UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

FACULTAD DE MEDICINA. DEPARTAMENTO DE CIENCIAS CLÍNICAS

“Epidemiology and characterization of Type 1 Diabetes Mellitus in children in Gran Canaria”

Tesis Doctoral

Yeray Nóvoa Medina

Las Palmas de Gran Canaria

Noviembre 2015

Anexo I

DR. JUAN FRANCISCO LORO FERRER DIRECTOR DEL DEPARTAMENTO DE CIENCIAS CLINICAS DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

CERTIFICA,

Que el Consejo de Doctores del Departamento en su sesión de fecha 14 de octubre de 2015, tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada "EPIDEMIOLOGY AND CHARACTERIZATION OF TYPE 1 DIABETES MELLITUS IN CHILDREN IN GRAN CANARIA" presentada por el doctorando D. Yeray Nóvoa Medina y dirigida por la Dra. Dña. Ana Wärgner Fahlin y el Dr. D. Francisco Nóvoa Mogollón.

Y para que así conste, y a efectos de lo previsto en el Artº 6 del Reglamento para la elaboración, defensa, tribunal y evaluación de tesis doctorales de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a 14 de octubre de dos mil quince.

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Facultad de Ciencias de la Salud. Departamento de Ciencias Clínicas

Programa de Doctorado

"Perspectivas actuales en la investigación pediátrica"

Título de la Tesis

"Epidemiology and characterization of Type 1 Diabetes Mellitus in children in Gran Canaria"

Tesis Doctoral presentada por

Don Yeray Nóvoa Medina

El presente trabajo ha sido realizado bajo mi dirección y autorizo su presentación ante el tribunal que la ha de juzgar.

Las Palmas de Gran Canaria, a 11 de Noviembre de 2015

Fdo. Los directores

Fdo. El doctorando

Prof. Dña. Ana Wägner Fahlin **Prof. D. Francisco Javier Nóvoa Mogollón** **D. Yeray Nóvoa Medina**

Dña. ANA WÄGNER FAHLIN, Profesora Titular vinculada de Medicina adscrita al Departamento de Ciencias Médicas y Quirúrgicas de la Universidad de Las Palmas de Gran Canaria y endocrinóloga adjunta al servivio de Endocrinología y nutrición del Complejo Hospitalario Universitario Insular Materno de Las Palmas de GC,

CERTIFICA:

Que Don Yeray Nóvoa Medina, licenciado en Medicina y especialista en Pediatría y sus Áreas Específicas, ha realizado bajo mi dirección y supervisión el trabajo de Tesis Doctoral titulado: "Epidemiology and characterization of Type 1 Diabetes Mellitus in children in Gran Canaria". La aportación más relevante de dicho trabajo es su contribución al conocimiento acerca de la epidemiología de la Diabetes Mellitus tipo 1 en Gran Canaria y la caracterización de los pacientes al debut. Confirma la elevada incidencia de diabetes tipo 1 en el archipiélago y ayuda a profundizar en el conocimiento de las características clínicas y genéticas de nuestros pacientes con diabetes.

Dado que la presente memoria reúne las condiciones para ser defendida como Tesis Doctoral ante tribunal y para optar a Mención Internacional, en cumplimiento de las disposiciones vigentes, firmo el presente certificado en Las Palmas, a 12 de noviembre de 2015.

Fdo.: Ana Wägner Fahlin

Don FRANCISCO JAVIER NÓVOA MOGOLLÓN, Profesor Titular de Medicina adscrito al departamento de Ciencias Médicas y Quirúrgicas de la Universidad de Las Palmas de Gran Canaria y Jefe de sección de Endocrinología y nutrición del Complejo Hospitalario Universitario Insular Materno de Las Palmas de GC,

CERTIFICA:

Que Don Yeray Nóvoa Medina, licenciado en Medicina y especialista en Pediatría y sus Áreas Específicas, ha realizado bajo mi dirección y supervisión el trabajo de Tesis Doctoral titulado: "Epidemiology and characterization of Type 1 Diabetes Mellitus in children in Gran Canaria". La aportación más relevante de dicho trabajo es su contribución al conocimiento acerca de la epidemiología de la Diabetes Mellitus tipo 1 en niños en Gran Canaria y la caracterización de los pacientes al debut. Confirma la elevada incidencia de diabetes tipo 1 en el archipiélago y ayuda a profundizar en el conocimiento de las características clínicas y genéticas de nuestros pacientes con diabetes.

Dado que la presente memoria reúne las condiciones para ser defendida como Tesis Doctoral ante tribunal y para optar a Mención Internacional, en cumplimiento de las disposiciones vigentes, firmo el presente certificado en Las Palmas, a 12 de noviembre de 2015.

Fdo.: Francisco Javier Nóvoa Mogollón

Anexo I

DR. JUAN FRANCISCO LORO FERRER DIRECTOR DEL DEPARTAMENTO DE CIENCIAS CLINICAS DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

CERTIFICA,

Que el Consejo de Doctores del Departamento en su sesión de fecha 14 de octubre de 2015, tomó el acuerdo de dar el consentimiento para su tramitación, con mención internacional a la tesis doctoral titulada "EPIDEMIOLOGY AND CHARACTERIZATION OF TYPE 1 DIABETES MELLITUS IN CHILDREN IN GRAN CANARIA" presentada por el doctorando D. Yeray Nóvoa Medina y dirigida por la Dra. Dña. Ana Wärgner Fahlin y el Dr. D. Francisco Nóvoa Mogollón.

Y para que así conste, y a efectos de lo previsto en el Artº 6 del Reglamento para la elaboración, defensa, tribunal y evaluación de tesis doctorales de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a 14 de octubre de dos mil quince.

Nicolás M Suárez, Ph.D.
464 Bearsden Road
Glasgow, G61 1QH
(0141) 330 4638
nicolas.suarez@glasgow.ac.uk

To whom it may concern:

I, Nicolás M Suárez, as a Research Associate at the MRC- University of Glasgow Centre for Virus Research, Glasgow, United Kingdom, hereby declare that after reading and assessing the potential contribution to the scientific community of the Ph.D. thesis **"Epidemiology and Characterization of Type 1 Diabetes Mellitus in Children in Gran Canaria"**, by the Ph.D. candidate Yeray Nóvoa Medina, I consider it to be of international standard, and therefore I support its qualification for an International Mention.

Yours truly,

9th November 2015

National Healthcare Programme for Rare Endocrine Diseases
Cochin Hospital, 27 rue de Faubourg Saint-Jacques, 75014
Paris, France
maria.givony@aphp.fr, 01 58 41 33 77

Paris, 10/11/2015

To whom it may concern :

I, Maria Givony, Project Manager at the " FIRENDO : National Healthcare Program for Rare Endocrine Diseases " in France, hereby declare that I read and assessed the scientific quality of the Ph.D. thesis "Epidemiology and Characterization of Type 1 Diabetes Mellitus in Children in Gran Canaria", by the Ph.D. candidate Yeray Nóvoa Medina. In my opinion, this manuscript has a potential of bringing an added value to the quality and organization of care for Type 1 Diabetes Mellitus pediatric patients in Gran Canaria. I therefore consider it to be of international standard, and therefore I support its qualification for an International Mention.

Best regards,

Maria Givony, PhD

COLUMBIA UNIVERSITY | MAILMAN SCHOOL of PUBLIC HEALTH HEILBRUNN DEPARTMENT OF POPULATION & FAMILY HEALTH

September 28, 2015

To Whom It May Concern,

This letter is to confirm that Yeray Novoa Medina matriculated in September 2014 into Columbia University Mailman School of Public Health's Accelerated Master's in Public Health (MPH) program. Mr. Novoa Medina has satisfied all degree requirements, and his MPH will be formally conferred on October 1, 2015.

Thank you for your time and attention to this matter, and please do not hesitate to contact me if you have any further questions.

Sincerely,

Courtney Hooper
Associate Director, Academic Programs
Columbia University Mailman School of Public Health
Heilbrunn Department of Population and Family Health
60 Haven Avenue Suite B2 #208
New York, NY 10032
212-304-5703
clh2155@columbia.edu

AGRADECIMIENTOS,

Este proyecto no hubiera sido posible sin la colaboración de muchas personas,

Me gustaría agradecer a todos los compañeros de la Unidad de Endocrinología Pediátrica del Hospital Materno Infantil de Canarias su ayuda y apoyo incondicional en el desarrollo del Proyecto: Sofia Quinteiro, Angela Domínguez, Maria Teresa Ruiz de Gauna y, especialmente, a Araceli de la Cuesta y Elisabeth Caballero por su inestimable apoyo en la recogida de datos. Sin su ayuda no se podría haber realizado este proyecto.

A Angelo Santana, del departamento de Matemáticas y Estadística de la ULPGG, y Howard Andrews, del departamento de Bioestadística de "Mailman School of Public Health", Columbia University, por su colaboración en el análisis de los datos.

A Lucas Fernando González, de la Oficina de Epidemiología y Prevención del Departamento de Salud Pública del Gobierno de Canarias, por aportar los datos acerca de la incidencia de gripe en Gran Canaria.

A Jorge Martínez, del departamento de Inmunología del Hospital Insular de Canarias por su ayuda y consejo en la determinación e interpretación del análisis del HLA.

A Mercedes Navarro, del departamento de Análisis Clínico del Hospital Materno Infantil, por su apoyo a nuestra unidad y su colaboración en la descripción de la metodología empleada para los análisis.

A Juan Eugenio Jiménez González, Catedrático de Psicología Evolutiva y de la Educación, Facultad de Psicología de la Universidad de La Laguna, por su apoyo y sugerencias en la edición del manuscrito.

Al Colegio Oficial de Médicos de Las Palmas y la Sociedad Española de Endocrinología Pediátrica por su apoyo para el desarrollo del presente proyecto.

A Sandoz Farmaceútica S.A por su generoso apoyo y contribución durante la etapa de recopilación de datos.

A mis directores de tesis, Ana Wägner Fahlin y Fco. Javier Nóvoa Mogollón, por su apoyo y consejo durante la elaboración y desarrollo del estudio.

Me gustaría enfatizar mi agradecimiento a todos los pacientes con diabetes y sus familias por la confianza que depositan en nosotros cada día. Ellos son los verdaderos protagonistas de esta historia. Gracias por enseñarnos.

LIST OF ABBREVIATIONS

AAIDs: associated autoimmune diseases

Anti-GAD antibodies: anti Glutamic Acid Decarboxylase antibodies

Anti-IA2 antibodies: anti tyrosine-phosphatase antibodies

Anti-TPO antibodies: Anti-Thyroid Peroxidase antibodies

ATD: Autoimmune Thyroid Disease

CSII: Continuous Subcutaneous Insulin Infusion

DAISY: Diabetes Autoimmunity Study in the Young

DCCT: Diabetes Control and Complications Trial

DKA: Diabetic Ketoacidosis

EDIC: The Epidemiology of Diabetes Interventions and Complications.

FPG: Fasting Plasma Glucose

GWAS: Genome Wide Association Studies

HbA1C: Glycated Hemoglobin. A1C hemoglobin

HLA: Human Leucocyte Antigen

IAAs: Anti-insulin antibodies

IDF: International Diabetes Federation

MDI: Multiple Daily Injections

NGSP: National Glycohemoglobin Standardization Program

OGTT: Oral Glucose Tolerance Test

OR: Odds Ratio

PG: Plasma Glucose

SSP-PCR: Single Specific Primer-Polymerase Chain Reaction

SWEET: is an acronym standing for ‘Better control in paediatric and adolescent diabetes´

TEDDY: The Environmental Determinants of Diabetes in the Young.

TSH: Thyroid-Stimulating Hormone

T1D: Type 1 Diabetes Mellitus

T1DGC: Type 1 Diabetes Genetic Consortium.

WHO: World Health Organization

95% CI: 95% Confidence Interval

TABLE OF CONTENTS

1. **Introduction**

1.1 Diabetes Mellitus: History, definition and diagnostic criteria 1

1.1.1 Type 1 Diabetes Mellitus 6

1.1.2 Natural History.....10

1.1.3 Genetic determinants 13

1.1.4 Environmental factors 16

1.1.5 Epidemiology 20

1.1.6 Clinical Presentation 21

1.1.7 Associated Autoimmune Diseases 22

1.2 Ethnic origin of the study population 23

1.3 Justification for the research.....24

2. Hypothesis and Objectives

2.1 Hypothesis 26

2.2 Objectives 27

3. Material and Methods

3.1 Sampling 29

3.2 Incidence 30

3.3 Characterization of T1D at onset.....32

4. Results

4.1 Incidence 37

4.2 Age and sex distribution at onset 42

4.3 HbA1c values at onset 43

4.4 Acute complications at onset: Diabetic ketoacidosis 44

4.5 HLA characterization 46

4.6 Autoantibodies 52

4.7 Associated autoimmunity.....54

4.8 Regression analysis 55

5. Discussion 57

6. **Conclusion** 68

7. **References** 122

Tables

Table 1. Diagnostic Criteria for Diabetes 4
Table 2. Types of insulin and pharmacodynamics.....8
Table 3. Multicenter studies regarding diabetes 9
Table 4. Summary of risk and protective haplotypes 14
Table 5. Differences in the microbiome of seroconverted vs High-risk non diabetic individuals.....17
Table 6. Incidence rates. Temporal trends. Age and sex distribution 38
Table 7. Monthly distribution of cases 39
Table 8. Number of onsets and cases of flu for the 2006-2013 period 40
Table 9. Distributions of onset in the different municipalities.....41
Table 10. Summary of descriptive features of participants at onset 42
Table 11. Age distribution 42
Table 12. HbA1C at onset 43
Table 13. Distribution of DKA at onset distributed by age groups 44
Table 14. 2x3 table. Distribution of DKA among age groups 44
Table 15. Distribution of DKA among gender 44
Table 16. DKA at onset and increased risk haplotype.....45
Table 17. DKA and auto-antibodies 45
Table 18. 2x2 table for DRB*03 & DQB*02 46
Table 19. 2x2 table for DRB*04 & DQB*03 46
Table 20. Increased risk alleles and age-groups (3 categories) 47
Table 21. Increased risk alleles and age-groups (2 categories) 47
Table 22. Increased risk alleles and gender 47
Table 23. Protective alleles and age-groups 48
Table 24. Protective alleles and gender 48
Table 25. Distribution of DR 49
Table 26. Distribution of DQ.....50
Table 27. Distribution of increased risk alleles/genotypes 51
Table 28. Distribution of anti-islet antibodies 52
Table 29. Sex distribution of anti-pancreatic autoantibodies.....53

Table 30. Distribution of anti-IA2 antibodies among the three age categories 53
Table 31. Relationship between DRB*04 and anti-IA2 53
Table 32. AAIDs 54
Table 33. Results from the model studying relationship between age of onset and risk HLA, antibodies and gender 55
Table 34. Results from model studying relationship between presenting with DKA and risk HLA, antibodies, gender and age of onset. 55
Table 35. OR 55

Figures

Figure 1. Physiological secretion of insulin in response to circulating glucose levels.3
Figure 2. Schematic representation of normal and affected islets in the pancreas 6
Figure 3. Physiologic insulin secretion 7
Figure 4. . Histopathology of islets of Langerhans from a two year old female patient with recent onset T1D 10
Figure 5. Schematic representation of the pathogenesis of T1D 11
Figure 6. Natural History of T1D 12
Figure 7. Schematic representation of the HLA region in Cr. 6 13
Figure 8. Human Type 1 diabetes susceptibility regions 15
Figure 9. Frequency of T1D worldwide 18
Figure 10. Incidence of childhood diarrheal diseases and prevalence of tuberculosis worldwide 18
Figure 11. Symptoms of T1D 21
Figure 12. Municipalities in Gran Canaria 40
Figure 13**.** Distribution of AAIDs among sex 54

Annex tables

Annex I. Comparison of frequency (%) of HLA DR in different populations 70
Annex II. Comparison of frequency (%) of HLA DQ in different populations 71
Annex III. Comparison of frequency (%) of increased risk HLA genotypes in different populations 71
Annex IV. Comparison of antibodies frequencies 72
Annex V. Traducción al español 73

Érase una vez un niño muy dulce…

I. INTRODUCTION

The first written records regarding Diabetes were found in the Eber´s Papyrus in Egypt, and date back to 1500 BC. The name comes from the Greek word Diabetes (meaning to pass through) and the Latin word Mellitus (meaning honeyed or sweet) and clearly refers to the most common symptom of the disease. The term was first used by the Greek physician Aretaeus, who was the first to recognize the excessive sugar levels in the urine. However, these high sugar levels in the urine where not confirmed until 1776 by Matthew Dobson.

The late 1800s and early 1900s witnessed the discovery of important landmarks in Diabetes research like the recognition of the role of the pancreas in glucose metabolism by Joseph von Mering and Oscar Minkowski in 1889 or the first reference to the deficiency of a single product from the pancreas as the origin of Diabetes by Edward Albert Sharpey-Schafer in 1910. He hypothesized that diabetes was caused by the deficiency of a single product from the pancreas, and called it Insulin, from the Latin Insula making reference to the islet cells of Langerhans. But it wasn't until the Nobel Prize winning discovery of Insulin in 1921 by Frederick Banting and Charles Best, that diabetes due to insulin deficiency stopped being a fatal disease within weeks to months after diagnosis. Soon after their discovery, they reversed diabetes induced in dogs with an extract from the pancreatic islet cells of healthy dogs. Treatment in humans was started soon after that, and marked the start of a new era in the management of insulin deficiency[1].

Current technological advances have allowed for the use of more efficient monitoring and treatment methods. From urine testing to glucose meters and continuous glucose monitoring, measurement of blood glucose has changed dramatically. Even more impressive advances have led to the use of synthetic insulin (resulting in a decrease of adverse local reactions), more comfortable and efficient delivery methods (pens and pumps) and better detection and treatment of complications secondary to chronic hyperglycemia (kidney transplantation for diabetic renal disease, laser therapy for retinopathy….). All of them have contributed to increased life expectancy and improved quality of life in patients with diabetes.

However, the overall threat diabetes represents for public health has only increased. One-two hundred years ago, symptoms of insulin deficiency were the main clinical presentation of diabetes, with milder forms mostly escaping clinical detection. Advances in biochemistry and genomics have resulted in the recognition of other forms of diabetes produced by single gene disorders (monogenic diabetes) that affect the pancreatic β-cell, but only account for about 1-2%

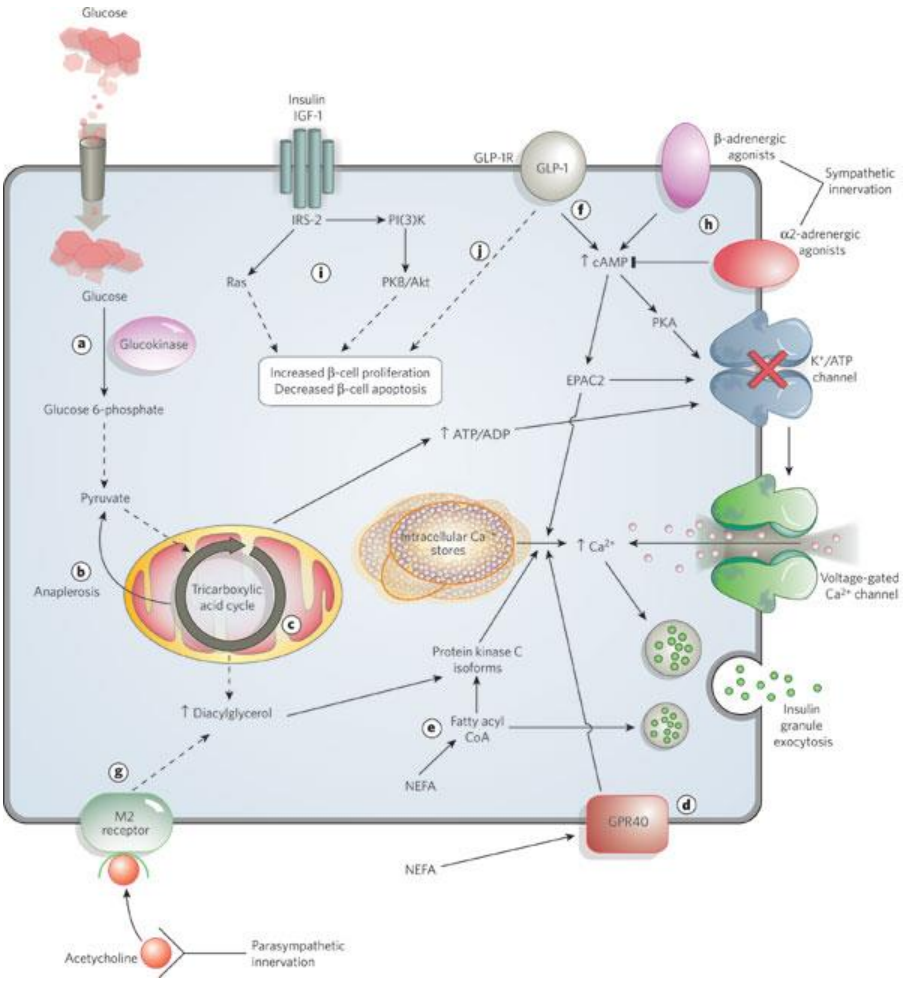
of cases. Changes in lifestyle in developed countries, increased life expectancy and the great surge in obesity have enormously increased the prevalence of diabetes and changed the spectrum of the overall clinical presentation. As a result, severe insulin deficiency only accounts for about 10% of all cases of diabetes. Most other cases are due to a combination of insulin resistance and impaired insulin secretion resulting from increased body fat[1]. The result is a worldwide epidemic that has made diabetes one of the most common and most serious threats to public health.

1.1 Diabetes Mellitus: Definition and diagnostic criteria

According to the American Diabetes Association[2], diabetes can be defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The resulting hyperglycemia can have acute and chronic consequences.

Insulin is normally secreted by the β-cells located in the islets of Langerhans in the pancreas. Its secretion is normally regulated by their ability to "sense" circulating blood glucose levels and respond with an adequate insulin secretion in order to maintain glycemic levels within the normal range. Different mechanisms can alter this physiologic process, resulting in increased insulin secretion than can produce hypoglycemia, or decreased insulin secretion in the case of diabetes. Glucose normally enters the β-cells through specific channels (GLUT-2 transporters) and is then phosphorylated by the enzyme glucokinase. Phosphorylation prevents glucose from leaving the cell, and allows the phosphorylated glucose molecule to enter the Krebs cycle, resulting in an increased ATP/ADP ratio inside the cell. This increased ratio results in closure of K+ channels, producing membrane depolarization that in turn opens calcium channels in the cell membrane. Increased intracytoplasmic calcium levels result in the liberation of stored insulin into the blood stream (Figure 1). Inability to normally produce and secrete insulin in response to increased blood glucose levels can result in abnormally high glycemic values.

Figure 1. Physiological secretion of insulin in response to circulating glucose levels.



Taken from Steven E. Kahn, Rebecca L. Hull and Kristina M. Utzschneider. Mechanisms linking obesity to insulin resistance and type 2 diabetes. 2006. *Nature* 444, 840-846.

Different pathogenic mechanisms can alter the pancreatic ability to secrete insulin. In recent guidelines, the ADA classifies diabetes into 4 general categories according to the underlying mechanisms producing it:

1. **Type 1 diabetes** (T1D): due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency. It appears more frequently in children and requires insulin replacement.

2. **Type 2 diabetes**: Due to progressive insulin secretory defect secondary to prior insulin resistance. This resistance is related to the presence of increased adiposity, especially the presence of abdominal fat.

3. **Gestational diabetes mellitus** (GDM): diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes

4. **Specific types of diabetes** due to other causes: e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation).

Diagnosis of diabetes is made when fasting plasma glucose (FPG) is ≥126 mg/dL, when a random plasma glucose (PG) is ≥200 mg/dL in the presence of symptoms of hyperglycemia, when PG is ≥200 mg/dL after an oral glucose tolerance test (OGTT) or when HbA1C is ≥6.5% when performed in a laboratory using methods standardized to the Diabetes Control and Complications Trial (DCCT) assay (Table 1).

Table1. Diagnostic Criteria for Diabetes. One of the 4 criteria must be met.

***HbA1C ≥6.5%.** The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
***FPG ≥126 mg/dL** (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
***2-h PG ≥200 mg/dL** (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
*In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L)
*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

In acute severe deficit of insulin action, metabolic derangement can derive in a severe form of presentation called diabetic ketoacidosis (DKA) that can lead to death if left untreated. It appears more frequently in Type 1 Diabetes Mellitus (T1D), especially in younger children, but can also appear in other types of diabetes. Chronic exposure to hyperglycemia is associated with long term micro- and macro vascular damage. Microvascular complications imply damage of small blood vessels resulting from impaired autoregulation of blood flow, altered permeability, inflammation, extracellular matrix accumulation, hypoxia, cell loss, neovascularization and fibrosis. It can result in dysfunction of organs like the eyes (retinopathy), kidneys (nephropathy)

and nerves (diabetic neuropathy) leading to blindness, renal insufficiency and nerve damage (causing gastrointestinal motility disorders, diaphoresis, orthostathic intolerance, “diabetic foot” and/or erectile dysfunction) respectively. Macrovascular complications are related to damage of the bigger vessels as a consequence of arterial endothelial and smooth muscle inflammation and dysfunction leading to accelerated atherosclerosis and resulting in cardiovascular, cerebrovascular and peripheral vascular disease. They represent the major cause of diabetes-related death. Also, joint immobility, obstructive sleep apnea, some skin conditions and cognitive decline have been associated with diabetes[3].

Good metabolic control, that is, maintaining glucose levels in the normal range, has been proven to delay the onset and evolution of these complications[4]. Recent reports describe a decrease in the appearance of complications, especially myocardial infarction, in the last 20 years, showing an improvement in overall management of the disease. Still, the increasing prevalence of diabetes worldwide makes the appearance of complications derived from poor glycemic control a growing burden for our health systems[5].

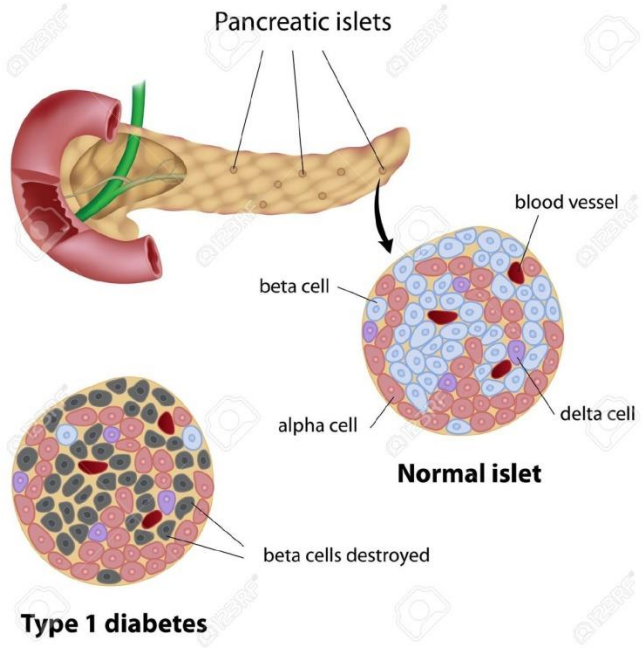
Depending on the subtype of diabetes and the stage of development, treatment will vary from weight loss, adjustment of dietary habits and an increase in exercise to the use of anti-hyperglycemic drugs or daily insulin replacement.

The object of study of this thesis is related to the epidemiology of Type 1 diabetes and the characterization of patients at the early clinical stages of the disease.

1.2 Type 1 Diabetes Mellitus.

Type 1 diabetes mellitus is an autoimmune disease caused by T-cell mediated autoimmune destruction of insulin-producing β-cells located in the islets of Langerhans in the pancreas (Figure 2). Albeit representing only 5-10% of the total cases of diabetes, it is the type most frequently found in children[2].

Figure 2. Schematic representation of normal and affected islets in the pancreas.



https://domainofscience.wordpress.com/2015/02/05/diabetes/. October 2015

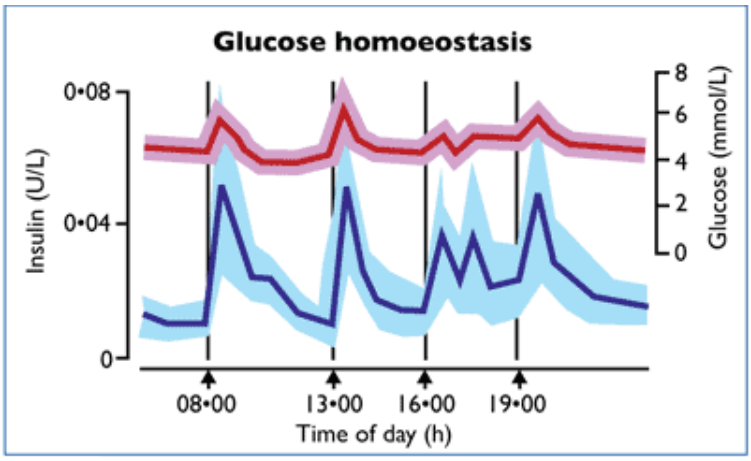
Its development is greatly influenced by individual genetic susceptibility and not well known environmental factors[6].Variation regarding geographical and temporal patterns of appearance, age of onset, sex distribution and degrees of metabolic derangement have been widely described. There is also heterogeneity in the clinical presentation of the disease. Age of presentation, symptoms, severity of metabolic derangement, associated genetic predisposition, presence of autoantibodies and associated autoimmunity vary among patients.

Due to the severe lack of insulin resulting from the destruction of the β-cell mass in the pancreas, insulin replacement is the only available option for treatment at the moment. DCCT results

emphasized the need for intensive control of sugar levels and set the ground for current treatment strategies[4].

Physiological insulin secretion includes the presence of low basal levels that are part of the normal mechanisms responsible for maintaining normoglycemia during the fasting state. After food ingestion, insulin secretion increases proportionally to the amount of ingested carbohydrates in order to favor the entrance of glucose into the cells for their utilization and to help maintain normal blood levels (figure 3).

Figure 3. Physiologic insulin secretion[7].



Twenty-four-hour plasma glucose and insulin profiles in healthy subjects. Mean values with 95% confidence interval. Red line: plasma glucose; blue line: insulin.

Insulin replacement tries to mimic physiological pancreatic secretion. Modifications to the insulin molecule have allowed for delayed or faster absorption after their injection into the subcutaneous tissue, thus creating faster or slower acting insulin molecules that can act as "basal" or "prandial" insulins. Current standard of care includes the use of "basal" acting insulins that are supplemented with "quick" acting insulins administered right before the meals[8] (table 2), along with the administration of extra doses in between meals to correct elevated glucose values if needed.

Table 2. Types of insulin used in children and pharmacodynamics[8].

Type of insulin	Insulin name	Time to start having an effect	Peak of action	Duration of effect
Rapid–acting	Lispro Glulisine Aspart	<15 minutes	30 minutes – 3 hours	3 – 5 hours
Short-acting	Regular	30 - 60 minutes	2 – 5 hours	4 – 8 hours
Intermediate-acting	NPH	1.5 - 4 hours	4 – 12 hours	10 – 18 hours
Long-acting	Glargine Detemir	0.8 - 4 hours	Minimal peak	≤ 24 hours 12-16 h (dose dependent)

T1D is widely studied given the severe repercussions it produces on the lives of those diagnosed with the disease. Collaborative multicenter studies allow for more meaningful conclusions, and many of them are currently underway or have recently finished. Table 3 summarizes many of the most relevant ones that are mentioned throughout the current paper.

Table 3. Multicenter studies regarding diabetes

BABYDIAB[9]. Started in Germany in 1989 it follows islet autoantibody and diabetes development prospectively in newborn offspring of parents with T1D.
DIPP[10]. Finish type 1 Diabetes Prediction and Prevention project. Started in 1994, it follows newborn infants with increased genetic risk screened from the general population.
DAISY[11]. Diabetes Autoimmunity Study in the Young. It recruited 2 groups of children between 1993-2004: newborns with a genetically increased risk of type 1 diabetes from the general population and relatives of patients with type 1 diabetes (aged 0-8 years).
TEDDY[12]**.** The Environmental Determinants of Diabetes in the Young was founded in 2002 and is an international extension of DAISY. It includes six clinical centers in the United States and Europe and a data coordinating center in an attempt to identify environmental factors predisposing to, or protective against, islet autoimmunity and T1D. http://www.teddycolorado.org/
Type 1 Diabetes Trialnet[13]. Its main goal is to counter the T1D disease process. Researchers are working to better understand the natural history of the disease, to identify persons at risk, and to evaluate novel therapies that balance potential risks and benefits.
SEARCH[14] for Diabetes in the Youth. Initiated in 2000 to address major knowledge gaps in the understanding of childhood diabetes. It is the largest and most diverse study among US youth. https://www.searchfordiabetes.org/public/dsphome.cfm
SWEET[15]: is an acronym standing for 'Better control in pediatric and adolescent diabete**S**: **W**orking to cr**E**ate c**E**n**T**res of Reference' and is based on a partnership of established national and European diabetes organizations (www.sweet-project.eu)
T1DGC[16]. The type 1 Diabetes Genetic Consortium is an international, multicenter program organized to promote research to identify asses the genes and alleles that determine an individual's risk for type 1 diabetes. https://www.niddkrepository.org/studies/t1dgc/
Type 1 Diabetes Exchange Clinic Registry[17]. Based in the US, it includes both adults and children. Bolsters research and development projects and programs in type 1 diabetes by helping researchers characterize individuals living with the disease, conduct exploratory or hypothesis-generating analyses, and identify participants for future clinical studies. https://t1dexchange.org/pages/clinic-registry/
DCCT[4]. Diabetes Control and Complications Trial. Ground breaking research that changed the way we treat patients with diabetes, emphasizing the importance of intensive therapy to delay the onset and slow the progression of diabetes complications.
EDIC[18]**.** The Epidemiology of Diabetes Interventions and Complications. EDIC is a multi-center, longitudinal, observational study designed to utilize the well- characterized Diabetes Control and Complications Trial (DCCT) cohort of 1297 patients.
DIAMOND[19]. Multinational Project for Childhood Diabetes (Diabetes Mondiale or DIAMOND). Developed by WHO in 1990 to investigate incidence, mortality and health care related to childhood diabetes.
EURODIAB[20]. Collaborative European study set up to assess incidence of childhood T1D in Europe (EUROpe and DIABetes) and to gather information to determine the causes and pathogenesis of the disease.
ENDIA[21]. Environmental determinants of islet autoimmunity (ENDIA): a pregnancy to early life cohort study in children at-risk of type 1 diabetes.

1.2.1 Natural History

T1D is a complex chronic disease triggered by not well known factors that exert their effect on genetically predisposed individuals. It starts with a preclinical T-cell mediated autoimmune destruction of the insulin producing β-cells in the islets of Langerhans in the pancreas. This can be seen in the form of characteristic lymphocytic infiltration limited to the islets and more prominent in early stage disease in children[22] (Figure 4).

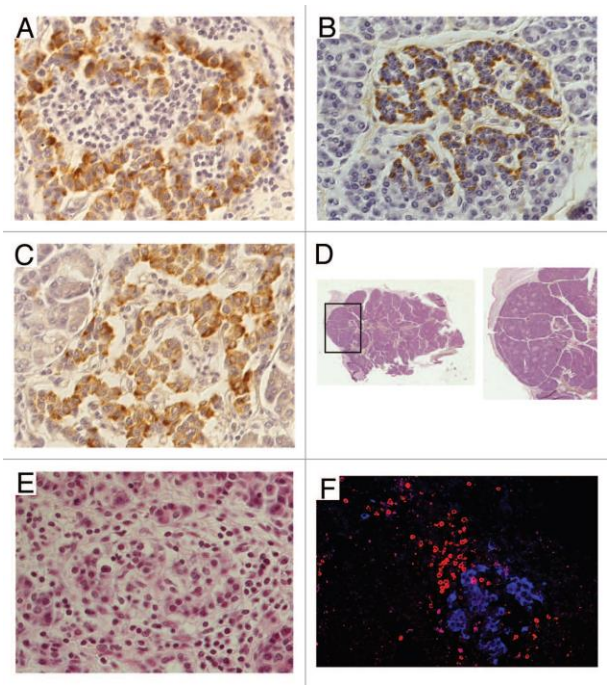


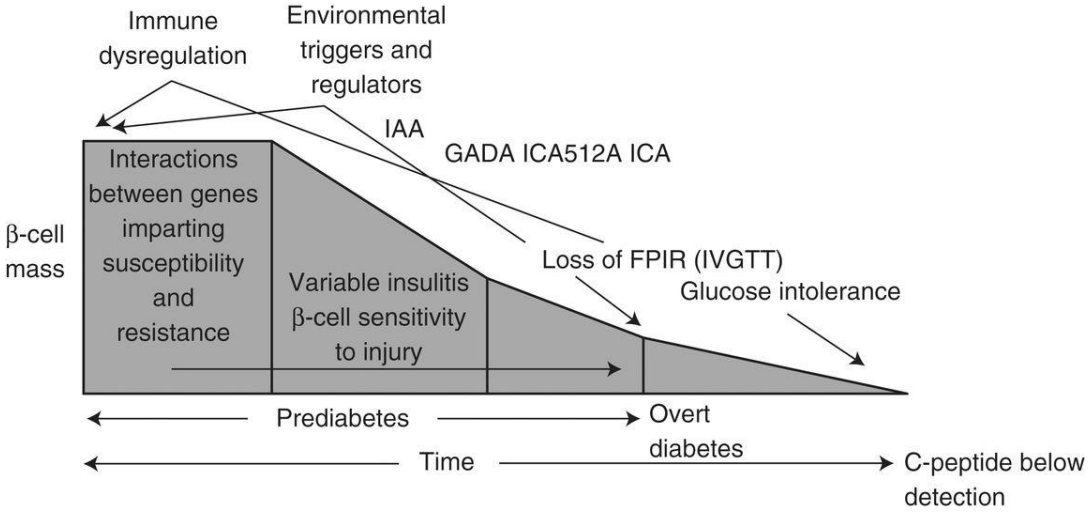
Figure 4. Histopathology of islets of Langerhans from a two year old female patient with recent onset (9 days) type 1 diabetes (case SP57/130 from W Gepts collection): insulitis in an islet immunohistochemically stained for insulin (A), pseudoatrophic islet stained for glucagon (B), islet with normal architecture stained for insulin (C). Section of pancreas from a three year old male patient with recent onset (60 days) type 1 diabetes (case ChHB 60/184 from W Gepts collection), showing marked islet hyperplasia in a single lobe (D). Insulitis in a 59 year old potentially pre-diabetic male organ donor with serum positivity for multiple autoantibodies against islet cell antigens and a susceptible HLA-DQ genotype (case two from ref. 40) (E). Immunofluorescent staining showing infiltrating $CD8^+$ T-cells (red) and residual b-cells stained for insulin (blue) in islets from case two (F)[22].

After the immune attack has started, Islet autoantibodies are measurable in most individuals and their presence is a helpful tool in the differential diagnosis of T1D[23]. They are believed to be markers of the disease, appearing as a result of the exposure of intracellular β-cell proteins to the defensive cells after their destruction during the autoimmune attack. Anti-GAD (anti Glutamic Acid Decarboxylase), anti-IA2 (anti tyrosine-phosphatase), anti-insulin (IAAs) and, in more

recent years, Zinc transporter 8 antibodies (ZnT8A)[24] are the most frequently measured ones. They can appear as early as six months of age, with a peak of appearance between 9 months and two years of age in children at an increased genetic risk[25]. Also, the order of appearance varies, with IAA appearing earlier than GADA. Testing for at least two of them at diagnosis is now considered standard of care in T1D[23]. Their presence can also be used to predict the risk of developing T1D: The TEDDY study follows children at increased risk for developing T1D. They have recently reported expression of two or more autoantibodies in children progressing to T1D, and an association between high levels of IAA and IA-2 and an increased risk of presenting the disease[26].

The appearance of the immune response precedes the destruction of the insulin secreting β-cells in the pancreas. The rate of destruction is determined by unknown environmental and genetic factors, presenting great variation among individuals. This asymptomatic phase can last from months to years. When β-cell destruction reaches such a degree (usually 80-90% of β-cell mass) that it impairs insulin response, blood sugar levels start to rise until reaching overt diabetes (Figure 5). It is important to be aware of the fact that the presence of autoimmunity doesn´t necessarily predict the development of T1D. In fact, only about 5% of individuals who express a single autoantibody go on to develop it[27].

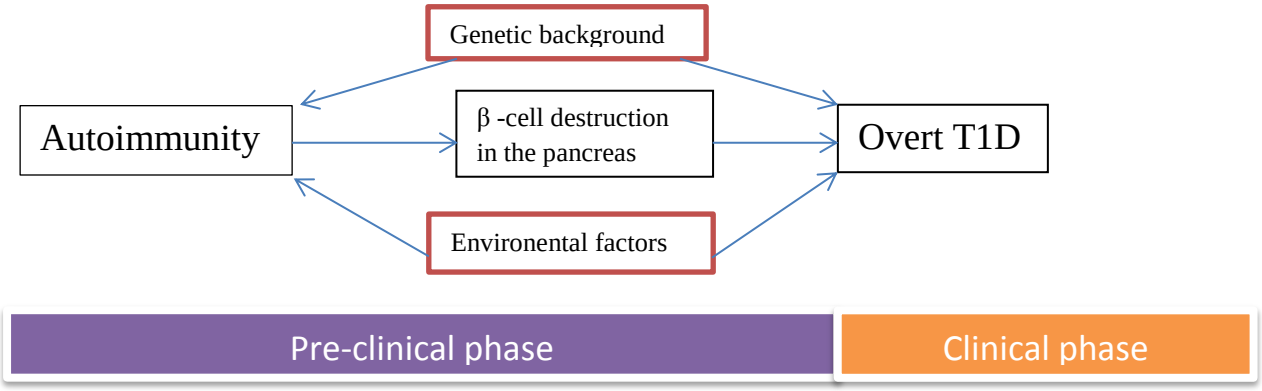
Figure 5. Schematic representation of the pathogenesis of T1D. Taken from Atkinson et al[28]



There are still important gaps in knowledge regarding the autoimmune attack and the progression to overt diabetes. Studies examining twin siblings have not shown complete concordance in the appearance of diabetes among monozygotic twins and, in cases were both twins develop T1D, the timing of the onset of the disease varies among them[29]. These findings suggest the presence of environmental factors influencing the appearance and the rate of progression of the disease.

What triggers the autoimmune response? What influences the progression to disease? Recent epidemiological studies show an increase in the incidence of T1D worldwide and link it to the growing influence of environmental factors[30]. Some authors propose an accelerated progression from islet autoimmunity to overt T1D as the underlying mechanism[31] (opposed to an increase in the incidence of islet autoimmunity). Birth cohort studies (see table 3) are providing new insights into the natural history of autoimmunity and T1D (Figure 6).

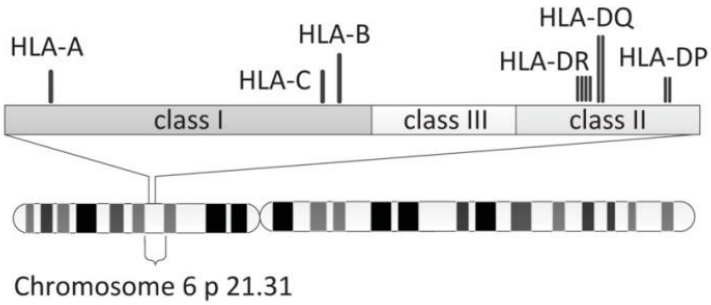
Figure 6. Natural History of T1D



1.2.2 Genetic determinants

T1D is partly determined by genetic factors, as shown by association studies that prove the increased risk of acquiring diabetes for first degree relatives of patients with the disease[32]. Recent studies suggest that the weight of the genetic load in determining the acquisition of T1D is as high as 80%[29]. Different loci have been associated with an increased risk of acquiring T1D[33]. The Human Leucocyte Antigen (*HLA*), encoded in chromosome 6, accounts for 40-50% of that genetic susceptibility according to data from the T1DGC[34]. The *HLA* region has three main coding areas: *HLA* I, coding for A, B and C molecules; *HLA* II region, coding for DQ, DR and DP molecules; and the *HLA* III region, coding for some immunologically relevant genes, but not classical *HLA* genes (Figure 7). Proteins encoded mainly by *HLA* regions I and II are responsible for binding antigens and presenting them to defensive T-cells, and have an influential role in the development of autoimmune diseases like T1D[35].

Figure 7. Schematic representation of the HLA region in Cr. 6[36]



HLA type II Molecules DR and DQ account for most of the risk encoded in *HLA* (Table 4), even though the role other *HLA* molecules play is gaining recognition[37]. Not just their presence but the combination of certain DR-DQ haplotypes, their interaction with other *HLA* and non-*HLA* molecules and the ethnic background are factors influencing the risk for the disease[38].

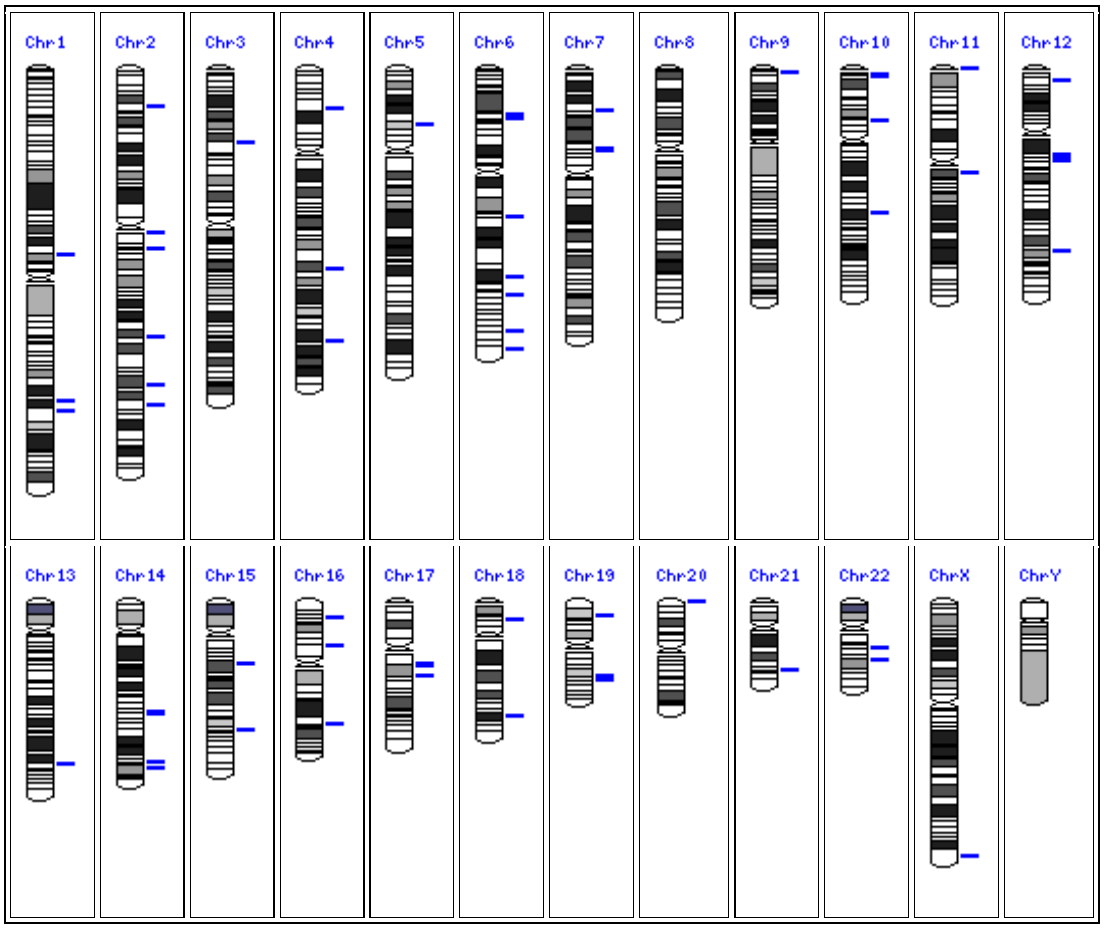
Table 4. Summary of risk and protective haplotypes[34]

Risk Haplotypes	OR	Protective Haplotypes	OR
DRB1*0405-DQA1*0301-DQB1*0302 (DR4)	11.37	DRB1*0701-DQA1*0201-DQB1*0303 (DR7)	0.02
DRB1*0401-DQA1*0301-DQB*0302 (DR4)	8.39	DRB1*1401-DQA1*0101-DQB1*0503 (DR6)	0.02
DRB1*0301-DQA1*0501-DQB1*0201 (DR3)	3.64	DRB1*1501-DQA1*0102-DQB1*0602 (DR2)	0.03
DRB1*0402-DQA1*0301-DQB1*0302 (DR4)	3.63	DRB1*1104-DQA1*0501-DQB1*0301 (DR11)	0.07
DRB1*0404-DQA1*0301-DQB1*0302 (DR4)	1.59	DRB1*1303-DQA1*0501-DQB1*0301	0.08
DRB1*0801-DQB1*0401-DQB1*0402 (DR8)	1.25		

Other genes linked to T1D and described based on a candidate gene approach are the Insulin gene (*INS*) and *PTPN22*. The insulin promoter region was linked with predisposing or protecting genotypes to T1D depending on the genotype. Their effect is speculated to affect susceptibility by modulating thymic expression of insulin and affecting T-cell "education". The *PTPN22* gene encodes for Protein tyrosine phosphatase, non-receptor type 22. It has an effect on responsiveness of T and B cell receptors, and mutations are associated with increases or decreases in risk of autoimmune diseases (rheumatoid arthritis, Grave´s disease, vitiligo…).

Genome-Wide Association Studies (GWAS) allow for testing of thousands of candidate genes at the same time. Since achieving significance for such a large number of genes requires very large numbers of cases and controls, large collaborative studies are the best strategy to achieve significant results. Type 1 Diabetes Genetics Consortium (T1DGC) is the one generating the largest body of data up to date. They have identified more than 40 different genetic regions with significant association with T1D, and confirmed the importance of the *INS* gene, finding it presents the second highest OR (2.38) for T1D[39]. They have also proven useful to confirm the relevance of the *HLA* region, showing the biggest association with T1D in the DR-DQ region, followed by the *HLA*-B, *HLA*-A and *HLA* DP regions[40]. A schematic representation of chromosomes and regions described as predisposing to T1D can be found in figure 8.

Figure 8. Human Type 1 diabetes susceptibility regions[41]



From www.t1dbase.org (September 2015)

1.2.3 Environmental factors

The appearance of T1D is not completely genetically determined. The influence environmental factors have on the development of T1D is being increasingly acknowledged. The fact that the concordance rate for T1D for monozygotic twins is only about 20-50%, reveals that other factors different to genetics play a role in the development of T1D. Environmental factors can exert their influence even before birth[42], and are currently thought to be responsible for the increasing trend in incidence in T1D worldwide[43,44].

The DAISY study group (Diabetes Autoimmunity Study in the Young) recruited children at increased risk of developing T1D from 1993 to 2004 and has followed them since, measuring the appearance of autoimmunity and overt T1D. They have reported a relationship between the age of food introduction in toddlers[45] and the presence of infections in susceptible individuals and the development of autoimmunity[46]. Not surprisingly, factors that increase the demand for insulin like increased intake of sugars in the diet[47] or a greater height growth velocity[48] have also been related to a faster progression from autoimmunity to T1D.

Vitamin D has also been extensively studied in relationship with T1D with varying results. A recent meta-analysis reports lower 25(OH) vitamin D concentrations in children with T1D than in healthy controls[49] whereas other authors report no association between the development of pancreatic autoimmunity or the progression from pancreatic autoimmunity to overt T1D with vitamin D intake or 25(OH) vitamin D levels in children[50]. Vitamin D supplementation has also been associated with an improved suppressive capacity of regulatory T-cells in children with new onset T1D[51], thus adding new lines of research in the therapy of the disease and showing the influence of nutritional factors in the progression of autoimmune diabetes.

The influence of the microbiome on the development of T1D is being increasingly researched. The presence of certain bacteria in the gut has been related to the appearance of autoimmunity and T1D, but it is not yet clear whether their presence is the cause of the appearance of the disease or it is simply a marker of the development of diabetes (table 5). The presence of healthy microbiota in the gastrointestinal tract has been linked to the education and maturation of the immune system for self/non-self immunoregulation early in life and also to the “leakiness” of the gut epithelial barrier. Alteration in these two necessary functions for the adequate development

of the immune system are two of the main mechanisms by which alterations in the microbiome could influence the appearance of autoimmune diseases such as T1D[52].

Table 5. Differences in the microbiome of seroconverted vs high-risk non diabetic individuals[52]

Property	Seroconverted subjects	High Risk controls
Dominant Phylum	Bacteroides	Firmicutes
Short Chain Fatty Acid producers	Succinate, Acetate	Butyrate
Bacterial diversity	Low	High
Functional diversity	Low	High
Genus differences	Bacteroides Clostridium Veillonella	Bifidobacterium Faecalibacterium Lactobacillus
Community stability	Low	High

Also, the increase of allergic and autoimmune diseases in developed countries has led to increasing recognition of the hygiene hypothesis as a possible model that could partially account for the increase in T1D[53]: lower exposure to pathogens during early years in life favors the increase in number of lymphocyte subtypes that predispose to the development of autoimmune disorders. This hypothesis is supported by the distribution of T1D and other autoimmune diseases worldwide. It contrasts widely with the countries with the highest prevalence of infectious disease (figures 9&10).

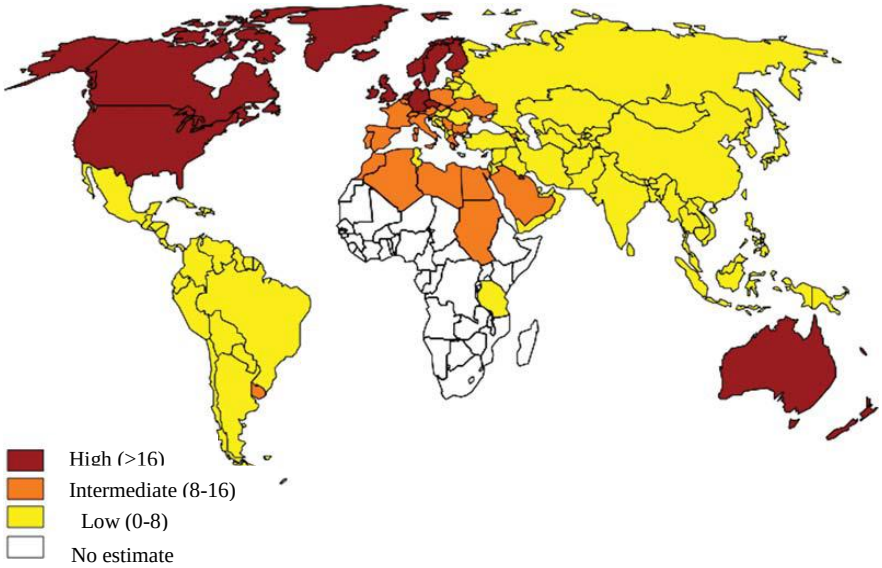


Figure 9. Frequency of T1D worldwide. Incidence per 100,000 children 0-14 yr. Data from www.eatlas.idf.org. October-2015

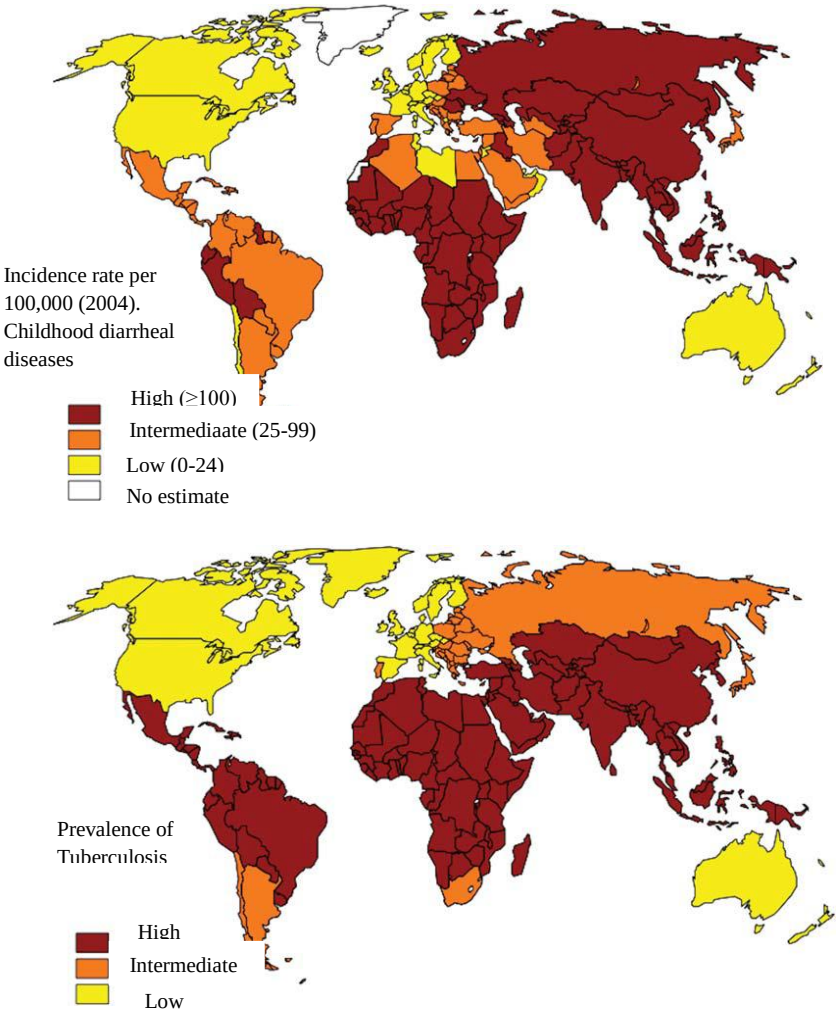


Figure 10[53]. Incidence of childhood diarrheal diseases and prevalence of tuberculosis worldwide. Data used in map creation was obtained from www.cdc.gov. October-2015.

Careful design of prospective studies with long term follow up is needed to gain deeper understanding of the role environmental factors play in the appearance of T1D. A large international study group, the Environmental Determinants of Diabetes in the Young (TEDDY)[12], is currently researching their role in predisposing or protecting the development of autoimmunity and T1D. They screened newborns for high-risk HLA DR and DQ genes and follow them long term to study the development of islet autoantibodies and T1D. Participants are carefully assessed for environmental exposures with questionnaires and carefully kept records for events like vaccinations, allergies, diet, school… Their final goal is to identify environmental triggers of T1D that could be targeted in primary prevention trials.

1.2.4 Epidemiology

Globally, the incidence of T1D presents substantial geographical variation, as shown by international registries (mainly EURODIAB[54] and DIAMOND[55]) as well as state, regional or locally based publications, with incidence values ranging from 0.1 to 1.5/100,000 in China or Japan[56] to over 60/100,000 cases in Finland[57]. The locations with the highest reported incidence to date are Finland, Sweden (44/100,000)[58] and the Italian island of Sardinia (40/100,000)[59]. Temporal fluctuation has also been reported, showing an annual increase in incidence of 2-3% during the last decades in some countries[54], but the tendency seems to be receding in some locations since 2000-2005[57,60,61]. Also, seasonal variation has been reported by Moltchanova et al after looking at data from DIAMOND, with most cases appearing in autumn and winter[62].

In Spain, the mean incidence is 17.7 cases/100,000 inhabitants[63], also showing great heterogeneity in the published results with incidence values ranging from 9.5 to 32/100,000. The highest value to date has been reported in the Island of La Palma by Belinchón et al[64] for the 1993-2007 period (32/100,000 (95% CI: 29.1-34.9)) in children aged 0-14 years. Data from Carrillo et al[65] looking at the overall incidence values also for children aged 0-14 years for the seven Canary Islands during the 1995-96 period (23.2/100,000 (95% CI: 19.4-27.1)) supports the high incidence of childhood onset T1D in the Canary Islands.

There are differences in the reported ages of onset for different countries. Most multicenter (DIAMOND[55]) or large national studies (Finland[57], Sweden[58] or Australia[61]) report increasing incidence with age, with a greater number of children presenting with T1D after 5 years of age. At the same time, they report that the recent increase in incidence is at the expense of the younger age groups[54]. Regarding sex distribution, DIAMOND[55] reports no overall differences. A 2001 review by Gale et al concludes that overall there seems to be no differences in T1D incidence in children <15 years, even though in populations with the highest incidence there seems to be a slight excess in males (3:2 male:female ratio) and the lowest risk populations tend to show a female bias[66]. Recent reports from Finland[57] support that conclusion. Evidence from Sweden adds to the great heterogeneity in the presentation of T1D by supporting the absence of differences in sex distribution in children aged less than 15 years[67], but supporting it in patients aged 15-40 years[68].

1.2.5 **Clinical presentation**

Clinical presentation at onset is important because its severity has been related to the preservation of β-cell mass as well as to future insulin requirements and metabolic control[69]. The classical clinical presentation consists of polyuria, polydipsia, polyphagia and weight loss, but it can also present with other symptoms (figure 11).

Figure 11. Symptoms of T1D.

From http://www.signsdiabetes.org/early-symptoms-of-type-1-diabetes-mellitus/. October 2015.

All of them derive from the lack of insulin action that results in poor glucose utilization and an elevation in its blood concentrations, increased glucose loss through the urine producing an osmotic effect that can lead to dehydration and an increased catabolic state with increased fat mobilization[70]. If this catabolic state is maintained, it can derive in the most severe form of presentation, called Diabetic Ketoacidosis (DKA). According to recent ISPAD guidelines[70], its presence is defined by the presence of hyperglycemia, acidosis (defined as pH<7.3 and/or Bicarbonate <15 mmol/L) and ketosis. It frequently appears at the onset of T1D, with widely varying incidence (16-67%)[71], but it can also develop during acute metabolic decompensation in previously diagnosed patients due to lack of insulin. A recent review reports a prevalence of DKA of 5-7% among patients with known T1D below 18 years of age, with higher odds for females, ethnic minorities and patients with poor metabolic control[72]. Adequate recognition and treatment of DKA is very important because of its possible consequences and sequelae. If left untreated, it can result in cerebral edema and death.

1.2.6 Associated Autoimmune Diseases

Metabolic control and presence of associated autoimmune diseases (AAIDs) also varies among studies. The risk of presenting celiac disease, autoimmune thyroid disease, vitiligo and other autoimmune conditions is increased in patients with T1D, especially in women, patients with later onset of T1D, presence of anti-GAD antibodies and family history of AAIDs[73]. By far, the most commonly AAIDs are celiac disease and thyroid dysfunction. Thyroid dysfunction has been classically described as the most frequent AAID associated with T1D, especially in girls, older children and longer time span since diagnosis[74].

Celiac disease is an autoimmune condition in which the presence of gluten in the diet (a protein found in wheat, rye and barley) launches an immune response that damages the small intestine. This attack results in a malabsortive syndrome that can affect normal growth and development depending on the degree of severity. Diagnosis is made by repeatedly positive Ig A auto-transglutaminase antibodies (titer > 4-10 U/mL) and confirmation by intestinal biopsy when required[75]. Treatment is simple: avoidance of gluten containing foods. Autoimmune thyroid dysfunction can result in autoimmune hypothyroidism (also called Hashimoto´s thyroiditis) or hyperthyroidism. The first one is more frequently associated with T1D. It is produced by an autoimmune infiltration and destruction of thyroxin producing thyroid cells, resulting in hypofunction of the thyroid gland and requiring treatment with thyroid hormone. Diagnosis is made by the presence of persistently elevated Thyroid-Stimulating Hormone (TSH) values (higher than 5 mIU/L) and positive anti-thyroid peroxidase (anti-TPO) antibodies[75]. Autoimmune hyperthyroidism results from the abnormal activation of the thyroid cells due to the presence of anti-TSH receptor antibodies. Diagnosis is made by the presence of repeatedly low TSH values (lower than 0.5 mIU/L) along with positive anti-TSH receptor antibodies[75].

The genetic load that predisposes to T1D also predisposes patients to other autoimmune disorders[76,77]. For example, the presence of the haplotype DRB*03-DQB*02, which has been described as one of the allelic combinations that increases risk of acquiring T1D, has been associated with an increased risk of celiac disease[78]. Overall frequency of appearance of AAIDs in association with T1D as well as the distribution of AAIDs varies depending on the population[78] and diagnostic criteria. Similarly to what happens with T1D, genetic as well as environmental factors influence their appearance.

1.3 Ethnic origin of the study population

Current inhabitants of the Canary Islands are mostly Caucasian with a mixed background. Aboriginal population from the Canary Islands originally came from the North of Africa, giving its inhabitants a common ancestry with the current Berber population. Several waves of immigration had an impact on the current genetic pool of the islanders. After the first settlements from Castilla started in the late 1400s, a growing Iberian and European influx began to appear. The need for farm labor force derived in the importation of slaves from West Africa. All these influenced the current genetic load of the inhabitants of the islands. Some studies have described a growing Iberian influence in the genetic pool since the Castilian settlements[79]. Indeed, analysis of the recent population has shown 62-78% of the genetic load coming from Iberian contribution, 23-38% related to current northwest African population and a small percentage related to Sub-Saharan populations (3%)[80]. These findings are consistent for all the islands except for El Hierro and La Gomera, which present a bigger NW African component[81]. The population assessed in the current thesis is from Gran Canaria and has the genetic background described above, with Iberian, NW African and Sub-Saharan influence.

1.4 Justification of the study

T1D is a chronic condition that requires affected individuals and their families to completely change their habits and lifestyle. It requires life-long treatment, and carries an increased risk of chronic complications that can affect the quality of life and the lifespan of affected individuals as well as an excess of unexplained mortality before the onset of late complications[82,83]. In the Canary Islands, end-stage renal disease related to diabetes is the highest reported to date in Spain[84]. Acute complications like DKA can also have dire consequences.

For the last 8-9 years, we have been impressed by the apparently growing number of patients presenting with T1D at our Hospital. Recent international and local reports regarding the increasing incidence of T1D worldwide prompted us to evaluate the incidence during the last 9 years in Gran Canaria and to characterize our patients in an attempt to gain knowledge about current incidence trends, to better understand epidemiological factors and to describe clinical and analytical characteristics at onset.

Given the important load diabetes imposes on the Canary Islands, we find it imperative to increase research and knowledge in the field. Gaining a deeper understanding of the root causes of T1D could allow us to increase our knowledge regarding possible mechanisms leading to the increased incidence in our region and to better target prevention and treatment strategies in an attempt to decrease the number of cases and complications[85]. Knowledge is the first step to improving quality of care, to advocate for change in current practice and to guide health policy regarding the care of patients with T1D.

As a first step, we present the incidence of childhood-onset T1D during the last 9 years in Gran Canaria and the characterization of our current patients at the moment of their T1D onset.

II. HYPOTHESIS AND OBJECTIVES

2.1 **Hypothesis**

2.1.1 Null Hypothesis:

- Incidence of T1D is not higher than in the rest of Spain.
- There hasn't been an increase in incidence in recent years in Gran Canaria.
- Our values are similar to those expected by looking at the rest of Spain and neighboring European and African countries and support the classically described North-South gradient for the incidence of T1D in Europe.
- Distribution of cases among different municipalities in Gran Canaria is similar
- There are no differences in the presentation of T1D when looking at gender and different age groups
- There is no relationship between flu the previous or the same year and the onset of T1D in Gran Canaria.
- There is no seasonality in the appearance of cases
- Genetic and immune characterization of our patients is not different from what has been reported for Caucasian populations.
- The presence of AAIDs in our populations is similar to that described in the literature.

2.1.2 Alternative Hypothesis:

- Incidence of T1D is higher in Gran Canaria than in the rest of Spain
- There has been an increasing trend in the number of cases with T1D presenting in Gran Canaria for the last 9 years.
- Our values are different to those expected by looking at the rest of Spain and neighboring European and African countries.
- Our incidence values do not support the classically described North-South gradient for the incidence of T1D in Europe.
- Distribution of cases differs throughout the island
- Distribution of cases and characteristics at onset among gender and age groups differ significantly.
- The presence of flu the previous or the same year influences the appearance of T1D in Gran Canaria.
- There is seasonality in the appearance of cases.
- Genetic and immune characterization of our patients is different from what has been reported for Caucasian population.
- The presence of AAIDs in our populations is different to that described in the literature

2.2 **Objectives**

The main objective is to learn about the incidence of T1D in Gran Canaria and to better comprehend factors leading to T1D in order to be able to more effectively decrease morbidity and mortality. The main operative objectives for the study are:

2.2.1 Incidence.

- To define the incidence of T1D in the pediatric population (children under 14 years of age) of Gran Canaria for the last 9 years (2006-2014) and evaluate temporal trends.
- To compare our incidence to that of the rest of Spain and neighboring European and African countries.
- To evaluate if our incidence values respect the classically described North-South gradient for European countries.
- To determine the possible existence of variability among 3 different age groups (0-4; 5-9 and 10-14 years of age) and between boys and girls.
- To determine the distribution of onsets among the different municipalities in an attempt to find factors that might be influencing its appearance.
- To evaluate the existence of a possible relationship between incident cases of flu and onset of T1D.
- To evaluate the influence of seasonality in the appearance of cases throughout the 9 year period.

2.2.2 Clinical characterization at onset

- To describe the following characteristics at onset: age of diagnosis, sex, mean HbA1C, severity of onset (DKA) and presence of AAID.

2.2.3 Genetic (HLA) and Autoimmune (anti-islet autoantibodies) characterization.

- To assess the frequency of previously described high-risk *HLA* alleles in our T1D population.
- To assess the frequency of anti-GAD, anti-IA2 and anti-insulin antibody positivity in our T1D population.
- To assess possible factors influencing age of onset and severity of presentation.

III. MATERIAL AND METHODS

3.1 Sampling

We used two different sampling procedures for the study:

- First, we used "census sampling" to describe the incidence of T1D in Gran Canaria for the 2006-2014 period, description of its distribution among age groups and sex, and to evaluate the relationship between incident cases of flu the previous year and the onset of T1D. This sample is representative and has enough size to reach meaningful conclusions regarding the population that is the object of our study, since to the best of our knowledge, we had the complete census of all children diagnosed with T1D in the island during the given period.

- In order to ensure availability of records, to describe the analytical characteristics of children with T1D in Gran Canaria at onset (HLA, anti-islet autoantibodies, prevalence of DKA and AAIDs) we decided to take a convenience sample: cross-sectional sample of patients with T1D under our care during the 2013-2014 period and retrospectively examined their characteristics at onset. After age 14, patients and their records are moved for care to a different hospital. Until recently, records were kept only in paper format.

The study was accepted by the Ethics Committee for Clinical Research from our Hospital.

3.2 Incidence

In order to evaluate incidence, we included all patients living on the island at the time of diagnosis during the 2006-2014 period. Record of new T1D patients for each year was kept in our Unit and was contrasted with data obtained from the hospital's pharmacy (new insulin prescriptions) and from the local Diabetes Association as described by the capture-recapture method[86]. Diagnosis was made according to ADA's diagnostic criteria. The date in which the first insulin dose was administered was considered as the date of onset. Data was collected retrospectively from hospital records. Incidence was calculated as the number of new cases identified per year for every 100,000 inhabitants younger than 14 years of age. For some of those whose onset was in 2006, we lacked access to the exact month of diagnosis. Thus, seasonality was assessed for the whole period except for 2006.

Census data was provided by the Canarian Institue of Statistics. For the assessment of climate parameters and flu incidence, we used data from the Meteorological Agency and from the Office of Epidemiology and Prevention of the Department of Public Health of the Canary Islands respectively. Geographical distribution was assessed by classifying onsets by municipality and evaluating possible differences in incidence throughout the island.

Statistical analysis

- SPSS vs 22 (SPSS Inc, Chicago, IL) was used for most of the analysis of data.. Results for continuous variables are described as mean (SD) or median (range), depending on distribution (Gaussian or not, respectively) and qualitative variables, as N or percentage. 95% Confidence Intervals were computed when considered appropriate.

- Temporal trend analysis was performed using Poisson regression, with the following modeling:

$$\log(\mu t)=\log(N)+\beta 0+\beta 1t$$

where “t” is the year, “μt” is the expected number of cases for that year and “N” is the exposed population. We assume that the number of new cases in the year “t” follows a Poisson distribution of “μt” value. This model can be expressed as: $\log(\mu_t N)=\beta_0+\beta_1 t$

meaning that the log of the expected incidence is a lineal function of time.

- Analysis of seasonality was performed using Cosinor test. Both Poisson regression analysis and Cosinor test were performed using computing environment R v3.0.2 (R Foundation for Statistical Computing, Vienna, Austria)

- Correlation analysis was performed to look for relationship between number of new T1D cases and flu prevalence the previous year as well as to look for relationships between the monthly onsets and possible relationship with temperature, humidity and hours of sunlight. Number of cases per month was computed by obtaining the mean value for each month throughout the whole 2006-2014 period. Mean temperature, humidity and hours of sunlight values used were the mean values per month for the 2000-2008 period.

- Chi-squared was used to assess differences in the distribution of cases (in the different municipalities). Observed cases were calculated counting the total number of cases for each municipality for the 2006-2013 period only (due to lack of data for all municipalities for the year 2014). Expected number of cases was calculated for each municipality using the overall incidence for the island and the census for the population of the island of 2013. We only had access to the total population per municipality, no data for the under 14 year old population per municipality was found. Thus, we are assumed that the distribution in the number of the children less than 14 is similar to the distribution of the total population per municipality.

- Chi-squared was also used to assess the differences in cases across age groups. The observed value used was the global % of cases found for each age group, and the expected value was 33% for each group. A bilateral $p < 0.05$ was considered significant.

- Yearly incidence was computed dividing the number of cases by the total population for the age group and adjusting for 100,000 children at risk. Incidence for the 9 year period was computed by dividing the total number of cases by the total number of children at risk for the whole period and adjusting for 100,000 children.

3.3 Characterization of T1D at onset

Data at onset of patients with T1D was collected retrospectively from 277 patients followed at the time of the study in the only Pediatric Endocrinology Unit in the Island of Gran Canaria, located at the "Hospital Materno-Infantil de Canarias" (Table 6). To calculate the mean number of years with T1D we used the last day at which data was entered (august 1st 2014). Patients' records were examined and the following variables were recorded: Date of birth, sex, date of diabetes onset, weight and height at onset, analytical data from the onset: HbA1c; pH; Class II HLA DQ-DR; anti-GAD, anti-insulin and anti-IA2 antibodies; TPO and anti-Transglutaminase Antibodies. BMI was calculated by dividing weight in Kg by squared-height in meters (Kg/m^2). T1D was defined according to ADA diagnostic criteria. Monogenic diabetes was excluded by performing genetic characterization in those patients in which we had reasons to suspect its presence: a 3-generation family history of diabetes and negative autoimmunity. Associated autoimmune diseases were defined by repeatedly elevated TSH values (higher than 5 mIU/L) and positive anti-TPO antibodies for autoimmune hypothyroidism; repeatedly low TSH values (lower than 0.5 mIU/L) along with positive anti-TSH receptor antibodies for hyperthyroidism; repeatedly positive Ig A auto-transglutaminase antibodies (titer > 4-10 U/mL) and confirmation by intestinal biopsy when required for celiac disease[87]. The presence of clinical symptoms was not required for diagnosis.

- Genomic DNA was extracted (QIAamp, Qiagen) and *HLA-DRB1 / DQB1* was genotyped by Single Specific Primer-Polymerase Chain Reaction (SSP-PCR) (INNOTRAIN, DiagnostikGmbH). We have results for *HLA-DRB1* in 114 patients and for *HLA-DQB1* in 118 patients. Data is presented at the two digit level (i.e DRB*03) so synonymous polymorphisms which are documented in the 3rd, 4th, 5th and 6th digit are not reported. Given the absence of parental genotyping, no haplotypes were inferred. Characterization is presented in two forms. 1. The number and the percentage of patients presenting the corresponding allele at least once. 2. On the 2N column, allele frequencies are based on the occurrence of a given allele out of the total number of alleles (2N), as seen in the analysis done by Black et al in the SEARCH for diabetes in the Youth study[88]. For analysis, high risk *HLA* was defined by the presence of the DRB*03 and/or DRB*04 alleles. Protective *HLA* was defined by the presence of the DRB*07, DRB*11, DRB*13 or DRB*15 alleles. When doing regression analysis looking at the impact of

"high risk HLA" on different variables, both the presence of the risk genotype and the risk alleles were evaluated separately. We performed hypothesis testing in order to evaluate the existence of significant differences in the frequency of appearance of DRB*03 and DRB*04 alleles and between DQB*02 and DQB*03 alleles in our population.

- Anti-islet autoantibodies were measured using radioimmunoanalysis by Reference Laboratory (Barcelona). IA2 autoantibody RIA kit from RSR with ^{125}I-labeled IA2 was used for the detection of IA2 antibodies[89]. GAD autoantibody RIA kit from RSR with ^{125}I-labeled GAD was used for the detection of GAD antibodies[90]. DIAsource AIA-100 kit was used for the detection of anti-insulin antibodies via determination of the binding of ^{125}I-Tyr-A14-insulin to the serum fraction precipitated by the polyethylene glycol. In the 2005 DASP study[91], RSR´s RIA kit showed 100% specificity (n=100) and 70% sensitivity (n=50) for IA-2 autoantibodies and 95% specificity (n=100) and 84% sensitivity (n=50) for GAD autoantibodies. Normal reference values for anti-GAD/64k and anti-IA2 are considered < 1 U/ml. For anti-insulin antibodies, positivity is considered when binding is > 8.2% (mean binding value + 3 SDS). Frequencies were determined taking into account the number of positive antibodies in the population number for which that antibody was measured: antiGAD and anti-insulin were measured in 261 patients, anti-IA2 only in 231 of our children and all three of them only in 228 of our patients. Absence of antibodies as a proportion of the population was computed using 228 as the denominator (number of children with all 3 antibodies measured). Anti-TPO antibodies were measured using electrochemiluminescence immunoassay. Anti-transglutaminase recombinant IgA was measured using enzyme-immunoassay.

- HbA1c was measured using turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood in auto analyzer "Cobas 6000" (Roche Diagnostics). EDTA was used as anticoagulant. The method meets DCCT standards. Overall mean values as well as mean values for subgroups with and without DKA were determined.

Statistical analysis

SPSS vs 22 (SPSS Inc, Chicago, IL) was used for most of the analysis of data. Data at onset was described using basic descriptive tools (mean, standard deviation and 95% CI when appropriate). Chi square was used to evaluate the existence of relationship among categorical variables.

Fisher´s exact test was used when 1 observed value was <5. Linear and logistic regression to evaluate relationship between independent and dependent variables were performed using computing environment R v3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

95% CI and hypothesis testing were calculated with the following assumptions and formulas[92]:

1. 95% CI for one sample, continuous variables

Formula	Assumptions to be met
$X \pm z^{*}\frac{s}{\sqrt{n}}$	$N \geq 30$

2. 95% CI for population proportions (one sample, dichotomous outcome):

Formula	Assumptions to be met
$p \pm z^{*}\sqrt{\frac{p*(1-p)}{n}}$	At least five successes (n*p) and at least five failures (n(1-p)) in the sample

3. Hypothesis testing with a continuous outcome variable in a single population:

Test statistic	Assumptions to be met
$n \geq 30$ $z = \frac{X-\mu 0}{s/\sqrt{n}}$ $n<30$ $t = \frac{X-\mu 0}{s/\sqrt{n}}$	For both, the mean specified in the Ho is a fair and reasonable comparator. Appropriate use of the t distribution assumes that the outcome of interest is approximately normally distributed.

4. Hypothesis testing with a dichotomous outcome variable in a single population:

Test statistic	Assumptions to be met
$Z = \frac{p-p0}{\sqrt{\frac{p0(1-p0)}{n}}}$	The smallest of $n*p_o$ or $n(1-p_o) \geq 5$

5. Hypothesis testing with a categorical outcome variable in a single population:

Test statistic	Assumptions to be met
$X^2 = \sum \frac{(O-E)^2}{E}$	Expected frequency in each response category is ≥5

Other variables analyzed:

- Mean age, age category distribution, weight, height and BMI at onset. Mean values and 95% CI were determined. Percentiles for weight, height and BMI were calculated using the 2008 growth charts developed by Carrascosa et al[93]. Chi-square was used to evaluate differences in distribution among age categories and gender.

- Overall mean HbA1c and values for children presenting with and without DKA. 95% CI were determined for all three groups.

- Presence/absence of DKA. Data was presented as % of cases and 95% CI for the 3 described age groups. Differences among groups were evaluated using chi-square.

- Prevalence of other autoimmune diseases associated with T1D and distribution among males and females. 95% CI were determined. Chi-square was used to evaluate the existence of significance in the distribution of AAIDs among males and females.

IV. RESULTS

4.1 Incidence.

-During the 2006-2014 period, a total of 316 children presented with T1D in Gran Canaria. Number of new onsets per year during the whole period, number of children under 14 years of age in Gran Canaria, annual and mean incidences are shown in table 6. Secondary sources did not provide any new cases (degree of ascertainment from the Hospital is close to 100%). As a result, the capture-recapture method did not provide additional information in our case.

-We did not find any statistically significant temporal trend in the number of new onsets from 2006 to 2014 (F=0.4982; df= 1 and 6; p=0.507; r=0077; adjusted r^2=-0.077).

-When comparing with the data from 1995-1996 (1995: 24.9/100,000 (95%CI:19.34-30.46); 1996: 21.5/100,000 (95%CI:16.23-26.77)) provided by Carrillo et al[65], we were not able to find any significant trends in the number of cases from the 1995-96 to the 2006-2014 period. The initial model showed significant results in the trend (z=2.467; df:10; AIC: 81.86; p=0.014). When we checked the goodness of fit, we observed that the model didn't have a good fit with the data (p-value of goodness of fit test, X^2=0.044). The lack of fit could be due to excessive dispersion of the data. To make up for it, we readjusted the model taking into account the excessive dispersion. After adjusting, coefficient values were the same, but the standard error changed. This resulted in a different p-value, which is nonsignificant (t=1.744; df=10; AIC:=NA; p=0.115).

-We didn't find any significant differences in sex or age-group distribution at onset for the whole period (Table 6).

- When looking at the number of onsets per month during the whole period, we did not find any significant trends in the distribution of cases. (Cosinor test; amplitude=0.37; Phase:Month=4.3; Low point: Month=10.3; p>0.05) (table 7).

- No significant correlation was found between the number of total onsets per month and the mean temperature, humidity or hours of sunlight for the same month (Temperature: r= -0.166; p=0.606. Humidity: r=0.064; p=0.844. Hours of sunlight: r=-0.301; p=0.341).

Table 6. Incidence rates. Age and sex distribution

	2006	2007	2008	2009	2010	2011	2012	2013	2014
Nº of new onsets	24	35	33	39	53	31	44	27	30
Nº of children under 14	117,299	118,064	119,177	119,827	119,867	118,757	117,699	116,063	113,304
Annual Incidence* (cases/100,000) (95%CI)	20.46 (12.28-28.64)	29.64 (19.82-39.46)	27.69 (18.24-37.14)	32.55 (22.34-42.76)	44.22 (32.32-56.12)	26.10 (16.91-35.29)	37.38 (26.34-48.42)	23.26 (14.49-32.03)	26.48 (17.01-35.95)
Girls (%)	63	49	40	49	53	52	50	59	43.3
Age groups (%) (0-4, 5-9, 10-14)	29/21/50	32/34/34	28/39/33	31/49/20	30/38/ 32	26/35/39	27/34/39	18/41/41	28/38/34
Global Incidence (95% CI)	**29.75/100,000 children** (26.5-33.09)								
Global female %**	**51**								
Global age distribution* %** (0-4, 5-9, 10-14)	**27.6 / 36.5 / 35.8**								

*Nosignificant trend was found. ** No significant difference between females and males *** Difference in cases between age groups is not significant.

Table 7. Monthly distribution of cases

	Jan	Febr	Mar	Abr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
2007	4	5	3	3	3	1	3	1	4	5	3	0
2008	2	2	2	1	2	5	4	2	4	4	1	4
2009	4	1	3	6	0	5	2	5	2	2	4	5
2010	3	6	5	3	7	5	3	5	7	4	2	3
2011	4	4	5	0	2	3	1	2	5	2	1	2
2012	4	6	3	3	2	2	3	4	2	5	3	7
2013	2	3	3	4	5	0	2	1	4	0	3	0
2014	3	3	3	3	2	2	2	1	4	0	5	2
TOTAL	**26 (8.2%)**	**30 (9.5%)**	**27 (8.5%)**	**23 (7.3%)**	**23 (7.3%)**	**23 (7.3%)**	**20 (6.3%)**	**21 (6.6%)**	**32 (10.1%)**	**22 (7%)**	**22 (7%)**	**23 (7.3%)**

*No significant difference in the number of cases/month

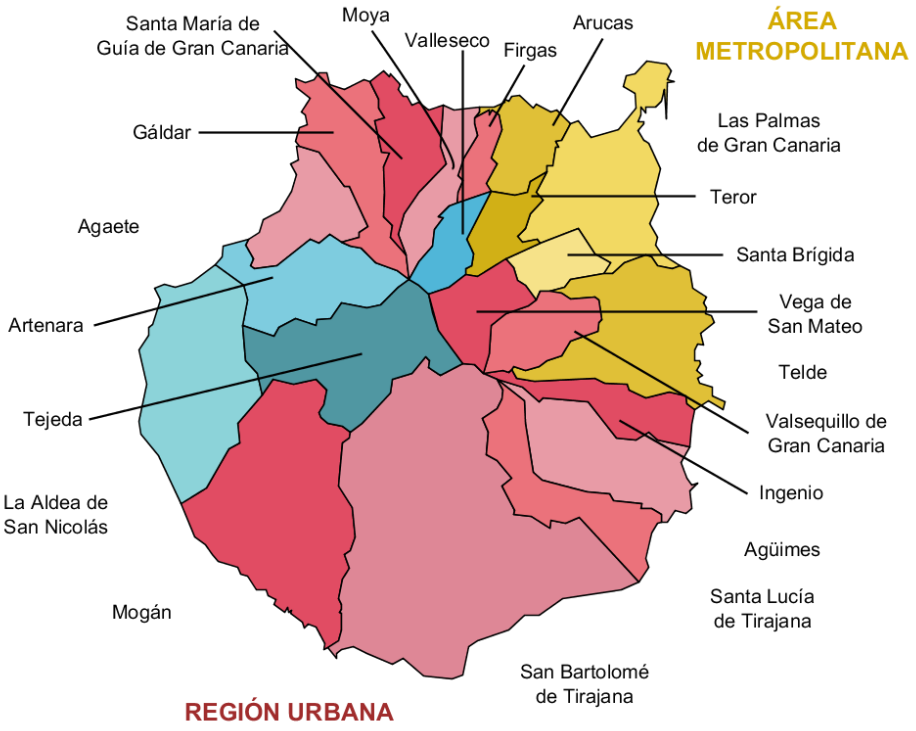
- We found a positive correlation between new T1D onsets and the incidence of flu the previous year, but without reaching statistical significance (r=0.554; p=0.197). No correlation was found for the incidence of flu the same year as the onsets (r= - 0.454; p=0.162) (Table 8)

Table 8. Number of onsets and cases of flu for the 2006-2013 period

	2006	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
T1D incident cases (n)	24	35	33	39	53	31	44	27
Cases of flu in children < 14 years (n)	41480	45608	34667	50526	12635	24935	19909	23980

- Geographical distribution also proved to have no influence in the appearance of new cases. No significant differences were found among municipalities (Figure 12 & table 9).

Figure 12. Municipalities in Gran Canaria



http://www.mapasdelmundo.org/20-mapa-gran-canaria-provincia/ October-2015

Table 9. Distributions of onset in the different municipalities

	Number of inhabitants per municipality	% of total population for Gran Canaria	Nº onsets per municipality	% of total onsets in each municipality	Expected result	Observed result	$X^2 = (Oi - Ei)^2 / Ei$
GRAN CANARIA	852.723	100	222	100	238.762	222	
Agaete	5.796	0.7	0	0	1.62288	0	0*
Agüimes	30.214	3.5	8	3.6	8.45992	8	0.025
Artenara	1.198	0.14	0	0	0.33544	0	0*
Arucas	36.852	4.3	10	4.5	10.3186	10	0.0098
Firgas	7.628	0.9	1	0.45	2.13584	1	0.604
Gáldar	24.227	2.8	10	4.5	6.78356	10	1.525
Ingenio	29.978	3.5	7	3.15	8.39384	7	0.2314
Mogán	21.782	2.5	7	3.15	6.09896	7	0.133
Moya	7.977	0.9	2	0.9	2.23356	2	0.024
Palmas de Gran Canaria (Las)	383.050	44.5	89	40.1	107.254	89	3.106
San Bartolomé de Tirajana	56.698	6.6	9	4	15.8754	9	2.977
Aldea de San Nicolás (La)	8.228	0.97	3	1.35	2.30384	3	0.210
Santa Brígida	18.971	2.2	6	2.7	5.31188	6	0.089
Santa Lucía	68.506	8	26	11.8	19.1817	26	2.42
Santa María de Guía	13.811	1.6	2	0.9	3.86708	2	0.901
Tejeda	2.028	0.2	0	0	0.56784	0	0*
Telde	102.170	12	32	14.4	28.6076	32	0.402
Teror	12.761	1.5	4	1.8	3.57308	4	0.051
Valsequillo	9.170	1	5	2.25	2.5676	5	2.30
Valleseco	3.904	1.46	0	0	1.09312	0	0*
Vega de San Mateo	7.774	0.9	1	0.45	2.17672	1	0.636
						X^2	15.658
						α= 0.05	26.3
						α= 0.01	32.0

*Values from Agaete, Artenara, Tejeda and Valleseco were not added to computed X^2 since they presented no cases (df=16).

4.2 Age and sex distribution and physical characteristics

In the cross-sectional study, a total of 277 children were included (134 boys and 143 girls), aged 6.4 years at onset, with a mean duration of T1D of 4.6 years by the time the study was started. We found that there was no significant difference in gender distribution. Also, we found no significant differences regarding weight, height or BMI at onset (Table 10). Looking at age groups, we found significantly more children presenting with T1D in each of the categories 0-4.9 years and 5-9.9 years than in the 10-13.9 category ($p < 0.01$) (Table 11).

Table 10. Summary of descriptive features of participants at onset

	Overall	Age	Weight	Height	BMI	HbA1c
Overall Value (95% CI)		6.4 years (5.99– 6.81)	24.38 kg (22.89 – 25.87)	1.16 mt (1.13 – 1.19)	16.3 Kg/m2 (15.78 – 16.8)	10.5% (10.3 – 10.8)
Boys	48.4% (43%-54%)	6.2	24.8 (P67)	1.16 (P48)	16.15 (P48)	10.5%
Girls	51.6% (46%-57%)	6.44	23.92 (P51)	1.17 (P39)	16.4 (P52)	10.6%

Table 11. Age distribution

	0 – 4.99 years	5 – 9.99 years	10-13.99 years
% (95% CI)	40.3 (34 – 46)	41.8 (36 – 48)	17.9* (13.5 – 22.5)

*p < 0.01

4.3 HbA1c values at onset

-We found that, on average, HbA1c at onset was 10.55%, higher for children with than for children without DKA at onset (table 12).

Table 12. HbA1C at onset

	Mean HbA1c	Mean HbA1c without DKA	Mean HbA1c with DKA
HbA1C (95%CI)	10.55% (10.3-10.8)	10.2% (9.99 – 10.63)	11.23% (10.96 – 11.44)

4.4 Acute complications at onset: Diabetic ketoacidosis

-In our sample, we found that 34.2% (95% CI= 29% – 40%) of our patients presented with DKA at onset.

-We found a statistically significant difference in the proportion of DKA among age groups (X^2=8.201; df=2; p<0.05), pointing at a higher incidence in children aged less than 5 years (table 13&14).

Table 13. Distribution of DKA at onset distributed by age groups

	Age 0-4.99	**Age 5-9.99**	**Age 10-13.99**
% of children presenting with DKA in each age-group (95% CI)	43.7 (34 - 53)	28.1 (19.6 - 36.4)	27.6 (14.6 - 40)

Table 14. 2x3 table. Distribution of DKA among age groups

		age category			
		age 0-4	**age 5-9**	**age 10-13**	**Total**
Presence of DKA	**yes**	49	31	12	92
	no	62	79	35	176
Total		111	110	47	268

-We found no significant difference when comparing distribution by gender (X^2=0.109; df=1; p=0.741) (Table 14).

Table 15. DKA and gender

		sex		
		Boys	**Girls**	**Total**
Presence of DKA	**yes**	43	49	92
	no	86	90	176
Total		129	139	268

-Also, we found no significant relationship between the presence of DKA at onset and having increased risk HLA (Fisher´s exact test, p=1 (2 sided)) (Table 16)

Table 16. DKA at onset and increased risk haplotype

		Presence of DKA		Total
		yes	no	
Increased risk HLA	no	4	8	12
	yes	32	67	99
Total		36	75	111

-Nor with the presence of islet auto-antibodies (X^2=3.124; df=5; p=0.681) (Table 17).

Table 17. DKA and auto-antibodies

		Anti-IA2 only	Anti-GAD only	antiGAD+anti-insulina	antigad+anti-IA2	three antibodies present	Total
DKA	yes	26	12	9	16	12	87
	no	38	19	20	40	31	171
Total		64	31	29	56	43	258

4.5 **HLA characterization**

-HLA characterization for DR (table 25), DQ (table 26) and "increased risk genotypes" (table 27) are described below. DRB*03 and DRB*04 are clearly the most frequent ones among the DR alleles (63.16% of the possible alleles), with significantly more children presenting at least one DRB*04 allele than one DRB*03. However, when looking at the overall allelic frequency, the difference between DRB*04 and DRB*03 is not significant. DRB*03, DRB*04 or both are present in 89.47% of our patients. Regarding DQ: DQ*02 and DQ*03 are the most frequent ones, with overlapping CIs and no significant differences in their appearance. A total of 96.61% of our children present either one or both of them. The presence of at risk genotypes is described in table 19. Regarding protective alleles (DRB*07, DRB*11, DRB*13, DRB*15), we found that they add up to 19.73% of the total alleles reported for our patients.

-When looking at the co-appearance of DRB*03 with DQB*02, we found that the presence of DRB*03 is accompanied by the appearance of DQB*02 in 100% of our patients (table 18). When looking at DRB*04 with DQB*03, we found that the presence of DRB*04 predicts the presence of DQB*03 in 87.5% of our patients (table 19).

Table 18. 2x2 table for DRB*03 & DQB*02

		HLA DQB*02		**Total**
		No	**Yes**	
HLA DRB*03	**No**	34	23	57
	Yes	0	57	57
Total		34	80	114

Table 19. 2x2 table for DRB*04 & DQB*03

		HLA DQB*03		**Total**
		No	**Yes**	
HLA DRB*04	**No**	31	5	36
	Yes	8	70	78
Total		39	75	114

-We found a non-significant difference in the distribution of higher risk alleles among age categories, with a slightly bigger proportion in the younger group (Freeman-Halton extension of Fisher´s exact test; p=0.25) (Table 20). We then categorized age into children younger and older than 5 years of age, and still found no significant differences (Fisher´s exact test; p= 0.125) (table 21)

Table 20. Increased risk alleles and age-groups (3 categories)

		age category			
		age 0-4	**age 5-9**	**age 10-13**	**Total**
Increased risk HLA alleles	No	2	6	4	12
	Yes	41	35	24	100
Total		43	41	28	112

Table 21. Increased risk alleles and age-groups (2 categories)

		age category		
		age 0-4	**age 5-13**	**Total**
Increased risk HLA alleles	No	2	10	12
	Yes	41	59	100
Total		43	69	112

-We didn´t find any differences when stratifying by sex (X^2=0.004; df=1; p=0.949) (Table 22).

Table 22. Increased risk alleles and gender

		sex		
		Male	**Female**	**Total**
Increased risk HLA alleles	**No**	6	6	12
	Yes	52	50	102
Total		58	56	114

-When looking at the protective alleles, we found no difference in their distribution among age categories (X^2= 0.761 ; df=2; p= 0.684) (table 23) or gender (X^2= 0.846; df=1 ; p=0.358) (table 24).

Table 23. Protective alleles and age-groups

		age category			
		age 0-4	**age 5-9**	**age 10-13**	**Total**
Protective HLA alleles	No	29	24	17	70
	Yes	14	17	11	42
Total		43	41	28	112

Table 24. Protective alleles and gender

		sex		
		Male	**Female**	**Total**
Protective HLA alleles	No	39	33	72
	Yes	19	23	42
Total		58	56	114

Table 25. Distribution of DR

	Individuals with at least one of the following DR values	% (95% CI)	allele present	% (2N)
DR*1	24	21.05 (13.5 - 28.5)	24	10.53
DR*3	57	50 (41 - 59)	63	27.63
DR*4	78	68.42 (59 – 77)	81	35.53
DR*7	19	16.67	19	8.33
DR*8	6	5.26	6	2.63
DR*9	5	4.39	5	2.19
DR*10	1	0.88	1	0.44
DR*11	6	5.26	6	2.63
DR*13	15	13.16	15	6.58
DR*15	5	4.39	5	2.19
DR*16	3	2.63	3	1.32

Table 26. Distribution of DQ

	Individuals with at least one of the following DQ values	**%** (95C% CI)	**% (2N)**
DQ*02	81	69.49 (61.5 -77.5)	40.25
DQ*03	78	66.1 (58 - 74)	36.44
DQ*04	6	5.08	2.54
DQ*05	33	27.97 (20 – 36)	14.83
DQ*06	14	11.86	5.93

Table 27. Distribution of increased risk alleles/genotypes

	Increased risk alleles and genotypes	N	% (95C% CI)
	Individuals with DR3 or DR4 or both	102	89.47 (83.9 – 95)
	Individuals with DQ2 or DQ3 or both	114	96.61
Genotype	**Individuals with DR3-DQ2 in homozygosis**	5	4.39
Genotype	**Individuals with DR4-DQ3 in homozygosis**	4	3.51
Genotype	**Individuals with DR3-4/DQ2-3**	31	27.19 (19 – 35)

4.6 Autoantibodies

Distribution of positive results is described below (table 28). Of note, is the fact the anti-Insulin antibodies always appeared in combination with anti-GAD or both anti-GAD & anti-IA2, but never by themselves or with anti-IA2.

* 62.1% of patients in whom antiGAD was measured were positive.

* 72.3% of patients in whom anti-IA2 was measured were positive.

* 74% of patients in whom anti-Insulin was measured were positive.

* 19.7% of patients in whom all 3 antibodies were measured were positive for all three of them.

* 24.7% of patients in whom anti-GAD and anti-IA2 were measured were positive for both of them.

* 11.1% of patients in whom anti-GAD and anti-insulin were measured were positive for both of them.

Table 28. Distribution of anti-islet antibodies

	Patients	**%** (95% CI)
AntiGAD	162	62.1 (56 - 68)
Anti-IA2	167	72.3 (66 - 78)
Anti-insulin	74	28.4 (22 - 34)
All 3 present	45	19.7 (14.5 - 25)
AntiGAD + anti-IA2	57	24.7 (19 - 31)
AntiGAD + anti- insulin	29	11.1
Anti-IA2 + anti-insulin	0	0
Antibody negative	30	13.2

- We found that the presence of antibodies (all types) was significantly increased in women (X^2=16.889; df=5; p<0.05) (Table 29).

Table 29. Sex distribution of anti-pancreatic autoantibodies

		Anti-IA2 only	Anti-GAD only	antiGAD + anti-insulin	Anti-GAD + anti-IA2	three antibodies present	Total
sex	**male**	39	16	10	23	15	128
	female	26	15	19	34	30	136
Total		65	31	29	57	45	264

- Also our data suggests that in our population, the presence of anti-IA2 significantly increases in children older than 5 years of age (X^2=7.87; df=2; p<0.05) (Table 30).

Table 30. Distribution of anti-IA2 antibodies among the three age categories

		Presence/Absence of anti-IA2 Ab		
		Absence of anti-IA2 Ab	**Presence of anti-IA2 Ab**	**Total**
age category	**age 0-4**	36	60	96
	age 5-9	20	77	97
	age 10-13	8	30	38
Total		64	167	231

- We looked at the possible relationship between DRB*03 and DRB*04 alleles (separately) and the positivity of autoantibodies (individually or combined), and found a significant relationship between DRB*04 and anti-IA2 (X^2=8.592; df=1; p= 0.03) (Table 31). No relationship was found between DRB*03 and any of the possible combinations of antibodies. Also, no relationship was found between the two alleles and the negativity of islet antibodies.

Table 31. Relationship between DRB*04 and anti-IA2

		Anti-IA2 positive		
		No	**Yes**	**Total**
HLA DRB*04	**No**	12	20	32
	Yes	9	63	72
Total		21	83	104

4.7 Associated autoimmunity

-At the time of the study, 7.9% of our patients (95% CI=4.8%– 11%) suffered from AAIDs, including celiac disease, autoimmune Hepatitis, hypothyroidism and hyperthyroidism (table 32).

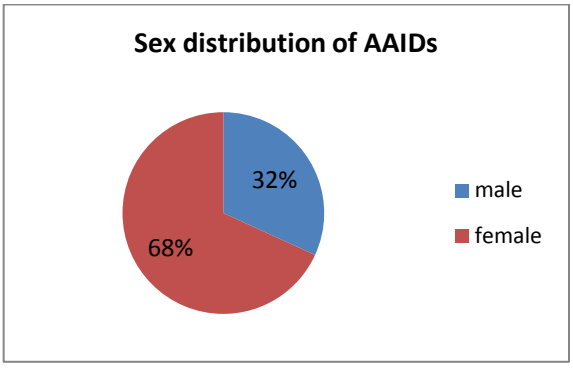
Table 32. AAIDs

	Celiac Disease	Autoimmune Hepatitis	Hypothyroidism	Hyperthyroidism
Number (M/F)	16 (7/9)	1 (0/1)	3 (0/3)	2 (0/2)
%	5.7	0.36	1.1	0.7

M: male. F: female

-We found the proportion male:female for the presence of AAIDs to be 7:15, with 31.8% males (95% CI= -10 – 73.8%) and 68.2% females (47.2 – 89.2%). The difference is not statistically significant (figure 14).

Figure 13**.** Distribution of AAIDs among sex



4.8 Regression analysis

-We found no relationship between the age of onset and having "high risk" HLA, presence/absence of antibodies and gender (F= 0.137 on 3 and 85 DF, p-value: 0.9377. Residual standard error = 44.77; df=85.Multiple R^2 =0.004812;Adjusted R^2=-0.03) (Table 33).

Table 33. Results from the model studying relationship between age of onset and risk HLA, antibodies and gender.

Coefficients

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	75.38	14.19	5.31	8.52e-07 ***
Risk_HLA_yes	7.14	13.92	0.51	0.609
three_antibodies_+	0.01	12.08	0.001	0.999
Female	3.90	9.72	0.402	0.689

-When modeling the relationship between presenting with DKA at onset and having "high risk" HLA, presence/absence of antibodies, sex and age at onset, we found that the risk of having DKA increases with age after adjusting for having high risk HLA, positivity of 3 antibodies and female sex (Null deviance: 107.77 on 86 df. Residual deviance: 102.44 on 82 df. AIC: 112.44). (Table 34). The OR increases 1.16 times for every one year increase in age (Table 35).

Table 34. Results from model studying relationship between presenting with DKA and risk HLA, antibodies, gender and age of onset.

Coefficients

	Estimate	Std. Error	z value	Pr(>|z|)	2.5 %	97.5%
(Intercept)	0.12	0.82	0.15	0.88	-1.45	1.86
RIsk_HLA_yes	-0.30	0.74	-0.41	0.68	-1.93	1.1
three_antibodies_+	0.09	0.62	0.15	0.88	-1.1	1.4
sexoFemale	-0.01	0.49	-0.024	0.98	-0.98	0.95
ONSET_AGE_YRS	0.15	0.07	2.2	0.029*	0.02	0.29

Table 35.OR

	OR	2.5%	97.5%
(Intercept)	1.13	0.23	6.43
RIsk_HLAyes	0.74	0.14	2.95
three_antibodies_+	1.1	0.33	4.01
sexoFemale	0.99	0.37	2.6
ONSET_AGE_YRS	1.16	1.02	1.34

V. **DISCUSSION**

Our results confirm the high incidence of T1D previously described in the Canary Islands by Carrillo et al[65] and Belinchón et al[64]. Our findings and their published results report incidence values much higher than those reported by EURODIAB[54] and DIAMOND[55] for Spain (13/100,000) and higher than the mean (17.7/100,000) and the highest (27.6/100,000) incidence reported by Conde Barreiro et al for the rest of the country[63]. Our incidence values places us immediately behind Finland, Sweden and Sardinia, and in front of the rest of European[60] countries. Indeed, the Canary Islands seem to be an additional exception to the North-South gradient hypothesis. However, even though it is not likely that there will be major differences, given that we measured the incidence in children aged less than 14 years of age and that EURODIAB, DIAMOND, previous data from the Canary Islands, and average data for the rest of Spain were computed for children aged less than 15 years of age, we must be careful when establishing comparisons and before reaching conclusions regarding possible differences in the findings.

When it comes to comparing with African countries, we are aware that poor health systems and high infant mortality due to infectious diseases and conflict make it difficult to effectively determine the real number of incident cases in the majority of countries in the continent. Looking at available data, we report an incidence greater than that found in Sudan (10.1 / 100,000) in 1990[94] , Tanzania (1.5 / 100,000)[95] and the rest of the African continent (6.4 / 100,000), according to recent data published by the International Diabetes Federation (IDF)[96]. Outside of Europe and Africa, Lawrence et al[97], on behalf of the SEARCH Study Group, recently reported similar incidence rates to ours for the Non-hispanic white under 15 population in the US for the 2002-2009 period. Haynes et al report estimates somewhat lower than ours for the Australian pediatric population during the 2000-2011 period (23.6/100,000)[98].

Particularly striking is the large inter-annual variability we found for Gran Canaria, with values ranging from 20.46 to 44.22/100,000. Similar variability was reported by Belinchón et al[64] for the island of La Palma, with incidences ranging from 12.7 to 67.2/100,000 throughout the 15 year period. These results cast doubt on the reliability of reported incidences of T1D from isolated annual values.

Our data shows no significant trend in the annual incidence of diabetes from 2006 to 2014. Furthermore, when adding data from 1995-96 to the analysis, there still seem to be no trends in

the number of cases. This last part of the analysis must be interpreted with care, since in order to be able to analyze the trend we are assuming that the incidence of T1D in Gran Canaria in 1995-96 is the same as the mean incidence described for the archipelago by Carrillo et al[65].

In the rest of Spain we find heterogeneity in the reports[63]. Some regions like Malaga[99], Aragón[100] and Cantabria[101] report increasing trends whereas others maintain uniform rates[102]. Similar heterogeneity is reported internationally. Patterson et al report non-uniformity in rates of increase over Europe in a recent review of data from Eurodiab[60]. Countries like Czech Republic[103], Finland[57], and Sweden[58] report plateaus in their previously increasing trends after 2005. Australia[61] reported a plateau after 2005, but a more recent analysis by Hynes et al[98] reports a sinusoidal pattern with 5-yearly peaks and troughs for the 2000-2011 period. On the other hand, countries like Germany[104] and the US[97] recently reported an ongoing increasing trend.

We found no significant difference in gender distribution, in line with data published from international reviews for children[55,66], or other population studies from Australia[61], Sweden[67] and Germany[104]. Some studies seem to show a slight difference in this respect, and always reporting a higher incidence in boys: in Sweden, in patients aged between 15-35[68]; in Finland with boys over 13 years of age[105] ; in Massachusetts with children under 6[106] and in other reports from Italy[59] and other parts of Spain[100].

Mean age at onset of our patients is younger than that reported by other authors, but effective comparisons cannot be done due to differences in the age of patients included in the studies. Belinchón et al[64] reported a mean age at onset of 8.5 years for the study in La Palma, including children aged less than 15 years. Lawrence et al presented data from the SEARCH study group in 2014[97] reporting a mean age of diagnosis of 9.9 years in patients younger than 15 years of age; Fröhlich-Reiterer et al reported a mean age at diagnosis onset of 8.7 years in Austrian/German patients from the DPV database aged less than 20 years[107]. In Korea, Jung et al[108] reported a mean age at diagnosis of 8.3±3.7 years for a sample including both children and adolescents.

Weight, height and BMI have been studied as both a determinant and a consequence of the appearance of T1D[48,112]. Both girls and boys with T1D onset in our population present, on average, height and BMI in the 50th percentile. Comparison with a control group would be

necessary to reach meaningful conclusions regarding the possible influence of BMI in the development of T1D for our population.

Regarding age distribution at onset, we found different results for the two sampling methods: while we found no differences using the census sample, we did find significantly less children aged 10-13.99 than in either of the 0-4.9 and 5-9.99 groups when looking at the cross-sectional sample. The difference is probably due to the fact that older children spend less time in our Unit since they are switched to adult care after they turn 14 years of age. By taking a cross-sectional sample, the probability of children aged 10-13.99 years at onset still being in the unit is smaller than for younger children. Other authors have classically described the age distribution as bimodal, with peaks at 4-6 years and 10-14 years[109,110]. Data from national registries from Finland[57] , Australia[61] and Germany[104] report greater incidence in children older than 4 years of age. Our results do show a greater number of children in the older age groups, but without reaching statistical significance. Belinchón et al[64] reported similar findings for the island of La Palma. Our conclusions are limited by the low number of subjects in the study. Perhaps greater numbers would have allowed for more significant results.

We found no significant difference in seasonality, with small non-significant peaks in September and February. Similar findings were reported by Carrillo et al[65] and Belinchon et al[64] in the Canary Islands. International reports describe a non-generalized seasonal pattern in the distribution of cases[62], especially in areas of high incidence. In our case, perhaps the number of cases wasn't enough to reach statistical significance, or maybe the climatic stability allows for less variability in Gran Canaria. In any case, studies including larger number of patients would be needed to reach meaningful conclusions.

Viral infections have been previously described as a potential trigger of T1D[111] and some reports mention increased numbers of T1D in children after H1N1 epidemics[112]. We found an interesting, albeit non-significant, correlation between the number new T1D cases and the incidence of flu the previous year. Once again, we are limited by the low number of years analyzed. Kondrashova et al[113], from the DIPP study group in Finland, recently published a paper looking into the relationship between antibodies against influenza A and islet autoimmunity and concluded that in children with increased genetic susceptibility for T1D, influenza A infections were not associated with the development of islet autoimmunity. Studies

in children with a different genetic predisposition to T1D need to be done before any definitive conclusions can be reached regarding the influence of flu in the appearance of T1D. Like Fourlanos et al[43] concluded in their paper, perhaps it is in children without high genetic risk where the environmental factors have a greater impact.

Regarding the severity of presentation, we found that 34.2% of our patients presented with DKA at onset. In Spain, Oyarzabal et al recently published the results of a multicenter study, reporting a mean incidence of DKA during T1D onset in children of 39.5%[114]. Lévy-Marchal et al report similar incidence in a 2001 report from EURODIAB[115], finding its appearance in about 40% of new onsets in 90% of the centers that are part of EURODIAB. Other multicenter studies in European countries show great variation, with incidence ranging from 7.3% in Sweden to 21.3% in Lithuania[116], 37.2% in Austria[117] and 43.9% in France[118]. Other studies from reference centers within countries show values ranging from 15.2% in northern Finland[119], 18.2% in Bulgaria[120], 23% in Poland[121], 26.3% in Germany[122], 27% in New Zealand[123], around 30% in the US[124], 31% in Oman[125], 41.9% in China[126], 48.5% -50.8% in Turkey[127],[128] and 55.2% in Saudi Arabia[129].

We found a significantly greater proportion of DKA in the younger age group, similarly to other authors[117,120,121,122,124]. We were surprised by the results obtained in the regression analysis looking at factors influencing the appearance of DKA. When adjusting for sex, having high risk HLA and three antibodies positive, the OR of DKA increased 1.16 times for every one year increase in age. We have found no authors reporting similar findings.

The lack of difference regarding sex distribution contrasts to that reported by other authors who report a greater incidence of DKA in females[122,124,127]. Also, we found no significant relationship between the presence of DKA at onset and having increased risk HLA or with the presence of antibodies.

Comparison of our results regarding the genetic component of our patients with those reported for different ethnic groups is aligned with results from studies reporting the complex ancestry influencing the inhabitants of Gran Canaria. Regarding DRB* (Annex 1), we found that DRB*03 and DRB*04 are the two most frequent alleles, with no statistically significant

difference in their allelic frequency. However, different proportions have been reported in different ethnic groups. Studies in Caucasians and Hispanics by the SEARCH study[88] and in Caucasians by the T1DGC[34] show DRB*04 as the most common allele in those groups, followed by DRB*03, with slight differences in their frequency. On the other hand, studies done in African Americans[130] and T1D patients from the north of Africa (Tunisia)[38] report reversed results, with DRB*03 presenting more frequently. Japanese patients[131] present DRB*04 as their most frequent risk-allele, but almost non-existing presence of DRB*03 (and this is true for both acute-onset and fulminant T1D[132]). Philippines' patients present a lower presence of both DRB*03 and DRB*04, and a greatly increased presence of DRB*15[34]. Our results are aligned with those reported for the Caucasian and Hispanic population by the SEARCH study[88], and only hold small differences in proportion with those reported by the T1DGC[34] (27.63% for DRB*03 vs 34.1% in T1DGC data and 35.53% for DRB*04 vs 42.7%). DRB*07, however, presents more similarities with results reported from African Americans[130] and T1D patients from Tunisia[38] (8.33% in Gran Canaria vs 3.6% in the T1DGC study, 10.2% in African Americans and 6.82% in Tunisia).

Regarding DQB* (Annex 2), our population presents DQB*02 and DQB*03 as the most frequent alleles. The third more frequent allele is DQB*05, followed by DQB*06 with a similar presence. The least frequently found allele in our population was DQB*04. When compared to other ethnic groups, we find that DQB*02 and DQB*03 are still the most frequent ones in Caucasian, Hispanic[88], African American[130] and North African populations[38]. Caucasian and Hispanic populations seem to have a higher frequency of DQB*03. However, African American and North African groups seem to show an inversion in frequency, with DQB*02 clearly being the most frequent one in African Americans, and not so clearly in North African populations. Similarly to what happened with DRB*, Japanese population[131] presents a distinct haplotype, with DQB*03 being the most frequent allele, followed by DQB*04. Philippines' patients[34] present DQB*05 as their most frequent allele (38%), followed by DQB*02 and DQB*03 (with lower frequencies than in the rest of the non-Asian populations). Also, they present a high prevalence of DQB*04 (11.9%), even though not as high as that presented by Japanese population.

When looking at genotypes (Annex 3), we observed that we present a lower prevalence for the DRB1*03-DQB1*02/DRB1*04-DQB1*03 genotype than reported for Caucasian population[34] (27% vs 38-40%) and higher than reported for Tunisian[38] and African American patients[130] (27% vs 11% and 10.8% respectively) (this comparison is done under the assumption of a linkage disequilibrium of 100% whenever we have DRB1*03-04 and DQB1*02-03. This assumption is based on non-published data analyzed by the T1DGC from T1D children from Gran Canaria showing 100% linkage disequilibrium between DRB*03 and DQB*02 and DRB*04 and DQB*03).

In general, our results present similarities to those reported for Caucasian population[34,88] but also to African American[130] and North African reports[38], in line with reports from other studies supporting the mixed genetic pool for inhabitants of the Canary Islands[79,80,81]. However, differences in the selection methods limit the ability to reach meaningful conclusions due to the possibility of selection bias that could interfere in the comparisons. Age of participants varies among studies: patients from Gran Canaria presented with T1D before age 14, while patients from the SEARCH study did so before 20 years of age; patients from the T1DGC were under 35 years of age; African American patients (coming from the SEARCH, T1DGC, DCT/EDIC and GoKinD databases) were both adults and children also with onset under 35 years of age; the study from Tunisia also included children and adults with a mean age of 16 years; and, finally, patients from Japan also included children and adults, with a mean age of 16 years. Furthermore, patients from the T1DGC were selected only if they had siblings with T1D, which might add further bias to the age difference among the studies.

Regarding distribution of high risk alleles between sex and age groups, we found a non-significant difference in the distribution of high risk alleles among the three age categories, with a slightly higher proportion of children in the younger age group presenting with high risk alleles (95.4% for children aged 0-4.9 years vs 85.7% for children aged 5-9.9 and 10-14). Other authors have reported higher incidence of high risk alleles and haplotypes in younger children[30]. Differences in results might be due to the low number of children in our sample. We found no difference in the distribution between males and females.

Regarding anti-islet autoantibodies, and similarly to reported values in the literature[108], 86.8% of our patients were positive for at least one autoantibody. Savola et al[133] reported 97.8% of patients from the Childhood Diabetes in Finland Study Group presenting with at least one positive anti-islet antibody at onset, but they screened for 4 antibodies (GADA, IA-2, IAA and ICA) whereas we only did for three. We found positivity for two or more autoantibodies in about 55-60% of our study population. Data from the DAISY study differs from ours, with 89% of their selected children[134] presenting it.

Data from TEDDY (Annex 4) is also somewhat different[135]: they report higher levels of anti-insulin antibodies (81% vs 28.4%) and lower levels of GAD-antibodies (44% vs 62.1% ($p<0.001$)) than those obtained for patients in Gran Canaria. Also, in their population sample insulin-antibodies appear in isolation (as well as in combination with others), whereas in patients from Gran Canaria they only do so with GAD or with both GAD and IA-2 antibodies. In TEDDY they only appear once in isolated combination with IA-2, not very different from our results in which it did not appear in isolated combination with IA-2 at all. The difference in findings might be due to the fact that the population for DAISY and TEDDY is selected based on their high risk of developing T1D and our sample is taken from the general T1D population. Also, their mean age at diagnosis is younger (2.3 vs 6.4 years of age). Both factors might explain the lower presence of autoimmunity in our population. The presence of different environmental factors might also be an influencing factor.

In the U.S. SEARCH for Diabetes in Youth study, 52% of newly diagnosed children were positive for GAD-antibodies, 60% were positive for IA–2-antibodies, and 38% were positive for both[136]. The Childhood Diabetes in Finland Study Group (DIPP) found that 91% of children with newly diagnosed T1D were positive for at least two autoantibodies, and 71% for three or more. IA–2-antibodies were detected in 86% of cases[133]. ZnT8-antibodies are present in 60–80% of new-onset T1D patients, and in 25% of those who are negative for GAD, IA2, anti-insulin and islet cell autoantibodies (ICA)[24]. We cannot provide our own estimates since the measurement of ZnT8 antibodies was not part of our protocol.

T1DGC data shows a prevalence of 47.4% for GADA and 45.7% for IA-2 antibodies[73], both of them lower than those reported for our population. Differences in selection criteria (siblings with

T1D for the T1DGC sample), likely differences in environmental factors in both populations, and longer time since diagnosis probably account for the reported differences.

We found no relationship between high risk alleles and genotypes and the presence of anti-islet autoantibodies. Finnish studies present opposite results. The DIPP study reports a relationship between high risk HLA (defined for this analysis as DQB*02) and autoimmunity in children that haven't developed T1D[137]. Similar results are reported by the Childhood Diabetes study group in children that have gone to develop T1D[138]. Differences might be due to the inherent differences and environmental factors surrounding both populations and/or the small number of cases in our study.

Regarding the higher frequency of anti-islet autoimmunity in women, we haven't found other authors reporting similar findings. However, autoimmunity in general and in association with T1D has been often associated with female gender[73].

When looking at AAIDs, the nonsignificant predominance we found in girls vs boys is aligned with findings reported by the majority of authors, stablishing a clear predominance of Celiac disease[139] and autoimmune thyroid dysfunction in females[140]. The low number of subjects with T1D and autoimmunity in our sample might explain the lack of significance in our results.

In adults thyroid dysfunction has been described as the most frequent AAID associated with T1D. Its presence grows in frequency with longer duration of T1D[140], later age at diagnosis, and female gender[73]. In pediatric population celiac disease and thyroid disease have been described as the most frequent ones[141].

Prevalence of celiac disease ranges from around 0.5 to 4% of children in the general population, according to reports from Sweden[142] and the USA[143], depending on the presence (increased risk) or absence of family relatives with the disease. In children with T1D the prevalence ranges from 4 to 11%[139], and the risk has been characterized as greater for those diagnosed younger than 5 years of age[141]. In Saudi Arabia, Al-Agha et al recently reported results from a cross-sectional study in which they found celiac disease in 19.7% of patients aged 0-18 years of age[144]. In Spain,

a 2008 report from Madrid reported co-occurrence of T1D and celiac disease in 8% of their patients[145].

Regarding autoimmune thyroid disease, the study regarding Arab population from Al-Algha et al reported a prevalence of 4.8%[144]. Brazilian studies report a prevalence of 7.9%[146], and European multicenter studies report a prevalence of 10% among children with T1D[140]. Some report prevalence as high as 25%[76,147]. All those studies present higher prevalence than the one found in our population. The results might be explained by the fact that they have an older population and with longer duration of T1D compared to ours.

Overall, we present a lower prevalence of both celiac disease and autoimmune thyroid dysfunction than that reported by other authors. The fact that celiac disease presents a higher prevalence than thyroid dysfunction in our sample (5.7% vs 1.8%) compared to other reports might be explained by the young age of our patients and the short time since diagnosis. Recent publications reinforce the evidence supporting the increased development of thyroid dysfunction with older age[148].

Our study has several strengths, as well as weaknesses. We found that the utilization of two different sampling methods ("census sampling" to compute the incidence and cross-sectional sampling to characterize T1D onset in our patients) allowed for better exploitation of the strengths and minimization of the weaknesses of our study. Regarding the study of the incidence in the island of Gran Canaria, the main strengths of our study are the utilization of "census sampling" (allowing for a high degree of ascertainment) and the inclusion of a 9 consecutive years cycle. On the other hand, the low number of cases overall limits the conclusions that can be drawn about age distribution, temporality, seasonality and association with flu. Also, we used the total population per municipality to compute the expected number of cases per municipality, since no data for less than 14 years of age was available at the time of the analysis. Thus, in order for the derived conclusion to be true we are assuming that the distribution in the number of the children less than 14 is similar to the distribution of the total population per municipality.

The utilization of a cross-sectional sample for the characterization of T1D patients from Gran Canaria at onset allows us to obtain information from a larger group of children, allowing for more significant conclusions in the analysis. On the other hand, it limits our ability to reach

conclusions given the possible influence of bias affecting our results. A clear example is the difference in age group distribution observed between the sampling methods. Careful interpretation of our results is required, and confirmation from prospective studies would give more reliability to the obtained results.

Lack of use of high resolution HLA genotyping and lack of results from a control group limits our ability to reach conclusions regarding the genetic background of the whole population as well as our capacity to identify the most at risk and protective alleles/genotypes present in our sample.

VI. **CONCLUSIONS**

Conclusions

1. We report for the first time the incidence of T1D during a 9 year cycle for the island of Gran Canaria, showing the highest value reported for Spain and one of the highest values reported worldwide.
2. Distribution among age groups and gender is similar.
3. We do not find any temporal trends nor seasonality in the onset of cases.
4. Our results do not support the classically described north-south gradient described for the incident cases of T1D in Europe.
5. The proportion of cases presenting with DKA at onset is 34.2%. DKA is more frequent in the youngest age group (0-4.9 years of age). However, after adjusting for high risk HLA, positivity of three antibodies and sex, we found a significant increase in the OR of developing DKA with increasing age.
6. Our findings regarding the genetic load of our patients (HLA) show that DRB*03 and DRB*04 are clearly the most frequent ones among the DR alleles, with significantly more children presenting at least one DRB*04 allele than one DRB*03. Regarding DQ, DQ*02 and DQ*03 are the most frequent ones, with overlapping CIs and no significant differences in their frequencies. 96.61% of our children present with either one (DQ*02 or DQ*03) or both of them. The genotype with the highest risk for T1D (DR3-4/DQ2-3) appears in 27.2% of our patients.
7. Islet autoimmunity in our patients appears in similar percentages to those described in the literature. We present a higher prevalence of appearance in girls. Regarding the relationship between islet autoimmunity and *HLA*, we found no relationship between its appearance and the presence of high risk alleles. However, we did find a significant relationship between the presence of DRB*04 and anti-IA2 antibodies. Regarding distribution, we present lower levels of insulin-antibodies than those reported by other authors, as well as lower frequency of combinations between 2 or three antibodies.
8. Our patients present a low prevalence of AAIDs, with a non-significantly greater frequency in girls and a predominance of celiac disease.

VII. ANEX TABLES AND TRANSLATION

Annex I. Comparison of frequency (%) of HLA DR in different populations.

HLA-DR	**1. Gran Canaria** (n=114)	**2.NonHipanic Whites**[88] (n=1285)	**3.Caucasian**[34] (n=1214)	**4.Hispanic**[88] (n=211)	**5.NonHipan Blacks**[88] (n=166)	**6.African American**[130] (n=772)	**7.Tunisia**[38] (n=88)	**8.Japan**[131] (n=132)	**9.Asian**[34] (Philipines) (n=76)
DRB*01	10.53	8.06	7.5	3.79	6.63	5.3	4.55	4.5	---------
DRB*03	27.63	28.44	34.1	27.01	28.31	26.83	38.63	---------	22.4
DRB*04	35.53	38.76	42.7	41.47	21.98	23.32	23.86	33.7	21
DRB*07	8.33	4.98	3.6	5.21	9.34	10.2	6.82	---------	1.3
DRB*08	2.63	2.42	3.5	4.5	0.3	2.56	1.7	11.36	---------
DRB*09	2.19	1.67	1	1.42	7.53	9.1	2.84	25.4	11.8
DRB*10	0.44	---------	0.3	---------	---------	0.7	2.27	---------	---------
DRB*11	2.63	1.4	1.9	1.89	1.81	3.75	2.84	---------	1.3
DRB*12	0	0	0.3	0	1.51	1.8	1.7	4.9	5.9
DRB*13	6.58	5.61	3.1	2.85	3.7	10	9.66	6.8	1.3
DRB*15	2.19	---------	0.6	---------	---------	1.69	2.27	3	31.5
DRB*16	1.32	1.01	1.1	0.71	0.6	1.32	2.84	---------	---------

(1) Gran Canaria. Under 14 years of age; (2, 4, 5) Under 20 years of age. 2013 US SEARCH study; (3, 9) T1DGC. Caucasians and Asians. Under 35 years of age. Chosen as 2 siblings from the same family; (6) Under 35 years of age. 2013. HLA in African americans. Data from SEARCH, T1DGC, DCT/EDIC and GoKinD; Children and adults. Mean age 16 years. HLA in Tunisia (7); Children and adults. Mean age 16 years. HLA in Japanese (8).

Annex II. Comparison of frequency (%) of HLA DQ in different populations

HLA-DQ	**1. Gran Canaria** (n=114)	**2.NonHipanic Whites**[88] (n=1285)	**3.Caucasians**[34] (n=1214)	**4.Hispanics**[88] (n=211)	**5.NonHipanic Blacks**[88] (n=166)	**6.African Americans**[130] (n=772)	**7.Tunisia**[38] (n=88)	**8.Japan**[131] (n=132)	**9.Asians**[34] (Philipines**)** (n=76)
DQB*02	40.25	33.42	38.5	32.93	44.58	46.81	42.61	---------	23.7
DQB*03	36.44	41.83	45.1	44.07	24.39	29.36	38.64	40.9	24.8
DQB*04	2.54	2.42	3.5	4.5	0.3	2.26	2.27	29.9	11.9
DQB*05	14.83	8.1	9	9.48	9.93	10.42	8.52	---------	38
DQB*06	5.93	5.57	3.7	2.61	4.82	7.75	7.95	7.2	1.3

Annex III. Comparison of frequency (%) of increased risk HLA genotypes in different populations

HLA-risk genotypes	**1. Gran Canaria** (n=114)	**3.Caucasians**[34] (n=1214)	**6.African Americans**[130] (n=772)	**7.Tunisia**[38] (n=88)	**9.Asians**[34] (Philipines**)** (n=76)
DRB*03-DQB*02 / DRB*03-DQB*02	4.39	---------	8.9	14	---------
DRB*04-DQB*03 / DRB*04-DQB*03	3.51	---------	----------	6	---------
DRB*03-DQB*02 / DRB*04-DQB*03	27.19	38.1	10.9	11	14.6

Annex IV. Comparison of antibodies frequencies

	% (95% CI)		DIPP[133]	TEDDY[135]	SEARCH[136]	Korea[108]
AntiGAD	62.1	(56 - 68)	----------	44	52	74
Anti-IA2	72.3	(66 - 78)	85.9	----------	60	20.5
Antiinsulin	28.4	(22 - 34)	----------	81	----------	24.7
All 3 present	19.7	(14.5 - 25)	71	3	----------	----------
AntiGAD + anti-IA2	24.7	(19 – 31)	----------	----------	38	----------
AntiGAD + anti- insulin	11.1		----------	28	----------	----------
Antibody negative	11.6		2.1	6	----------	----------

ANEXO V. TRADUCCIÓN AL CASTELLANO

Introducción

De acuerdo con la Asociación Americana de Diabetes (AAD), la Diabetes Mellitus se define como un grupo de trastornos metabólicos caracterizados por la presencia de hiperglucemia secundaria a defectos en la secreción de insulina, a defectos en su acción o a ambos. La AAD clasifica la Diabetes en 4 categorías, de acuerdo a los mecanismos subyacentes que la producen:

1. **Diabetes Tipo 1** (T1D): secundaria a la destrucción de las células-β pancreáticas, normalmente desencadenando un déficit absoluto de insulina.

2. **Diabetes Tipo 2**: debida a un defecto secretor progresivo de insulina secundario a la presencia previa de resistencia a su acción.

3. **Diabetes Gestacional**: diabetes diagnosticada en el segundo o tercer trimestre del embarazo, sin llegar a ser un caso claro de diabetes manifiesta.

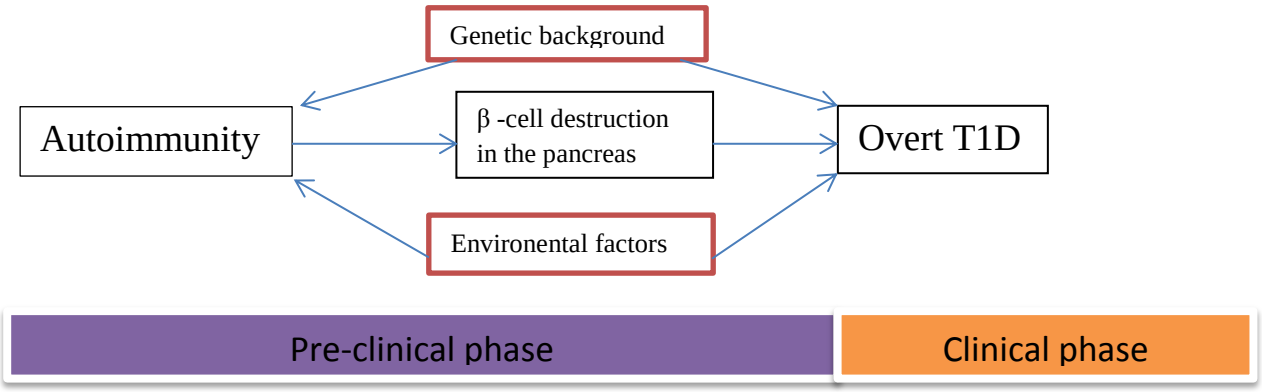
4. **Tipos de diabetes específicos,** debidos a otras causas: por ejemplo, diabetes monogénicas (como la diabetes neonatal o la diabetes tipo MODY), enfermedades del páncreas exocrino (como la fibrosis quística), y la diabetes secundaria a tratamiento farmacológico o a sustancias químicas (como en el tratamiento del VIH/SIDA o tras trasplante de órganos).

La Diabetes Mellitus Tipo 1 (DM1) es el tipo de diabetes más frecuentemente encontrado en la edad pediátrica, y el objeto de estudio del presente trabajo. La DM1 es una enfermedad crónica compleja, desencadenada por factores no del todo conocidos que ejercen su efecto en individuos con una cierta predisposición genética. Comienza con una fase preclínica, que puede durar desde meses hasta años, en la que aparece una destrucción autoinmune de las células β productoras de insulina en los Islotes de Langerhans en el páncreas. El ritmo de destrucción presenta gran variabilidad entre individuos, dependiendo de factores genéticos y ambientales no del todo conocidos. Cuando la destrucción de la masa de células-β alcanza un nivel lo suficientemente elevado como para afectar la secreción de insulina (destrucción del 80-9% de las células), los niveles de azúcar en sangre comienzan a elevarse hasta alcanzar niveles de diabetes manifiesta. La elevación crónica de los niveles de azúcar en sangre conlleva la aparición de complicaciones micro- y macrovasculares a medio y largo plazo como la retinopatía, nefropatía y neuropatía diabéticas, así como un aumento en la incidencia de problemas cardiovasculares.

Estudios previos en nuestro archipiélago sugieren que la incidencia de DM1 en población pediátrica en Canarias se encuentra entre las más altas publicadas para España y para el resto de Europa. Con el presente estudio se pretende profundizar en el conocimiento de la DM1 en nuestro entorno, con la finalidad de identificar

posibles factores desencadenantes y mejorar la asistencia que se presta a nuestros pacientes.

Figure 6. Natural History of T1D



Justificación del estudio

La DM1 es una enfermedad crónica que requiere que los individuos afectos y sus familias cambien por completo sus hábitos y estilo de vida. Requiere tratamiento mantenido, y conlleva riesgo de aparición de complicaciones crónicas que pueden afectar la calidad y la esperanza de vida de las personas afectadas. También se ha descrito un exceso de mortalidad en estos pacientes antes de la aparición de complicaciones tardías,. En las Islas Canarias, la prevalencia de enfermedad renal terminal relacionada con la diabetes es la más alta reportada hasta la fecha en España. Las complicaciones agudas como la cetoacidosis diabética también pueden tener consecuencias nefastas.

Durante los últimos 8-9 años, nos ha impresionado el aparentemente creciente número de pacientes con DM1 en nuestro Hospital. El volumen de publicaciones internacionales y locales haciendo referencia a la creciente incidencia de la diabetes tipo 1 en todo el mundo nos llevaron a evaluar la incidencia durante los últimos 9 años en Gran Canaria y caracterizar a nuestros pacientes en un intento por adquirir conocimientos sobre las tendencias de incidencia actual, entender mejor los factores epidemiológicos y describir características clínicas y analíticas al debut de nuestros pacientes.

Dada la importante carga que la diabetes supone en las Islas Canarias, nos parece necesario aumentar la investigación y el conocimiento en este campo. Un conocimiento más profundo de la diabetes tipo 1 nos permitirá aumentar nuestro conocimiento sobre los posibles mecanismos que conducen al aumento de la incidencia en nuestra región, así como diseñar mejores estrategias de prevención y tratamiento. El conocimiento es el primer paso para mejorar la calidad de la atención, para abogar por el cambio en la práctica actual y para orientar las políticas de salud en relación con el cuidado de pacientes con DM1.

Como primer paso, se presenta la incidencia de la DM1 en la población pediátrica de Gran Canaria durante los últimos 9 años así como la caracterización clínica de nuestros pacientes en el momento de su debut.

Hipótesis y Objetivos

Hipótesis

1. Hipótesis nula

- La incidencia de diabetes tipo 1 no es más alta que en el resto de España.

- No ha habido un aumento de la incidencia en los últimos años en Gran Canaria.

- Nuestros valores son similares a los encontrados en el resto de España y países europeos y africanos vecinos, y apoyan el gradiente norte-sur se describe clásicamente para la incidencia de diabetes tipo 1 en Europa.

- La distribución de casos entre los diferentes municipios de Gran Canaria es similar

- No hay diferencias en la presentación de diabetes tipo 1 en relación al sexo y los diferentes grupos etarios.

- No existe relación entre la incidencia de gripe el mismo año o el previo y la incidencia de diabetes tipo 1 en Gran Canaria.

- No existe estacionalidad en la aparición de casos

- La caracterización genética e inmune de los pacientes no es diferente de lo que se ha reportado en poblaciones caucásicas.

- La presencia de AAIDs en nuestras poblaciones es similar a la descrita en la literatura.

2. Hipótesis alternativa

- La incidencia de diabetes tipo 1 es mayor en Gran Canaria que en el resto de España

- Ha habido una tendencia creciente en el número de casos de diabetes tipo 1 en Gran Canaria durante los últimos 9 años.

- Nuestros valores son diferentes a los encontrados en el resto de España así como en el resto de países europeos y africanos vecinos.

- Nuestros valores de incidencia no son compatibles con el gradiente norte-sur clásicamente descrito para la incidencia de diabetes tipo 1 en Europa.

- La distribución de casos no es homogénea en todos los municipios de la isla.

-La distribución de los casos y las características al debut entre los grupos de sexo y edad difiere significativamente.

- La incidencia de gripe en el año previo o el mismo año influye en la aparición de diabetes tipo 1 en Gran Canaria.

- Existe estacionalidad en la aparición de casos.

- La caracterización genética e inmune de nuestros pacientes es diferente de lo que se ha reportado para población caucásica.

- La presencia de AAIDs en nuestras poblaciones es diferente a la descrita en la literatura.

Objetivos

El objetivo principal del presente trabajo es conocer la incidencia de diabetes tipo 1 en Gran Canaria, así como obtener una comprensión más profunda sobre sus características al debut. Todo ello, con la finalidad de comprender mejor los factores que conducen a la aparición de diabetes tipo 1 en nuestro medio y ser capaces de disminuir de manera más efectiva la morbilidad y mortalidad que se le asocian. Los principales objetivos operativos para el estudio son:

1. **Incidencia.**

• Definir la incidencia de diabetes tipo 1 en la población pediátrica (menores de 14 años de edad) de Gran Canaria en los últimos 9 años (2006-2014) y evaluar las tendencias temporales.

• Comparar nuestra incidencia a la del resto de España así como a la de países europeos y africanos vecinos.

• Evaluar si nuestros valores de incidencia respetan el gradiente norte-sur clásicamente descrito para los países europeos.

• Determinar la posible existencia de variabilidad en la aparición de diabetes tipo 1 entre los 3 grupos etarios (0-4, 5-9 y 10-14 años de edad) así como entre niños y niñas.

• Determinar su distribución entre los diferentes municipios en un intento de encontrar factores que pueden estar influyendo en su aparición.

• Evaluar la existencia de una posible relación entre los casos incidentes de gripe y la aparición de diabetes tipo 1.

• Evaluar la influencia de la estacionalidad en la aparición de casos durante el período de 9 años.

2. **Caracterización clínica al debut**

• Describir las características siguientes al inicio: la edad del diagnóstico, sexo, HbA1C media, la gravedad al debut (CAD) y la presencia de enfermedades autoinmunes asociadas.

3. **Caracterización genética (HLA) y autoinmune (autoanticuerpos anti-islote).**

• Evaluar la frecuencia de aparición de alelos *HLA* de riesgo en nuestra población DM1.

• Evaluar la frecuencia de aparición de anticuerpos anti-GAD, anti-IA2 y anti-insulina en nuestra población con diabetes tipo 1.

• Evaluar los posibles factores que puedan influir en la edad de inicio y la gravedad de la presentación de la diabetes tipo 1 en la isla de Gran Canaria.

Material y Métodos

1. Métodos de muestreo

Se han utilizado dos procedimientos de muestreo diferentes para el estudio:

- En primer lugar, hemos utilizado "muestreo censal" para describir la incidencia de diabetes tipo 1 en Gran Canaria durante el período 2006-2014, para la descripción de su distribución entre los diferentes grupos etarios y entre varones y hembras, y para evaluar la relación entre los casos incidentes de gripe del año anterior y los debuts de diabetes tipo 1. Esta muestra es representativa y tiene suficiente tamaño para llegar a conclusiones significativas con respecto a la población objeto de nuestro estudio.

- Con el fin de garantizar la disponibilidad de registros, para describir las características analíticas de los niños con diabetes tipo 1 en Gran Canaria al debut (HLA, autoanticuerpos anti-islotes, la prevalencia de la CAD y AAIDs) decidimos tomar una muestra de conveniencia: muestra transversal de pacientes con DM1 bajo nuestro cuidado durante el período de 2013 a 2014 y retrospectivamente examinar sus características al debut.

El estudio cuenta con el visto bueno del Comité Ético de Investigación Clínica de nuestro Hospital.

2. Incidencia

Con el fin de evaluar la incidencia, se incluyeron todos los pacientes que vivían en la isla en el momento del diagnóstico durante el período 2006-2014. En nuestra unidad contamos con el registro de todos los niños que debutan con diabetes tipo 1 desde el año 2006. Dicha información se contrastó con los datos obtenidos a partir de la farmacia hospitalaria (recetas nuevas de insulina) y de la asociación local de diabetes, según está descrito por el método de captura-recaptura. El diagnóstico se realizó de acuerdo con los criterios de la Asociación Americana de Diabetes. La fecha en que se administró la primera dosis de la insulina fue considerada como la fecha de debut. Los datos se recogieron de forma retrospectiva a partir del registro hospitalario. La incidencia fue calculada como el número de nuevos casos identificados por año por cada 100.000 habitantes menores de 14 años de edad. Para algunos de aquellos cuya aparición fue en 2006, nos faltaba el acceso al mes exacto de diagnóstico. Por lo tanto, la estacionalidad se evaluó durante todo el período a excepción de 2006.

Los datos del censo fueron obtenidos a partir de la información publicada por el Instituto Canario de Estadística. Para la evaluación de los parámetros climáticos y la incidencia de gripe, se utilizaron los datos de la Agencia de Meteorología y de la Oficina de Epidemiología y Prevención del Departamento de Salud Pública de Canarias, respectivamente. La distribución geográfica se evaluó mediante la

clasificación de los debuts por municipio y evaluando posibles diferencias en incidencia en la isla.

Análisis estadístico

SPSS vs 22 (SPSS Inc, Chicago, IL) fue utilizado para la mayor parte del análisis de los datos . Resultados para las variables continuas se describen como media (SD) o mediana (rango), dependiendo de la distribución (gaussiana o no, respectivamente). Las variables cualitativas se describen como N ó porcentaje. Se calcularon intervalos de confianza del 95% cuando se consideró apropiado.

- El análisis de la tendencia temporal se realizó mediante regresión de Poisson, con el siguiente modelado:

$$\log(\mu t) = \log(N) + \beta 0 + \beta 1t$$

donde "t" es el año “μt " es el número esperado de casos de ese año y "N" es la población expuesta. Suponemos que el número de nuevos casos en el año "t" sigue una distribución de Poisson de valor " μt ". Este modelo se puede expresar como:

$$\log(\mu tN) = \beta 0 + \beta 1t$$

lo que significa que el log de la incidencia esperada es una función lineal del tiempo.

- El análisis de la estacionalidad se realizó mediante el test de Cosinor. Tanto el análisis de regresión de Poisson como el test de Cosinor se realizaron con el entorno informático R v3.0.2 (Fundación R para la Computación de Estadística, Viena, Austria)

- Se realizó un análisis de correlación para buscar relación entre el número de casos nuevos diabetes tipo 1 y la prevalencia de la gripe el año anterior, así como para buscar relaciones entre los debuts mensuales y la posible relación con la temperatura, la humedad y las horas de luz solar. El número de casos por mes se calculó obteniendo el valor medio para cada mes durante todo el período 2006-2014. La temperatura media, la humedad y las horas de luz solar valores utilizados fueron los valores medios mensuales para el periodo 2000-2008.

- Chi-cuadrado se utilizó para evaluar las diferencias en la distribución de los casos (en los diferentes municipios). Los casos observados se calcularon contando el número total de casos para cada municipio para el período 2006-2013 (debido a la falta de datos para todos los municipios para el año 2014). Se calculó el número esperado de casos para cada municipio utilizando la incidencia general de la isla y el censo de la población de la isla de 2013. Sólo tuvimos acceso a la población total por municipio, no hay datos para la población menor de 14 años de edad por

municipio. Por lo tanto, asumimos que la distribución en el número de niños menores de 14 es similar a la distribución de la población total por municipio.

- Chi-cuadrado también se utilizó para evaluar diferencias en la aparición de casos entre los diferentes grupos etarios. El valor observado utilizado para el cálculo fue el % global de los casos encontrados para cada grupo de edad, y el valor esperado fue de 33% para cada grupo. Un valor de p bilateral <0,05 se consideró significativo.

- La incidencia anual se calculó dividiendo el número de casos por la población total para el grupo de edad, y se ajustó por 100.000 niños expuestos a desarrollar diabetes tipo 1. La incidencia de todo el período se calculó dividiendo el número total de casos por el número total de niños en situación de riesgo para todo el período, ajustando el cálculo por 100.000 niños.

3. Caracterización de la diabetes tipo 1 al debut

Los datos al debut de los pacientes con diabetes tipo 1 se recogieron retrospectivamente de 277 pacientes seguidos en el momento del estudio en la única Unidad de Endocrinología Pediátrica en la Isla de Gran Canaria, localizada en el "Hospital Materno-Infantil de Canarias" (Tabla 6). Para calcular el número medio de años con diabetes, se utilizó el último día en el que se introdujeron datos para el estudio (01 de agosto 2014). Examinamos las historias clínicas de los pacientes y se registramos las siguientes variables: fecha de nacimiento, sexo, fecha de inicio de la diabetes, el peso y la altura al debut, los datos analíticos al debut: HbA1c; pH; Clase II HLA DQ-DR; anticuerpos anti-GAD, anti-insulina y anti-IA2; anticuerpos anti-TPO y anti-transglutaminasa. El IMC se calculó dividiendo el peso en Kg por la altura en metros al cuadrado (kg / m2). El diagnóstico de diabetes tipo 1 se realizó según los criterios diagnósticos de la Asociación Americana de Diabetes. El diagnóstico de diabetes monogénica fue excluido mediante la realización de estudio genético en aquellos pacientes que presentaron una historia familiar con presencia de diabetes en al menos tres generaciones y con autoinmunidad negativa. La presencia de enfermedades autoinmunes asociadas se definió ante la presencia de valores elevados de TSH en repetidas ocasiones (superior a 5 mUI / L) y anticuerpos anti-TPO positivos para el hipotiroidismo autoinmune; valores de TSH en repetidas ocasiones bajas

(inferiores a 0,5 mUI / L), junto con los anticuerpos anti-receptor de TSH positivos para el hipertiroidismo; valores de Ig A anti-transglutaminasa repetidamente positivos (título> 4-10 U / ml) y la confirmación mediante biopsia intestinal cuando fue considerado necesario por la unidad de Digestivo pediátrico para la enfermedad celíaca. La presencia de síntomas clínicos no se consideró necesaria para el diagnóstico.

- Usamos genotipado mediante "Single Specific Primer-Polymerase Chain Reaction" (SSP-PCR) (INNOTRAIN, DiagnostikGmbH) para el estudio de los genes *HLA-DRB1 / DQB1* tras extracción de ADN genómico (QIAamp, Qiagen). Obtuvimos resultados para HLA-DRB1 en 114 pacientes y para HLA-DQB1 en 118 pacientes. Los datos se presentan a nivel de dos dígitos (por ejemplo: DRB * 03) por lo que polimorfismos sinónimos documentadas en el tercero, cuarto, quinto y sexto dígito no se reportan. Dada la ausencia de determinación del genotipo de los padres, no realizamos inferencias sobre los haplotipos. La caracterización se presenta en dos formas. 1. El número y el porcentaje de pacientes que presentan los alelos correspondientes al menos una vez. 2. En la columna "2N" se presentan frecuencias alélicas basadas en la presencia de un alelo con respecto al número total de alelos (2N), de forma similar a la realizada por Black et al en el estudio SEARCH en EEUU. Para el análisis, HLA de riesgo se definió por la presencia de los alelos DRB * 03 y / o DRB * 04. Realizamos tests de hipótesis con el fin de

evaluar la existencia de diferencias significativas en la frecuencia de aparición de alelos DRB * 03 y DRB * 04 y entre los alelos DQB * 02 y DQB * 03 en nuestra población.

-Los anticuerpos anti-islotes pancreáticos se midieron utilizando radioinmunoanálisis en el Laboratorio Reference (Barcelona). Para la detección de anticuerpos anti- IA2 se usó el "IA2 autoantibody RIA kit" de RSR con IA2 marcado con ^{125}I. Para la detección de anticuerpos anti- GAD se usó el "GAD autoantibody RIA kit" de RSR con GAD marcado con ^{125}I. Kit DIAsource AIA-100 se utilizó para la detección de anticuerpos anti-insulina. En el estudio DASP 2005, el RIA kit de RSR mostró 100% de especificidad (n = 100) y 70% de sensibilidad (n = 50) para autoanticuerpos IA-2 y 95% de especificidad (n = 100) y 84% de sensibilidad (n = 50) para autoanticuerpos GAD. Se consideran valores de referencia normales para anti-GAD / 64k y anti-IA2 <1 U / ml. Para los anticuerpos anti-insulina, la positividad se define mediante una unión > 8,2%. Las frecuencias se determinaron teniendo en cuenta el número de anticuerpos positivos en la población en la que se midió el anticuerpo: antiGAD y anti-insulina se midieron en 261 pacientes, anti-IA2 sólo en 231 de nuestros niños y los tres anticuerpos sólo en 228 de nuestros pacientes. La ausencia de anticuerpos como proporción de la población se calculó utilizando 228 como denominador (número de niños con los 3 anticuerpos medidos). Los anticuerpos anti-TPO se midieron

utilizando inmunoensayo de electroquimioluminiscencia. Anti-transglutaminasa IgA recombinante se midió utilizando enzima-inmunoensayo.

- La HbA1c se midió utilizando inmunoensayo de inhibición turbidimétrica (Tinia) a partir de sangre entera hemolizada en autoanalizador "Cobas 6000" (Roche Diagnostics). Se utilizó EDTA como anticoagulante. El método cumple con los estándares DCCT. Se determinaron los valores medios, así como los valores medios de los subgrupos con y sin cetoacidosis diabética al debut.

Análisis estadístico

Utilizamos SPSS vs 22 (SPSS Inc, Chicago, IL) para la mayor parte del análisis de los datos. Los datos al debut se describieron utilizando herramientas básicas descriptivas (media, desviación estándar y IC del 95% en su caso). Chi cuadrado se utilizó para evaluar la existencia de relación entre las variables categóricas. Se utilizó la prueba exacta de Fisher cuando uno de los valores observados fue <5. Utilizamos regresión lineal y regresión logística para evaluar la relación entre las variables independientes y dependientes usando entorno informático R v3.0.2 (Fundación R para la Computación de Estadística, Viena, Austria).

Test de hipótesis e intervalos de confianza del 95% se calcularon usaron las siguientes fórmulas y asunciones:

1. Intervalo de confianza del 95% para una muestra, con variables continuas:

Formula	Asunciones
$X \pm z^* \frac{s}{\sqrt{n}}$	$N \geq 30$

2. Intervalo de confianza del 95% para proporciones poblacionales (una muestra, resultado dicotómico):

Formula	Asunciones
$p \pm z^* \sqrt{\frac{p*(1-p)}{n}}$	Al menos 5 éxitos (n*p) y al menos 5 fracasos (n(1-p)) en la muestra

3. Test de hipótesis con una variable de resultado continua en una población:

Test statistic	Asunciones
$n \geq 30$ $z = \frac{X-\mu 0}{s/\sqrt{n}}$ $n < 30$ $t = \frac{X-\mu 0}{s/\sqrt{n}}$	Para los dos: la media especificada en Ho es un comparador justo y fiable. El uso apropiado de la distribución "t· assume que el resultado de interés sigue una distribución aproximadamente normal.

4. Hypothesis testing with a dichotomous outcome variable in a single population:

Test statistic	Asunciones
$Z = \frac{p-p0}{\sqrt{\frac{p0(1-p0)}{n}}}$	The smallest of $n*p_o$ or $n(1-p_o) \geq 5$

5. Hypothesis testing with a categorical outcome variable in a single population:

Test statistic	Asunciones
$X^2 = \sum \frac{(O-E)^2}{E}$	Expected frequency in each response category is ≥ 5

Otras variables analizadas:

- La edad media, la distribución según categorías de edad, el peso, la altura y el índice de masa corporal al debut. Se determinaron los valores medios y los intervalos de confianza del 95%. Los percentiles de peso, la altura y el IMC se calcularon utilizando las tablas de crecimiento de 2008 desarrollados por Carrascosa et al. Chi-cuadrado se utilizó para evaluar las diferencias en la distribución entre las categorías de edad y de sexo.

- Valores medios de HbA1c así como para los niños que presentan con y sin CAD. Los intervalos de confianza del 95% se determinaron para los tres grupos.

- Presencia / ausencia de la CAD. Los datos se presentan como% de los casos e IC 95% para los 3 grupos de edad descritos. Las diferencias entre grupos fueron evaluados usando chi-cuadrado.

- La prevalencia de otras enfermedades autoinmunes asociadas con DM1 y la distribución entre hombres y mujeres. También se determinaron los IC del 95%. Chi-cuadrado se utilizó para evaluar la existencia de diferencias significativas en la distribución de enfermedades autoinmunes entre niños y niñas.

Resultados

1. Incidencia

-Durante el período 2006-2014, un total de 316 niños debutaron con diabetes mellitus tipo 1 en Gran Canaria. El número de debuts por año durante todo el período, el número de niños menores de 14 años de edad en Gran Canaria, las incidencias anuales y medias se muestran en la Tabla 6. Las fuentes secundarias no proporcionaron nuevos casos (el grado de "seguridad" del Hospital es próximo al 100%). Como resultado, el método de captura-recaptura no proporcionó información adicional en nuestro caso.

-No encontramos ninguna tendencia temporal estadísticamente significativa en el número de debuts desde 2006 hasta 2014 (F = 0,4982; gl = 1 y 6; p = 0,507; r = 0,077; r^2 ajustado = -0,077).

-Cuando comparamos con los datos de 1995-1996 (1995: CI 24,9 / 100.000 (95%: 19,34-30,46); 1996: CI 21,5 / 100.000 (95%: 16,23-26,77)) proporcionado por Carrillo et al, no encontramos tendencias significativas en el número de casos desde 1995 a 1996 hasta el período 2006-2014. El modelo inicial mostró resultados significativos en la tendencia (z = 2,467; df: 10; AIC: 81,86; p = 0,014). Cuando registramos la bondad del ajuste, se observó que el modelo no tenía un buen ajuste con los datos (p-valor de la bondad de ajuste de prueba, X^2 = 0,044). La falta de ajuste podría ser debida a la dispersión excesiva de los datos. Para compensarlo,

reajustamos el modelo teniendo en cuenta el exceso de dispersión de los datos. Después de realizar el ajuste, los valores de los coeficientes no sufrieron cambios, pero se modificó el error estándar. Esto dio lugar a un valor de p diferente, que no resulta significativo (t = 1,744; df = 10; AIC: NA =; p = 0,115).

-No encontramos diferencias significativas en la distribución por sexo o por grupos etarios al debut durante el período 2006-2014 (Tabla 1).

- Al mirar el número de debuts por mes durante todo el período para evaluar la estacionalidad en la aparición de casos, no encontramos ninguna tendencia significativa en la distribución de los mismos. (Test de Cosinor; amplitud = 0,37; Fase: Mes = 4,3; Baja punto: Mes = 10,3; p> 0,05) (Tabla 2).

- No se encontró correlación significativa entre el número de debuts por mes y la media de la temperatura, la humedad o la horas de luz solar para el mismo mes (Temperatura: r = -0,166; p = 0,606 Humedad: r = 0,064; p = 0,844.. horas de sol: r = -0,301; p = 0,341).

Tabla 1. Incidencia. Distribución por sexo y grupos etarios.

	2006	2007	2008	2009	2010	2011	2012	2013	2014
Nº de debuts	24	35	33	39	53	31	44	27	30
Población < 14 años	117,299	118,064	119,177	119,827	119,867	118,757	117,699	116,063	113,304
Incidencia anual* (casos/100,000) (95%CI)	20.46 (12.28-28.64)	29.64 (19.82-39.46)	27.69 (18.24-37.14)	32.55 (22.34-42.76)	44.22 (32.32-56.12)	26.10 (16.91-35.29)	37.38 (26.34-48.42)	23.26 (14.49-32.03)	26.48 (17.01-35.95)
Niñas (%)	63	49	40	49	53	52	50	59	43.3
Grupos etarios (%) (0-4, 5-9, 10-14)	29/21/50	32/34/34	28/39/33	31/49/20	30/38/ 32	26/35/39	27/34/39	18/41/41	28/38/34
Incidencia media (95% CI)	**29.75/100,000 children** (26.5-33.09)								
**** % medio de niñas**	**51**								
Distribución etaria global* %** (0-4, 5-9, 10-14)	**27.6 / 36.5 / 35.8**								

*Noseencontró tendencia significativa. ** Ausencia de diferencia significativa entre nios y niñas *** La diferencia entre grupos etarios no es significativa

Tabla 2. Distribución mensual de casos

	Enero	Febr	Marz	Abr	May	Jun	Jul	Agost	Sept	Oct	Nov	Dic
2007	4	5	3	3	3	1	3	1	4	5	3	0
2008	2	2	2	1	2	5	4	2	4	4	1	4
2009	4	1	3	6	0	5	2	5	2	2	4	5
2010	3	6	5	3	7	5	3	5	7	4	2	3
2011	4	4	5	0	2	3	1	2	5	2	1	2
2012	4	6	3	3	2	2	3	4	2	5	3	7
2013	2	3	3	4	5	0	2	1	4	0	3	0
2014	3	3	3	3	2	2	2	1	4	0	5	2
TOTAL	**26 (8.2%)**	**30 (9.5%)**	**27 (8.5%)**	**23 (7.3%)**	**23 (7.3%)**	**23 (7.3%)**	**20 (6.3%)**	**21 (6.6%)**	**32 (10.1%)**	**22 (7%)**	**22 (7%)**	**23 (7.3%)**

*No hay diferencias significativas en el número de casos/mes

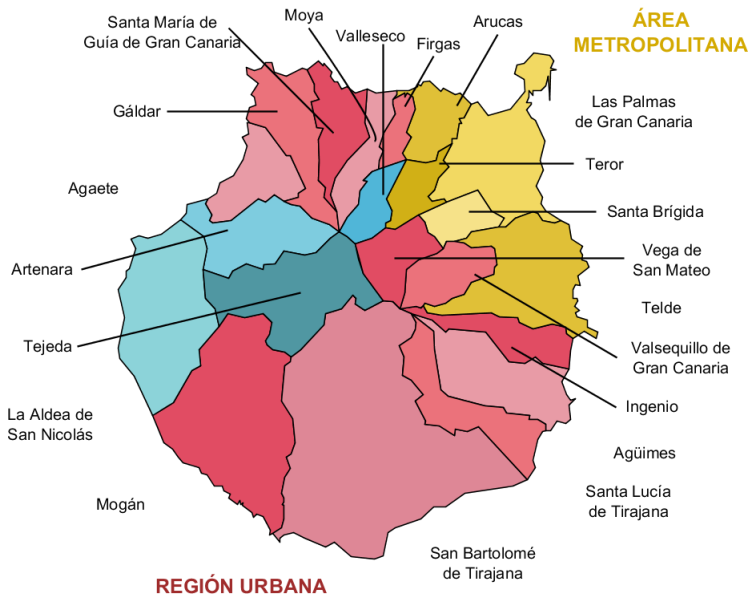
- Encontramos una correlación positiva entre los casos incidentes de DM1 y el número de casos declarados de gripe para el mismo grupo etario el año anterior, aunque sin alcanzar significación estadística (r=0.554; p=0.197). No encontramos correlación entre el número de debuts y los casos de gripe el mismo año (r= -0.454; p=0.162) (Tabla 3)

Tabla 3. Número de debuts y casos y de gripe para el período 2006-2013 period

	2006	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Casos incidentes de DM1 en <14 años (n)	24	35	33	39	53	31	44	27
Casos de gripe en < 14 años (n)	41480	45608	34667	50526	12635	24935	19909	23980

-No encontramos diferencias significativas en la distribución de los casos entre los diferentes municipios de la isla (Figure 1 & tabla 4).

Figure 1. Municipios en Gran Canaria



http://www.mapasdelmundo.org/20-mapa-gran-canaria-provincia/ Octubre-2015

Tabla 4. Distribución de los debuts por municipios.

	Habitantes por municipio	% de la población total Gran Canaria	Nº debuts por municipio	% de los debuts por municipio	Resultado esperado	Resultado observado	$X^2 = (Oi - Ei)^2 / Ei$
GRAN CANARIA	852.723	100	222	100	238.762	222	
Agaete	5.796	0.7	0	0	1.62288	0	0*
Agüimes	30.214	3.5	8	3.6	8.45992	8	0.025
Artenara	1.198	0.14	0	0	0.33544	0	0*
Arucas	36.852	4.3	10	4.5	10.3186	10	0.0098
Firgas	7.628	0.9	1	0.45	2.13584	1	0.604
Gáldar	24.227	2.8	10	4.5	6.78356	10	1.525
Ingenio	29.978	3.5	7	3.15	8.39384	7	0.2314
Mogán	21.782	2.5	7	3.15	6.09896	7	0.133
Moya	7.977	0.9	2	0.9	2.23356	2	0.024
Palmas de Gran Canaria (Las)	383.050	44.5	89	40.1	107.254	89	3.106
San Bartolomé de Tirajana	56.698	6.6	9	4	15.8754	9	2.977
Aldea de San Nicolás (La)	8.228	0.97	3	1.35	2.30384	3	0.210
Santa Brígida	18.971	2.2	6	2.7	5.31188	6	0.089
Santa Lucía	68.506	8	26	11.8	19.1817	26	2.42
Santa María de Guía	13.811	1.6	2	0.9	3.86708	2	0.901
Tejeda	2.028	0.2	0	0	0.56784	0	0*
Telde	102.170	12	32	14.4	28.6076	32	0.402
Teror	12.761	1.5	4	1.8	3.57308	4	0.051
Valsequillo	9.170	1	5	2.25	2.5676	5	2.30
Valleseco	3.904	1.46	0	0	1.09312	0	0*
Vega de San Mateo	7.774	0.9	1	0.45	2.17672	1	0.636
						X^2	15.658
						α= 0.05	26.3
						α= 0.01	32.0

*Valores de Agaete, Artenara, Tejeda y Valleseco no se añadieron al calcular X^2 ya que no aportaron casos (df=16).

2. Distribución por sexo y edad y características físicas.

En el estudio transversal, se incluyeron un total de 277 niños (134 niños y 143 niñas), con una edad media de 6,4 años al debut, y con una duración media de diabetes tipo 1 de 4,6 años en el momento en que se inició el estudio. No se encontraron diferencias significativas en la distribución de sexo. Tampoco encontramos diferencias significativas en cuanto al peso, talla o IMC al debut (Tabla 5). En cuanto a los grupos de edad, se encontraron significativamente más niños con diabetes tipo 1 en las categorías 0-4.9 y 5-9.9 años que en la de 10 a 13,9 años (p <0,01) (Tabla 6).

Tabla 5. Summary of descriptive features of participants at onset

	Global	Edad	Peso	Talla	IMC	HbA1c
Valor global (95% CI)		6.4 years (5.99– 6.81)	24.38 kg (22.89 – 25.87)	1.16 mt (1.13 – 1.19)	16.3 Kg/m2 (15.78 – 16.8)	10.5% (10.3 – 10.8)
Niños	48.4% (43%-54%)	6.2	24.8 (P67)	1.16 (P48)	16.15 (P48)	10.5%
Niñas	51.6% (46%-57%)	6.44	23.92 (P51)	1.17 (P39)	16.4 (P52)	10.6%

Tabla 6. Distribución etaria

	0 – 4.99 años	5 – 9.99 años	10-13.99 años
% (IC 95%)	40.3 (34 – 46)	41.8 (36 – 48)	17.9* (13.5 – 22.5)

*p < 0.01

3. HbA1c al debut

-La HbA1c media al debut en nuestra muestra de pacientes es de 0.55%. Los pacientes que debutaron con cetoacidosis presentaron valores significativamente más elevados que los que no la presentaron (tabla 7).

Tabla 7. HbA1C al debut

	HbA1c media	HbA1c media sin CAD	HbA1c media con CAD
HbA1C	10.55%	10.2%	11.23%
(IC 95%)	(10.3-10.8)	(9.99 – 10.63)	(10.97 – 11.44)

4. Complicaciones agudas al debut: cetoacidosis diabética

-34.2% (IC 95%= 29% – 40%) de nuestros pacientes presentaron cetoacidosis al debut.

-Encontramos una diferencia estadísticamente significativa en la distribución de CAD entre los diferentes grupos etarios (X^2=8.201; df=2; p<0.05), señalando a una mayor incidencia en los niños menores de 5 años (table 8 & 9).

Tabla 8. Distribución de CAD al debut por grupos etarios

	0-4.99 años	5-9.99 años	10-13.99 años
% de niñ@s con CAD por grupo etario (IC 95%)	43.7 (34 - 53)	28.1 (19.6 - 36.4)	27.6 (14.6 - 40)

Tabla 9. Tabla 2*3. Distribución de CAD por grupos etarios.

		Categoría de edad			
		0-4 años	**5-9 años**	**10-13 años**	**Total**
Presencia de CAD	**Si**	49	31	12	92
	No	62	79	35	176
Total		111	110	47	268

-No encontramos diferencias significativas en la distribución de CAD entre niños y niñas (X^2=0.109; df=1; p=0.741) (Tabla 10).

Tabla 10. Distribución de CAD por sexo

		sexo		
		Niños	**Niñas**	**Total**
Presencia de CAD	**Si**	43	49	92
	No	86	90	176
Total		129	139	268

-No encontramos diferencias significativas entre la presencia de CAD y tener HLA de riesgo (Fisher´s exact test, p=1 (2 sided)) (Tabla 11)

Tabla 11. CAD al debut y HLA de riesgo

		Presenia de CAD		Total
		si	**no**	
HLA de riesgo	**no**	4	8	12
	si	32	67	99
Total		36	75	111

-Tampoco con la presencia de anticuerpos antipancreáticos (X^2=3.124; df=5; p=0.681) (Tabla 12).

Tabla 12. CAD y anticuerpos anti-islotes pancreáticos

		Anti-IA2	Anti-GAD	antiGAD+anti-insulina	antigad+anti-IA2	Presencia de los 3 anticuerpos	Total
CAD	**si**	26	12	9	16	12	87
	no	38	19	20	40	31	171
Total		64	31	29	56	43	258

5. Caracterización HLA

-La caracterización HLA para DR (tabla 20), DQ (tabla 21) y "genotipos de riesgo aumentado" (tabla 22) se describen a continuación. DRB * 03 y DRB * 04 son claramente los alelos más frecuentes entre los alelos DR (63,16% de los posibles alelos), con un número significativamente mayor de pacientes presentando al menos un alelo DRB * 04 que DRB * 03. Sin embargo, cuando se mira la frecuencia alélica en general, la diferencia entre DRB * 04 y DRB * 03 no es significativa. DRB * 03, DRB * 04 o ambos están presentes en 90,35% de nuestros pacientes. En cuanto a DQ: DQ * 02 y DQ * 03 son los más frecuentes, con solapamiento de los IC y sin diferencias significativas en su aparición. Un total de 96,61% de nuestros niños presentan uno o ambos alelos. La presencia de genotipos de riesgo se describe en la tabla 19. Con respecto a los alelos protectores (DRB * 07, DRB * 11, DRB * 13, DRB * 15), encontramos que suponen un 19,73% de los alelos totales reportados para nuestros pacientes.

-Cuando miramos la co-aparición de DRB * 03 con DQB * 02, encontramos que la presencia de DRB * 03 se acompaña de la aparición de DQB * 02 en el 100% de nuestros pacientes (tabla 13). Al mirar DRB * 04 con DQB * 03, apreciamos que la presencia de DRB * 04 predice la presencia de DQB * 03 en el 87,5% de nuestros pacientes (tabla 14).

Tabla 13. Tabla 2*2 para ver concordancia entre DRB*03 & DQB*02

		HLA DQB*02		Total
		No	**Si**	
HLA DRB*03	**No**	34	23	57
	Si	0	57	57
Total		34	80	114

Tabla 14.Tabla 2*2 para ver concordancia entre DRB*04 & DQB*03

		HLA DQB*03		Total
		No	**Si**	
HLA DRB*04	**No**	31	5	36
	Si	8	70	78
Total		39	75	114

-Encontramos una diferencia no significativa en la distribución de alelos de riesgo para el desarrollo de DM1 entre las categorías de edad, con una proporción ligeramente mayor en el grupo más joven (extensión de la prueba exacta de Fisher Freeman-Halton, p = 0,25) (Tabla 15). Tras categorizar los grupos de edad en dos grupos (niños menores y mayores de 5 años de edad), tampoco encontramos diferencias significativas (prueba exacta de Fisher, p = 0,125) (tabla 16).

Tabla 15. HLA de riesgo y grupos etarios (3 categorias)

		Categorías de edad			
		0-4 años	**5-9 años**	**10-13 años**	**Total**
HLA de riesgo	No	2	6	4	12
	Si	41	35	24	100
Total		43	41	28	112

Tabla 16. HLA de riesgo y grupos etarios (2 categorias)

		Categorías de edad		
		0-4 años	**5-13 años**	**Total**
HLA de riesgo	No	2	10	12
	Si	41	59	100
Total		43	69	112

-No encontramos diferencias significativas al estratificar en función del sexo (X^2=0.004; df=1; p=0.949) (Tabla 17).

Tabla 17. HLA de riesgo y relación con sexo

		sexo		
		Niños	**Niñas**	**Total**
HLA de riesgo	**No**	6	6	12
	Si	52	50	102
Total		58	56	114

-Al estudiar la distribución de alelos protectores, no encontramos diferencias en su distribucion por categorías de edad (X^2= 0.761 ; df=2; p= 0.684) (tabla 18) ni en su distribución en función del sexo (X^2= 0.846; df=1 ; p=0.358) (tabla 19).

Tabla 18. Alelos protectores y grupos etarios

		Categoría de edad			
		0-4 años	**5-9 años**	**10-13 años**	**Total**
HLA protector	No	29	24	17	70
	Si	14	17	11	42
Total		43	41	28	112

Table 19. Alelos protectores y sexo

		sex		
		Male	**Female**	**Total**
HLA protector	No	39	33	72
	Si	19	23	42
Total		58	56	114

Tabla 20. Distribution de DR

	Individuos con al menos uno de los siguientes valores DR	% (IC 95%)	allelo presente	% (2N)
DR*1	24	21.05 (13.5 - 28.5)	24	10.53
DR*3	57	50 (41 - 59)	63	27.63
DR*4	78	68.42 (59 – 77)	81	35.53
DR*7	19	16.67	19	8.33
DR*8	6	5.26	6	2.63
DR*9	5	4.39	5	2.19
DR*10	1	0.88	1	0.44
DR*11	6	5.26	6	2.63
DR*13	15	13.16	15	6.58
DR*15	5	4.39	5	2.19
DR*16	3	2.63	3	1.32

Tabla 21. Distribution de DQ

	Individuos con al menos uno de los siguientes valores DQ	**%** (IC 95C%)	**% (2N)**
DQ*02	81	69.49 (61.5 -77.5)	40.25
DQ*03	78	66.1 (58 - 74)	36.44
DQ*04	6	5.08	2.54
DQ*05	33	27.97 (20 – 36)	14.83
DQ*06	14	11.86	5.93

Table 22. Distribution of alelos/genotipos de riesgo

	Alelos de riesgo y genotipos	N	% (IC 95%)
	Individuos con DR3 o DR4 o los dos	102	89.47 (83.9 – 95)
	Individuos con DQ2 o DQ3 o los dos	114	96.61
Genotypo	**Individuos con DR3-DQ2 in homozygosis**	5	4.39
Genotypo	**Individuos con DR4-DQ3 in homozygosis**	4	3.51
Genotypo	**Individuos con DR3-4/DQ2-3**	31	27.19 (19 – 35)

6. Autoanticuerpos

La distribución de resultados positivos se describe a continuación (tabla 23). Es de destacar el hecho de que los anticuerpos anti-insulina siempre aparecieron en combinación con anti-GAD o junto con anti-GAD y anti-IA2, pero nunca por sí mismos o con anti-IA2.

* 62,1% de los pacientes en los que se midió antiGAD fueron positivos.

* 72,3% de los pacientes en los que se midió anti-IA2 fueron positivos.

* 74% de los pacientes en los que se midió anti-insulina fueron positivos.

* 19,7% de los pacientes en los que se midieron los 3 anticuerpos presentaron positividad para los tres.

* 24,7% de los pacientes en los que se midieron anti-GAD y anti-IA2 presentaron positividad para ambos.

* 11,1% de los pacientes en los que se midieron anti-GAD y anti-insulina presentaron positividad para ambos.

Tabla 23. Distribuciónn de anticuerpos anti-pancreáticos

	Pacientes	**%** (95% CI)
AntiGAD	162	62.1 (56 - 68)
Anti-IA2	167	72.3 (66 - 78)
Anti-insulina	74	28.4 (22 - 34)
3 anticuerpos presentes	45	19.7 (14.5 - 25)
AntiGAD + anti-IA2	57	24.7 (19 - 31)
AntiGAD + anti- insulina	29	11.1
Anti-IA2 + anti-insulina	0	0
Anticuerpos negativos	30	13.2

- Encontramos que la presencia de anticuerpos (todos los tipos) es significativamenet mayor en niñas (X^2=16.889; df=5; p<0.05) (Table 24).

Tabla 24. Distribución de anticuerpos antipancreáticos por sexo

		Anti-IA2	**Anti-GAD**	**antiGAD + anti-insulin**	**Anti-GAD + anti-IA2**	**3 anticuerpos presentes**	**Total**
sexo	**niños**	39	16	10	23	15	128
	niñas	26	15	19	34	30	136
Total		65	31	29	57	45	264

- Nuestros datos también sugieren que, en nuestra población, la presencia de anticuerpos anti-IA2 es significativamente mayor en niños >5 años (X^2=7.87; df=2; p<0.05) (Tabla 25).

Tabla 25. Distribución de anticuerpos anti-IA2 en los tres gurpos de edad

		Presencia/Ausencia de anticuerpos anti-IA2		
		Ausencia de anti-IA2	**Presencia de anti-IA2**	**Total**
Categoría de edad	**0-4 años**	36	60	96
	5-9 años	20	77	97
	10-13 años	8	30	38
Total		64	167	231

- Al estudiar la posible relación entre DRB * 03 y DRB * 04 alelos (por separado) y la positividad de autoanticuerpos (individualmente o en combinación), encontramos una relación significativa entre el alelo DRB * 04 y el anticuerpo anti-IA2 (X2 = 8,592; gl = 1; p = 0,03) (Tabla 26). No encontramos relación entre el alelo DRB * 03 y cualquiera de las posibles combinaciones de anticuerpos. Tampoco encontramos relación entre los dos alelos y la ausencia de autoinmunidad.

Tabla 26. Relación entre DRB*04 y anti-IA2

		Anti-IA2 positivo		
		No	**Si**	**Total**
HLA DRB*04	**No**	12	20	32
	Si	9	63	72
Total		21	83	104

7. Autoimmunidad asociada

-En el momento del studio, 7.9% de nuestros pacientes (IC 95%=4.8%– 11%) presentaban enfermedades autoimmunes asociadas, incluyendo enfermedad celíaca, hepatitis autoinmune, hipotiroidismo e hipertiroidismo (tabla 27).

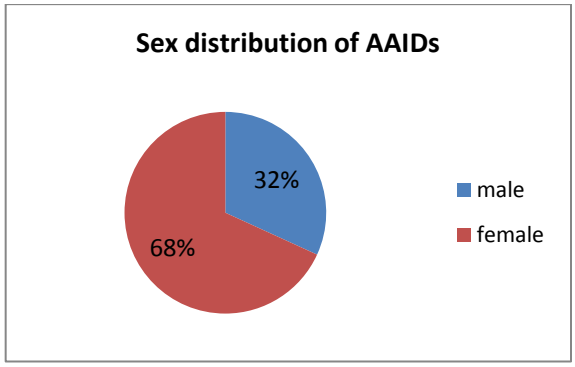
Tabla 27. Enfermedades autoimmunes asociadas

	Enfermedad celíaca	Hepatitis autoimmune	Hipotiroidismo	Hipertiroidismo
Número (V/H)	16 (7/9)	1 (0/1)	3 (0/3)	2 (0/2)
%	5.7	0.36	1.1	0.7

V: varón. H: hembra

-Encontramos que la proporción niños:niñas para la asociación con enfermedades autoimmunes era de 7:15, con 31.8% en niños (IC 95%= -10 – 73.8%) y 68.2% en niñas (47.2 – 89.2%). La diferencia no es estadísticamente significativa (figura 2).

Figura 2**.** Distribucion de enfermedades autoimmunes por sexo



8. Análisis de regresión

-No se encontró relación entre la edad de inicio y la presencia de HLA de riesgo, la presencia / ausencia de anticuerpos y el sexo (F = 0.137 días 3 y 85 DF, p=0,9377 error estándar residual = 44,77; gl = 85.Multiple R2 = 0,004812; R^2 ajustado = -0,03) (Tabla 27).

Tabla 27. Resultados de modelo de regression valorando la relación entre la edad de debut y HLA de riesgo, anticuer pos y sexo.

Coeficientes

	Estimate	**Std. Error**	**valor de t**	**Pr(>|t|)**
(Intercept)	75.38	14.19	5.31	8.52e-07 ***
Risk_HLA_yes	7.14	13.92	0.51	0.609
three_antibodies_+	0.01	12.08	0.001	0.999
Female	3.90	9.72	0.402	0.689

-Cuando modelamos la relación entre la presencia de CAD al debut y la presencia de HLA de riesgo, la presencia / ausencia de anticuerpos, el sexo y la edad de inicio, encontramos que el riesgo de debutar con CAD aumenta con la edad después de ajustar por HLA de riesgo, positividad de 3 anticuerpos y el sexo femenino (desviación nula: 107.77 en 86 df desviación residual; 102,44 en 82 df AIC: 112.44). (Tabla 28). El OR aumenta 1,16 veces por cada aumento de un año en la edad (Tabla 29).

Tabla 28. Resultados del modelo que estudia la relación la presencia de CAD al debut y la presencia de HLA de riesgo, anticuerpos, sexo y edad del debut.

Coefficients						
	Estimate	**Std. Error**	**valor de z**	**Pr(>|z|)**	**2.5 %**	**97.5%**
(Intercept)	0.12	0.82	0.15	0.88	-1.45	1.86
RIsk_HLA_yes	-0.30	0.74	-0.41	0.68	-1.93	1.1
three_antibodies_+	0.09	0.62	0.15	0.88	-1.1	1.4
sexoFemale	-0.01	0.49	-0.024	0.98	-0.98	0.95
ONSET_AGE_YRS	0.15	0.07	2.2	0.029*	0.02	0.29

Tabla 29. OR

	OR	**2.5%**	**97.5%**
(Intercept)	1.13	0.23	6.43
RIsk_HLAyes	0.74	0.14	2.95
three_antibodies_+	1.1	0.33	4.01
sexoFemale	0.99	0.37	2.6
ONSET_AGE_YRS	1.16	1.02	1.34

Conclusiones

Conclusiones

1. Se presenta por primera vez la incidencia durante un ciclo de 9 años de la isla de Gran Canaria, mostrando el valor más alto reportado en España y uno de los más altos valores reportados en todo el mundo.

2. La distribución de casos por género y grupos de edad es similar.

3. No encontramos tendencias temporales ni estacionalidad en la aparición de casos.

4. Nuestros resultados no apoyan el gradiente norte-sur clásicamente descrito para los casos incidentes de DM1 en Europa.

5. La proporción de casos que presentan CAD al debut es el 34,2%. La CAD es más frecuente en el grupo de edad más joven (0-4.9 años de edad). Sin embargo, después de ajustar por HLA de riesgo, la positividad de tres anticuerpos y el sexo, se encontró un aumento significativo del OR de desarrollar CAD al debut al ir aumentando la edad (por cada año de edad, aumenta la OR de desarrollar CAD al debut en 1.16).

6. Nuestras conclusiones respecto a la carga genética de nuestros pacientes (HLA) muestran que los alelos DRB * 03 y DRB * 04 son claramente los más frecuentes entre los alelos DR, con significativamente más niños presentando al menos un

alelo DRB * 04 que DRB * 03. En cuanto a los alelos DQ, observamos que DQ * 02 y DQ * 03 son los más frecuentes, con solapamiento de los IC y sin diferencias significativas en sus frecuencias de aparición. El 96.61% de nuestros niños presentan uno de ellos (DQ * 02 o DQ * 03) o ambos. El genotipo con el mayor riesgo para el desarrollo de de diabetes tipo 1 (DR3-4 / DQ2-3) aparece en el 27,2% de nuestros pacientes.

7. La autoinmunidad antipancreática aparece en nuestros pacientes en porcentajes similares a los descritos en la literatura. Encontramos mayor prevalencia de aparición en las niñas. En cuanto a la relación entre la presencia de autoinmunidad de forma global y HLA, no encontramos relación entre su aparición y la presencia de alelos de alto riesgo. Sin embargo, encontramos una relación significativa entre la presencia de anticuerpos DRB * 04 y anticuerpos anti-IA2. En cuanto a la distribución, encontramos niveles de anticuerpos anti-insulina más bajos que los reportados por otros autores, así como una menor frecuencia de combinación entre 2 ó 3 anticuerpos.

8. Nuestros pacientes presentan una baja prevalencia de enfermedades autoinmunes asociadas, con una frecuencia no significativamente mayor en las niñas y un predominio de la enfermedad celíaca.

VII. REFERENCES

[1] Polonsky K.The Past 200 Years in Diabetes. N Engl J Med 2012;367:1332-40.

[2] American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. Volume 37, supplement 1, January 2014.

[3] Russell N, Cooper M. 50 years forward: mechanisms of hyperglycaemia-driven diabetic complications. Diabetologia (2015) 58:1708–1714

[4] The Diabetes Control and Complications Trial Research Group. The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. NEJM. September 30, 1993 Vol. 329 No. 14:977-86.

[5] Gregg E, Li Y, Wang J, Rios N, Ali M, Rolka D, Williams D, and Geiss L. Changes in Diabetes-Related Complications in the United States, 1990–2010. N engl j med 370;16:1514-23.

[6] Hermann R, Knip M, Veijola R, et al. Temporal changes in the frequencies of HLA genotypes in patients with Type 1 diabetes-indication of an increased environmental pressure?. Diabetologia 2003; 46:420-25

[7] White J. Insulin analogs: What are the clinical implications of structural differences? US Pharm. 2010;35(5)(Diabetes suppl):3-7.

[8] American Diabetes Association. Approaches to glycemic treatment. Sec. 7. In Standards of Medical Care in Diabetes-2015. Diabetes Care 2015;38(Suppl. 1):S41–S48

[9] Ziegler AG, Hillebrand B, Rabl W, Mayrhofer M, Hummel M, Mollenhauer U, Vordemann J, Lenz A, Standl E. On the appearance of islet associated autoimmunity in offspring of diabetic mothers: a prospective study from birth. Diabetologia. 1993 May;36(5):402-8.

[10] Kupila A, Muona P, Simell T, Arvilommi P, Savolainen H, Hämäläinen AM, Korhonen S, Kimpimäki T, Sjöroos M, Ilonen J, Knip M, Simell O; Juvenile Diabetes Research Foundation Centre for the Prevention of Type I Diabetes in Finland. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. Diabetologia. 2001 Mar;44(3):290-7.

[11] Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, McDuffie RS Jr, Hamman RF, Klingensmith G, Eisenbarth GS, Erlich HA. Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). Diabetologia. 1996 Jul;39(7):807-12.

[12] TEDDY Study Group.The Environmental Determinants of Diabetes in the Young (TEDDY) Study. Ann N Y Acad Sci. 2008 Dec;1150:1-13.

[13] Skyler J, Greenbaum C, Lachin J, Leschek E, Rafkin-Mervis L, Savage P, Spain L, and Type 1 Diabetes TrialNet Study Group. Type 1 Diabetes TrialNet – An International Collaborative Clinical Trials Network. Ann N Y Acad Sci. 2008 December ; 1150: 14–24.

[14] Hamman RF, Bell RA, Dabelea D, D'Agostino RB Jr, Dolan L, Imperatore G, Lawrence JM, Linder B, Marcovina SM, Mayer-Davis EJ, Pihoker C, Rodriguez BL, Saydah S; SEARCH for Diabetes in Youth Study Group.The SEARCH for Diabetes in Youth study: rationale, findings, and future directions. Diabetes Care. 2014 Dec;37(12):3336-44.

[15] Danne T, Lion S, Madaczy L, Veeze H, Raposo F, Rurik I, Aschemeier B, Kordonouri O; SWEET group. Criteria for Centers of Reference for pediatric diabetes--a European perspective. Pediatr Diabetes. 2012 Sep;13 Suppl 16:62-75.

[16] Rich SS, Concannon P, Erlich H, Julier C, Morahan G, Nerup J, Pociot F, Todd JA.The Type 1 Diabetes Genetics Consortium. Ann N Y Acad Sci. 2006 Oct;1079:1-8.

[17] Beck R, Tamborlane W, Bergenstal R, Miller K,DuBose S, and Hall C, for the T1D Exchange Clinic Network. The T1D Exchange Clinic Registry. J Clin Endocrinol Metab 97: 4383– 4389, 2012

[18] Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. Diabetes Care. 1999 Jan;22(1):99-111.

[19] WHO Multinational Project for Childhood Diabetes. WHO Diamond Project Group. Diabetes Care. 1990 Oct;13(10):1062-8.

[20] Green A, Gale EA, Patterson CC. Incidence of childhood-onset insulin-dependent diabetes mellitus: the EURODIAB ACE Study. Lancet. 1992 Apr 11;339(8798):905-9.

[21] Penno M, Couper J, Craig M, Colman P, Rawlinson W, Cotterill A, Jones T, Harrison L and ENDIA Study Group 2013 BMC PEDIATRICS

[22] In't Veld P. Insulitis in human type 1 diabetes: The quest for an elusive lesion. Islets. 2011 Jul-Aug;3(4):131-8.

[23] Nokoff N, Rewers M. Pathogenesis of type 1 diabetes: lessons from natural history studies of high-risk individuals. Ann N Y Acad Sci. 2013 Apr;1281:1-15.

[24] Wenzlau, J.M., K. Juhl, O. Moua, et al. 2007. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc. Natl. Acad. Sci. USA 104: 17040–17045

[25] Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Simell OG, Toppari J, Ziegler AG, Akolkar B, Bonifacio E; TEDDY Study Group. The 6

year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia. 2015 May;58(5):980-7.

[26] Steck AK, Vehik K, Bonifacio E, Lernmark A, Ziegler AG, Hagopian WA, She J, Simell O, Akolkar B, Krischer J, Schatz D, Rewers MJ; TEDDY Study Group. Predictors of Progression from the Appearance of Islet Autoantibodies to Early Childhood Diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). Diabetes Care. 2015 May;38(5):808-13.

[27] Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014 Jan 4;383(9911):69-82. doi: 10.1016/S0140-6736(13)60591-7.

[28] Atkinson M. The pathogenesis and Natural History of Type 1 Diabetes. Cold Spring Harb Perspect Med 2012;2:a007641

[29] Hyttinen V, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. Diabetes. 2003 Apr;52(4):1052-5.

[30] Gillespie K, Bain S, Barnett A, Bingley P, Christie M, Gill G, Gale E. The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes. Lancet 2004; 364: 1699–700

[31] Zieglera A, Pfluegerb M, Winklerb C, Achenbacha P, Akolkarc B, Krischerd J, and Bonifacioe E. Accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children. J Autoimmun. 2011 August ; 37(1): 3–7.

[32] Wägner A.M, Wiebe J.C. Genetics of type 1 diabetes: Recent progress and future perspectives. Av. Diabetologia. 2009; 25: 78-89

[33] Cooper JD, Howson JM, Smyth D, Walker NM, Stevens H, Yang JH, She JX, Eisenbarth GS, Rewers M, Todd JA, Akolkar B, Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Pociot F, Rich SS; Type 1 Diabetes Genetics Consortium. Confirmation of novel type 1 diabetes risk loci in families. Diabetologia. 2012 Apr;55(4):996-1000.

[34] Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, Mychaleckyj JC, Todd JA, Bonella P, Fear AL, et al. 2008. HLA DR-DQ haplotypes and genotypes and Type 1 diabetes risk: Analysis of the Type 1 Diabetes Genetics Consortium families. Diabetes 57: 1084–1092.

[35] Noble J and Erlich H. Genetics of Type 1 Diabetes. Cold Spring Harb Perspect Med 2012;2:a007732

[36] http://www.biomedcentral.com/1471-2105/11/S11/S10/figure/F1?highres=y. September 2015

[37] Noble J, Martin A, Valdes A, Lane J, Galgani A, Petrone A, Lorini R, Pozzilli P, Buzzetti R, and Erlich H. Type 1 diabetes risk for HLA-DR3 haplotypes depends on genotypic context: Association of DPB1 and HLA class I loci among DR3 and DR4 matched Italian patients and controls. Hum Immunol. 2008; 69(4-5): 291–300.

[38] Stayoussef M, Benmansour J, Al-Irhayim A-Q, Said H, Rayana C, Mahjoub T and Almawi W. Autoimmune Type 1 Diabetes Genetic Susceptibility Encoded by Human Leukocyte Antigen DRB1 and DQB1 Genes in Tunisia. Clinical and vaccine immunology, Aug. 2009, p. 1146–1150.

[39] Pociot F, Akolkar B, Concannon P, Erlich HA, Julier C, Morahan G, Nierras CR, Todd JA, Rich SS, Nerup J. 2010. Genetics of type 1 diabetes: What's next? Diabetes 59: 1561–1571.

[40] Howson JM,Walker NM, Clayton D, Todd JA. 2009a. Confirmation of HLA class II independent type 1 diabetes associations in the major histocompatibility complex including HLA-B and HLA-A. Diabetes Obes Metab 11 (Suppl 1): 31–45.

[41] Burren OS, Adlem EC, Achuthan P, Christensen M, Coulson RM. T1DBase: update 2011, organization and presentation of large-scale data sets for type 1 diabetes research. Nucleic Acids Res. 2011 Jan;39(Database issue):D997-1001.

[42] Badenhoop k., Kahles H., Seidl C., Kordonouri O., Lopez E. R., Walter M., Rosinger S., Ziegler A., and Böhm B. O. The Type 1 Diabetes Genetics Consortium. MHC–environment interactions leading to type 1 diabetes:feasibility of an analysis of HLA DR-DQ alleles in relation to manifestation periods and dates of birth. Diabetes Obes Metab. 2009 February ; 11(Suppl 1): 88–91.

[43] Fourlanos S, Varney MD, Tait BD, Morahan G, Honeyman MC, Colman PG, Harrison LC. The Rising Incidence of Type 1 Diabetes Is Accounted for by Cases With Lower-Risk Human Leukocyte Antigen Genotypes. Diabetes Care, Volume 31, Number 8, August 2008.1546-49.

[44] Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith GJ, Rewers M, Dabelea D. Trends in High-Risk HLA Susceptibility Genes Among Colorado Youth With Type 1 Diabetes. Diabetes Care, Volume 31, Number 7, July 2008:1392-96.

[45] Frederiksen B, Kroehl M, Lamb M, Seifert J, Barriga K. Infant Exposures and Development of Type 1 Diabetes Mellitus: The Diabetes Autoimmunity Study in the Young (DAISY). JAMA Pediatr. 2013 September ; 167(9): 808–815.

[46] Snell-Bergeon JK, Smith J, Dong F, Barón AE, Barriga K, Norris JM, Rewers M. Early childhood infections and the risk of islet autoimmunity: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetes Care. 2012 Dec;35(12):2553-8.

[47] Lamb MM, Frederiksen B, Seifert JA, Kroehl M, Rewers M, Norris JM. Sugar intake is associated with progression from islet autoimmunity to type 1 diabetes: the Diabetes Autoimmunity Study in the Young. Diabetologia. 2015 Sep;58(9):2027-34.

[48] Lamb MM, Yin X, Zerbe GO, Klingensmith GJ, Dabelea D, Fingerlin TE, Rewers M, Norris JM. Height growth velocity, islet autoimmunity and type 1 diabetes development: the Diabetes Autoimmunity Study in the Young. Diabetologia. 2009 Oct;52(10):2064-71.

[49] Feng R, Li Y, Li G, Li Z, Zhang Y, Li Q, Sun C.Lower serum 25 (OH) D concentrations in type 1 diabetes: A meta-analysis. Diabetes Res Clin Pract. 2015 Jun;108(3):e71-5.

[50] Simpson M, Brady H, Yin X, Seifert J, Barriga K, Hoffman M, Bugawan T, Barón AE, Sokol RJ, Eisenbarth G, Erlich H, Rewers M, Norris JM. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia. 2011 Nov;54(11):2779-88.

[51] Treiber G, Prietl B, Fröhlich-Reiterer E, Lechner E, Ribitsch A, Fritsch M, Rami-Merhar B, Steigleder-Schweiger C, Graninger W, Borkenstein M, Pieber TR. Cholecalciferol supplementation improves suppressive capacity of regulatory T-cells in young patients with new-onset type 1 diabetes mellitus - A randomized clinical trial. Clin Immunol. 2015 Aug 12;161(2):217-224.

[52] Dunne JL, Triplett EW, Gevers D, Xavier R, Insel R, Danska J, Atkinson MA. The intestinal microbiome in type 1 diabetes. Clin Exp Immunol. 2014 Jul;177(1):30-7.

[53] Bach JF, Chatenoud L. The hygiene hypothesis: an explanation for the increased frequency of insulin-dependent diabetes. Cold Spring Harb Perspect Med. 2012 Feb;2(2):a007799.

[54] Patterson C, Dahlquist G, Gyürüs E, Green A, Soltész G, and the EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicenter prospective registration study. Lancet 2009; 373: 2027–33

[55] The DIAMOND Project Group. Incidence and trends of childhood Type 1 Diabetes worldwide 1990-1999. Diabetes UK. Diabetic medicine, 23, 857-866. 2006

[56] Tajima N, Morimoto A. Epidemiology of childhood diabetes mellitus in Japan. Pediatr Endocrinol Rev. 2012 Oct;10 Suppl 1:44-50.

[57] Harjutsalo V, Sund R, Knip M, Groop P-H. Incidence of Type 1 Diabetes in Finland. JAMA July 24/31, 2013 Volume 310, Number 4:427-8.

[58] Berhan Y, Waernbaum I, Lind T, Mollsten A, Dahlquist G. Thirty years of prospective nationwide incidence of childhood type 1 diabetes: the accelerating increase by time tends to level off in Sweden. Diabetes. 2011; 60(2):577–81.

[59] Bruno G, Maule M, Merletti F, Novelli G, Falorni A, Iannilli A, et al. Age-period-cohort analysis of 1990–2003 incidence time trends of childhood diabetes in Italy: the RIDI study. Diabetes. 2010; 59(9):2281–7.

[60] Patterson C. et al. Trends in childhood type 1 diabetes incidence in Europe during 1989–2008: evidence of non-uniformity over time in rates of increase. Diabetologia (2012) 55:2142–2147

[61] Australian Institute of Health and Welfare 2010. Incidence of Type 1 diabetes in Australian children 2000–2008. Diabetes series no. 13. Cat. no. CVD 51. Canberra: AIHW.

[62] Moltchanova EV, Schreier N, Lammi N, Karvonen M. Seasonal variation of diagnosis of Type 1 diabetes mellitus in children worldwidwole. Diabet Med. 2009 Jul;26(7):673-8.

[63] Conde S. et al. Epidemiología de la diabetes mellitus tipo 1 en menores de 15 años en España. An Pediatr (Barc). 2014. Sep;81(3):189.e1-189.e12. Epub 2014 Jan 24

[64] Belinchón BM, Hernández Bayo JA, Cabrera Rodríguez R. Incidence of childhood type 1 diabetes (0-14yrs) in La Palma Island. Diabetologia. 2008; 51(Suppl 1:S5-564)

[65] Carrillo Dominguez A. [Incidence of type 1 diabetes mellitus in the Canary Islands (1995-1996). Epidemiologic Group of the Canary Society of Endocrinology and Nutrition]. Rev Clin Esp. 2000; 200:257-60.

[66] Gale EA, Gillespie KM. Diabetes and gender. Diabetologia. 2001; 44(1):3–15.

[67] Wändell PE, Carlsson AC. Time trends and gender differences in incidence and prevalence of type 1 diabetes in Sweden. Curr Diabetes Rev. 2013 Jul;9(4):342-9.

[68] Ostman J, Lönnberg G, Arnqvist HJ, Blohmé G, Bolinder J, Ekbom Schnell A, Eriksson JW, Gudbjörnsdottir S, Sundkvist G, Nyström L. Gender differences and temporal variation in the incidence of type 1 diabetes: results of 8012 cases in the nationwide Diabetes Incidence Study in Sweden 1983-2002. J Intern Med. 2008 Apr;263(4):386-94.

[69] Fredheim S, Johannesen J, Johansen A, Lyngsøe L, Rida H, Andersen ML, Lauridsen MH, Hertz B, Birkebæk NH, Olsen B, Mortensen HB, Svensson J; Danish Society for Diabetes in Childhood and Adolescence. Diabetic ketoacidosis at the onset of type 1 diabetes is associated with future HbA1c levels. Diabetologia. 2013 May;56(5):995-1003.

[70] Wolfsdorf JI, Allgrove J, Craig ME, Edge J, Glaser N, Jain V, Lee WWR, Mungai LNW, Rosenbloom AL, Sperling MA, Hanas R. ISPAD Clinical Practice Consensus Guidelines 2014 Compendium. Diabetic ketoacidosis and hyperglycem ic hyperosmolar state. A Consensus Statement from the International Society for Pediatric and Adolescent Diabetes: Diabetic ketoacidosis and hyperglycemic hyperosmolar state. Pediatric Diabetes 2014: 15 (Suppl. 20): 154–179.

[71] Usher-Smith JA, Thompson M, Ercole A, Walter FM. Variation between countries in the frequency of diabetic ketoacidosis at first presentation of type 1 diabetes in children: a systematic review. Diabetologia. 2012 Nov;55(11):2878-94.

[72] Maahs DM, Hermann JM, Holman N, Foster NC, Kapellen TM, Allgrove J, Schatz DA, Hofer SE, Campbell F, Steigleder-Schweiger C, Beck RW, Warner JT, Holl RW; National Paediatric Diabetes Audit and the Royal College of Paediatrics and Child Health, the DPV Initiative, and the T1D Exchange Clinic Network. Rates of Diabetic Ketoacidosis: International Comparison With 49,859 Pediatric Patients With Type 1 Diabetes From England, Wales, the U.S., Austria, and Germany. Diabetes Care. 2015 Aug 17. pii: dc150780. [Epub ahead of print]

[73] Wägner AM, Santana A, Herńndez M, Wiebe JC, Nóvoa J, Mauricio D; T1DGC. Predictors of associated autoimmune diseases in families with type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium. Diabetes Metab Res Rev. 2011 Jul;27(5):493-8.

[74] Mantovani RM, Mantovani LM, Dias VM. Thyroid autoimmunity in children and adolescents with type 1 diabetes mellitus: prevalence and risk factors. J Pediatr Endocrinol Metab. 2007 Jun;20(6):669-75.

[75] American Diabetes Association. Children and adolescents. Sec.11. In Standards of Medical Care in Diabetes-2015. Diabetes Care 2015;38(Suppl. 1):S70–S76.

[76] Hanukoglu A, Mizrachi A, Dalal I, Admoni O, Rakover Y, Bistritzer Z, Levine A, Somekh E, Lehmann D, Tuval M, Boaz M, Golander A. Extrapancreatic autoimmune manifestations in type 1 diabetes patients and their first-degree relatives: a multicenter study. Diabetes Care. 2003 Apr;26(4):1235-40.

[77] Hemminki K, Li X, Sundquist J, Sundquist K. Familial association between type 1 diabetes and other autoimmune and related diseases. Diabetologia. 2009 Sep;52(9):1820-8.

[78] Liu E, Lee HS, Aronsson CA, Hagopian WA, Koletzko S, Rewers MJ, Eisenbarth GS, Bingley PJ, Bonifacio E, Simell V, Agardh D; TEDDY Study Group. Risk of pediatric celiac disease according to HLA haplotype and country. N Engl J Med. 2014 Jul 3;371(1):42-9.

[79] Fregel R, Betancor E, Suárez NM, Cabrera VM, Pestano J, Larruga JM, González AM. Temporal evolution of the ABO allele frequencies in the Canary Islands: the impact of the European colonization. Immunogenetics. 2009 Sep;61(9):603-10.

[80] Maca-Meyer N, Villar J, Pérez-Méndez L, Cabrera de León A, Flores C. A tale of aborigines, conquerors and slaves: Alu insertion polymorphisms and the peopling of Canary Islands. Ann Hum Genet. 2004 Nov;68(Pt 6):600-5

[81] Fregel R, Maca-Meyer N, Cabrera VM, González AM, Larruga JM. Description of a simple multiplex PCR-SSCP method for AB0 genotyping and its application to the peopling of the Canary Islands. Immunogenetics. 2005 Sep;57(8):572-8.

[82] Patterson CC, Dahlquist G, Harjutsalo V, Joner G, Feltbower RG, Svensson J, Schober E, Gyürüs E, Castell C, Urbonaité B, Rosenbauer J, Iotova V, Thorsson AV, Soltész G.Early mortality in EURODIAB population-based cohorts of type 1 diabetes diagnosed in childhood since 1989. Diabetologia. 2007 Dec;50(12):2439-42.

[83] Lind M, Svensson AM, Kosiborod M, Gudbjörnsdottir S, Pivodic A, Wedel H, Dahlqvist S, Clements M, Rosengren A. Glycemic control and excess mortality in type 1 diabetes. N Engl J Med. 2014 Nov 20;371(21):1972-82.

[84] Lorenzo V, Boronat M, Saavedra P, Rufino M, Maceira B, Novoa Francisco J. and Torres A. Disproportionately high incidence of diabetes-related end-stage renal disease in the Canary Islands. An analysis based on estimated population at risk. Nephrol Dial Transplant (2010) 25: 2283–2288.

[85] Elding H, Vehik K, Bell R, Dabelea D, Dolan L, Pihoker C, Knip M, Veijola R, Lindblad B, Samuelsson U, Holl R, Haller MJ; TEDDY Study Group; SEARCH Study Group; Swediabkids Study Group; DPV Study Group; Finnish Diabetes Registry Study Group. Reduced prevalence of diabetic ketoacidosis at diagnosis of type 1 diabetes in young children participating in longitudinal follow-up. Diabetes Care. 2011 Nov;34(11):2347-52.

[86] LaPorte et al. Counting diabetes in the next millenium. Application of capture-recapture technology. Diabetes Care, 1993 Feb;16(2):528-34.

[87] Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP; ESPGHAN Working Group on Coeliac Disease Diagnosis; ESPGHAN Gastroenterology Committee; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012 Jan;54(1):136-60.

[88] Black M, Lawrence J, Pihoker C, Dolan L, Anderson A, Rodriguez B, Marcovina S, Mayer-Davis E, Imperatore G and Dabelea D, for the SEARCH for Diabetes in Youth Study Group. HLA-Associated Phenotypes in Youth with Autoimmune Diabetes. Pediatr Diabetes. 2013 March ; 14(2): 121–128.

[89] Masuda M, Powell M, Chen S, Beer C, Fichna P, Rees Smith B, Furmaniak J. Autoantibodies to IA-2 in insulin-dependent diabetes mellitus. Measurements with a new immunoprecipitation assay. Clin Chim Acta. 2000 Jan 20;291(1):53-66.

[90] Powell M, Prentice L, Asawa T, Kato R, Sawicka J, Tanaka H, Petersen V, Munkley A, Morgan S, Rees Smith B, Furmaniak J. Glutamic acid decarboxylase autoantibody assay using 125I-labelled recombinant GAD65 produced in yeast. Clin Chim Acta. 1996 Dec 30;256(2):175-88.

[91] Törn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ & Participating Laboratories. Diabetes Antibody Standardization Program: evaluationof assays for autoantibodies to glutamic acid decarboxylaseand islet antigen-2. Diabetologia (2008) 51:846–852

[92] Sullivan L. 2012. Essentials of Biostatistics in Public Health. 2nd Edition. Burlington (MA): Jones & Bartlett Learning.

[93] Carrascosa A, Fernández JM, Fernández C, Ferrández A, López-Siguero JP, Sánchez E, Sobradillo B, Yeste D. Spanish growth studies 2008. New anthropometric standards. Endocrinol Nutr. 2008 Dec;55(10):484-506

[94] Elamin A, Omer MI, Zein K, Tuvemo T. Epidemiology of childhood type I diabetes in Sudan, 1987-1990. Diabetes Care 1992 Nov;15(11):1556-9.

[95] Swai AB, Lutale J, McLarty DG. Diabetes in tropical Africa: a prospective study 1981-7. I. Characteristics of newly presenting patients in Dar es Salaam, Tanzania, 1981-7. BMJ 1990;300(6732):1103–6

[96] Nasheeta Peer, Andre-Pascal Kengne, Ayesha A. Motalac, Jean Claude Mbanyad. IDF Diabetes Atlas. Diabetes in the Africa region: An update. Diabetes Research and Clinical Practice Volume 103, Issue 2 , Pages 197-205, February 2014

[97] Lawrence J, Imperatore G, Dabelea D, Mayer-Davis E, Linder B, Saydah S, Klingensmith G, Dolan L, Standiford D, Pihoker K, Pettitt D,Talton J,Thomas J, Bell R, and D'Agostino R, Jr.for the SEARCH for Diabetes in Youth Study Group*. Trends in Incidence of Type 1 Diabetes Among Non-Hispanic White Youth in the U.S. 2002–2009 Diabetes 2014;63:3938–3945

[98] Haynes A, Bulsara MK, Bower C, Jones TW, Davis EA. Regular peaks and troughs in the Australian incidence of childhood type 1 diabetes mellitus (2000-2011). Diabetologia. 2015 Jul 31. [Epub ahead of print]

[99] Lopez-Sigueiro et al. Increased Incidence of Type 1 Diabetes in the South of Spain. Diabetes Care, 2002; Volume 25, number 6: 1099

[100] Conde S, Rigual M, Bueno G, Rodrigo Mª Pilar, Compés MªL, Soria J, González B. Registro de Diabetes Mellitus Tipo 1 en Aragón: 20 años de seguimiento. Rev Esp Endocrinol Pediatr 2013; Volumen 4. Número 1

[101] Escribano A, Martos JM, Valcárcel I, Gutiérrez A. Revisión del Debut de Diabetes Tipo 1 en Hospital terciario: 2003-2007. Abstract. XXX Congreso de la Sociedad Española de Endocrinología Pediátrica. Madrid. 2008. An Pediatr 2008; 68 (Suppl 1): 111-112.

[102] Zorrilla Torras B, Cantero Real JL, Barrios Castellanos R, Ramírez Fernández J, Argente Oliver J, González Vergaz A; en nombre del Registro de Incidencia de Diabetes Tipo 1 en Menores de 15 años (Consejería de Sanidad, Comunidad de Madrid). [Incidence of type 1 diabetes mellitus in children: results from the population registry of the Madrid Region, 1997-2005]. Med Clin (Barc). 2009 Apr 18;132(14): 545-8.

[103] Cinek O, Kulich M, Sumnik Z. The incidence of type 1 diabetes in Young Czech children stopped rising. Pediatric Diabetes. 2012. Nov; 13(7):559-63.

[104] Bendas A, Rothe U, Kiess W, Kapellen TM, Stange T, Manuwald U, et al. (2015) Trends in Incidence Rates during 1999-2008 and Prevalence in 2008 of Childhood Type 1 Diabetes Mellitus in GERMANY – Model-Based National Estimates. PLoS ONE. 2015 Jul 16; 10(7): e0132716.

[105] Harjutsalo V, Sjöberg L, Tuomilehto J.Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. Lancet. 2008 May 24;371(9626):1777-82.

[106] Quinn M, Fleischman A, Rosner B, Nigrin DJ, Wolfsdorf JI. Characteristics at diagnosis of type 1 diabetes in children younger than 6 years. J Pediatr. 2006;148(3):366

[107] Fröhlich-Reiterer EE, Rosenbauer J, Bechtold-Dalla Pozza S, Hofer SE, Schober E5, Holl RW6; DPV-Wiss Study Group and German BMBF Competence Networks Diabetes mellitus and Obesity. Predictors of increasing BMI during the course of diabetes in children and adolescents with type 1 diabetes: data from the German/Austrian DPV multicentre survey. Arch Dis Child. 2014 Aug;99(8):738-43.

[108] Jung ES, Han DK, Yang EM, Kim MS, Lee DY, Kim CJ. Thyroid autoimmunity in children and adolescents with newly diagnosed type 1 diabetes mellitus. Ann Pediatr Endocrinol Metab. 2014 Jun;19(2):76-9.

[109] Felner EI, Klitz W, Ham M, Lazaro AM, Stastny P, Dupont B, White PC. Genetic interaction among three genomic regions creates distinct contributions to early- and late-onset type 1 diabetes mellitus. Pediatr Diabetes. 2005;6(4):213-20.

[110] Durruty P, Ruiz F, García de los Ríos M. Age at diagnosis and seasonal variation in the onset of insulin-dependent diabetes in Chile (Southern hemisphere). Diabetologia. 1979;17(6):357-60.

[111] Van der Werf N, Kroese F, Rozing J, Hillebrands J-L. Viral infections as potential triggers of type 1 diabetes. Diabetes/Metabolism Research and Reviews Diabetes Metab Res Rev 2007; 23: 169–183.

[112] Valdés C, Unanue N, Hernández M, García R, Castro M, Vásquez L, Torres JP, Mericq V. Is there a link between influenza and type I diabetes? Increased incidence of TID during the pandemic H1N1influenza of 2009 in Chile. Pediatr Endocrinol Rev. 2013 Dec;11(2): 161-6.

[113] Kondrashova A, Nurminen N, Patrikainen M, Huhtala H, Lehtonen J, Toppari J, Ilonen J, Simell OG, Veijola R, Knip M, Hyöty H. Influenza A virus antibodies show no association with pancreatic islet autoantibodies in children genetically predisposed to type 1 diabetes. Diabetologia. 2015 Aug 8. [Epub ahead of print]

[114] Oyarzabal M, García B, Barrio R, Torres M, Gómez AL, González I, Hermoso F, Luzuriaga C, Rica I, López MJ, Rodríguez M. Ketoacidosis at onset of type 1 diabetes mellitus in pediatric age in Spain and review of the literature. Pediatr Endocrinol Rev. 2012 Mar;9(3):669-71.

[115] Lévy-Marchal C, Patterson CC, Green A; EURODIAB ACE Study Group. Europe and Diabetes. Geographical variation of presentation at diagnosis of type I diabetes in children: the EURODIAB study. European and Dibetes. Diabetologia. 2001 Oct;44 Suppl 3:B75-80.

[116] Sadauskaite-Kuehne V, Samuelsson U, Jasinskiene E, Padaiga Z, Urbonaite B, Edenvall H, Ludvigsson J; DEBS Study Group. Severity at onset of childhood type 1 diabetes in countries with high and low incidence of the condition. Diabetes Res Clin Pract. 2002 Mar;55(3):247-54.

[117] Fritsch M, Schober E, Rami-Merhar B, Hofer S, Fröhlich-Reiterer E, Waldhoer T; Austrian Diabetes Incidence Study Group. Diabetic ketoacidosis at diagnosis in Austrian children: a population-based analysis, 1989-2011. J Pediatr. 2013 Nov;163(5):1484-8.e1.

[118] Choleau C, Maitre J, Filipovic Pierucci A, Elie C, Barat P, Bertrand AM, de Kerdanet M, Letallec C, Levy-Marchal C, Nicolino M, Tubiana-Rufi N, Cahané M, Robert JJ; AJD Study Group. Ketoacidosis at diagnosis of type 1 diabetes in French children and adolescents. Diabetes Metab. 2014 Apr;40(2):137-42.

[119] Hekkala A, Knip M, Veijola R. Ketoacidosis at diagnosis of type 1 diabetes in children in northern Finland. Temporal changes over 20 years. Diabetes care 30:861-866. 2007

[120] Savova R, Popova G, Koprivarova K, Konstantinova M, Angelova B, Atanasova M, Goranova S, Christov V, Kalinov K. Clinical and laboratory characteristics of type I (insulin

dependent) diabetes mellitus at presentation among Bulgarian children. Diabetes Res Clin Pract. 1996 Oct;34 Suppl:S159-63.

[121] Chumięcki M, Prokopowicz Z, Deja R, Jarosz-Chobot P. Frequency and clinical manifestation of diabetic ketoacidosis in children with newly diagnosed type 1 diabetes]. Pediatr Endocrinol Diabetes Metab. 2013;19(4):143-7.

[122] Neu A, Willasch A, Ehehalt S, Hub R, Ranke MB; DIARY Group Baden-Wuerttemberg. Ketoacidosis at onset of type 1 diabetes mellitus in children--frequency and clinical presentation. Pediatr Diabetes. 2003 Jun;4(2):77-81.

[123] Jefferies C, Cutfield SW, Derraik JG, Bhagvandas J, Albert BB, Hofman PL, Gunn AJ, Cutfield WS. 15-year incidence of diabetic ketoacidosis at onset of type 1 diabetes in children from a regional setting (Auckland, New Zealand). Sci Rep. 2015 May 19;5:10358.

[124] Dabelea D, Rewers A, Stafford JM, Standiford DA, Lawrence JM, Saydah S, Imperatore G, D'Agostino RB Jr, Mayer-Davis EJ, Pihoker C; SEARCH for Diabetes in Youth Study Group. Trends in the prevalence of ketoacidosis at diabetes diagnosis: the SEARCH for diabetes in youth study. Pediatrics. 2014 Apr;133(4):e938-45.

[125] Al-Yaarubi S, Ullah I1, Sharef SW2, Al Shidhani A1, Al Hanai S3, Al Kalbani R3, Al Jamoodi S3. Demographic and clinical characteristics of type 1 diabetes mellitus in omani children - single center experience. Oman Med J. 2014 Mar;29(2):119-22.

[126] Xin Y, Yang M, Chen XJ, Tong YJ, Zhang LH. Clinical features at the onset of childhood type 1 diabetes mellitus in Shenyang, China. J Paediatr Child Health. 2010 Apr;46(4):171-5.

[127] Demir F, Günöz H, Saka N, Darendeliler F, Bundak R, Baş F, Neyzi O. Epidemiologic Features of Type 1 Diabetic Patients between 0 and 18 Years of Age in İstanbul City.J Clin Res Pediatr Endocrinol. 2015 Mar 5;7(1):49-56.

[128] Ardicli D, Kandemir N, Alikasifoglu A, Ozon A, Gonc N. Clinical characteristics of type 1 diabetes over a 40 year period in Turkey: secular trend towards earlier age of onset. J Pediatr Endocrinol Metab. 2014 Jul;27(7-8):635-41.

[129] Al-Magamsi MS, Habib HS. Clinical presentation of childhood type 1 diabetes mellitus in the Al-Madina region of Saudi Arabia. Pediatr Diabetes. 2004 Jun;5(2):95-8.

[130] Noble J, Johnson J, Lane J. and Valdes A. HLA Class II Genotyping of African American Type 1 Diabetic Patients Reveals Associations Unique to African Haplotypes. Diabetes, Vol. 62, September 2013:3292-3299.

[131] Kawabata Y, Ikegami H, Kawaguchi Y, Fujisawa T, Shintani M, Ono M, Nishino M, Uchigata Y, Lee I, Ogihara T. Asian-specific HLA haplotypes reveal heterogeneity of the

contribution of HLA-DR and -DQ haplotypes to susceptibility to type 1 diabetes. Diabetes. 2002 Feb;51(2):545-51.

[132] Kawabata Y, Ikegami H, Awata T, Imagawa A, Maruyama T, Kawasaki E, Tanaka S, Shimada A, Osawa H, Kobayashi T, Hanafusa T, Tokunaga K & Makino H on behalf of the Committee on Type 1 Diabetes, Japan Diabetes Society. Differential association of HLA with three subtypes of type 1 diabetes: fulminant, slowly progressive and acute-onset. Diabetologia (2009) 52:2513–2521

[133] Savola K, Bonifacio E, Sabbah E, Kulmala P, Vähäsalo P, Karjalainen J, Tuomilehto-Wolf E, Meriläinen J, Akerblom HK, Knip M. IA-2 antibodies--a sensitive marker of IDDM with clinical onset in childhood and adolescence. Childhood Diabetes in Finland Study Group. Diabetologia. 1998 Apr;41(4):424-9.

[134] Steck AK, Johnson K, Barriga KJ, Miao D, Yu L, Hutton JC, Eisenbarth GS, Rewers MJ. Age of islet autoantibody appearance and mean levels of insulin, but not GAD or IA-2 autoantibodies, predict age of diagnosis of type 1 diabetes: diabetes autoimmunity study in the young. Diabetes Care. 2011 Jun;34(6):1397-9.

[135] Elding Larsson H, Vehik K, Gesualdo P, Akolkar B, Hagopian W, Krischer J, Lernmark Å, Rewers M, Simell O, She JX, Ziegler A, Haller MJ; TEDDY Study Group. Children followed in the TEDDY study are diagnosed with type 1 diabetes at an early stage of disease. Pediatr Diabetes. 2014 Mar;15(2):118-26.

[136] Dabelea, D., C. Pihoker, J.W. Talton, et al. 2011. Etiological approach to characterization of diabetes type: the SEARCH for diabetes in youth study. Diabetes Care 34: 1628–1633

[137] Kukko M, Kimpimäki T, Kupila A, Korhonen S, Kulmala P, Savola K, Simell T, Keskinen P, Ilonen J, Simell O, Knip M. Signs of beta-cell autoimmunity and HLA-defined diabetes susceptibility in the Finnish population: the sib cohort from the Type 1 Diabetes Prediction and Prevention Study. Diabetologia. 2003 Jan;46(1):65-70.

[138] Sabbah E, Savola K, Kulmala P, Reijonen H, Veijola R, Vähäsalo P, Karjalainen J, Ilonen J, Akerblom HK, Knip M. Disease-associated autoantibodies and HLA-DQB1 genotypes in children with newly diagnosed insulin-dependent diabetes mellitus (IDDM). The Childhood Diabetes in Finland Study Group. Clin Exp Immunol. 1999 Apr;116(1):78-83.

[139] Akirov A, Pinhas-Hamiel O. Co-occurrence of type 1 diabetes mellitus and celiac disease. World J Diabetes. 2015 Jun 10;6(5):707-14

[140] Kordonouri O, Klinghammer A, Lang EB, Grüters-Kieslich A, Grabert M, Holl RW. Thyroid autoimmunity in children and adolescents with type 1 diabetes: a multicenter survey. Diabetes Care. 2002 Aug;25(8):1346-50.

[141] Kordonouri O, Klingensmith G, Knip M, Holl RW, Menon PSN, Aanstoot HJ, Craig ME. Other complications and diabetes-associated conditions in children and adolescents. Pediatric Diabetes 2014: 15 (Suppl. 20): 270–278.

[142] Ivarsson A, Myléus A, Norström F, van der Pals M, Rosén A, Högberg L, Danielsson L, Halvarsson B, Hammarroth S, Hernell O, Karlsson E, Stenhammar L, Webb C, Sandström O, Carlsson A. Prevalence of childhood celiac disease and changes in infant feeding. Pediatrics. 2013 Mar;131(3):e687-94.

[143] Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 2003 Feb 10;163(3):286-92.

[144] Al-Agha AE, Alafif MM, Abd-Elhameed IA. Glycemic control, complications, and associated autoimmune diseases in children and adolescents with type 1 diabetes in Jeddah, Saudi Arabia. Saudi Med J. 2015 Jan;36(1):26-31.

[145] Nóvoa Y, López-Capapé M, Lara E, Alonso M, Camarero C, Barrio R. [Impact of diagnosis of celiac disease on metabolic control of type 1 diabetes]. [Article in Spanish]. An Pediatr (Barc). 2008 Jan;68(1):13-7.

[146] Machado R, Machado L and Alves V.M. Thyroid Autoimmunity in Children and Adolescents with Type 1 Diabetes Mellitus: Prevalence and Risk Factors. Journal of Pediatric Endocrinology & Metabolism, 20,669-675 (2007)

[147] Kakleas K, Soldatou A, Karachaliou F, Karavanaki K. Associated autoimmune diseases in children and adolescents with type 1 diabetes mellitus (T1DM). Autoimmun Rev. 2015 Sep;14(9):781-97.

[148] Kahles H, Fain PR, Baker P, Eisenbarth G, Badenhoop K. Genetics of Autoimmune Thyroiditis in Type 1 Diabetes Reveals a Novel Association With DPB1*0201: Data From the Type 1 Diabetes Genetics Consortium. Diabetes Care. 2015 Oct;38(Suppl 2):S21-S28.