symptoms prior to diagnosis, and more severe metabolic decompensation, including a higher incidence of diabetic ketoacidosis.

Unlike the immunohistological findings of Leete et al. (141, 143), in our study the distribution of the characteristics did not segregate discretely between the age groups. For example, though HLA-DR/DQ genotype related increased genetic risk and IAA-dominance were more common in the youngest group, not all of those diagnosed before the age of 7 years had a high-risk genotype or IAA positivity. This is not surprising and was not expected, as the characteristics studied cannot be considered to be directly related to immunohistological findings. Rather, the continuum reflects the extent of the heterogeneity, as the disease process is affected by complex and ongoing actions and interactions of genetic and environmental factors.

Some of the clustering of characteristics within the age groups may reflect logical interrelations rather than effects of the disease pathogenesis. For instance, genetic risk and familial disease are naturally interconnected, whilst familial disease increases disease awareness, reducing diagnostic delays and influencing clinical presentation at diagnosis. In addition, some of the differences observed, rather than being an expression of specific age-related biopathological mechanisms, may instead be related to age through social factors. For example, the greater extent of metabolic decompensation and higher proportion of ketoacidosis we observed in the oldest age group may be due to delays in seeking medical attention due to decreased parental awareness.

The results of this study reinforce the understanding of the age-related heterogeneity of type 1 diabetes, but we cannot claim that the observed differences are the result of the specific pathological pathways proposed to represent T1DE1 and T1DE2.

6.5 Age and sex in type 1 diabetes

The specific reasons behind age- and sex-related differences in incidence trends are unclear but are likely related to differing pathways of pathogenesis and interactions with environmental factors. The more pronounced changes which we observed in the incidence in the youngest age group could be related in part to the fact that disease onset at an early age often indicates rapid progression from seroconversion to clinical disease (194), so the effects of environmental changes could be seen more rapidly and intensely. The greater fluctuation in incidence in young boys, together with the fact that incidence is higher in boys in high incidence countries, suggest that boys may be more susceptible to certain environmental factors which trigger or otherwise affect the autoimmune process leading to type 1 diabetes. This could

be the result of sex-specific genetic and hormonal factors resulting in sex-related differences in immunity and in the microbiota (195). Mini puberty occurring at a young age may be responsible for some of the sex-related differences occurring in young children, as at this time testosterone levels increase in boys and estrogen levels in girls (196). Boys are generally more susceptible to infections (197, 198), a finding we confirmed for rotavirus infections, so changes in exposure to infections that affect the pathogenesis of type 1 diabetes could be more prominently reflected in the incidence among boys.

6.6 Strengths and limitations

This thesis was carried out in Finland, which has the highest incidence of type 1 diabetes globally. As a nationwide register-based study, the study population is thus large, enabling the detection of subtle differences and allowing for detailed subgroup analyses. The comprehensive data recorded in the FPDR further allows for the examination of a wide range of characteristics. Strengths of such population-level observational studies include the ability to explore epidemiological phenomena in a real-world setting, with the possibility for studying long-term trends, in an ethically feasible and cost-effective way. However, in this type of study the ability to establish causality and account for biased is limited. Efforts were made to address these issues by performing sensitivity analyses and by cautious interpretation of the results.

When studying population-level data, inferences cannot be made about individual-level associations as this would be ecological fallacy. In Publication II, using birth cohorts allowed for analysis of the association differentiating between children with high and low risk of exposure based on introduction and coverage of the rotavirus vaccine. However, only population-level association between exposure to rotavirus and type 1 diabetes could be explored, due to the lack of individual-level data to inform whether rotavirus infection and type 1 diabetes occurred in the same individual, and it was not possible to establish the time order of effects (i.e. whether rotavirus infection preceded diabetes diagnosis). On the other hand, a strength in Publication III was the ability to assess individual level-data on Covid-19 infections preceding diabetes diagnosis. Confounding is another challenge; adjustment for age, sex and period was performed in analyses when considered appropriate, but it was not for instance possible to account for changes in other environmental factors changing simultaneously with exposure to rotavirus, which might have affected type 1 diabetes incidence.

Potential for bias, including information bias and selection bias is also a factor to consider. When comparing present and historical cohorts such as was done in Publications I, II and III, it is important to consider potential differences in case detection and medical practices between the periods. However, to our knowledge,

there have been no major changes in the register practices for data collection, verification and recording, nor any changes in diagnostic criteria over the 20-year study period, so this is unlikely to cause information bias when comparing the incidence of type 1 diabetes between periods or birth cohorts. The FPDR was established specifically to compile data on pediatric diabetes from all pediatric units treating children with newly diagnosed diabetes. It collected a wide range of data allowing for reliable distinction of diabetes type based on clinical presentation as well as diabetes-associated autoantibodies and HLA-defined genetic disease susceptibility. Misclassification of diabetes type is thus unlikely in the FPDR. However, as the FPDR was based on recruitment and parental informed consent, it did not cover all cases of pediatric diabetes in Finland. The multi-register-based FinDM database on the other hand has access to data on the entire population. It relies on entries in national administrative registries of good coverage and quality. However, misclassification of diabetes type is a possibility, as for example monogenic and other atypical types of pediatric diabetes cannot be reliably identified and excluded. The possibility of comparing the coverage of the FPDR and the FinDM database was interesting for the validation of the results. This highlighted one of the inherent weaknesses of this study and register-based studies in general, i.e. that no database is likely to represent 100% of the target population, even when based on data sources of high quality and coverage like the FPDR and the FinDM databases. The difference between the two registers was observed to be approximately 19%. The observed difference is greater than the 9,2% difference previously observed between cases of type 1 diabetes recorded in the FPDR compared with hospital records in 2003–2005. The incidence of type 1 diabetes calculated based on the FinDM data (average 70.2 per 100,000 PY between 2003 and 2022) was also higher than previously estimated in Finland. These factors would imply that the FinDM database may to some extent overestimate the incidence of type 1 diabetes in children. A more in-depth analysis of the differences between the diabetic populations covered by these two registers would be informative. However, paramount for the conclusions of this thesis, the trends in incidence were similar between the two registers, and the differences in incidence rates calculated from the two different sources have remained relatively consistent over time, indicating that they are both stable data sources and reliable for the study of incidence trends.

As not all families with children newly diagnosed with type 1 diabetes choose to participate in the FPDR, it is possible that this may lead to some selection bias, as families with certain backgrounds may be more or less likely to participate. In the comparison between the FPDR and the FinDM database the percentage difference was largest for children aged 10–14, but we cannot claim that this is because this age group participates in the FPDR less, as these children could also be overexpressed in the FinDM database due to for example misclassification.

Selection bias could also be a factor in Publication IV, as analyses were limited to those participating in the Sample Repository with HLA- and autoimmune data, resulting in inclusion of about 70% of register subjects. A previous study on FPDR data compared Sample Repository participants with those not participating in the Sample Repository and found that those not participating were younger and had more severe metabolic decompensation at diagnosis (199). Similarly, in Publication III, serum samples for analysis of SARS-CoV-2 were available from 74% of cases diagnosed during the pandemic, and it has been reported that children with mild COVID-19 infection may not develop SARS-CoV-2 antibodies (200). Thus, more cases may have had infection than we were able to ascertain. To control for this, we performed additional analyses, comparing those with serum samples to those without, as well as those testing positive for SARS-CoV-2 to those testing negative, and found no indication that these groups differed in a way that would suggest bias. The NIDR, used in Publication II, is on the other hand known not to provide data on all rotavirus infections, but rather to represent "the tip of the iceberg", as it records those infections confirmed by laboratory testing. In our study, we assumed that this correlates with the magnitude of, and changes in, the prevalence. The validity of this assumption is supported by the fact that the reduction we observed in cases of rotavirus between pre- and post-vaccine cohorts was in line with the changes reported by Leino et al. (99).

Limited follow-up time is a common limitation in research. The 20-year period studied for incidence trends was relatively long, though it remains interesting to see how incidence rates will change after 2022. Limited follow-up time was, however, an issue in Publication II, as it resulted in incomplete age-ranges, especially regarding the post-vaccine birth cohorts. At the time of the study, data was available from the FinDM database until 2016, and the least complete age-range was observed for the 2010–2015 birth cohort, which was the most interesting cohort in terms of the dramatically decreased exposure to rotavirus infection. Sensitivity analyses using imputed data were performed to attempt to address the limitations and only results significant in both main and sensitivity analyses were considered robust. However, as the incidence of type 1 diabetes fluctuates, imputed data is a 'best guess' with risk for inaccuracy, as demonstrated by the conspicuous change in incidence observed at the start of the COVID-19 pandemic, which was not factored into the imputed data. But as post-2019 incidence of type 1 diabetes is affected by the COVID-19 pandemic, including this data in studies such as Publication II could overshadow or confound the impacts of other changes in exposure. This is a factor to consider in future research on population or group-level associations between exposures and outcomes spanning the pre- and post-pandemic period.

6.7 Implications

Whilst in Finland and other high incidence countries the incidence of type 1 diabetes has seemed to plateau, incidence has kept increasing in other regions, particularly in low incidence regions. Attempting to uncover what drives changes in incidence is thus still very relevant. Finland remains the country with the highest incidence of type 1 diabetes globally (201), and is thus an interesting setting for type 1 diabetes research. Changes in the incidence in Finland should be continued to be monitored regularly. It will be interesting to see whether the decreasing trend observed before the pandemic might continue, once the "disruption" caused by the pandemic has passed, as the latest data from Finland may imply, or whether a state of fluctuating plateau will continue.

Whilst the fluctuation we observed in incidence demonstrates the necessity for long-term follow-up to gain a reliable view of possible long-term trends, it is also of interest to follow the short-term changes in incidence, as they may provide clues as to the underlying environmental factors affecting the disease process and incidence. Causality is, however, challenging to uncover, due to the vast heterogeneity of type 1 diabetes. It is obscured in part by the varying duration of prediabetes before clinical diagnosis, as well as by the fact that multiple environmental factors are likely to act and interact even simultaneously to influence progression. In other words, real-life data is both valuable and challenging to interpret. Further research from both Finland and other countries should be undertaken to test whether incidence changes are indeed most pronounced in young boys, because this could be a valuable clue towards etiology.

A possible association between rotavirus and type 1 diabetes had been implied for many decades (104), but the matter remains ambiguous. Based on the population level association found in this thesis, as well as the results of the meta-analysis (119), an association at the individual level seems possible, supporting the idea that further individual level studies with sufficiently large samples sizes for statistical power would be recommended to hopefully clarify the matter. On the other hand, since widespread distribution of the rotavirus vaccine is recommended due to its efficiency in preventing rotavirus gastroenteritis, any potential benefit of the vaccine for the prevention of type 1 diabetes will be obtained regardless.

The full impact of the SARS-CoV-2 virus on the pathogenesis and incidence of type 1 diabetes may yet be undetermined, as future research may reveal. However, as the increase in type 1 diabetes incidence and ketoacidosis observed in Finland during the beginning of the pandemic cannot be attributed to the direct effects of the SARS-CoV-2 virus, it is important to consider and assess other pandemic-related changes which could have come together to affect these changes. Weighing the overall benefits and risks of lockdown should, in turn, guide future decision-making in pandemic circumstances. Given the considerable increase we observed in life-threatening ketoacidosis, particular efforts should be made to raise

awareness of the symptoms of new-onset diabetes and to ensure that access to healthcare professionals is readily available and encouraged, also in pandemic circumstances.

Based on this thesis, there is clear age-related heterogeneity in the characteristics and incidence trends of type 1 diabetes in children. As this thesis showed most pronounced incidence changes in young boys, it would be interesting to further explore possible differences in the age-related heterogeneity between boys and girls. In the age of precision medicine, with the potential to revolutionize type 1 diabetes care, understanding the reasons behind the heterogeneity and identifying distinct disease endotypes would have significant implications for both research and clinical practice. The two proposed endotypes, T1DE1 and T1DE2, could have important implications for interventional immunotherapy, as studies have suggested that immune therapy with teplizumab and GAD-alum might be more beneficial in T1DE1 and T1DE2 respectively (140, 202-204). The use of precision medicine approaches in type 1 diabetes offers exciting possibilities for improved patient outcomes and to enable this progress further research into the heterogeneity and disease endotypes is highly paramount.

7 Conclusions

The main conclusions of this study are summarized below:

1. In Finland, the incidence of type 1 diabetes in children aged under 15 years fluctuated between 2003 and 2022, and the fluctuation seemed not to occur by chance alone. After the plateau period previously described between 2006 and 2011, the incidence initially decreased. The incidence subsequently increased during the first 18 months of the COVID-19 pandemic, after which it again leveled off. Interestingly, the changes in incidence rate were most pronounced in boys aged under 5 years, and during the last two years of the follow-up, the incidence in this group decreased again. Overall, over the 20-year follow-up period, there was no clear long-term trend in incidence.

2. At the population level, by studying birth cohorts, we observed an association between a reduction in exposure to rotavirus infections and a decrease in the incidence of type 1 diabetes in children aged under 5 years.

3. During the first 18 months of the COVID-19 pandemic, both the incidence of type 1 diabetes and the frequency of ketoacidosis at diagnosis of new-onset type 1 diabetes increased in children in Finland. As very few of those diagnosed during the pandemic tested positive for infection-induced SARS-CoV-2 antibodies, the changes observed were not a result of COVID-19 infection.

4. There were significant age-related differences in demographic data, clinical characteristics, autoantibody profiles and HLA class II-associated disease risk in children diagnosed with type 1 diabetes in Finland. Our observations contribute to a better understanding of the age-related heterogeneity of type 1 diabetes, which may be related to endotypes of type 1 diabetes.

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