DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
47

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
47

IMMUNE AND GENETIC FACTORS OF CHILDHOOD ONSET IDDM IN ESTONIA

An epidemiological study

BELA ADOJAAN

TARTU UNIVERSITY
PRESS

Department of Internal Medicine, University of Tartu

Dissertation is accepted for the commencement of the degree of Doctor of Medical Science on October 21, 1998 by the Doctoral Committee of the Faculty of Medicine, University of Tartu

Opponents: Professor Hele Everaus, MD, PhD, DMSci,
University of Tartu, Tartu, Estonia
Doctor Sirje Velbri, MD, PhD, DMSci,
Tallinn Central Hospital, Tallinn, Estonia

Commencement: January 27, 1999

Publication of this dissertation is granted by the University of Tartu

Tartu Ülikooli Kirjastuse trükikoda
Tiigi 78, Tartu 50410
Tellimus nr. 466

CONTENTS

LIST OF ORIGINAL PUBLICATIONS 7
ABBREVIATIONS 9
INTRODUCTION 10
BACKGROUND OF THE STUDY 12
1. Immune factors of IDDM 12
1.1. Islet cell antibodies 12
1.2. Glutamic acid decarboxylase antibodies 14
1.3. Tyrosine phosphatase antibodies 16
2. Genetic factors of IDDM 17
2.1. HLA-DRB1 alleles 17
2.2. HLA-DQB1 alleles 19
2.3. HLA-DQA1 alleles 21
2.4. HLA-DQ and HLA-DR allele combinations 22
PURPOSE OF THE STUDY 24
MATERIAL AND METHODS 25
1. Study subjects 25
1.1. Childhood onset IDDM patients 25
1.2. Healthy siblings of childhood onset IDDM patients 25
1.3. Controls 26
2. Detection of antibodies 26
2.1. Islet cell antibodies 26
2.2. Glutamic acid decarboxylase antibodies 26
2.3. Tyrosine phosphatase antibodies 27
3. Detection of HLA-DQ and HLA-DR alleles 28
3.1. Extraction of DNA 28
3.2. Detection of alleles 28
4. Statistical methods 29
RESULTS AND DISCUSSION 30
1. Immune factors of IDDM 30
1.1. Antibodies in newly diagnosed childhood onset IDDM patients 30
1.2. Antibodies in healthy siblings of childhood onset IDDM patients 32
1.3. Antibodies in controls 34
1.4. Discussion 35
2. Genetic factors of IDDM 39
2.1. Selected HLA-DQB1 alleles in childhood onset IDDM patients and controls 39

2.2. Selected HLA-DQ and HLA-DR allele combinations in childhood onset IDDM patients and controls 41
2.3. Discussion 42
3. Immune and genetic factors of IDDM in healthy siblings of childhood onset IDDM patients followed for three years 44
CONCLUSIONS 46
REFERENCES 47
SUMMARY IN ESTONIAN: Lapseeas alanud insuliinisõltuva suhkurtõve immunoloogilised ja geneetilised tegurid Eestis 57
ACKNOWLEDGEMENTS 62
PUBLICATIONS 63

LIST OF PUBLICATIONS

I Adojaan B, Knip M, Vähäsalo P, Karjalainen J, Kalits I, Åkerblom HK. Relationship between the incidence of childhood IDDM and the frequency of ICA positivity in nondiabetic children in the general population. Diabetes Care 1996; 19: 1452–1454.

II Ilonen J, Koskinen S, Nejentsev S, Sjöroos M, Knip M, Schwartz EI, Adojaan B, Kovalchuk L, Sochnevs A. HLA-DQB1*0304-DRB1*0408 haplotype associated with insulin-dependent diabetes mellitus in populations in the Eastern Baltic region. Tissue Antigens 1997; 49: 532–534.

III Nejentsev S, Reijonen H, Adojaan B, Kovalchuk L, Sochnevs A, Schwartz E, Åkerblom HK, Ilonen J. The effects of HLA-B allele on the IDDM risk defined by DRB1*04 subtypes and DQB1*0302. Diabetes 1997; 46: 1888–1892.

IV Nejentsev S, Koskinen S, Sjöroos M, Reijonen H, Schwartz EI, Kovalchuk L, Sochnev A, Adojaan B, Podar T, Knip M, Simell O, Koskenvuo M, Åkerblom HK, Ilonen J. Distribution of insulin-dependent diabetes (IDDM)-related HLA alleles correlates with the difference in IDDM incidence in four populations of the Eastern Baltic Region. Tissue Antigens 1998; 52: 473–477.

V Adojaan B, Podar T. Lapseeas alanud insuliinisõltuva suhkurtõve immunoloogilised ja geneetilised tegurid Eestis. Eesti Arst 1998; N. 6: 488–491.

RELATED PAPERS

VI Podar T, Adojaan B, Kalits I. Eesti insuliinsõltuva suhkurtõvega laste register. Eesti Arst 1990; 3: 179–180.

VII Tuomilehto J, Podar T, Reunanen A, Kalits I, Lounamaa R, Tuomilehto-Wolf E, Adojaan B, Neff B, LaPorte RE. Comparison of incidence of IDDM in childhood between Estonia and Finland, 1980–1988. Diabetes Care 1991; 14: 982–988.

VIII Tuomilehto J, Podar T, Brigis G, Urbonaite B, Rewers M, Adojaan B, Cepaitis Z, Kalits I, King H, LaPorte R, Lounamaa R, Padaiga Z, Reu-

nanen A, Tuomilehto-Wolf E, Walczak M. Comparison of the incidence of insulin-dependent diabetes mellitus in childhood among five Baltic populations during 1983–1988. International Journal of Epidemiology 1992; 21: 518–527.

IX Podar T, Tuomilehto-Wolf E, Tuomilehto J, LaPorte RE, Adojaan B. Insulin-dependent diabetes mellitus in native Estonians and immigrants to Estonia. American Journal of Epidemiology 1992; 135: 1231–1236.

X Tillmann V, Adojaan B, Shor R, Price DA, Tuvemo T. Physical development in Estonian children with Type 1 diabetes. Diabetic Medicine 1996; 13: 97–101.

XI Adojaan B. Insuliinisõltuva suhkurtõve etiopatogenees. Eesti Arst 1996; 4: 322–324.

XII Einberg Ü, Podar T, Adojaan B, Rägo T. Lapseea insuliinisõltuva suhkurtõve sümptomaatikast. Eesti Arst 1997; 2: 129–132.

ABBREVIATIONS

Arg	arginine
Asp	aspartate
AU	arbitrary unit(s)
Ci	Curie
cpm	counts per minute
DNA	desoxyribonucleic acid
EDTA	ethylene diamine tetra acetic acid
f	female(s)
GAD	glutamic acid decarboxylase
GADA	glutamic acid decarboxylase antibodies
HLA	human leucocyte antigen
IA-2A	tyrosine phosphatase antibodies
ICA	islet cell antibodies
ID	identification number
IDDM	insulin-dependent diabetes mellitus
IFL	immunofluorescence
IgG	immunoglobulin(s) class G
JDFU	Juvenile Diabetes Foundation unit(s)
kD	kilodalton(s)
M	mole(s)
m	male(s)
ml	milliliter(s)
N	total number of individuals
ND	not detected
nm	nanometer(s)
OR	odds ratio
p	p value
PCR	polymerase chain reaction
r	correlation coefficient
RNA	ribonucleic acid
rpm	rounds per minute
RU	relative unit(s)
U	unit(s)
µl	microliter(s)
µg	microgram(s)

INTRODUCTION

Insulin-dependent diabetes mellitus (IDDM) is one of the most severe and common metabolic diseases of childhood. It has major impact on the society as for cost. IDDM occurs most frequently in persons of Northern European descent. The incidence of IDDM among children ranges from 1.7 per 100,000 per year in Japan to 35 per 100,000 per year in Finland (Karvonen *et al.*, 1993). In Estonia the incidence of IDDM is about 10 per 100,000 per year (VII, VIII).

IDDM is a chronic disease with yet unknown aetiology. Therefore, no effective measures for the prevention of the disease are available. Evidence accumulated over the last two decades has strongly supported the hypothesis that IDDM develops in genetically susceptible individuals by an autoimmune mechanism.

To support this hypothesis, antibodies to pancreatic islet cells have been found (Bottazzo *et al.*, 1974; Atkinson and Maclaren, 1993). Not just humoral immune abnormalities, but also cell-mediated ones have been found in the peripheral blood of patients (Drell and Notkins, 1987; Atkinson *et al.*, 1992; Harrison *et al.*, 1993; Rabinovitch, 1994). The Langerhans islets of persons with IDDM have been infiltrated with mononuclear cells, and histologically insulitis and beta-cell destruction documented (Bottazzo *et al.*, 1974, 1986; Tisch *et al.*, 1993; Atkinson and Maclaren, 1994). IDDM is often concurrent with other autoimmune abnormalities such as autoimmune thyroid disease, pernicious anaemia, Addison's disease, idiopathic hypoparathyroidism, primary hypogonadism, myasthenia gravis, vitiligo, alopecia totalis, coeliac disease (Bottazzo *et al.*, 1974, 1986; Drell and Notkins, 1987; Wagner *et al.*, 1994; Björk *et al.*, 1994).

IDDM is strongly associated with certain HLA-linked immune response genes (Raum *et al.*, 1979; Vardi *et al.*, 1987; Deschamps *et al.*, 1991; Tuomilehto-Wolf *et al.*, 1991; Todd, 1995; Routsias and Papadopuolus, 1995). Genes in the HLA region on chromosome six confer the major genetic susceptibility to IDDM (Todd *et al.*, 1987; Tuomilehto-Wolf and Tuomilehto, 1991). The role of HLA in IDDM was first indicated by association with HLA-B8 and B15 (Singal and Blajchman, 1973). Subsequently a stronger association was found with HLA-DR3 and HLA-DR4 antigens encoded at the HLA-DRB1 locus (Thomsen *et al.*, 1975; Svejgaard *et al.*, 1980; Overbach *et al.*, 1983; Wolf *et al.*, 1983). More recently, HLA-DQB1 and HLA-DQA1 genes were shown to be even more strongly associated with IDDM (Todd *et al.*, 1987; Rønningen *et al.*, 1991; Thorsby and Rønningen, 1993). The proteins encoded by HLA class II genes are antigen-presenting molecules and may confer protection or susceptibility for the development of IDDM (Thorsby and Rønningen, 1993).

According to widely accepted concept of the etiopathogenesis of IDDM, environmental factors such as microbial agents and chemicals act as triggers of an autoimmune response against pancreatic islet beta-cells in genetically IDDM susceptible individuals (Yoon, 1990; Vreugdenhil *et al.*, 1998; Saukkonen *et al.*, 1998). Certain viruses have been associated with the induction of IDDM in humans, notably rubella, mumps, Coxsackie and cytomegalovirus (Rubinstein *et al.*, 1982; Helmke *et al.*, 1986; Guberski *et al.*, 1991; Gaskins *et al.*, 1992). Molecular mimicry between epitopes common to microbial antigens and host proteins has often been implicated in induction of autoimmune disease in man (Hyöty *et al.*, 1995; Vreugdenhil *et al.*, 1998).

However, there is still confusion concerning the interpretation of the complex, often discrepant, immune and genetic associations with IDDM. For that reason, studies of different populations of varying genetic and environmental backgrounds are needed for better understanding of the aetiology and pathogenesis of IDDM. Until now, insufficient information is available on the prevalence of IDDM associated immune and genetic factors in Estonia.

BACKGROUND OF THE STUDY

1. Immune factors of IDDM

The humoral autoimmunity is extensively studied in IDDM. The most commonly reported immunological abnormalities in IDDM are autoantibodies reacting with pancreatic islet-cell antigens. Many autoantibodies have been described in IDDM. It has become harder to identify those antibodies that are the most important and involved in the primary immunological events of the autoimmune disease process. The following is an incomplete list of the autoantigens against which antibodies have been detected in IDDM — insulin, sialoganglioside of islet cells, glutamic acid decarboxylase, insulin receptor, islet tyrosine phosphatase, glucose transporter, carboxypeptidase H, cow milk protein (Bottazzo *et al.*, 1974; Baekkeskov *et al.*, 1982; Borch-Johnsen *et al.*, 1984; Atkinson and Maclaren, 1993, 1994; Christie *et al.*, 1990; Christie, 1993; Martin *et al.*, 1991; Saukkonen *et al.*, 1998).

1.1. Islet cell antibodies

Islet cell antibodies (ICA) were first described in IDDM patients who had other coexistent autoimmune endocrine disorders by Bottazzo and colleagues in 1970s (Bottazzo *et al.*, 1974). The initial description of ICA provided strong evidence for an autoimmune etiology and pathogenesis of IDDM.

ICA react with antigens in the cytoplasm of all endocrine cells of pancreatic islets and are shown by conventional indirect immunofluorescence (IFL). They are exclusively of immunoglobulin G (IgG) class (Atkinson and Maclaren, 1994). The target antigen recognized by ICA in IFL assays has been suggested to be a sialoganglioside (Nayak *et al.*, 1985). Some ICA, in particular those of high titre, can fix complement. This has been shown predominantly in newly diagnosed IDDM patients and in individuals genetically predisposed to IDDM (Karjalainen *et al.*, 1986; Bonifacio *et al.*, 1989).

Natural history of IDDM has been defined through studies of nondiabetic subjects who had IDDM-associated autoantibodies years before the onset of hyperglycemia. Measurement of ICA in prospective studies of individuals at high risk to develop IDDM has established that the abnormal immunological state against beta-cells precedes the clinical onset of the disease by up to several years (Kobayashi *et al.*, 1987; Kuglin *et al.*, 1989; Bruining *et al.*, 1989; Bingley *et al.*, 1993). The standardization of ICA measurements and the introduction of reference Juvenile Diabetes Foundation units (JDFU) have enabled better comparison of data from different centers (Lernmark *et al.*, 1991).

ICA are found shortly after diagnosis in most of IDDM patients (Table 1). The percentage of ICA positive individuals ranges from 67 to 93 in different studies.

Table 1. Prevalence (%) of ICA in newly diagnosed IDDM patients (IDDM) and healthy first degree relatives of IDDM patients (Relatives)

IDDM	
Kolb *et al.* (1980)	83
Lernmark *et al.* (1981)	67
Notsu *et al.* (1985)	63
Karjalainen *et al.* (1986)	75
Landin-Olsson *et al.* (1989)	81
Genovese *et al.* (1992)	89
Bonifacio *et al.* (1995)	93
Gorus *et al.* (1997)	73
Wiest *et al.* (1997)	75
Savola *et al.* (1997)	84
Relatives	
Gorsuch *et al.* (1980)	4.0
Thivolet *et al.* (1988)	5.1
Bonifacio *et al.* (1989)	3.3
Kuglin *et al.* (1989)	3.5
Seissler *et al.* (1996)	6.9
Gorus *et al.* (1997)	5.0

In IDDM patients, ICA tend to disappear from the circulation a few weeks or months after diagnosis (Kolb *et al.*, 1980; Atkinson and Maclaren, 1993). Several reports have shown that some IDDM patients continue to be ICA positive with lower titers for a long period after diagnosis (Kobayashi *et al.*, 1987, 1993). The prevalence of ICA gradually decreases with time and eventually drops to 12–35% in IDDM patients with duration of five years or more (Kobayashi *et al.*, 1987, 1993; Kolb *et al.*, 1988). Considering the 10–30% of the IDDM patients negative for ICA at diagnosis, these might have been recruited from subjects with previous transient ICA positivity and disappearance of ICA from sera would occur when pancreatic beta-cells have been completely destroyed. ICA may reflect ongoing autoimmune beta-cell destruction and the ICA titer the extent of this beta-cell destruction. Patients with ICA positivity are considered to have some residual beta-cell function (Kolb *et al.*, 1988; Kobayashi *et al.*, 1993).

Table 2. Prevalence (%) of ICA in background population and annual incidence of childhood onset IDDM (per 100,000) in selected populations participating in International ICA Standardization Workshop. Data from Bergay *et al.*, 1983; Bingley and Gale, 1989; Landin-Olsson *et al.*, 1989; Nyström *et al.*, 1990; Karjalainen, 1990; Tuomilehto *et al.*, 1991; Green *et al.*, 1992; Muntoni *et al.*, 1992; Levy-Marchal *et al.*, 1992; Bingley *et al.*, 1993; Schatz *et al.*, 1994.

Population	Individuals studied	Age range (years)	Prevalence of ICA	Incidence of IDDM
Finland	1212	3–18	4.1	35
Sardinia	1894	6–17	3.2	30
Sweden	321	0–14	3.0	24
UK	242	5–17	2.2	13
France	8363	6–17	1.8	8
USA	9696	5–17	0.6	16
Spain	2291	3–15	0.4	12

ICA have been found also in nondiabetic first degree relatives of patients with IDDM. The prevalence of ICA in relatives of IDDM patients has been reported to be lower than in newly diagnosed patients (Table 1). It ranges usually from three to seven percent. It has been suggested that the progression to IDDM in relatives depends on the titer of ICA (Bonifacio *et al.*, 1989; Karjalainen, 1990). Bottazzo and colleagues have found that all first degree relatives with ICA≥80 JDFU developed IDDM within seven years of follow up. In those with ICA between 20 and 80 JDFU, the probability of being free of IDDM after ten years was only 27% (Bottazzo *et al.*, 1991). In other studies, IDDM was more likely to appear in relatives with high titre of ICA if they were young or from multiplex IDDM families (Riley *et al.*, 1990).

ICA are present in the serum of approximately 0.5–4 percent of healthy subjects (Table 2). Finland has reported the highest prevalence of ICA and the highest incidence of IDDM in the world (Karjalainen, 1990). There seems to be a close correlation between the incidence of the disease and the prevalence of ICA in nondiabetic children in various countries (Bergay *et al.*, 1983; Landin-Olsson *et al.*, 1989; Karjalainen, 1990; Levy-Marchal *et al.*, 1992; Muntoni *et al.*, 1992; Bingley *et al.*, 1993; Schatz *et al.*, 1994). The prevalence of ICA exceeds the prevalence of IDDM many times and ICA might, therefore, occur transiently without subsequent development of diabetes (Landin-Olsson *et al.*, 1989).

1.2. Glutamic acid decarboxylase antibodies

Autoantibodies to a 64 kD islet protein were first reported in 1982 by Baekkeskov and coworkers (Baekkeskov *et al.*, 1982). Later the 64 kD protein was identified as glutamic acid decarboxylase (GAD), the major antigen for autoan-

tibodies from IDDM patients (Baekkeskov *et al.*, 1990). GAD catalyzes the formation of gamma amino butyric acid, a neuroinhibitor in the central nervous system. GAD is also expressed in the islets of Langerhans and other tissues. Some factors suggest that GAD may play a role in the pathogenesis of IDDM. Mononuclear cells from IDDM patients have been shown to proliferate in response to GAD (Tisch *et al.*, 1993). The immunocytochemical ICA is likely a composite of antibodies directed at several pancreatic islet molecules, one of the target antigens being GAD (Martino *et al.*, 1991; Genovese *et al.*, 1992; Atkinson and Maclaren, 1993). A number of assays for GADA have been developed, including immunoprecipitation methods. The quantitative radiobinding assay has higher sensitivity than other methods and is more simple and reproducible (Hagopian *et al.*, 1993; Schmidli *et al.*, 1995).

GADA are found by radiobinding assay in 65–87% of individuals with newly diagnosed IDDM (Baekkeskov *et al.*, 1990; Karlsen *et al.*, 1992; Schmidli *et al.*, 1994; Bonifacio *et al.*, 1995) (Table 3). GADA precede the diagnosis of IDDM by several years (Genovese *et al.*, 1992; Thivolet *et al.*, 1992; Aanstoot *et al.*, 1994; Tuomilehto *et al.*, 1994; Yu *et al.*, 1996). GADA levels are reported to be influenced by age, sex and ICA status, and generally remain stable after the onset of clinical IDDM (Martino *et al.*, 1991; Bingley *et al.*, 1994; Schmidli *et al.*, 1994; Jaeger *et al.*, 1997). GADA have been found several years after diagnosis, in the majority of patients for up to 10–20 years after the diagnosis of IDDM with some decrease in antibody frequency (Rowley *et al.*, 1992; Tuomi *et al.*, 1993).

Table 3. Prevalence (%) of GADA in newly diagnosed IDDM patients (IDDM), healthy first degree relatives of IDDM patients (Relatives) and background population (Controls)

Study	IDDM	Relatives	Controls
Karlsen *et al.* (1992)	75		1.7
Petersen *et al.* (1994)	74		2.5
Bonifacio *et al.* (1995)	65		1.0
Seissler *et al.* (1996)	87	6.3	1.0
Kulmala *et al.* (1995)		9.6	
Gorus *et al.* (1997)	82	6.0	
Wiest *et al.* (1997)	75	6.4	
Savola *et al.* (1997)	73		
Zanone *et al.* (1997)	53		
Jaeger *et al.* (1997)	78		
Ortego *et al.* (1997)		5.6	
Vähäsalo *et al.* (1997)		6.5	
Velluzzi *et al.* (1997)			2.1

Among the healthy first degree relatives the prevalence of GADA has been 6.0–9.6% (Chen *et al.*, 1993; Roll *et al.*, 1994; Seissler *et al.*, 1996; Gorus *et al.*, 1997). The highest prevalence has been reported from Finland (Kulmala *et al.*, 1995). Population screenings for GADA have shown the frequency in healthy control subjects up to 2.5%, exceeding the prevalence of IDDM five to ten times (Petersen *et al.*, 1994; Leech *et al.*, 1995; Aanstoot, 1995).

1.3. Tyrosine phosphatase antibodies

Christie and coworkers described a novel antigen in the beginning of 1990s as an islet protein with molecular weight of 37 kD/40 kD (Christie *et al.*, 1990, 1992a, 1992b). Recently Christie and co-workers identified the 40 kD antigen as the intracytoplasmic domain of the tyrosine phosphatase IA-2 (Christie *et al.*, 1993). Several groups provided evidence that IA-2 is the precursor of the islet 37 kD and 40 kD polypeptide autoantigens and as one of the ICA specificities (Payton *et al.*, 1995; Rabin *et al.*, 1994; Passini *et al.*, 1995; Zimmet, 1996; Solimena *et al.*, 1996). Detection of antibodies to IA-2 by a radio-binding assay allows quantitative measurements of these autoantibodies (Grubin *et al.*, 1994; Wiest-Ladenburger *et al.*, 1997). It has been suggested that antibodies to tyrosine phosphatase (IA-2A) may be a sign of rapid progression to IDDM (Christie *et al.*, 1994; Leslie *et al.*, 1995).

Table 4. Prevalence (%) of tyrosine phosphatase antibodies (IA-2A) in newly diagnosed IDDM patients (IDDM), healthy first degree relatives of IDDM patients (Relatives) and background population (Controls)

Study	IDDM	Relatives	Controls
Gianani *et al.* (1995)	38		
Bonifacio *et al.* (1995)	62		0
Seissler *et al.* (1996)		4.4	0
Genovese *et al.* (1996)	61		
Gorus *et al.* (1997)	58	1.5	0.8
Kawasaki *et al.* (1997)	41		1
Wiest *et al.* (1997)	73	2.9	0
Savola *et al.* (1997)	73		
Zanone *et al.* (1997)	64		
Velluzzi *et al.* (1997)			2
De Leeuw *et al.* (1997)	49		
Kulmala *et al.* (1997)		5.1	

Few studies have investigated the prevalence of IA-2A (Table 4). IA-2A have been found in 38–73% of IDDM patients at the time of diagnosis. The highest frequency of these antibodies (73%) has been reported from Germany and Finland — 73% (Wiest-Ladenburger *et al.*, 1997; Savola *et al.*, 1997). Lower

values have been documented in Japan and Italy (Kawasaki *et al.*, 1997; Gianani *et al.*, 1995).

IA-2A have been found in 1.5–4.4% of healthy relatives of IDDM patients. Gorus and coworkers have detected IA-2A only in 1.5% of relatives (1997). Seissler's group found IA-2A in 4.4% of first degree relatives, which is comparable to the frequencies of ICA and GADA (1996). The few studies investigating the frequency of IA-2A in background population have documented these antibodies in 0–2% of individuals. The highest prevalence was reported by Velluzzi and colleagues, in 2%, also similar to prevalence data of ICA and GADA in the background population (1997).

2. Genetic factors of IDDM

Although a variety of genetic markers have been studied in relation with IDDM, most of the research has concentrated at the HLA system. The association of HLA antigens with IDDM was discovered more than 20 years ago. Singal and Blajchman found an increase of class I molecules B8 and B15 in IDDM patients compared with controls (1983). Since then many reports from different countries have confirmed and extended these findings (Raum *et al.*, 1979; Tuomilehto-Wolf and Tuomilehto, 1991; Fennesy *et al.*, 1994; Gemaine *et al.*, 1995). Family studies confirmed genetic linkage between HLA and IDDM, suggesting that genes within or near the HLA region are involved in the susceptibility for the disease (Overbach *et al.*, 1983; Thomson *et al.*, 1988; Deschamps *et al.*, 1991; Reijonen *et al.*, 1994; Honeyman *et al.*, 1995). Recent genome-wide mapping studies have confirmed that the HLA class II region encodes the most important determinants of protection against and susceptibility to IDDM (Tiwari and Terasaki, 1985; Todd *et al.*, 1987, 1989; Nepom, 1990; Rønningen *et al.*, 1991; Harrison and Tait, 1991; Deschamps *et al.*, 1993; Davies *et al.*, 1994; Routsias and Papadopuolus, 1995; She, 1996). However, data from different populations show that there is still confusion concerning the interpretation of the complex HLA associations with IDDM (She, 1996).

2.1. HLA-DRB1 alleles

Certain serologically determined HLA-DR locus antigens were shown to be associated with IDDM after the initial revelation of the link between the disease and HLA-B locus (Svejgaard *et al.*, 1980; Wolf *et al.*, 1983; Marshall *et al.*, 1994). After the discovery that some HLA-DQ locus alleles were more strongly related to IDDM, the importance of the contribution of the HLA-DR locus was temporarily underestimated. Recent studies have reconfirmed the importance of

the HLA-DR alleles in the determination of the risk of IDDM (Olerup *et al.*, 1992; Undlien *et al.*, 1995; Van der Auwera *et al.*, 1995; Honeyman *et al.*, 1995; Sanjeevi *et al.*, 1996). HLA-DR alleles can convey susceptibility or protection against IDDM (Van der Auwera *et al.*, 1995; Tait *et al.*, 1995) (Table 5).

Table 5. HLA-DRB1 alleles proposed to confer susceptibility to or protection against IDDM.

Susceptibility
*0301
*0401
*0402
*0405
Protection
*02
*0403
*0406
*0408
*11
*13
*14
*15

The HLA-DR antigens most often associated with the increased risk of the development of IDDM, are DR3 and DR4 (Deschamps *et al.*, 1984; MacMillan and Foster, 1991; Kockum *et al.*, 1995). In Caucasians, more than 90% of IDDM patients are HLA-DR3 or HLA-DR4 positive, compared with 40–50% of controls (Caillat-Zucman *et al.*, 1992). The same group has found that the greatest risk is in HLA-DR3/DR4 heterozygous individuals, who make up 30–50% of IDDM patients (1992). Frequencies of DR3 and DR4 were also significantly increased in both the IDDM cases and their unaffected siblings compared with the general Southeast US population (MacMillan and Foster, 1991). MacMillan's investigation showed that 46% of IDDM children possessed both DR3 and DR4 antigens while only 7% had neither (1991). Among Brazilian IDDM patients DR3 was detected in 57% of the patients versus 28% of the controls and DR4 in 54% of the patients versus 23% of the controls (Eizirik *et al.*, 1987). Eighty seven percent of IDDM patients had HLA-DR3 and/or DR4 versus 36% of the Venezuelan general population (Gunczler *et al.*, 1993). Honeyman and coworkers have shown that HLA-DR4 conveys a greater risk of IDDM than HLA-DR3 in familial cases (1995). In a Sardinian study the genotype analysis of the patients showed a strong increase of the DR3/DR4 heterozygotes with a relative risk higher than that of the DR3 and DR4 homozygotes (La Nasa *et al.*, 1990). Among Moroccan IDDM patients HLA-DR3 had a

strong positive association with the disease (68% of patients versus 33.3% of controls) (Izaabel *et al.*, 1996). The DR7 association with IDDM is specific for the Negroid race, and DR9 is only weakly associated in Caucasoid and Negroid subjects (Fletcher *et al.*, 1988). In the study from Korea the frequencies of HLA-DR3, DR4 and DR9 were also higher in IDDM patients compared with the control population (Lee *et al.*, 1996).

HLA-DR2 appears as protective against IDDM from multiple studies (Eizirik *et al.*, 1987; Thomson *et al.*, 1987; Lee *et al.*, 1996). Other HLA-DR antigens contributing to protection against IDDM are DR7 and DR15 (Eizirik *et al.*, 1987; Awata and Kanazawa, 1994; Kockum *et al.*, 1995). The frequency of DR2 was significantly decreased in Korean IDDM patients in comparison with controls (Lee *et al.*, 1996). Sanjeevi and coworkers found that DR2 was detectable in only 3% of IDDM patients and 28% of controls (1994). In Sardinia, the DR2 antigen was also negatively associated with IDDM in the central island districts (La Nasa *et al.*, 1990). In Brazil, DR2 was seen in 11% of the patients versus 31% of the controls and DR7 in 3% of the patients versus 21% of the controls (Eizirik *et al.*, 1987). Generally, HLA-DR2 appears to be inversely associated with IDDM in all races (Thomson *et al.*, 1988).

Recent studies have analyzed the subtypes of HLA-DR4 with molecular techniques. Interestingly, only some HLA-DR4 subtypes evidently convey susceptibility to IDDM, while others may even provide protection against the disease. Data is contradictory. The strongest susceptibility in some ethnic groups is related to HLA-DRB1*0405, followed by *0402, *0401 and *0404. HLA-DRB1*0403, *0406 and *0408 provide protection (Harrison and Tait, 1991; Caillat-Zucman *et al.*, 1992; Van der Auwera *et al.*, 1995; Sanjeevi *et al.*, 1996; She, 1996). Undlien and coworkers have shown that some DR4 subtypes, such as DRB1*0405, *0402, and *0401, confer little or no protection, while others, DRB1*0404, *0403, and *0406 cause an increasing degree of protection (1995). The most common HLA-DR4 subtype in the Swedish IDDM patients was HLA-DRB1*0401, found in 62%. DRB1*0404 was decreased in the IDDM subjects compared with controls (4.8% versus 19.0%) (Sanjeevi *et al.*, 1996). DRB1*0401 also conferred the highest risk of IDDM in Norway, followed by *0404 and *0403 (Yasunaga *et al.*, 1996).

2.2. HLA-DQB1 alleles

After the documentation of the association of IDDM with HLA-DR locus, susceptibility to or protection against IDDM was found to be even more strongly linked to some HLA-DQ alleles (Todd *et al.*, 1987, 1989; Thorsby and Rønningen, 1993; Tosi *et al.*, 1994) (Table 6). Todd and coworkers proposed the primary role for residues on the HLA-DQB1 gene in IDDM susceptibility (1987). While sequencing HLA-DQB1 alleles of IDDM patients and controls,

they noticed that non-aspartic acid residue in the 57th position (non-Asp-57) of the HLA-DQβ chain was associated with higher risk of IDDM (Todd *et al.*, 1989). Studies in several populations have correlated the frequency of the HLA-DQB1 non-Asp-57 alleles with differences in IDDM incidence (Awata *et al.*, 1990; Deschamps *et al.*, 1991; Cruickshanks *et al.*, 1994; Chauffert *et al.*, 1995; Sanjeevi *et al.*, 1995). Only 25.5% of the IDDM subjects were phenotyped as having aspartic acid in the 57th position of the DQβ chain compared with 82% of control subjects. This suggests that Asp-57 negativity is a definite risk marker for developing IDDM in Finnish patients (Reijonen *et al.*, 1991). The specific HLA-DQB1 alleles that do not code for aspartic acid in the 57th position of the DQβ chain, are DQB1*0201, *0302, *0501 and *0502 (Chauffert *et al.*, 1995). Caillat-Zucman with coauthors has found that among Caucasians 74.6% of IDDM patients had DQB1*0201 and 59.2% DQB1*0302 allele compared with 33.2% and 11.8% in controls, respectively (1992). Harfouch and colleagues found DQB1*0302 allele in most of IDDM patients (94.2%) compared with lower prevalence (64.7%) in controls (1996). In Sardinia, where the incidence of IDDM is very high, the DQB1 molecular analysis showed only three alleles in IDDM patients: DQB1*0201 (75.8%), DQB1*0302 (16.1%), and DQB1*0502 (8.1%). It seems that nearly if not all Sardinian IDDM patients are non-Asp-57 homozygotes. The DQB1*0502 allele, extremely rare in other Caucasian populations, represents in Sardinia about 70 per cent of the HLA-DR2 haplotypes, contributing to the increase of the pool of IDDM susceptible genes (La Nasa *et al.*, 1990).

Table 6. HLA-DQB1 alleles proposed to confer susceptibility to or protection against IDDM.

Susceptibility
*0201
*0302
*0501
Protection
*0301
*0401
*0402
*0601
*0602
*0603

Recent papers have informed that HLA-DQB1*0304 is a rare allele combining features of both HLA-DQB1*0301 and HLA-DQB1*0302 alleles. Similarly to HLA-DQB1*0302 the 57th amino acid of the β chain of HLA-DQB1*0304 is a non-Asp amino acid (alanine) suggested crucial in susceptibility to IDDM (Cucca *et al.*, 1994).

Some DQB1 alleles appear protective against IDDM. HLA-DQB1*0602 provides the strongest protection. Pugliese and coworkers have found that DQB1*0602 is protective from IDDM both in population studies and among relatives compared with IDDM patients (1995). Caillat-Zucman and co-investigators showed that DQB1*0602/0603 was present in 35% of unrelated Caucasian controls compared with 3% of IDDM cases (1992). Among Moroccan IDDM cases the frequency of DQB1*0602 was 2% compared with 14% in controls (Izaabel *et al.*, 1996). In Korea both DQB1*0301 and DQB1*0601 were negatively associated with IDDM (Lee *et al.*, 1996). Also in Japan and Norway DQB1*0301 was less frequent in IDDM patients (Yasunaga *et al.*, 1996). Ilonen's group has found that among healthy siblings of IDDM patients DQB1*0301 was associated with weaker protective effect against IDDM compared with those having DQB1*0602/0603 (Ilonen *et al.*, 1996)

2.3. HLA-DQA1 alleles

In 1990 Khalil and coworkers performed extensive HLA-DQA1 and HLA-DQB1 typing in Caucasian IDDM patients and found that arginine in the 52nd position of the HLA-DQα chain, corresponding to HLA-DQA1*0301 and *0501 alleles, was associated with higher risk for IDDM. During the years to follow, many authors have confirmed that arginine in the 52nd position of the HLA-DQα chain correlates highly significantly with IDDM (α chain, corresponding to HLA-DQA1*0301 and *0501 alleles, was associated with higher risk for IDDM. During the years to follow, many authors have confirmed that arginine in the 52nd position of the HLA-DQα chain correlates highly significantly with IDDM (s, respectively (1992). A study from Colorado found DQA1*0301 in 70–80% and DQA1*0501 in 58–61% of IDDM cases and about twice less frequently in control group (Cruickshanks *et al.*, 1994). Analysis of the frequency of the subtypes of DQA1 alleles among Japanese IDDM patients showed that the DQA1*0301 allele was the most strongly associated with the disease (prevalence of 97.4% in IDDM cases versus 56.7% in controls) (Tanaka *et al.*, 1992). Izaabel and colleagues have shown that DQA1*0501 was the most frequent HLA-DQA1 allele among IDDM patients (1996). They documented this allele in 72% of IDDM cases compared with 48% in controls and DQA1*0301 in 45% of IDDM cases compared with 26% in controls (Izaabel *et al.*, 1996). Lee *et al.* confirmed that DQA1*0301 and DQA1*0501 were positively associated with IDDM (1996).

Some HLA-DQA1 alleles have been suggested to confer protection against IDDM. Thus, DQA1*0102 and DQA1*0201 were negatively associated with IDDM in a study from Korea (Lee *et al.*, 1996). Also DQA1*0103 has been described as protective from IDDM (Awata, Kanazawa, 1994).

Table 7. HLA-DQA1 alleles proposed to confer susceptibility to or protection against IDDM.

Susceptibility
*0301
*0501
Protection
*0102
*0103
*0201

2.4. HLA-DQ and HLA-DR allele combinations

Not just single alleles, but their combinations on chromosome six appear to be important for the susceptibility to or protection against IDDM (She, 1996). Recent studies have shown certain associations between certain combinations of different DRB1, DQA1, and DQB1 alleles and risk of IDDM. Rønningen and coworkers have reviewed the known associations of candidate class II susceptibility alleles with IDDM in five large racial groups: Caucasians, Asian Indians, Negroids, Japanese and Chinese (1991) (Table 8). It has been shown that susceptibility to IDDM was most strongly associated with a particular combination of HLA-DQA1 and HLA-DQB1 alleles in different ethnic groups. DQA1*0301 together with DQB1*0302, and DQA1*0501 together with DQB1*0201 were found associated with IDDM susceptibility in Caucasians, Blacks and Japanese (Rønningen *et al.*, 1991). HLA-DR3-DQA1*0501-DQB1*0201/HLA-DRB1*0405-DQA1*0301-DQB1*0302 appears to be the strongest susceptibility genotype in Caucasians with IDDM, carrying relative risk between 20 and 45 (Van der Auwera *et al.*, 1995; Sanjeevi *et al.*, 1996). Caillat-Zucman studied Caucasian IDDM subjects and healthy controls for DRB1*03, DRB1*04, DQB1*0201, DQB1*0302, DQA1*0301, and DQA1*0501. The highest relative risk was observed in patients carrying both the DRB1*03-DQB1*0201 and the DRB1*0402 or DRB1*0405-DQB1*0302 haplotypes (1992). The most frequent genotypes in IDDM cases in Spain were DR3-DQB1*0201-DQA1*0501 and DR4-DQB1*0302-DQA1*0301 (Serrano-Rios *et al.*, 1996). Tait and colleagues have found that 95% of DRB1*0401 IDDM subjects were also DQB1*0302 positive (1995). Heimberg's group detected that DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype was present in 30% of the IDDM patients and only in 1% of the typed healthy controls, resulting in a relative risk of 35 (1992). Lampasona and coworkers have shown that 88% of IDDM patients from Northern-Italy and 20% of unrelated control subjects had either HLA-DQA1*0501-DQB1*0201 or HLA-DQA1*0301-DQB1*0302 in the absence of HLA-DQA1*0102-DQB1*0602 (1995). In Japanese IDDM patients DRB1*0405-DQA1*0301-

DQB1*0401, DRB1*0901-DQA1*0301-DQB1*0303 and DRB1*0802-DQA1*0301-DQB1*0302 were the major susceptibility haplotypes (Awata and Kanazawa, 1994). In a Korean study DQA1*0301-DQB1*0201, DQA1*0501-DQB1*0201 and DQA1*0501-DQB1*0302 were positively and DQA1*0102-DQB1*0601 negatively associated with IDDM. The frequencies of DR3-DQA1*0301-DQB1*0201 and DR3-DQA1*0501-DQB1*0201, DR4-DQA1*0301-DQB1*0201 and DR9-DQA1*0301-DQB1*0303 were significantly higher in IDDM patients (Lee *et al.*, 1996).

Table 8. HLA-DR-DQ allele combinations proposed to confer susceptibility to or protection against IDDM.

Susceptibility		
DRB1	DQA1	DQB1
	*0301	*0201
	*0301	*0302
	*0301	*0501
	*0501	*0201
	*0501	*0302
*0401		*0302
*0402		*0302
*0405		*0302
*03	*0501	*0201
*03	*0301	*0201
*04	*0301	*0201
*0405	*0301	*0302
Protection		
DRB1	DQA1	DQB1
	*0102	*0601
	*0102	*0602
*0403	*0501	*0301
*15	*0102	*0602
*15	*0103	*0602

As with single alleles, some allele combinations have been shown to confer protection against IDDM. Protection against IDDM is usually associated with HLA-DQB1*0602, in any allele combination. Resistance against development of IDDM has been found with HLA-DQA1*0102-DQB1*0602, less protection was associated with HLA-DRB1*0403-DQA1*0501-DQB1*0301 (Van der Auwera *et al.*, 1995). The major protective effect for IDDM in the population of Swedish children is conferred by the DR15-DQA1*0102-DQB1*0602 haplotype in a dominant manner. The DQB1*0602 was the allele most likely to be responsible for the protective effect of this haplotype, although an effect of the DR15 allele could not be excluded (Kockum *et al.*, 1995). DRB1*1501-DQA1*0103-DQB1*0602 and DRB1*1502-DQA1*0103-DQB1*0601 are the major resistance haplotypes in Japan (Awata and Kanazawa, 1994).

PURPOSE OF THE STUDY

This study was undertaken to establish the prevalence of some suggested immune and genetic factors associated with IDDM in Estonia in

1) newly diagnosed childhood onset IDDM patients,
2) healthy siblings of IDDM patients,
3) control group.

The specific aims of the study were:

1. To establish the prevalence of ICA, GADA and IA-2A in study groups.

2. To learn the frequency of selected HLA-DQ alleles associated with IDDM and their combinations in Estonian IDDM patients and control group, also the concomitant risk of IDDM.

3. To follow for three years the siblings of IDDM patients, initially investigated for the immune and genetic factors, for the development of IDDM.

4. To parallel the data from Estonia with that in other populations.

MATERIAL AND METHODS

1. Study subjects

1. 1. Childhood onset IDDM patients

Group one

Consecutive 29 newly diagnosed IDDM children under the age of 15 in Estonia in 1993–1994, were included in the study. The age ranged from two to 14 years (mean 7.9±3.8). The group consisted of 11 girls (37.9%) and 18 boys (62.1%). Blood specimens for the detection of autoantibodies was taken during the first week after the diagnosis.

Group two

Two hundred invitation letters were mailed to the patients from the Estonian Childhood Onset IDDM Registry (VI) in 1990. Ninety-seven patients responded and were recruited for the genetic investigation. The Estonian Childhood Onset IDDM Registry contains information on the cases diagnosed since 1980. It is at least 95% complete. This group consisted of 53 females (54.6%) and 44 males (45.4%) who had developed IDDM at less than 15 years of age (age at diagnosis 0–14 years, mean age 7.5±3.8). This group provided blood specimens for the detection of HLA-DR and HLA-DQ locus alleles.

1.2. Healthy siblings of childhood onset IDDM patients

Healthy siblings of IDDM cases from the Estonian Childhood Onset IDDM Registry were invited to participate in the study by mail in 1994. One hundred invitation letters were mailed to the families with an IDDM child. The consent to participate was received from 44 families. Seventy-one siblings from these families were included in the study. The prevalence of autoantibodies and HLA-DR and HLA-DQ locus alleles was estimated in them. There were 32 boys (45.1%) and 39 girls (54.9%) in this group, age two to 24 years (mean 10.2±4.8).

The same siblings were contacted in December 1997 by mail. Their status as for clinical IDDM was learned.

1.3. Controls

Group one

This group consisted of 614 non-diabetic kindergarten and schoolchildren, 314 girls (51.1%) and 300 boys (48.9%), age 3–18 years (mean 10.1±4.2). Children were chosen from six different regions of Estonia (Tallinn, Tartu, Paide, Põlva, Nõo and Jõgeva) to determine the prevalence of ICA and GADA. The age distribution of this group was following — 3–5 years (118), 6–8 years (131), 9–11 years (119), 12–14 years (124), 15–18 years (122). Samples from 614 healthy nondiabetic children and adolescents were analyzed in 1991 for ICA and GADA. In 1994 only those positive for ICA in the initial 1991 sample were retested for ICA and GADA.

Group two

One hundred and three first year medical students from the University of Tartu (mean age 20.5±1.7) and 166 healthy blood donors (mean age 32.4±11.2) provided blood for the assessment of the HLA-DR and HLA-DQ locus allele frequencies in the Estonian population.

2. Detection of antibodies

2.1. Islet cell antibodies

ICA were determined by a standard immunofluorescence method. Fluorescein-conjugated rabbit antihuman IgG (Behringwerke, Marburg, Germany) was used to detect ICA. Both laboratories participate in the international ICA Standardization Workshop. Endpoint dilution titers for all positive samples were expressed in JDFU relative to the above mentioned international reference standard. The detection limit was 2.5 JDFU. Assay sensitivity was 100%, specificity 98%, validity 98% and consistency 98% (Karjalainen, 1990). Three independent observers read the results.

2.2. Glutamic acid decarboxylase antibodies

GADA were measured according to the quantitative immunoprecipitation radioligand method described by Petersen *et al.* (1994). Human recombinant islet GAD65 cDNA was transcribed and translated in vitro according to the manufacturer's instruction (Promega, Madison, WI). The translation of transcribed RNA was performed in a methionine free rabbit reticulocyte lysate (Promega,

USA) in the presence of ^{35}S-methionine (Amersham International, UK). Aliquots containing approximately 30,000 cpm of labeled GAD65 were incubated overnight at 4°C with serum (final dilution 1:25) in a total volume of 50 µl. Competition analysis was carried out by adding an excess of cold purified recombinant GAD65. The immune complexes were isolated by adding 7.5 mg of protein A-Sepharose (Pharmacia, Sweden) to each tube. After incubation for two hours at 4°C the reaction volume was transferred to a 96-well filtration system. The units were placed on a vacuum device allowing rapid washing. After ten washes, the bottom of each well in the filtration units was punched into tubes. Subsequently 2.5 ml of scintillation fluid was added to each tube. The radioactivity was measured in the scintillation counter. The results were expressed in relative units (RU) representing specific binding as a percentage of that obtained with a positive standard serum. The cutoff limit for GADA positivity was set at 7.7 RU. The sensitivity of the assay was 80%, the specificity 94% based on the 101 samples included in the second international GADA workshop (Schmidli *et al.*, 1995).

2.3. Tyrosine phosphatase antibodies

IA-2A were measured by radioimmunoassay (Wiest-Ladenburger *et al.*, 1997). Plasmid cDNA of full-length IA-2 and cDNA encoding for the cytoplasmic part of IA-2 (aa 603–979) were cloned into the pSP64poly(A)-vector and were amplified in Escherichia coli XL1 blue and isolated by a QIAprep spin plasmid miniprep kit (QIAGEN, Germany). cDNA was transcribed into RNA and then translated into protein in the presence of ^{35}S methionine (40 µCi; 1,000 Ci/mmol, Amersham, Germany) using a SP6 coupled reticulocyte lysate system (Promega, Madison, WI). Protein-bound radioactivity was separated by gel filtration on Sephadex G25 columns (Pharmacia, Germany). 2.5 µl of serum was incubated in duplicates with 20,000 cpm of radioactive protein in 50 µl immunoprecipitation buffer in round-bottom microtiter wells at 4°C overnight. One µg of swollen prewashed protein A sepharose (Pharmacia, Sweden) was diluted in 50 µl of immunoprecipitation buffer and was incubated with samples for one hour at 4°C. Protein A Sepharose-bound immune complexes were separated from unbound protein by washing 10 times with 150 µl of immunoprecipitation buffer in membrane bottomed microtiter wells and transferred to scintillation vials. Counts per minute were determined in a beta counter. Quantification was achieved by including a standard dilution curve on each microtiter plate. For recently identified autoantigen IA-2, human monoclonal antibodies were not available. The quantification of IA-2A had to be performed relative to a positive-reference serum with unknown concentration of specific antibody. Selected reference sera was taken from a patient with newly diag-

nosed IDDM, which revealed an identical titer of IA-2A. Results were expressed in arbitrary units (AU). The radioactivity precipitated by a 1:8 dilution of the standard was set at 10 U, as this dilution was in the linear range of the standard curve in all established assays. IA-2A assay had the sensitivity of 62.7% and specificity of 93.1%.

3. Detection of HLA-DQ and HLA-DR alleles

3.1. Extraction of DNA

DNA was extracted from peripheral blood using the protocol of DNA salt extraction by Gustincich (1991). One ml of red cell lysis buffer was added to EDTA-blood, rotated for 10 minutes and leucocytes/nuclei spinned down by centrifuge at 10 minutes at 3500 rpm. Washing steps were repeated until the pellet was white and the pellet resuspended by adding three ml Proteidae K-lysis buffer. After overnight incubation at 37°C, one ml of saturated NaCl (~6M) was added at room temperature, tubes shaken vigorously and precipitated proteins spinned down for 15 minutes at 3500 rpm. The supernatant was poured into new polypropylene tubes and two volumes of cold absolute ethanol added. Tubes were inverted gently until DNA precipitated out in the form of a white stringy mass. Using a Pasteur pipette which tip had been heated to form a hook, DNA was picked up and washed once in 90% ethanol to remove traces of salt. Finally DNA was resuspended in a small volume 200 µl of TE buffer and incubated at 65°C until DNA was dissolved. The purity and concentration of DNA was estimated by spectrophotometric method. The readings of optical density were taken at wavelengths 260 nm and 280 nm.

3.2. Detection of alleles

The HLA-DQ alleles suggested associated with low or high IDDM risk were determined using two steps (Sjöroos *et al.*, 1995). The samples were first studied for the presence of the DQB1*0201, *0301, *0302 and *0602/0603 using a method based on binding of the biotinylated amplification product onto streptavidin coated microtiter plate wells, followed by hybridization with lanthanide labeled oligonucleotide probes. The fluorescence properties of the different lanthanides (europium, samarium and terbium) allowed simultaneous detection of three different reaction products in a single microtiter plate well with time-resolved fluorometry (Sjöroos *et al.*, 1995).

All samples positive for HLA-DQB1*0302 were selected for the further DR4 subtyping which was carried out by genomic amplification in two po-

lymerase chain reactions (PCRs), with the primer sequences as defined by Olerup and Zetterquist (1992). Primers permitted to detect DRB1*0401, *0402, *0403, *0404, *0405, *0408 alleles.

Samples positive for DQB1*0201 were further studied for DQA1*0501 and *0201 alleles. This assay was based on a modified method where biotinylated sequence specific probes and an europium labeled detection probe was utilized (Sjöroos *et al.*, 1996).

All samples that were found positive for both HLA-DQB1*0301 and *0302 in the screening test were later reanalyzed for the presence of the DQB1*0304 allele using sequence specific primers described by Olerup and colleagues (1993).

4. Statistical methods

For normally distributed continuous variables the mean (M) and the standard deviation (SD) were calculated. A chi-square test with Yates correction or two-sided Fisher's exact test when appropriate were used for the detection of significant differences in the prevalence of alleles and antibodies between study groups. Correlations between antibodies and other parameters were analyzed by the Spearman's test. Statistical significance was assumed at p less than 0.05.

Odds ratio (OR) was calculated according to the formula $(a \times d / b \times c)$ where a and b are the numbers of the IDDM patients that were positive and negative for the marker, respectively, and c and d, the respective numbers of control subjects. Haldane's correction for small samples was used when appropriate. Correction of the acceptance p level (0.05) was performed by the Bonferoni method for multiple comparisons (Armitage and Berry, 1994).

RESULTS AND DISCUSSION

1. Immune factors of IDDM

1.1. Antibodies in newly diagnosed childhood onset IDDM patients

All three antibodies (ICA, GADA and IA-2A) were measured in 29 newly diagnosed childhood onset IDDM cases (V). The values of individual cases are given in Table 9. Table 10 summarizes the prevalence of the different antibodies in newly diagnosed IDDM group.

Table 9. Individual values of ICA (JDFU), GADA (RU) and IA-2A (AU) in newly diagnosed childhood onset IDDM patients

ID	Sex	Age	ICA	GADA	IA-2A
E01	m	8	40	60.2	0
E02	m	3	20	0	83.1
E03	m	3	80	14.1	29.6
E04	m	7	80	61.4	0
E05	m	12	5	0	0
E06	m	14	20	0	4.7
E07	f	14	0	48.6	0
E08	m	9	0	0	0
E09	f	5	20	0	0
E10	m	14	≥160	0	0
E11	m	9	≥160	76.5	103.4
E12	f	11	2.5	0	0
E13	m	6	5	76	0
E14	m	5	≥160	109.3	0
E15	f	6	20	0	0
E16	f	9	80	0	109.5
E17	m	11	5	0	0
E18	m	3	20	0	70.2
E19	m	12	≥160	92.6	65.3
E20	f	4	80	44.1	73.3
E21	f	3	80	0	0
E22	m	4	0	0	8.5
E23	f	8	2.5	0	0
E24	f	9	40	0	0
E25	m	13	40	83.6	0
E26	f	2	0	0	0
E27	m	6	≥160	107.9	9.7
E28	m	0	0	0	0
E29	f	10	0	0	0

Twenty-three IDDM patients out of 29 (79.3%) were positive for ICA at the first week after diagnosis, 15 boys and eight girls. Three boys and three girls did not have detectable ICA. The range of ICA level in this group was from 2.5 to more than 160 JDFU. The median value of the whole group was 20 JDFU. Eighteen (78.3%) among the 23 ICA positive individuals had the titer greater than or equal to 20 JDFU, ten (43.7%) had the titer greater than or equal to 80 JDFU. Five children (21.7%), all boys, expressed a very high titer of ICA, greater than or equal to 160 JDFU. The association between ICA and age (r=–0.09) was weak in this cohort, between ICA and sex (r=–0.30) — moderately stronger.

Table 10. Prevalence of ICA, GADA and IA-2A in newly diagnosed childhood onset IDDM patients (IDDM), healthy siblings of IDDM patients (Siblings) and controls (Controls)

Antibody	IDDM N=29		Siblings N=71		Controls N=614	
	n	%	n	%	n	%
ICA	23	79.3[1,2]	5	7.0	10	1.6
ICA≥20 JDFU	18	62.7[1,2]	5	7.0	6	1.0
ICA≥80 JDFU	10	34.5[1,2]	5	7.0	2	0.3
ICA≥160 JDFU	5	17.2[2]	5	7.0	0	0.0
Only ICA	9	31.0[1,2]	1	1.4	0	0.0
GADA	11	37.9[2]	12	16.9[3]	14	2.3
ICA and GADA	10	34.5[2]	4	5.6	5	0.8
IA-2A	10	34.5	4	5.6	ND	ND
ICA and IA-2A	9	31.0	3	4.2	ND	ND
ICA, GADA and IA-2A	5	17.2[2]	3	4.2	ND	ND
GADA and IA-2A	5	17.2	4	5.6	ND	ND
GADA and/or IA-2A	16	55.2	12	16.9	ND	ND
ICA and GADA and/or IA-2A	14	48.3[1]	5	7.0	ND	ND
At least one antibody	25	86.2	13	18.4	19	3.1
No antibodies	4	13.8[1,2]	58	81.6[3]	595	96.9

[1] Statistically significant difference ($p<0.05$) between IDDM and Siblings

[2] Statistically significant difference ($p<0.05$) between IDDM and Controls

[3] Statistically significant difference ($p<0.05$) between Controls and Siblings

ND — not detected

GADA were found in this group about twice less frequently than ICA. Only 11 (37.9%) of newly diagnosed IDDM cases tested positive for GADA. Nine of the eleven GADA positive individuals were males. The positive range was from 14 to 109 RU. The median value was 76 RU among those with GADA. The association between the presence of GADA and age (r=0.01) was very weak, with sex (r=–0.32) somewhat stronger.

Ten children (34.5%) out of 29 had detectable IA-2A at the diagnosis of IDDM. Again the boys were more prone to express humoral autoimmunity. Eight boys and only two girls had IA-2A. The positive range was from 4.7 to 109.5 units, the median value 68 AU among those. The correlations between antibody positivity and sex (r=–0.27) and age (r=–0.23) were low.

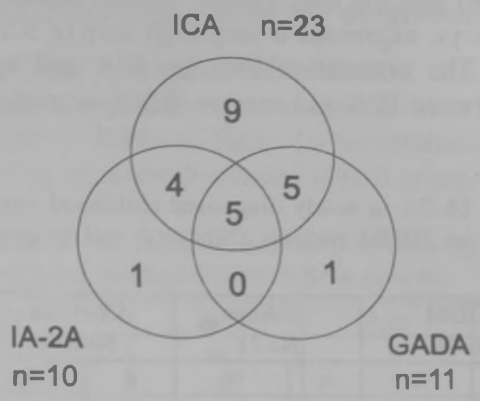


Figure 1. Concurrence of ICA, GADA and IA-2A in 29 newly diagnosed childhood onset IDDM patients

All three antibodies were positive only in five children with newly diagnosed IDDM (17.2%) and none were positive in four (13.8%) children. At least one antibody was positive in 25 (86.2%) of newly diagnosed patients. Nine (31.3%) had double antibody positivity. GADA and/or IA-2A were positive in 16 cases out of 29 (55.2%), 14 of these 16 were also ICA positive. The correlation between antibodies of newly diagnosed IDDM patients was low, between ICA and GADA (r=0.22), between ICA and IA-2A (r=0.37), between GADA and IA-2A (r=0.18).

1.2. Antibodies in healthy siblings of childhood onset IDDM patients

We studied 71 healthy siblings of childhood onset IDDM patients for the prevalence of IDDM related antibodies (V) (Table 10). The individual results of any antibody positive individuals are given in Table 11.

The frequency of ICA was 7.0% in healthy siblings C in five individuals out of 71. Four of them were females and one was male. All ICA positive individuals had a very high titer of antibodies C greater than or equal to 160 JDFU.
Twelve siblings (16.9%) had detectable GADA that exceeded the prevalence of ICA more than twice. The titer of GADA among them ranged from 10.7 to 115.3 RU. GADA were found among males and females with similar frequency. The GADA positive group consisted from five females and seven males. The highest GADA titers were documented in females in this group.

Table 11. Individual values of ICA (JDFU), GADA (RU) and IA-2A (AU) in antibody positive healthy siblings of IDDM patients.

ID	Sex	ICA	GADA	IA-2A
7a	f	0	115.3	0
13a	m	0	10.7	0
13b	m	0	39.9	0
16a	m	0	63.1	0
16b	m	0	10.7	76
17a	f	≥160	84.6	0
19a	m	0	74.6	0
20a	f	≥160	78.5	94
25a	m	0	65	0
32a	f	≥160	90.6	100
35a	f	0	45	0
37a	f	≥160	0	0
39a	m	≥160	68	95

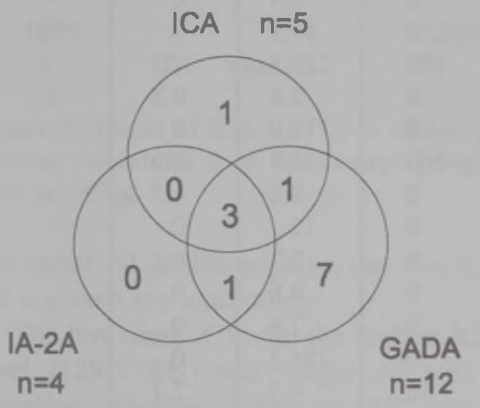


Figure 2. Concurrence of ICA, GADA and IA-2A in 71 healthy siblings of childhood onset IDDM patients

IA-2A was the least frequently found antibody studied by us. These were found in four (5.6%) siblings. Two males and two females were IA-2A positive. All four had high levels of IA-2A, exceeding 75 AU. We found that most of the healthy siblings C 58 (81.6%) C did not have any of the three studied antibodies. Among the 13 antibody positive individuals (18.3%), 12 tested positive for GADA (Figure 2). All three antibodies were positive only in three (4.2%) siblings. Four siblings out of five ICA positives had also GADA and/or IA-2A.

Table 12. Correlation coefficients between sex, age and IDDM-related antibodies in healthy siblings of childhood onset IDDM patients

	Sex	Age	ICA	GADA
ICA	0.14	0.11		0.46
GADA	–0.12	0.04	0.46	
IA-2A	–0.02	0.18	0.65	0.54

In the healthy siblings group the correlation between different antibodies was stronger than in the newly diagnosed childhood onset IDDM group (Table 12). The correlation between ICA and IA-2A was the strongest (r=0.65), followed by the one between GADA and IA-2A (r=0.54) and ICA and GADA (r=0.46).

1.3. Antibodies in controls

The analysis of the prevalence of IDDM associated antibodies in controls was performed in 614 children and adolescents (I, V). Nineteen of the 614 tested individuals (3%) were either ICA and/or GADA positive (Table 10, Table 13).

Table 13. Individual values of ICA (JDFU) and GADA (RU) in antibody positive individuals from the control group in 1991 and 1994. IDDM+ individuals developed IDDM between the two investigations.

ID	Sex	Age	ICA		GADA		IDDM
			1991	1994	1991	1994	
9	f	6	0	0	9.5	0	
23	f	11	0	0	97.7	0	
27	m	13	97	ND	129.3	ND	+
32	m	15	3	0	2.4	0.3	
60	m	15	0	0	11.9	0	
89	f	16	18	ND	125.9	ND	+
121	f	17	18	0	8.8	4.3	
141	f	10	0	0	12	0	
177	m	6	0	0	7.7	0	
257	m	15	0	0	9.6	0	
259	m	14	0	0	7.8	0	
268	f	17	0	0	154.2	0	
301	f	11	65	18	4.3	2.7	
369	f	11	0	0	7.8	0	
390	f	9	34	0	2.3	3.8	
405	f	10	81	18	123	80.4	
537	m	11	50	5	8.5	0.6	
570	f	3	34	36	4.9	0.9	
588	m	4	3	0	3.8	1.4	

ND — not detected

Ten children out of 614 tested positive for ICA (1.6%). The levels of ICA in the ICA positive subjects ranged from three to 97 JDFU, median 42 JDFU. Six children had ICA levels higher than 20 JDFU. Two of the six had the ICA level higher than 80 JDFU. We did not find any differences in ICA frequency between males and females (p=0.188) or between those younger and older than 10 years of age (p=0.333).

GADA were found more often in this group compared with ICA. Fourteen children out of 614 (2.3%) had detectable GADA. There was no significant difference in the frequency of GADA between boys and girls and between those younger and older than 10 years.

Five subjects tested positive for both antibodies (0.8%). The frequency of double antibody positivity was significantly lower than for GADA or ICA alone (p=0.03). The correlation between GADA and ICA levels was moderate (r=0.43).

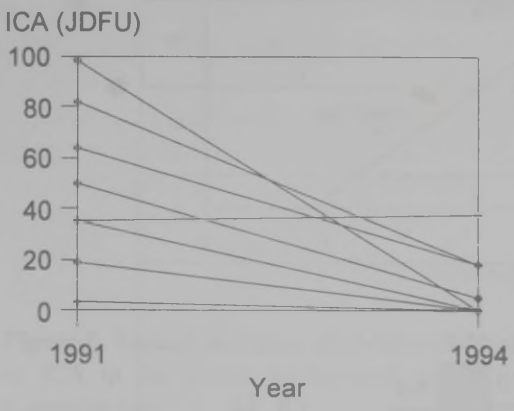


Figure 3. Values of ICA (JDFU) in antibody positive individuals from the control group, 1991 and 1994.

ICA and GADA were tested three years later, in 1994, only in initially ICA positive individuals. Four individuals out of ten remained positive for ICA in the repeated testing but their antibody levels decreased almost statistically significantly from the median of 42 JDFU to 18 JDFU (p=0.06). The median GADA level decreased significantly from 4.9 to 1.4 RU (p=0.04). Those children who remained ICA positive had the initial level of ICA greater than 20 JDFU. Among the four children initially positive for both antibodies, only one remained double positive, one was ICA positive at repeated testing and two did not have any antibodies.

The boy (case 27) with the highest ICA titer (97 JDFU) and highest GADA level (129.3 RU) was diagnosed with IDDM four months after the first sampling. Another child (case 89) with GADA 123 RU and ICA 18 JDFU developed diabetes two years after the first testing in 1991.

1.4. Discussion

Our study describes the prevalence of various autoantibodies associated with IDDM in Estonia, including ICA, GADA, IA-2A. Although ICA were described already in 1974, data on the prevalence of these antibodies are still missing for many populations. Data from Estonia are of particular interest as Estonia is geographically close to Finland, the country with the highest incidence of IDDM in the world.

The prevalence of the antibodies first described in IDDM patients, ICA, was the highest in the newly diagnosed IDDM group, 79.3%. The prevalence of ICA

in Estonian newly diagnosed IDDM patients was comparable to other studies from Europe. Thus, the prevalence of ICA in Finland has been reported to be 75% (Karjalainen *et al.*, 1986), in Germany 75% (Wiest-Ladenburger *et al.*, 1997), in Italy 89% (Genovese *et al.*, 1992), in Sweden 81% (Landin-Olsson *et al.*, 1989), in Belgium 73% (Gorus *et al.*, 1997). Data from Estonia confirm that most but not all newly diagnosed IDDM patients have detectable ICA.

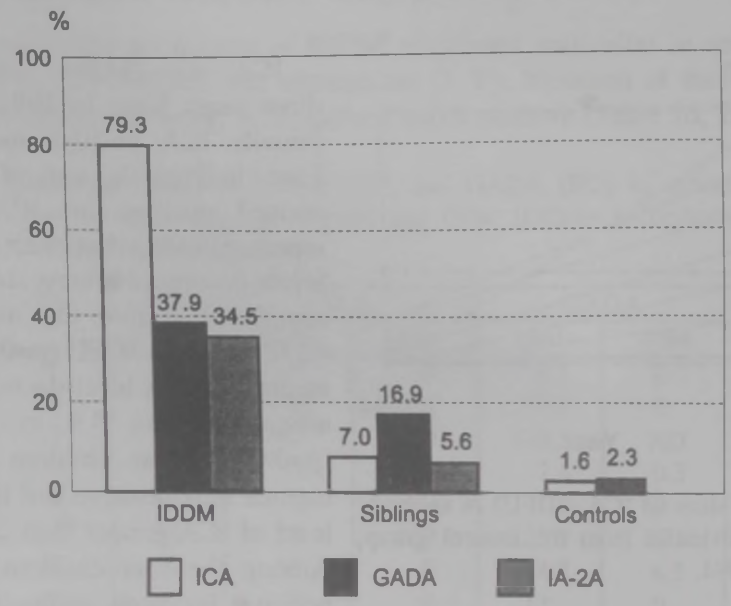


Figure 4. Prevalence (%) of ICA, GADA and IA-2A in newly diagnosed childhood onset IDDM patients (IDDM), healthy siblings of IDDM patients (Siblings) and controls (Controls)

The prevalence of ICA in Estonian siblings of IDDM patients is one of the highest reported until now in Europe. ICA were found in 7% of siblings of IDDM patients. The ICA prevalence in healthy siblings group was similar to that in Germany 6.9% (Seissler *et al.*, 1996), and higher than in France 5.1% (Thivolet *et al.*, 1988) and Belgium 5% (Gorus *et al.*, 1997). It has to be considered that all these data including ours are based on comparatively small numbers of subjects and the choice of the individuals might have had influence on the outcome.

The prevalence of ICA in the controls, aged 3–18 years, was 1.6%. Approximately, a tenfold difference has been found in the prevalence of ICA between European populations. ICA has been found in 0.4% to 4.1% of healthy general population. Estonian data are similar to the ones from France, both as to the prevalence of ICA and the incidence of IDDM (Levy-Marchal *et al.*, 1992; Green *et al.*, 1992; I).

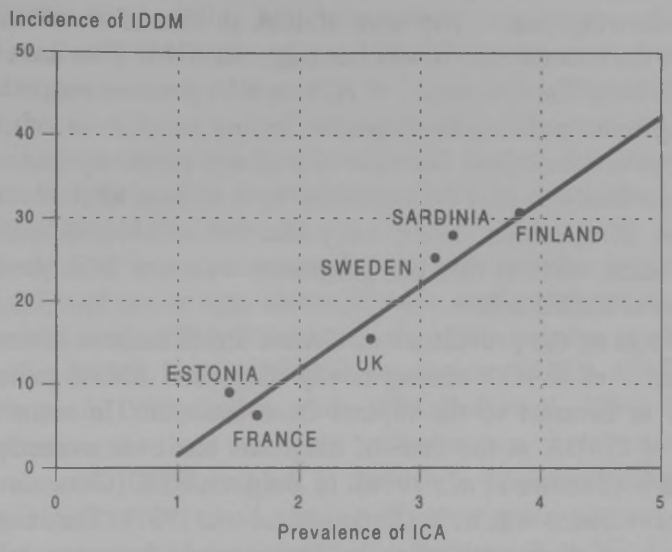


Figure 5. Annual incidence of childhood onset IDDM (per 100,000) and prevalence (%) of ICA in the young background population of selected countries, and the linear regression line.

The prevalence of ICA in the control group was about twelve times more than the prevalence of IDDM in Estonia in the respective age group. This indicates that the great majority of those positive for ICA will not progress to clinical IDDM. That was also confirmed by the decrease in the prevalence of ICA and their titer at the repeated investigation. Based on available data, we have compared the frequency of ICA in the young background populations in a series of six countries with the incidence of childhood onset IDDM in those countries (I). It appears there is a highly significant correlation ($r=0.97$, $p<0.01$), indicating that the proportion of ICA-positive children progressing to clinical disease is similar in different populations. That means the variation in disease incidence between countries can be partly explained by the frequency of ICA positivity in the non-diabetic population (I). In comparison with other populations, the prevalence of ICA in Estonian controls and the incidence of IDDM are intermediate.

The repeated testing of ICA in individuals positive for this antibody from the control group after three years showed a considerably reduced frequency. Of the eight repeatedly tested IDDM-free individuals, in three ICA had disappeared, and the titer was reduced in four. Only in one individual was seen a slight increase in the titer of ICA. The observation that about half of those testing positive for ICA in the initial sample did not have detectable antibodies three years later illustrates that ICA-positivity is often transient in nondiabetic children. High ICA level tends to be persistent more often than low level. Two

of the ten individuals testing positive for ICA in 1991 developed IDDM during the three following years. The titer of ICA in one of these cases exceeded 80 JDFU, in the second case it was not high, 18 JDFU. Few investigators have studied repeatedly the frequency of ICA in ICA positive individuals from the young background population. Repeated testing for ICA in other populations has given similar results, but decrease of antibody positivity was not so remarkable as in our study. It may be explained by a shorter interval to the repeated investigation (Karjalainen, 1990; Levy-Marchal *et al.*, 1992; Bingley *et al.*, 1993). Our data confirm that the prognostic value of ICA positivity for the development of IDDM is low.

Our findings on the prevalence of GADA are of interest in several respects. The prevalence of GADA among newly diagnosed IDDM patients was low, only 37.9% in contrast to the reports from literature. In some countries the prevalence of GADA at the time of diagnosis has even exceeded 80% — in Germany 87% (Seissler *et al.*, 1996), in Belgium 82% (Gorus *et al.*, 1997). In Latvia the prevalence was 67% (Shtauvere *et al.*, 1997). The reason for such a low frequency of these antibodies in Estonia could be methodological differences.

On the other hand, the prevalence of GADA in our healthy siblings cohort was higher (16.9%) than documented in other studies. The figure from our study was even higher than for Finland. Thus, in Finland the frequency of GADA in siblings was 9.6% (Kulmala *et al.*, 1995), in Germany 6.4% (Wiest-Ladenburger *et al.*, 1997). GADA were found in our siblings group twice as often as ICA and IA-2A. Our finding might be explained by the selection methods.

The frequency of GADA in the controls (2.3%) was similar to that of ICA. The frequency of GADA was similar to ours in Denmark, 2.5% (Petersen *et al.*, 1994) and Italy 2.1% (Velluzzi *et al.*, 1997). The highest prevalence of GADA has been reported in the state of Washington — 4.6% (Leech *et al.*, 1995).

The repeated tests of GADA in ICA positive individuals from the general population three years later showed that like ICA, also GADA levels and frequency tend to diminish. Three out of four seroconverted to GADA-negativity including one with a very high initial level. Only one individual remained positive in repeated testing in 1994. This shows that transiently strong humoral responses to GAD can be seen in normal children. Our observation lends support to the view that GADA levels fluctuate and a possibility of transient, relatively meaningless for the development of IDDM, positivity of GADA in general population.

The investigation carried out by us is one of the few to study the prevalence of IA-2A in the IDDM cohort. The method for the detection IA-2A was introduced only in 1990 and there are few reports on the association of IA-2A and IDDM. We found the prevalence of IA-2A of 34.5% among the newly diagnosed IDDM cases. These figures are somewhat lower than reported for other

European populations — in Germany 73% (Wiest-Ladenburger *et al.*, 1997), in Italy 58% (Gorus *et al.*, 1997), but close to data from Japan — 41% (Kawasaki *et al.*, 1997) and neighboring Latvia — 41% (Shtauvere *et al.*, 1997).

We investigated also the frequency of IA-2A in healthy siblings of IDDM patients. IA-2A were found in 5.6% of the healthy siblings of IDDM cases. The frequency of these antibodies was somewhat higher than reported in other European studies. Two of the three siblings of IDDM cases who later developed IDDM, had detectable IA-2A.

We did not find any strong correlations between the prevalence of ICA, GADA, IA-2A and sex or age, although some studies have seen such relationships (Schmidli *et al.*, 1994; Petersen *et al.*, 1994; Seissler *et al.*, 1996). Most studies, similar to ours, have not found associations of ICA, GADA with sex or age (Christie *et al.*, 1993; Grubin *et al.*, 1994; Roll *et al.*, 1994). Only moderate relationship among the three measured antibodies was found among the siblings of individuals with IDDM, but not in the newly diagnosed IDDM group. That may be explained by the small number of investigated patients. A number of studies have investigated the relationship between ICA, GADA and IA-2A. Strong association between IA-2A, GADA and ICA has been observed in some studies, in particular with high levels of ICA (Christie *et al.*, 1993; Bonifacio *et al.*, 1995; Ongagna *et al.*, 1995; Lan *et al.*, 1996; Seissler *et al.*, 1996). No correlation was found in the same antibody levels (Gorus *et al.*, 1997).

2. Genetic factors of IDDM

2.1. Selected HLA-DQB1 alleles in childhood onset IDDM patients and controls

The data of HLA-DQB1 alleles and their combinations with other HLA alleles (II, III, IV, V) is presented in Table 14 and Table 15.

Table 14. Prevalence of selected HLA-DQB1 alleles in the childhood onset IDDM patients (IDDM) and controls (Controls)

Allele	IDDM		Controls		OR
	n	%	n	%	
*0201	53	54.6	84	31.2	2.7*
*0301	10	10.3	89	33.1	0.2*
*0302	64	66.0	48	17.8	8.9*
*0304	5	5.2	2	0.7	7.3*
*0602/0603	2	2.1	119	44.2	0.03*

* OR statistically significantly different from 1 ($p<0.05$)

Of the five analyzed HLA-DQB1 alleles (*0201, *0301, *0302, *0304 and *0602/0603), the most frequent in the IDDM group was *0302 (66%). The prevalence of this allele was considerably higher than in the control group (17.8%), $p<0.05$. The next most common allele among the IDDM cases was *0201, present in 54.6% of IDDM cases, also occurring significantly more frequently than in the controls where its prevalence was 31.2%. Although rare among both IDDM cases and controls, the prevalence of the novel DQB1*0304 (5.2%) was significantly higher among IDDM cases compared with the controls (0.7%) ($p<0.05$).

The highest risk for IDDM of the studied HLA-DQB1 locus was conferred by *0302. The OR for was 8.9, $p<0.05$. DQB1*0304 appeared to confer the second highest susceptibility to IDDM in Estonia (OR=7.3, $p<0.05$). DQB1*0201 was also associated with significantly increased risk for the development of IDDM, OR — 2.7, $p<0.05$.

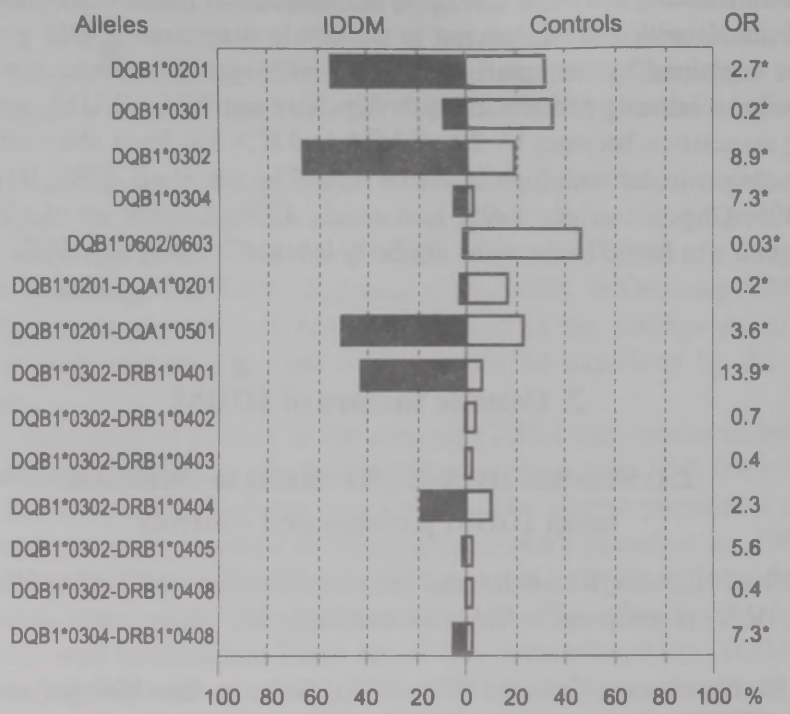


Figure 6. Prevalence (%) of selected HLA-DQB1 alleles and HLA-DQB1-DQA1 and HLA-DQB1-DRB1 allele combinations in the childhood onset IDDM patients (IDDM) and controls (Controls).
* OR statistically significantly different from 1 ($p<0.05$)

Two of the investigated HLA-DQB1 alleles were significantly less frequent in IDDM cases in Estonia than in the controls — *0301 and *0602/0603. More than 44% of the Estonian population was positive for HLA-DQB1*0602/0603.

The prevalence of DQB1*0602/0603 was about 20 times less among the IDDM group (2.1%) than in the controls, resulting in the OR of 0.03 ($p<0.05$). About one third of the Estonian population carried DQB1*0301. This allele was more than three times less common (10.3%) in the IDDM group compared with controls and appeared also to provide protection against IDDM, but not as strongly as *0602/0603. The OR for this allele was 0.2 ($p<0.05$).

2.2. Selected HLA-DQ and HLA-DR allele combinations in childhood onset IDDM patients and controls

We also studied the prevalence of different allele combinations and found that the frequency of four was significantly different between the IDDM patients and control group (Table 15).

Table 15. Prevalence of selected HLA-DQB1-DQA1 and HLA-DQB1-DRB1 allele combinations in IDDM patients (IDDM) and controls (Controls).

Allele combination		IDDM		Controls		OR
		n	%	n	%	
DQB1	DQA1					
*0201	*0501	49	50.5	59	22.0	3.6*
*0201	*0201	3	3.1	40	15.0	0.2*
DQB1	DRB1					
*0302	*0401	42	43.3	14	5.2	13.9*
*0302	*0402	1	1.0	4	1.5	0.7
*0302	*0403	0	0	3	1.1	0.4
*0302	*0404	18	18.6	24	8.9	2.3
*0302	*0405	2	2.1	1	0.4	5.6
*0302	*0408	0	0	3	1.1	0.4
*0304	*0408	5	5.2	2	0.7	7.3*

* OR statistically significantly different from 1 ($p<0.05$)

Of the allele combinations the most frequent among the IDDM patients from Estonia was DQB1*0201-DQA1*0501 (50.5%). This allele combination was more than twice as common in IDDM cases compared with the general Estonian population (22%), $p<0.05$. The combination conferred a moderately increased risk of IDDM, OR of 3.6. It appeared that all Estonian IDDM cases that carried DQB1*0201-DQA1*0501 in our particular study were also positive for DQB1*0302.

Of the two-allele combinations of the HLA-DQB1 and HLA-DRB1 alleles, the most common among IDDM patients was DQB1*0302-DRB1*0401. This two-allele combination was the second in frequency of those found in Estonian IDDM cases. It was present in 43.3% of IDDM cases and in only 5.2% of the

controls. The OR for this combination was the highest in our study, 13.9 ($p<0.05$). A high OR was also found for the two allele combination of HLA-DQB1*0304-DRB1*0408. The value of the OR was 7.3 ($p<0.05$), although both alleles were uncommon in the IDDM (5.2%) and control group (0.7%).

The combination of HLA-DQB1*0201-DQA1*0201 was significantly less common in IDDM cases compared with controls. The presence of this particular combination reduced the risk of IDDM about five times.

2.3. Discussion

We analyzed the prevalence of selected HLA-DQB1 locus alleles proposed to be related to the protection against or susceptibility to IDDM in Estonia. The study carried out by us is one of the first to establish the frequency of certain HLA-DQ locus alleles in the Estonian population. This was necessary to find out the alleles associated with the risk of IDDM in Estonia. Our study showed that the main features of HLA-related IDDM susceptibility in Estonia were similar to those found in most other European studies.

The most common HLA-DQ alleles of the ones studied in the Estonian IDDM patients were DQB1*0302 (66%) followed by DQB1*0201 (54.6%), both non-Asp-57. These data agree with the numerous reports from literature where these two alleles have been recognized as the most important single alleles responsible for the susceptibility to IDDM (Saukkonen *et al.*, 1998; Izaabel *et al.*, 1996; Sanjeevi *et al.*, 1995; Reijonen *et al.*, 1994).

Increased risk of IDDM was conferred also by HLA-DQB1*0304, OR of 7.3. HLA-DQB1*0304 is a rare DQB1*03 subtype that combines the features of both DQB1*0301 and DQB1*0302 alleles (Fenske and Baxter-Lowe, 1992). The first of the two alleles reduces the risk of IDDM while the second is an allele associated with and increased risk (Thorsby and Ronningen, 1993). HLA-DQB1*0304 evidently also confers higher risk for IDDM. Although of interest, this finding should not be clinically overestimated as the frequency of the HLA-DQB1*0304 was only a low of 5.2% among IDDM cases and 0.7% in the controls. In a comparative study that allele yielded the OR of 4.2 in Latvians, 3.6 in Russians (III). HLA-DQB1*0304 was found as a very rare allele in North-American and German Caucasian population, but more commonly in Czech gypsies (Fenske *et al.*, 1992; Tautz *et al.*, 1992; Cerna *et al.*, 1992). In Sardinia it was associated with protection against IDDM (Cucca *et al.*, 1993).

The least frequently found HLA-DQB1 allele among Estonian IDDM patients was *0602/0603, the allele postulated to be protective against IDDM (Pugliese *et al.*, 1995; Ilonen *et al.*, 1996). Protective influences are thought to be superior to the susceptibility to IDDM conferred by other alleles (Caillat-Zucman *et al.*, 1992; Lee *et al.*, 1996; Yasunaga *et al.*, 1996). The prevalence of

this allele in IDDM patients was only 2.1%. Although significantly lowering the risk of IDDM, the presence of this allele does not provide complete protection against IDDM in Estonian population. Two of the 97 IDDM cases still had this protective allele. In these two particular instances the other alleles at the HLA-DQ and HLA-DR loci were DQB1*0304-DRB1*0408 and DQB1*0302-DRB1*0401, both conferring susceptibility to IDDM.

The DQB1*0602/0603 allele was associated with the highest protection against IDDM of the investigated HLA-DQ locus alleles in Estonia. The factor of protection the presence of this allele conferred in Estonia was very high, about 38 (OR=0.03). The HLA-DQB1*0602 has appeared as protective from most of the studies of diverse populations — Caucasians, Hispanics, Orientals, Negroids (Caillat-Zucman *et al.*, 1992; Lee *et al.*, 1996; Yasunaga *et al.*, 1996). This allele has yielded OR-s of the magnitude of 0.12 among Finns (V), 0.15 among Moroccans (Izaabel *et al.*, 1996), 0.2 among Hispanics (Cruickshanks *et al.*, 1994).

Remarkable differences in the incidence of IDDM have been found in neighboring populations. The incidence of IDDM in Finland is 35 per 100,000 per year (Karvonen *et al.*, 1993), in Estonia only 10 per 100,000 per year (VII). To clarify the IDDM incidence variation HLA-typing may add important information. There are studies confirming the correlation between frequency of some HLA-DR, DQ alleles, their combinations and incidence of IDDM in different ethnic groups (Cruickshanks *et al.*, 1994; Van der Auwera *et al.*, 1995; She, 1996). The resistance against IDDM is often dominant over susceptibility (Van der Auwera *et al.*, 1995; Ilonen *et al.*, 1996). The high prevalence of the HLA-DQB1*0602/0603 in the population of Estonia and its high degree of protection against IDDM might be an important factor in explaining the considerably lower incidence of IDDM in Estonia compared with Finland where the incidence is the highest in the world.

The most frequent allele combination among Estonian IDDM patients was DQB1*0201-DQA1*0501, in half the cases. Similar findings have been reported in many other studies in Caucasians, Blacks and Japanese (Rønningen *et al.*, 1991; Lampasona *et al.*, 1995; Van der Auwera *et al.*, 1995; Serrano-Rios *et al.*, 1996). The OR associated with these combinations was 3.6. Similar relative risk of 3–5 has been confirmed in the analysis IDDM susceptibility or resistance in Black, Caucasian and Japanese subjects (Thorsby and Rønningen, 1993).

The most frequently encountered HLA-DR4 subtype of the varieties studied in the Estonian DQB1*0302 positive IDDM patients, was HLA-DRB1*0401. This allele combination was eight times more frequent in IDDM patients (43.3%) compared with the controls (5.2%). The HLA-DRB1*0401 allele has been found most frequently among IDDM cases also in Sweden and Norway (Sanjeevi *et al.*, 1996; Yasunaga *et al.*, 1996). The next most common allele combination was HLA-DQB1*0302-DRB1*0404, found in 18.6% of cases. The

prevalence of this combination in cases was about twice higher than in the controls, but did not reach statistical significance.

The DQB1*0304 concurred with DRB1*0408 allele in all Estonian IDDM patients (III). The same finding was documented for cases from Latvia and Russia (III). The DQB1*0304-DRB1*0408 combinations were found only in three subjects from Northern America (in the remaining three DQB1*0304 was associated with DRB1*0403) and in one from Czech gypsies. In Sardinia, DQB1*0304 was in combination with DRB1*0403 (Fenske *et al.*, 1992; Cerna *et al.*, 1992; Cucca *et al.*, 1993).

Of the allele combinations studied, four evolved as significantly associated with IDDM. Three of those were conferring susceptibility to IDDM and one protection. Our study confirms that the whole HLA-haplotype is important in the determination of the risk of IDDM, not just single alleles. The presence of HLA-DQA1*0201 in DQB1*0201 positive individuals decreased the risk for the development of IDDM while the association with DQA1*0501 increased the risk. Also, the combination of HLA-DQB1*0302 with different DRB1 locus alleles produced varying results in the risk of IDDM. While DRB1*0401 increased the risk conferred by DQB1*0302 alone, *0403 had a tendency to decrease the risk of IDDM.

3. Immune and genetic factors of IDDM in healthy siblings of childhood onset IDDM patients followed for three years

We followed the 71 healthy siblings of childhood onset IDDM patients for three years for the development of clinical IDDM. Three children (4%) were diagnosed with clinical IDDM by December 1997 (Table 16).

IDDM was diagnosed in case 16b six months after the investigation, in case 17a nine months later and case 20a one and a half years later.

All three children, who developed later IDDM, had at least double antibody positivity at the time of the investigation. Two of them were double antibody positive (GADA/IA-2A and ICA/GADA) and one sibling had all three antibodies detectable. One of the siblings carried DQB1*0302-DRB1*0401 allele combination, the second and third DQB1*0201-DQA1*0501. Both these combinations were found most often among Estonian IDDM patients. None of the alleles associated with protection against IDDM in Estonia were present in the three cases that went on to develop IDDM. After three years of follow-up, at the end of December 1997, the remaining two of the all antibody positive siblings were still IDDM-free. Both siblings were positive for an allele that confers protection against IDDM in Estonia, DQB1*0301 and DQB1*0602/0603.

One of these two siblings is remarkably a dizygotic twin brother of the IDDM case.

Table 16. Individual values of ICA (JDFU), GADA (RU) and IA-2A (RU) and occurrence of selected HLA-DR and HLA-DQ alleles in antibody positive healthy siblings of IDDM patients. IDDM+ individuals developed IDDM during the three-year follow-up.

ID	ICA	GADA	IA-2A	DRB1	DQA1	DQB1	IDDM
7a	0	115.3	0		*0501	*0201/ *0301	
13a	0	10.7	0	*0401	*0501	*0201/ *0302	
13b	0	39.9	0	*0401		*0302	
16a	0	63.1	0	*0401/ *0404		*0302	
16b	0	10.7	76	*0401		*0302	+
17a	≥160	84.6	0		*0501	*0201	+
19a	0	74.6	0		*0501	*0201	
20a	≥160	78.5	94		*0501	*0201	+
25a	0	65	0	*0401		*0302	
35a	0	45	0		*0501	*0201	
32a	≥160	90.6	100	*0401		*0302/ *0602/ *0603	
37a	≥160	0	0			*0602/ *0603	
39a	≥160	68	95	*0401		*0301/ *0302	

Our data corroborate that IDDM related antibodies are much more prevalent among first degree relatives of IDDM patients than general population and consequently, they are at a higher risk for the development of IDDM. An ICA level of 20 JDFU or more has been observed to be strongly predictive of progression to IDDM in first-degree relatives of IDDM patients, with a progression rate of 24–25% over five years (Riley *et al.*, 1990; Bonifacio *et al.*, 1989). There are data suggesting that ICA are more predictive in first-degree relatives than in non-diabetic unrelated children (Levy-Marchal *et al.*, 1992; Bingley *et al.*, 1993). The titer of ICA was very high in all five siblings from Estonia who were positive for ICA. The titer of ICA exceeded 160 JDFU in all of them. Two out of the five developed clinical IDDM shortly after investigation.

CONCLUSIONS

1. The most frequent IDDM associated antibody in the newly diagnosed childhood onset IDDM patients in Estonia is ICA (79%) followed by lower prevalence of GADA (38%) and IA-2A (35%). The prevalence of IDDM associated antibodies among healthy siblings of IDDM cases was less and the lowest in the healthy controls.

2. The frequency of ICA in the young background population is closely associated with the incidence of childhood onset IDDM, $r=0.97$, $p<0.05$. The proportion of ICA-positive children progressing to IDDM is similar in different populations.

3. The most frequent of the studied HLA-DQB1 alleles among Estonian IDDM patients is HLA-DQB1*0302 (present in 66%) followed by HLA-DQB1*0201 (55%). Both these alleles were significantly more prevalent in the IDDM patients than in the controls.

4. The single HLA-DQB1 allele carrying the highest risk for IDDM in Estonia, is HLA-DQB1*0302 (OR 8.9) followed by the novel HLA-DQB1*0304 allele (OR 7.3).

5. The allele carrying the lowest risk of IDDM in Estonia is HLA-DQB1*0602/0603. This allele is present in 44% of the Estonian healthy population. The risk of IDDM conferred by this allele is about four times less in Estonia than in Finland and this could partly explain the more than threefold difference in the incidence of IDDM between these two neighboring countries.

6. The most frequent double allele combination studied among Estonian IDDM patients is HLA-DQB1*0201-DQA1*0501, present in half the cases, and followed by HLA-DQB1*0302-DRB1*0401 (in 43%). This agrees with other studies in Caucasians.

7. The siblings of IDDM patients were at an increased risk of developing IDDM compared with the young background population. Four percent of siblings developed the disease during the three-year follow-up. Those who developed IDDM were all positive for at least two IDDM-related antibodies, including GADA, and carried the most frequent HLA double allele combinations of Estonian IDDM patients.

REFERENCES

Aanstoot HJ, Petersen JS, Batstra MR, *et al.* Low frequency of antibodies to glutamic acid decarboxylase in the general population. Diabetologia 1995; 38 (Suppl. 1): A54.

Aanstoot HJ, Sigurdsson E, Jaffe M, Shi J, Hofman A, Baekkeskov S. Value of antibodies to GAD65 combined with islet cell cytoplasmic antibodies for predicting IDDM in a childhood population. Diabetologia 1994; 37: 917–924.

Armitage P, Berry G. Statistical methods in medical research. 3rd ed. Blackwell, Oxford 1994; p.331.

Atkinson MA, Kaufman DL, Campbell L, Gibbs KA, Maclaren NK. Response of peripheral blood mononuclear cells to glutamate decarboxylase in insulin dependent diabetes. Lancet 1992; 339: 458–459.

Atkinson MA, Maclaren NK. Islet cell autoantigens in insulin-dependent diabetes. J Clin Invest 1993; 92:1608–1616.

Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 1994; 24: 1428–1436.

Awata T, Kanazawa Y. Genetic markers for insulin-dependent diabetes mellitus in Japanese. Diabetes Res Clin Pract 1994; 24(Suppl. 1): 83–87.

Awata T, Kuzuya T, Matsuda A, *et al.* High frequency of aspartic acid at position 57 of HLA-DQ b-chain in Japanese type 1 diabetic patients and nondiabetic subjects. Diabetes 1990; 39: 266–269.

Baekkeskov S, Aanstoot HJ, Christgau S, *et al.* Identification of the 64K autoantigen in insulin dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 1990; 347: 151–156.

Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark Å. Autoantibodies in newly diagnosed diabetic children immunoprecipitate specific human pancreatic islet cell proteins. Nature 1982; 298: 167–169.

Bergay M, Sole J, Marion G. Prevalence of islet cell antibodies, insulin antibodies and hyperglycemia in 2,291 schoolchildren. Diabetologia 1983; 30: 724–726.

Bingley PJ, Bonifacio E, Gale EAM. Can we really predict IDDM? Diabetes 1993; 42: 213–220.

Bingley P, Christie M, Cassidy D, *et al.* Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody positive relatives. Diabetes 1994; 43: 1304–1310.

Björk E, Velloso L, Kämpe O, Karlsson A. GAD autoantibodies in IDDM, Stiff-man syndrome and autoimmune polyendocrine syndrome type 1 recognize different epitopes. Diabetes 1994; 43: 161–165.

Bonifacio E, Bingley P, Shattock M *et al.* Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. Lancet 1989; 335: 147–151.

Bonifacio E, Genovese S, Braghi S, Bottazzo GF, Gale EAM, Bosi E. Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. Diabetologia 1995; 38: 816–822.

Borch-Johnsen K, Joner G, Mandrup-Poulsen T, *et al.* Relation between breast feeding and incidence rates of insulin dependent diabetes mellitus. Lancet 1984; II: 1083–1086.

Bottazzo GF, Florin-Christensen A, Doniach D. Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiency. Lancet 1974; II: 1279–1283.

Bottazzo GF, Genovese S, Bosi E, *et al.* Novel consideration on the antibody/autoantigen system in Type 1 diabetes mellitus. Ann Med 1991; 23: 453–461.

Bottazzo GF, Pujol-Borell R, Gale EAM. Autoimmunity and diabetes: progress, consolidation and controversy. Diabetes Ann 1986; 2: 13–29.

Bruining GJ, Molenaar JL, Grobbee DE, *et al.* Ten year follow up study of islet cell antibodies and childhood diabetes. Lancet 1989; I: 1100–1103.

Caillat-Zucman S, Garchon HJ, Timsit J, *et al.* Age dependent HLA genetic heterogeneity of type 1 insulin dependent diabetes mellitus. J Clin Invest 1992; 90: 2242–2250.

Cerna M, Fernandez-Vina M, Ivaskova E, Stastny P. Comparison of HLA class II alleles in Gypsy and Czech populations by DNA typing with oligonucleotide probes. Tissue Antigens 1992; 39: 111–116.

Chauffert M, Cisse A, Chevenne D, Parfait B, Michel S, Trivin F. HLA-DQB1 typing and Non-Asp 57 alleles in the Aborigene population of Senegal. Diabetes Care 1995; 18: 677–680.

Chen QY, Rowley MJ, Byrne C, Zimmet PZ, Macay IR. Antibodies to glutamic acid decarboxylase in Australian children with insulin dependent diabetes mellitus and their first-degree relatives. Pediatr Res 1993; 34: 785–790.

Christie M. Characterization of distinct islet protein autoantigens associated with type 1 diabetes. Autoimmunity 1993;15:1–3.

Christie MR, Genovese S, Cassidy D, *et al.* Antibodies to islet 37 kD antigen, but not to glutamic decarboxylase, discriminate rapid progression to IDDM in endocrine autoimmunity. Diabetes 1994; 43: 1254–1259.

Christie M, Hollands JA, Brown TJ, *et al.* Detection of pancreatic islet 64,000Mr autoantigens in insulin dependent diabetes distinct from glutamate decarboxylase. J Clin Invest 1992; 90: 240–248.

Christie M, Trun RYM, Lo SS, *et al.* Antibodies to GAD and tryptic fragments of islet 64 k antigen as distinct markers for development of IDDM, studies with identical twins. Diabetes 1992; 41: 782–787.

Christie MR, Vohra G, Champagne P, Danemen D, Delovitch DL. Distinct antibody specificities to a 64 kDa islet cell antigen in type 1 diabetes as revealed by trypsin treatment. J Exp Med 1990; 172: 789–795.

Cruickshanks K, Jobim L, Lawler J, *et al.* Ethnic differences in human leucocyte antigen markers of susceptibility to IDDM. Diabetes Care 1994; 17: 132–137.

Cucca F, Frau F, Lampis R, *et al.* HLA-DQB1*0303 and -DQB1*0304 alleles among Sardinians C evolutionary and practical implications for oligotyping. Hum Immunol 1994; 40: 143–149.

Cucca F, Muntoni F, Lampis R, *et al.* Combinations of specific DRB1, DQA1, DQB1 haplotypes are associated with IDDM in Sardinia. Hum Immunol 1993; 37(Suppl. 2): 85–94.

Davies JL, Kawaguchi Y, Bennett ST, *et al.* A genome wide search for human type 1 diabetes susceptibility genes. Nature 1994; 371: 130–136.

Delamaire M, Timsit J, Caillat-Zucman S, *et al.* HLA-associated heterogeneity of the humoral response to islet antigen in insulin-dependent diabetes. J Autoimmun 1995; 8: 645–657.

De Leeuw I, Vandewalle C and Belgian Diabetes Registry. In new-onset IDDM patients the presence of IA2-antibodies is not associated with increased thyroid autoimmunity. Diabetologia 1997; 40(Suppl. 1): A80.

Deschamps I, Beressi JP, Khalil I, Robert JJ, Hors J. The role of genetic predisposition to type 1 diabetes mellitus. Ann Med 1991; 23: 427–435.

Deschamps I, Boitard C, Hors J, *et al.* Life table analysis of the risk of type 1 diabetes mellitus in siblings according to islet antibodies and HLA-markers: an 8-year prospective study. Diabetologia 1993; 35: 951–957.

Deschamps I, Homberg JC, Janssen F, *et al.* Islet cell antibodies and HLA genotypes in 107 IDDM patients and their first degree relatives. Hum Immunol 1984; 10: 129–134.

Drell DW, Notkins AL. Multiple immunological abnormalities in patients with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1987; 30: 132–143.

Eizirik DL, Monteiro CM, Voltarelli JC, Foss MC. Frequency of HLA antigens in a Brazilian type I diabetic population. Brazilian J Med & Biol Res 1987; 20(Suppl. 5): 533–537.

Fennesy M, Metcalfe K, Hitman G, *et al.* A gene in the HLA class I region contributes to susceptibility to IDDM in the Finnish population. Diabetologia 1994; 37: 937–944.

Fenske TS, Baxter-Löwe LA. Characterization of a novel DQB1 allele associated with HLA-DQw3: implications for oligotyping. Hum Immunol 1992; 33: 224–227.

Fletcher J, Mijovic C, Odugbesan O, Jenkins D, Bradwell AR, Barnett AH. Trans-racial studies implicate HLA-DQ as a component of genetic susceptibility to type 1 (insulin-dependent) diabetes. Diabetologia 1988; 31: 864–870.

Gaskins HR, Prochazka M, Hamaguchi K, Serreze DV, Leiter EH. B-cell expression of endogenous xenotropic retrovirus distinguishes diabetes susceptible NOD/Lt from resistant NON/Lt mice. J Clin Invest 1992; 90: 2220–2227.

Gemaine AG, Hibbert ML, Mangles D, Millward BA. A new marker in the HLA class I region is associated with the age of onset. Diabetologia 1995; 38: 623–628.

Genovese S, Bonfanti R, Bazzigaluppi E, Bonifacio E. Association of IA-2 autoantibodies with HLA DR4 phenotypes. Diabetologia 1996; 39: 1223–1226.

Genovese S, Bonifacio E, McNally JM, *et al.* Distinct cytoplasmic islet cell antibodies with different risks for type 1 diabetes mellitus. Diabetologia 1992; 35: 385–388.

Gianani R, Rabin DU, Verge CF, Yu L, Babu SR, Pietropaolo M, Eisenbarth GS. ICA512 autoantibody radioassay. Diabetes 1995; 44: 1340–1344.

Gorsuch AN, Dean BM, Bottazzo GF, *et al.* Evidence that type 1 diabetes and thyrogastric autoimmunity have different genetic determinants. Br Med J 1980; 280: 145–147.

Gorus FK, Goubert P, Semakula C, *et al.* IA-2 autoantibodies complement GAD65-autoantibodies in new onset IDDM patients and help predict impending diabetes in their siblings. Diabetologia 1997; 40: 95–99.

Green A, Gale EAM, Patterson CC. Incidence of childhood-onset insulin dependent diabetes mellitus: the EURODIAB ACE study. Lancet 1992;33 9: 905–909.

Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Karlsen LA, Li L, Lernmark Å. A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 1994; 37: 344–350.

Guberski DL, Thomas VA, Shek WR, *et al.* Introduction of type 1diabetes by Kilhams rat virus in diabetes resistant BB/Wor rats. Science 1991; 254: 1010–1013.

Gunczler P, Lanes R, Layrisse Z, Esparza B, Salas R, Hernendez L, Arnaiz-Villena A. Epidemiology and immunogenetics in recently diagnosed Venezuelan children with insulin dependent diabetes mellitus. J Pediatr Endocrinol 1993; 6: 165–171.

Gustincich S, Manfiolett G, Del Sal G, Schneider C, Carninci P. A fast method for high quality genomic DNA extraction from whole human blood. Bio Techniques 1991; 11: 298–302.

Hagopian WA, Karlsen AE, Gottsater A, Landin-Olsson M, Lernmark Å. Quantitative assay using recombinant human islet glutamic acid decarboxylase shows that 64 K autoantibody positivity at onset predicts diabetes type. J Clin Invest 1993; 91: 368–374.

Harfouch-Hammoud E, Timsit J, Boitard C, Bach JF, Caillat-Zucman S. Contribution of DRB1*04 variants to predisposition to or protection from insulin dependent diabetes mellitus is independent of DQ. J Autoimmun 1996; 9: 411–414.

Harrison LC, Honeyman MC, Schmidli RS, Tait BD, Gram DS. Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin dependent diabetes. Lancet 1993; 341: 1365–1369.

Harrison LC, Tait BD. The genetics of diabetes. Clin Endocrinol Metab 1991; 5(Suppl. 2): 454–460.

Heimberg H, Nagy ZP, Somers G, De Leeuw I, Schuit FC. Complementation of HLA-DQA and -DQB genes confers susceptibility and protection to insulin-dependent diabetes mellitus. Hum Immunol 1992; 33: 7–10.

Helmke K, Otten A, Willems WR, *et al.* Islet cell antibodies and the development of diabetes mellitus in relation to mumps infection and mumps vaccination. Diabetologia 1986; 29: 30–33.

Honeyman MC, Harrison LC, Drummond B, Colman PG, Tait BD. Analysis of families at risk for insulin dependent diabetes mellitus reveals that HLA antigens influence progression to clinical disease. Mol Med 1995; 1: 576–582.

Hyöty H, Hiltunen M, Knip M, *et al.* A prospective study of the role of Coxsackie b and other enterovirus in the pathogenesis of IDDM. Diabetes 1995; 44: 652–657.

Ilonen J, Reijonen H, Herva E, *et al.* Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population. Diabetes Care 1996; 19: 795–800.

Izaabel H, Garchon H, Biga M, *et al.* Distribution of HLA class II alleles and haplotypes in insulin dependent Moroccan diabetics. Hum Immunol 1996; 49: 137–143.

Jaeger C, Allendorfer J, Hatziagelaki E, Federlin K and Bretzel R. High concordance of GAD65 antibodies and ICA in recent onset IDDM patients and IDDM of long duration. Diabetologia 1997; 40: A72.

Karjalainen JK. Islet cell antibodies as predictive markers for IDDM in children with high background incidence of disease. Diabetes 1990; 39: 1144–1150.

Karjalainen J, Knip M, Mustonen A. Relation between islet antibody and complement fixing islet cell antibody at clinical diagnosis of IDDM. Diabetes 1986; 35: 620–622.

Karlsen AE, Hagopian WA, Petersen JS, Boel E, Dyrberg T, Lernmark Å. Recombinant glutamic acid decarboxylase (representing the single isoform expressed in human islets) detects IDDM associated 64-Mr autoantibodies. Diabetes 1992; 41: 1355–1359.

Karvonen M, Tuomilehto J, Libman I, LaPorte R. A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1993; 36: 883–892.

Kawasaki E, Yu L, Gianani R, Verge CF, *et al.* Evaluation of islet cell antigens ICA512/IA-2 autoantibody radioassays using overlapping ICA512/IA-2 constructs. J Clin Endocrinol Metab 1997; 82: 375–380.

Khalil I, d'Auriol L, Gobet M, Morin L, *et al.* A combination of HLA-DQ beta Asp57-negative and HLA-DQ alpha Arg52 confers susceptibility to insulin-dependent diabetes mellitus. J Clin Invest 1990; 85: 1315–1319.

Kobayashi T, Itoh T, Osaka K, Sato K. Time course of islet cell antibodies and B-cell function in nonisulindependent stage of Type 1 diabetes. Diabetes 1987; 36: 510–517.

Kobayashi T, Tamemoto K, Nakanishi K, *et al.* Immunogenetic and clinical characterization of slowly progressive IDDM. Diabetes Care 1993; 16: 780–788.

Kockum I, Sanjeevi CB, Eastman S, Landin-Olsson M, Dahlquist G, Lernmark Å. Population analysis of protection by HLA-DR and -DQ genes from insulin-dependent diabetes mellitus in Swedish children with insulin-dependent diabetes and controls. Eur J Immunogenet 1995; 22: 443–465.

Kolb H, Dannehl K, Gruneklee D, Zielasek J. Prospective analysis of islet cell antibodies in children with type 1 diabetes. Diabetologia 1988; 31: 189–194.

Kolb H, Strocheker M, Biener J, *et al.* Analysis of the persistence of islet cell antibodies in type 1 diabetics children. In: Autoimmune aspects of endocrine diseases. Academic Press, London 1980, p. 291–294.

Kuglin B, Bertrams J, Linke C, Gries F, Kolb H. Prevalence of cytoplasmic islet cell antibodies and insulin autoantibodies is increased in subjects with genetically defined high risk for IDDM. Klin Wochenschr 1989; 67: 66–73.

Kulmala P, Petersen J, Reijonen H, *et al.* GAD65 autoantibodies in genetically high and low risk siblings of children with IDDM. Diabetologia 1995; 38: A55.

Lampasona V, Ferrari M, Bonifacio E, *et al.* HLA-DQ screening for risk assessment of insulin dependent diabetes in Northern Italy. Acta Diabetologia 1995; 32(Suppl. 3): 137–142.

La Nasa G, Carcassi C, Cirillo R, *et al.* Serological and molecular studies of HLA in insulin-dependent diabetes mellitus in Sardinia. Disease Markers 1990; 8: 333–340.

Lan MS, Wasserfall C, Maclaren NK, *et al.* IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. Proc Natl Acad Sci USA 1996; 93: 6367–6370.

Landin-Olsson M, Karlsson A, Dahlquist G, Blom L, Lernmark Å. Islet cell and other organ-specific autoantibodies in all children developing Type 1 (insulin-dependent) diabetes mellitus in Sweden during one year and in matched control children. Diabetologia 1989; 32: 387–395.

Lee HC, Ikegami H, Fujisawa T, Ogihara T, Park SW, Chung YS. Role of HLA class II alleles in Korean patients with IDDM. Diabetes Res Clin Pract 1996; 31: 9–15.

Leech NJ, McCulloch DK, Rowe RE, Palmer JP, Hagopjan WA. The role of GAD65Ab in the prediction of IDDM in the general population and it is relationship to other susceptibility markers. Autoimmunity 1995; 21: 63–64.

Lernmark Å, Hagglof B, Freedman Z, *et al.* A prospective analysis of antibodies reacting with pancreatic islet cells in insulin dependent diabetic children. Diabetologia 1981; 20: 471–475.

Lernmark Å, Molenaar JL, Beers WAM, Yamaguchi Y, Nagataki S, Ludvigsson L, Maclaren NK. The fourth international serum exchange workshop to standardize cytoplasmic islet cell antibodies. Diabetologia 1991; 34: 534–535.

Leslie RDG, Hawa M, Rowe W, *et al.* Antibodies to a tyrosine phosphatase-like molecule precede and predict IDDM: an identical twin study. Autoimmunity 1995; 21: A230.

Levy-Marchal C, Tichet J, Fajardy I, Gu XF, Dubois F, Czernichow P. Islet cell antibodies in normal French schoolchildren. Diabetologia 1992; 35: 577–582.

MacMillan DR, Foster MB. HLA type and the genetic risk for type 1 diabetes mellitus. J Kentucky Med Assoc 1991; 89: 192–193.

Marshall KW, Liu AF, Canales J, *et al.* Role of polymorphic residues in HLA-DR molecules in allele specific binding of peptide antigens. J Immunol 1994; 152: 4946–4957.

Martin J, Trink B, Daneman D, Dosch HM, Robinson B. Milk proteins in the etiology of IDDM. Ann Med 1991; 23: 447–452.

Martino GV, Tappaz ML, Braghi S, *et al.* Autoantibodies to glutamic acid decarboxylase detected by an immuno-trapping enzyme activity assay: relation to insulin dependent diabetes mellitus and islet cell antibodies. J Autoimmun 1991; 4: 915–923.

Muntoni S, Loviselli A, Martino P, *et al.* High prevalence of islet cell antibodies in healthy schoolchildren in Sardinia. Diabetologia 1992; 35(Suppl. 1): A32.

Nayak RC, Omar MA, Rabizadeh A, Srikanta S, Eisenbarth GS. Cytoplasmic islet cell antibodies: evidence that the target antigen is a sialoglycoconjugate. Diabetes 1985; 34: 617–619.

Nepom GT. A unified hypothesis for the complex genetics of HLA association with IDDM. Diabetes 1990; 35: 1153–1157.

Notsu K, Oka N, Note S, Sakurami T. Islet cell antibodies in the Japanese population and subjects with type 1 diabetes. Diabetologia 1985; 28: 660–662.

Nyström L, Dahlquist G, Rewers M, Wall S. The Swedish childhood diabetes study: an analysis of the temporal trend in diabetes incidence 1978–87. Int J Epidemiol 1990; 19: 141–146.

Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers in 2-hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantations. Tissue Antigens 1992; 39: 225–235.

Olerup O, Aldener A, Fogdell A. HLA-DQB1 and HLA-DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. Tissue Antigens 1993; 41: 119–134.

Ongagna JC, Levy-Marchal C. Anti-37kDa antibodies are associated with the development of IDDM in individuals with islet cell antibodies. Diabetologia 1995; 38: 370–375.

Ortego J, Guerrero F, Rodriguez C, *et al.* Glutamic acid decarboxylase and islet cell antibodies in IDDM and first-degree relatives in Andalusian. Diabetologia 1997; 40(Suppl. 1): A74.

Overbach D, Lernmark Å, Platz P, *et al.* HLA-D region beta-chain DNA endonuclease fragments differ between HLA-DR identical healthy and insulin dependent diabetic individuals. Nature 1983; 303: 815–817.

Passini N, Larigan DJ, Genovese S, *et al.* The 37/40 kilodalton autoantigen in insulin-dependent diabetes mellitus is the putative tyrosine phosphatase IA-2. J Immunol 1995; 153: 238–242.

Payton MA, Hawkes CJ, Christie MR. Relationship of the 37,000 and 40,000-Mr tryptic fragments of islet antigens in insulin dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). J Clin Invest 1995; 96: 1506–1511.

Petersen JS, Kim RH, Moody A, Karlsen AE, Dyrberg T. Detection of GAD 65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes 1994; 43: 459–467.

Pugliese A, Gianani R, Moromisato R, *et al.* HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM. Diabetes 1995; 44: 608–613.

Rabin DU, Plaesic SM, Shapiro JA, *et al.* Islet cell antigen 512 is a diabetes specific islet autoantigen related to protein tyrosine phosphatases. J Immunol 1994; 152: 3183–3187.

Rabinovitch A. Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Diabetes 1994; 43: 613–621.

Raum D, Alper CA, Stein R, Gabbay KH. Genetic marker for insulin dependent diabetes mellitus. Lancet 1979; I: 1208–1210.

Reijonen H, Ilonen J, Knip M, Åkerblom HK. HLA-DQB1 alleles and absence of Asp 57 as susceptibility factors of IDDM in Finland. Diabetes 1991; 40: 1640–1644.

Reijonen H, Vähäsalo P, Karjalainen J, Ilonen J, Åkerblom HK, Knip M. HLA-DQB1 genotypes and islet cell antibodies in the identification of siblings at risk for insulin-dependent diabetes (IDDM) in Finland. J Autoimmun 1994; 7: 675–686.

Riley WJ, Maclaren NK, Krischer J, *et al.* A prospective study of development of diabetes in relatives of patients with insulin dependent diabetes. N Engl J Med 1990; 323: 1167–1172.

Robinson BH, Dosch HM, Martin JM, Ilonen J. A model for the involvement of MHC class II proteins in the development of Type 1 diabetes mellitus in response to bovine serum albumin peptides. Diabetologia 1993; 36: 364–368.

Roll U, Christie MR, Standl E, Ziegler AG. Associations of anti-GAD antibodies with islet cell antibodies and insulin autoantibodies in first-degree relatives of type 1 diabetic patients. Diabetes 1994; 43: 154–160.

Rønningen KS, Spurkland A, Tait BD, *et al.* HLA class II associations in insulin-dependent diabetes mellitus among Blacks, Caucasoids and Japanese. In: HLA 1991. Oxford University Press, Oxford, p. 12–15.

Routsias J, Papadopuolus GK. Polymorphic structural features of modelled HLA-DQ molecules segregate according to susceptibility or resistance to IDDM. Diabetologia 1995; 38: 1251–1261.

Rowley MJ, MacKay IR, Chen QY, Knowles WJ, Zimmet PZ. Antibodies to glutamic acid decarboxylase discriminate major types of diabetes mellitus. Diabetes 1992; 41: 548–551.

Rubinstein P, Walker ME, Fedun B, Witt ME, Cooper LZ, Ginsberg-Felner F. The HLA system in congenital rubella patients with and without diabetes. Diabetes 1982; 31: 1088–1090.

Sanjeevi CB, Höök P, Landin-Olsson M, Kockum I, Dahlquist G, Lybrand TP, Lernmark Å. DR4 subtypes and their molecular properties in a population-based study of Swedish childhood diabetes. Tissue Antigens 1996; 47: 275–283.

Sanjeevi CB, Lybrand TP, Deweese C, *et al.* Polymorphic amino acid variations in HLA-DQ are associated with systematic physical property changes and occurrence of IDDM. Diabetes 1995; 44: 125–131.

Sanjeevi CB, Lybrand TP, Landin-Olsson M, *et al.* Analysis of antibody markers DRB1, DRB5, DQA1 and DQB1 genes and modelling of DR2 molecules in DR2-positive patients with insulin-dependent diabetes mellitus. Tissue Antigens 1994; 44: 110–119.

Saukkonen T, Virtanen SM, Karppinen M, *et al.* Significance of cow milk protein antibodies as risk factor for childhood IDDM: interactions with dietary cows milk intake and HLA-DQB1 genotype. Diabetologia 1998; 41: 72–78.

Savola K, Bonifacio E, Sabbah E, *et al.* IA2A antibodies and genetic risk markers in children with recent-onset of IDDM. Diabetologia 1997; 40: A70.

Schatz D, Krischer J, Horne G, Maclaren NK, *et al.* Islet cell antibodies predict IDDM in United States school age children as powerfully as in unaffected relatives. J Clin Invest 1994; 93: 2403–2407.

Schmidli RS, Colman PG, Bonifacio E. Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies. Diabetes 1995; 44: 636–640.

Schmidli RS, Colman PG, Bonifacio E, Bottazzo GF, Harrison LC. High level of concordance between assays for glutamic acid decarboxylase antibodies. Diabetes 1994; 43: 1005–1009.

Schmidli RS, DeAizpurua HJ, Harrison LC, Colman PG. Antibodies to glutamic acid decarboxylase in at-risk and clinical insulin-dependent diabetic subjects: relationship to age, sex and islet cell antibody status, and temporal profile. J Autoimmun 1994; 7: 55–66.

Seissler J, Morgenthaler NG, Achenbach P, *et al.* Combined screening for autoantibodies to IA-2 and GAD65 in first degree relatives of patients with IDDM. Diabetologia 1996; 39: 1351–1356.

Serrano-Rios M, Gutierrez-Lopez MD, Perez-Bravo F, *et al.* HLA-DR, DQ and anti-GAD antibodies in first degree relatives of type I diabetes mellitus. Diabetes Res Clin Pract 1996; 34: 133–139.

She JX. Susceptibility to type 1 diabetes: HLA-DQ and DR revisited. Immunol Today 1996; 17: 323–329.

Shtauvere A, Rumba I, Dzivite I, Farloni A, Sanjeevi CB. Antibodies to ICA512 and GAD65 in IDDM patients from Latvia. Diabetologia 1997; 40(Suppl. 1): A71.

Singal DP, Blajchman MA. Histocompatibility (HLA) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes 1973; 22: 429–432.

Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T. Triple-label hybridization assay for type-1 diabetes related HLA alleles. Bio Techniques 1995; 18: 870–877.

Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T. HLA-DQA1 typing using time-resolved fluorometry. Scand J Immunol 1996; 43: 722.

Solimena M, Dirkx RJ, Hermel JM, *et al.* ICA512, an autoantigen of type 1 diabetes, is an intrinsic membrane protein of neurosecretory granules. Baillieres Eur J 1996; 15: 2102–2114.

Svejgaard A, Platz P, Ryder LP. Insulin dependent diabetes mellitus. In: Histocompatibility testing. Ed. Terasaki PI, 1980, p. 638–656.

Tait BD, Drummond BP, Varney MD, Harrison LC. HLA-DRB1*0401 is associated with susceptibility to insulin-dependent diabetes mellitus independently of the DQB1 locus. Eur J Immunogenet 1995; 22: 289–297.

Tanaka M, Abe J, Kohsaka T, Tanae A. Analysis of HLA-DQA1 in Japanese patients with type 1 diabetes mellitus, using DNA-PCR-RFLP typing. Acta Pediatr Japonica 1992; 34: 46–51.

Tautz C, Zwollo P, Marsh DG, Baur X. Sequence of a novel HLA-DQB1 allele. Immunogenetics 1992; 35: 421–424.

Thivolet C, Beaufrere B, Betuel H, Francois R. Islet cell and insulin autoantibodies in subjects at high risk for development of Type 1 diabetes mellitus: the Lyon family study. Diabetologia 1988; 31: 741–746.

Thivolet C, Tappaz M, Durand A, *et al.* Glutamic acid decarboxylase autoantibodies are additional predictive markers of type 1 diabetes mellitus in high risk individuals. Diabetologia 1992; 35: 570–576.

Thomsen M, Platz P, Andersen OO, *et al.* MCL typing in juvenile diabetes mellitus and idiopathic Addison's disease. Transplantation Rev 1975; 22: 125–147.

Thomson G, Robinson W, Kuhner M, *et al.* Genetic heterogenity, modes of inheritance and risk estimates for a joint study of Caucasians with IDDM. Am J Hum Genet 1988; 13: 799–805.

Thorsby E, Rønningen KS. Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type 1 diabetes mellitus. Diabetologia 1993; 36: 371–377.

Tisch R, Yang XD, Singer SM, Liblau RS, Fugger L, McDevitt HO. Immune response to glutamic acid decarboxylase correlates with insulitis in nonobese diabetic mice. Nature 1993; 72: 366–370.

Tiwari JL, Terasaki PI. Juvenile diabetes mellitus HLA and disease associations. Springer Verlag 1985; 185–210.

Todd JA. Genetic analysis of type 1 diabetes using hole genome approaches. Proc Nat Acad Sci USA 1995; 92: 8560–8565.

Todd JA, Bell JI, McDevitt HO. HLA-DQB gene contributes to susceptibility and resistance to insulin dependent diabetes mellitus. Nature 1987; 329: 559–604.

Todd JA, Mijovic C, Fletcher J, Jenkins D, Barnett AH. Identification of susceptibility loci for IDDM by trans racial gene mapping. Nature 1989; 338: 587–589.

Tosi G, Facchin A, Pinelli L, Accolla R. Assessment of the DQB1-DQA1 complete genotype allows best prediction for IDDM. Diabetes Care 1994; 17: 1045–1049.

Tuomi T, Groop L, Zimmet P, Rowley M, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 1993; 42: 359–362.

Tuomilehto J, Zimmet P, Mackay IR, *et al.* Antibodies to glutamic acid decarboxylase as predictors of IDDM before clinical onset of disease. Lancet 1994; 343: 1383–1385.

Tuomilehto-Wolf E, Podar T, Adojaan B, Kalits I, Tuomilehto J. Can the difference in the incidence of type 1 diabetes between Estonia and Finland be partly explained by genetic reasons? Diabetologia 1991; 24(Suppl. 2): A65.

Tuomilehto-Wolf E, Tuomilehto J. HLA antigens in insulin-dependent diabetes mellitus. Ann Med 1991; 23: 481–488.

Undlien DE, Akselsen HE, Knutsen I, *et al.* DRB1*04 subtypes in IDDM. Implications for genetic risk assessment. Autoimmunity 1995; 21: 17–18.

Van der Auwera B, Van Waeyenberge C, Schuit F, *et al.* DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous HLA-DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. Diabetes 1995; 44: 527–530.

Vardi P, Dibella E, Timothy J. Islet cell autoantibodies: pathobiology and clinical applications. Diabetes Care 1987; 10: 645–652.

Veijola R, Vähäsalo P, Tuomilehto-Wolf E, *et al.* Human leukocyte antigen identity and DQ risk alleles in autoantibody-positive siblings of children with IDDM are associated with reduced early insulin response. Childhood Diabetes in Finland (DiMe) Study Group. Diabetes 1995; 44: 1021–1028.

Velluzzi F, Loviselli A, Mele P, *et al.* The Sardinian school children IDDM study. Predictive value of IDDM-related autoantibodies in a cohort of healthy Sardinian school children. Diabetologia 1997; 40(Suppl. 1): A80.

Vreugdenhil GR, Geluk A, Ottenhoff THM, *et al.* Molecular mimicry in diabetes mellitus: the homologous domain in Coxsackie B virus protein 2C and islet autoantigens GAD65 is highly conserved in the Coxsackie B-like enteroviruses and binds to the diabetes associated HLA-DR3 molecule. Diabetologia 1998; 41: 40–46.

Vähäsalo P, Miettinen A, Kulmala P, *et al.* GAD antibodies are associated with thyroid antibodies in children with IDDM and in their siblings. Diabetologia 1997; 40(Suppl. 1): A77.

Wagner R, Genovese S, Bosi E, *et al.* Slow metabolic deterioration towards diabetes islets cell antibody positive patients with autoimmune polyendocrine disease. Diabetologia 1994; 37: 365–371.

Wiest-Ladenburger U, Hartmann R, Hartmann U, Berling K, Boehm B, Richter W. Combined analysis and single step detection of GAD65 and IA-2A autoantibodies in IDDM can replace the histochemical ICA test. Diabetes 1997; 46: 565–571.

Wolf E, Spencer KM, Cudworth AG. The genetic susceptibility to type 1 diabetes: analysis of the HLA-DR associations. Diabetologia 1983; 24: 224–228.

Yasunaga S, Kimura A, Hamaguchi K, Rønningen KS, Sasazuki T. Different contribution of HLA-DR and -DQ genes in susceptibility and resistance to insulin-dependent diabetes mellitus (IDDM). Tissue Antigens 1996; 47: 37–48.

Yoon JW. The role of viruses and environmental factors in the introduction of diabetes. Curr Top Microbiol Immunol 1990; 164: 95–124.

Yu L, Rewers M, Gianani R, Kawasaki E, *et al.* Antiislet autoantibodies usually develop sequentially rather than simultaneously. J Clin Endocrinol Metab 1996; 81: 4264–4267.

Zanone M, Pietropaolo M, Catalfamo E, *et al.* GAD-65 and ICA-512 autoantibodies as disease marker and their natural history in a population of young insulin-dependent diabetic patients. Diabetologia 1997; 40(Suppl. 1): A71.

Zimmet P. ICA512: another beta-cell antigen. Intern Diabetes Monit 1996; 8: 2–3.

LAPSEEAS ALANUD INSULIINISÕLTUVA SUHKURTÕVE IMMUNOLOOGILISED JA GENEETILISED TEGURID EESTIS

Kokkuvõte

Insuliinisõltuv suhkurtõbi (ISST) on sageli lapseeas algav raske krooniline haigus. Haiguse etioloogia ei ole senini teada. Seetõttu puudub ISST profülaktika võimalus. Viimase kahekümne aasta jooksul kogunenud andmed lubavad järeldada, et ISST tekib haigusele geneetiliselt vastuvõtlikel indiviididel autoimmuunse mehhanismiga. Paraku on immunoloogiliste ja geneetiliste tegurite seos ISST-ga komplitseeritud. Seetõttu on ISST etioloogia ja patogeneesi paremaks mõistmiseks vaja uurida erisuguse geneetilise ja keskkondliku taustaga populatsioone. Praeguseni puudub Eestis informatsioon ISST-ga seotud immunoloogiliste ja geneetiliste tegurite levimuse kohta.

Uurimuse eesmärgid

Ülaltoodust lähtudes oli käesoleva uuringu peaeesmärgiks kindlaks teha mitmete teadaolevate ISST-ga seotud immunoloogiliste ja geneetiliste tegurite levimus järgmistes rühmades:

1) lapseeas alanud ISST-ga patsiendid,
2) lapseeas alanud ISST-ga patsientide terved õed-vennad,
3) kontrollgrupp.

Töö konkreetsed eesmärgid

1. Leida uuringurühmades Langerhansi saarekeste vastaste (ICA), glutamaadi dekarboksülaasi vastaste (GADA) ja türosiini fosfataasi vastaste antikehade (IA-2A) levimus.
2. Teha kindlaks HLA-DQB1 lookuse ISST riskiga seotud alleelide ning mõnede teiste HLA-alleelide kombinatsioonide sagedus ISST-ga patsientidel ja kontrollgrupis, samuti nendega kaasnevad haiguse riskimäärad.
3. Jälgida ISST-ga patsientide terveid ja immunoloogiliste ning geneetiliste tegurite suhtes uuritud õdesid-vendi haiguse tekke suhtes kolm aastat.
4. Kõrvutada Eesti andmeid teistes riikides saadutega.

Materjal ja meetodid

1. Uuritavad

Lapseeas alanud ISST-ga patsiendid

1. rühm — 29 Eestis 1993.–1994. aastal järjestikuliselt esmasavastatud patsienti vanuses kuni 15 aastat, 18 poeglast ja 11 tütarlast. Sellelt rühmalt võeti veri antikehade määramiseks esimese nädala jooksul pärast diagnoosimist.

2. rühm — 97 lapseeas alanud ISST-ga patsienti, kes võeti Eesti laste ISST suhkurtõve registrist. Kahesajale registris olevale lapsele saadeti 1990. aastal kutse uuringus osalemiseks, millega 97 soostus. Nendelt võeti veri HLA-DR- ja HLA-DQ-alleelide levimuse määramiseks.

Lapseeas alanud ISST-ga patsientide terved õed-vennad

Lapseeas alanud ISST-ga patsientide 71 tervet õde-venda, kellel uuriti nii antikehade kui ka HLA-DR- ja HLA-DQ-alleelide levimust. Eesti ISST-ga laste registrist võeti saja lapse aadressid ja perekonnale saadeti 1994. aastal kutse, kus paluti terveid õdesid ja vendi osaleda uuringus. Neljakümne neljalt perekonnalt saadi nõusolek.

Kontrollgrupp

1. rühm

614 lasteaia- ja koolilast vanuses 3–18 aastat kuuest Eesti piirkonnast (Tallinn, Tartu, Paide, Põlva, Nõo ja Jõgeva), keda uuriti Langerhansi saarekeste ja glutamaadi dekarboksülaasi vastaste antikehade suhtes. 1991. aastal kogutud kontrollgrupp jaotus viide vanuserühma: 3–5 aastat (118), 6–8 aastat (131), 9–11 aastat (119), 12–14 (124), 15–18 aastat (122).

2. rühm

103 Tartu Ülikooli arstiteaduskonna 1993. aasta esimese kursuse üliõpilast ja 166 järjestikust doonorit Maarjamõisa Polikliiniku verekeskusest, kellel uuriti HLA-DQ- ja HLA-DR-alleelide levimust.

2. Meetodid

ICA määrati immunofluorestsentsmeetodil, GADA kvantitatiivse immunopretsipitatsiooni radioligandsel meetodil. IA-2A leiti radioimmunoloogilisel meetodil. HLA-DR- ja HLA-DQ-alleele määrati originaalmeetodil M. Sjöroosi järgi (*simultaneus triple-label hybridization assay for HLA alleles*). HLA-alleele määrati kahes etapis. Esmalt uuriti DQB1*0201, *0301, *0302, *0602/0603

suhtes. DQB1*0201-positiivsetel määrati ka DQA1*0201 ja *0501. Neid, kel esines DQB1*0302, uuriti DRB1*0401, *0402, *0403, *0404, *0405, *0408. DQB1*0301- ja *0302-positiivseid indiviide testiti DQB1*0304 suhtes.

Alleelide ja antikehade levimuse erinevust hinnati χ^2- ja Fisheri testiga. Normaalse jaotumusega muutujate kirjeldamisel kasutati keskmist ja standardhälvet. Korrelatsioonanalüüs teostati Spearmani meetodil. Statistiliselt oluliseks peeti $p<0,05$. Iga uuritud alleeli jaoks leiti šansside suhe (SS). Vajadusel kasutati Haldane'i korrektsiooni. Multiiplite võrdluste korral kasutati p väärtuse korrektsiooni Bonferoni järgi.

Tulemused ja arutelu

Lapseeas alanud vastavastatud ISST haigetel oli kõige sagedasem antikeha ICA (79%). Järgnesid GADA 38%-l ja IA-2A 35%-l juhtudest. Vähemalt üks uuritud antikehadest leiti 86%-l vastavastatud ISST haigetest, kõik kolm uuritud antikeha ainult 17%-l.

ISST patsientide tervetel õdedel-vendadel leiti üks või rohkem uuritud antikeha 13-l (18,4%). Kõige sagedamini leitud antikeha oli GADA — 17%-l. Järgnesid ICA 7%-ga ja IA-2A 5,6%-ga. Enamusel sellest rühmast — 81,6%-l — ei leitud ühtegi antikeha.

Tervete lasteaia- ja koolilaste seas leiti ICA 10-l (1,6%) ja GADA 14-l (2,3%). Kaks ICA- ja GADA-positiivset indiviidi haigestus edaspidi ISST-sse. Samu antikehi uuriti 1994. aastal korduvalt nendel 8-l, kel 1991. aastal leiti seerumis ICA ning kes polnud haigestunud ISST-sse. Selgus, et pooltel neist oli ICA kadunud, GADA tiiter aga vähenenud.

ISST haigetel leidus meie uuritud alleelidest kõige sagedamini HLA-DQB1*0302 — 66%-l. Järgnes HLA-DQB1*0201 (55%). Mõlemad alleelid esinesid ISST haigetel statistiliselt oluliselt sagedamini kui kontrollrühmas. Meie poolt uuritud HLA-DQB1 alleelidest kandis kõrgeimat ISST riski *0302 (SS 8,9), mis ületas HLA-DQB1*0304 poolt edastatava haigestumisriski (SS 7,3). Viimatinimetatud alleeli oli ISST haigetel harva — 5%-l juhtudest. Statistiliselt oluliselt kõrgema ISST riskiga oli seotud ka HLA-DQB1*0201, SS 2,7 ($p<0,05$).

Madalaima ISST riskiga alleel Eestis oli HLA-DQB1*0602/0603. See alleel oli 44%-l kontrollrühmast ja ainult 2%-l ISST haigetest. DQB1*0602/0603 vähendas ISST riski 38 korda, võrreldes nendega, kellel seda alleeli polnud. Samuti oli madala ISST riskiga DQB1*0301, SS 0,2 ($p<0,05$).

Kõige sagedasem ISST haigetel esinev meie uuritud kahe alleeli kombinatsioon oli HLA-DQB1*0201-DQA1*0501, mis leiti pooltel. Tervete kontrollrühmas esines seda kombinatsiooni ka märkimisväärselt — 22%-l. ISST haigete hulgas oli sageduselt järgmine HLA-DQB1*0302-DRB1*0401, mida leiti

43%-l uuritutest, kontrollrühmas aga oluliselt harvemini — 5,2%-l. Nende kahe alleelikombinatsiooniga kaasnenud ISST risk oli vastavalt 3,6 ja 13,9. DQB1*0304-DRB1*0408 oli ISST rühmas 5%-l ja üldpopulatsioonis ainult 0,7%-l, SS 7,3 ($p<0,05$).

71-st õdede-vendade rühma liikmest haigestus kolmeaastase jälgimise käigus ISST-sse kolm (4%). Neist kahel oli HLA-DQB1*0201-DQA1*0501 alleelide kombinatsioon, Eesti ISST haigete sagedasim, ja ühel HLA-DQB1*0302-DRB1*0401. Kõik kolm haigestunud lähisugulast kuulusid nende 13 hulka, kellel leiti antikehad. Kõigil kolmel oli leitud vähemalt kahte liiki antikehi, ühel isegi kõiki uuritud kolme. Neil kõigil kolmel olid antikehad GADA vastu.

Käesolev uurimus kirjeldab ISST-ga seotud antikehade ICA, GADA ja IA-2A levimust Eestis vastavastatud ISST-ga laste, ISST-ga laste tervete õdede-vendade hulgas ja noorte kontrollrühmas. Kõige kõrgem kolme antikeha levimus oli ootuspäraselt vastavastatud ISST-ga laste seas, mis kinnitab aktiivselt käigus olevat autoimmuunset protsessi. Antikehade levimusmääralt järgnes õdede-vendade grupp, kelle seas omakorda oli antikehade sagedus kõrgem kui kontrollrühmas. ISST haigete esimese astme sugulaste suurem risk haigestuda ISST-sse võrreldes üldpopulatsiooniga leidis kinnitust: 4% nendest haigestus kolme jälgitud aasta jooksul.

On leitud, et ICA levimus populatsioonides on umbes kümme korda kõrgem ISST levimusest, s.t. enamusel ICA-positiivsetest isikutest ei arene kunagi haigust. Üldpopulatsiooni ICA levimuse kõrvutamisel meie omaga sarnaselt tehtud uurimustega Soomes, Prantsusmaal, Rootsis, Sardiinias ja Rootsis selgus seos ISST haigestumuse ja sama üldpopulatsiooni ICA levimuse vahel. Nendes populatsioonides, kus haigestumus ISST-sse oli kõrgem, oli suurem ka ICA levimusmäär ($r=0,97$, $p<0,05$), mis viitab sellele, et ICA-positiivsetest lastest haigestub erinevates populatsioonides umbes sama osa.

Käesoleva uuringuga täienes teave mitmete HLA-regiooni alleelide levimuse kohta Eestis, nii kontrollrühmas kui ka ISST haigete seas. Eesti ISST haigete kõrge ja madala riski alleelid ei erinenud teiste Euroopa populatsioonide. ISST suhtes madala riskiga alleelideks on Eestis DQB1*0301 ja DQB1*0602/0603, sarnaselt mitmetele teistele populatsioonidele. Märkimisväärne on madala riski alleeli DQB1*0602/0603 suur levimus Eestis, eriti aga temaga kaasnev väga madal riskimäär (neli korda madalam kui Soomes). See võib olla üheks põhjuseks, miks Eestis on haigestumus ISST-sse üle kolme korra väiksem kui Soomes.

Järeldused

1. Kõige sagedasem ISST-ga seotud antikeha vastavastatud patsientidel oli ICA (79%), sellele järgnes GADA (38%) ja IA-2A (35%). ISST patsientide tervetel õdedel-vendadel leiti samu antikehi oluliselt harvemini, ent sagedamini kui kontrollrühmas.

2. ICA esinemissagedus üldpopulatsioonis on lähedases seoses ISST haigestumusega ($r=0,97$; $p<0,05$). Tervete ICA-positiivsete laste osa, kel tekib ISST, on eri maades sarnane.

3. Kõige sagedasem esinev HLA-DQB1 alleel ISST patsientide seas oli HLA-DQB1*0302 (66%), sellele järgnes HLA-DQB1*0201 (55%). Mõlemad alleelid esinesid statistiliselt oluliselt sagedamini ISST patsientide seas, võrreldes kontrollgrupiga.

4. Kõige kõrgema ISST riskiga alleel oli HLA-DQB1*0302, mille SS oli 8,9, järgnes HLA-DQB1*0304 (SS 7,3).

5. Kõige madalama ISST riskiga alleel Eestis oli HLA-DQB1*0602/0603. See alleel esines kontrollgrupis 44%-l. Selle alleeliga kaasnev ISST risk on Eestis ligikaudu neli korda väiksem kui Soomes. Antud tulemus võib osaliselt seletada üle kolmekordset ISST haigestumuse erinevust Eestis ja Soomes.

6. Kõige sagedasem alleelide kombinatsioon ISST patsientide seas oli HLA-DQB1*0201-DQA1*0501, mis esines pooltel, järgnes HLA-DQB1*0302-DRB1*0401 (43%). See tulemus on kooskõlas teiste valge rassi seas läbi viidud uuringutega.

7. ISST patsientide tervetel õdedel-vendadel oli suurem risk haigestuda ISST-sse võrreldes üldpopulatsiooniga. 4% ISST haigete õdedest-vendadest haigestus kolmeaastase jälgimise vältel. Jälgimise algul leiti neil vähemalt kahte liiki antikehad ja neil olid Eestis kõige sagedamini ISST põdejatel esinevad HLA-alleelide kombinatsioonid.

ACKNOWLEDGEMENTS

I thank professor Toomas Podar for supervision, support and encouragement during the years.

I thank professor Vello Salupere, Head of the Department of Internal Medicine, University of Tartu, for important support and useful criticism.

I am indebted to my collaborators from the Hospital of Endocrinology, Clinicum of the University of Tartu, especially to Andrei Solntsev.

My special thanks go to B. Boehm, W. Richter, M. Knip, H.K. Åkerblom, J. Ilonen, E. Sabbah, P. Kulmala. Parts of the research were performed at the University of Ulm, University of Oulu and University of Turku.

Constructive comments were given by professors Raivo Uibo and Andres Metspalu.

Large help was provided by dr. Riina Shor from the Tallinn Central Children's Policlinic in collecting blood samples.

I acknowledge the financial support from the Estonian Science Foundation, grant 1836. This research was also supported by Deutsche Forschungsgemeinschaft, Finnish Academy and Foundation for Pediatric Research in Finland.

My gratitude also goes to my family for their ever-present support.

PUBLICATIONS

I

Adojaan B, Knip M, Vähäsalo P, Karjalainen J, Kalits I, Åkerblom HK.
Relationship between the incidence of childhood IDDM and
the frequency of ICA positivity in nondiabetic children in the general population.
Diabetes Care 1996; 19: 1452–1454.

Relationship Between the Incidence of Childhood IDDM and the Frequency of ICA Positivity in Nondiabetic Children in the General Population

There is a considerable difference in the incidence of IDDM among children in the two neighboring countries Finland and Estonia with an approximately three times lower rate in Estonia (1). On the other hand, there are marked ethnic similarities between Estonians and Finns, suggesting that environmental factors may make a crucial contribution to the observed difference in incidence. Cytoplasmic islet cell antibodies (ICAs) are considered to be secondary markers of ongoing β-cell damage, which eventually leads to the clinical manifestation of IDDM in some individuals once a majority of the β-cells have been destroyed (2). Assessments of the frequency of ICA positivity among nondiabetic children have shown clear geographic variation (3–5) and have resulted in speculations regarding a possible relationship between the prevalence of ICAs in the background population and the incidence of the disease. The highest ICA frequency, 4.1%, has been observed in nondiabetic Finnish children (3). To test the hypothesis that the incidence of childhood IDDM is related to the frequency of ICA positivity in nondiabetic children, we decided to study the latter among nondiabetic Estonian children and compare it with that found in Finland.

The study populations included 614 healthy Estonian children and adolescents with a mean age of 10.6 ± 4.3 years (range, 2.6–17.9 years) and 1,212 Finnish subjects with a mean age of 10.7 ± 5.1 years (range, 3–18 years). The Finnish population has been described in detail previously (3). The Estonian population included 314 boys (50.9%). The subjects represented five age-groups (3–5, 6–8, 9–11, 12–14, and 15–17 years) and came from six regions of Estonia. Blood samples were obtained by venipuncture, and the serum samples were stored at −20°C until analyzed. ICAs were determined in the Research Laboratory of the Department of Pediatrics at the University of Oulu, Oulu, Finland, by a standard immunofluorescence method from sections of a frozen human group-O pancreas (6). Fluorescein-conjugated rabbit anti-human IgG (Behringwerke, Marburg, Germany) was used to detect ICAs. All the Finnish samples were screened with the same substrate in 1987–1988, and the Estonian samples were analyzed in 1991 using another substrate that gave a standard curve similar to that obtained with the first one using an international reference standard that was derived from the Stage IV ICA Standardization Workshop (7). Endpoint dilution titers for all positive Estonian and Finnish samples were examined using the same substrate and the results expressed in Juvenile Diabetes Foundation units (JDF U), relative to the above-mentioned international reference standard. The detection limit was 2.5 JDF U. Our laboratory has participated in the International Workshops on the Standardization of Cytoplasmic ICAs, in which its sensitivity was 100%, specificity 98%, validity 98%, and consistency 98% in the fourth round.

Other countries were selected for the comparison between the frequency of ICA positivity in the nondiabetic child population and the incidence of childhood IDDM on the following criteria: 1) reliable incidence data available from a time that was close to or the same as the period during which the serum samples for the ICA assay were collected and 2) ICA assays that

were carried out in a laboratory participating in the International Workshops on the Standardization of Cytoplasmic ICAs and having reliable performance characteristics. The results were evaluated statistically using cross-tabulation and χ^2 statistics or the Fisher's exact probability test, the Mann-Whitney *U* test for the comparison of the ICA levels between two groups, and linear regression analysis.

Ten Estonian children (1.63%; 95% CI 0.63–2.63) tested positive for ICAs, compared with 50 Finnish children (4.13%, 95% CI 3.01–5.25; $P < 0.01$), and 6 children from Estonia (0.98%, 95% CI 0.20–1.76) and 23 from Finland (1.90%, 95% CI 1.13–2.67; $0.05 < P < 0.1$) had an ICA level of ≥20 JDF U. Four Estonian boys (1.27%, 95% CI 0.03–2.51) and 27 Finnish boys (4.80%, 95% CI 3.04–6.57; $P < 0.05$) were observed to be ICA$^+$, whereas there was no significant difference in the frequency of ICAs between Estonian (6 of 300; 2.00%, 95% CI 0.42–3.58) and Finnish girls (23 of 649; 3.54%, 95% CI 2.12–4.96). The levels of ICAs in the positive Estonian subjects (median, 34 JDF U; range, 3–97 JDF U) were similar to those in their Finnish counterparts (median, 16 JDF U; range, 3–128 JDF U; $P = 0.28$). Comparison of the frequency of ICA positivity in nondiabetic children with the incidence of childhood IDDM in six countries showed a highly significant correlation (Fig. 1), indicating that 94% of the variation in disease incidence between countries could be explained by the frequency of ICA positivity in the nondiabetic child population.

The present study shows that the frequency of ICA positivity in nondiabetic Estonian children is about one third of that in nondiabetic Finnish children, a ratio which is of the same magnitude as that observed in the incidence of IDDM. There seems to be a close correlation between the incidence of the disease and the prevalence of ICAs in nondiabetic children in various countries, which suggests that a similar proportion of ICA$^+$ children will eventually present with IDDM in all countries. Since close to 0.6% of Finnish children will contract IDDM before the age of 20 (A. Reunanen, unpublished observations), the frequency of ICA positivity is almost seven times higher than the cumulative incidence of the disease. In addition, 10–20% of subjects with IDDM test negative for ICAs at diagnosis. Taken together, this implies that a maximum one out of eight nondiabetic ICA$^+$ children will eventually present with the disease. What will happen to the remaining seven? One or two of them may present with IDDM later in life, but a majority of them will remain unaffected. In the previous study of the Finnish population, four out of 39 initially ICA$^+$ children (10.3%) became negative over an observation period of 6 years (3), suggesting that the immune attack against the β-cells may be turned off in a portion of the subjects.

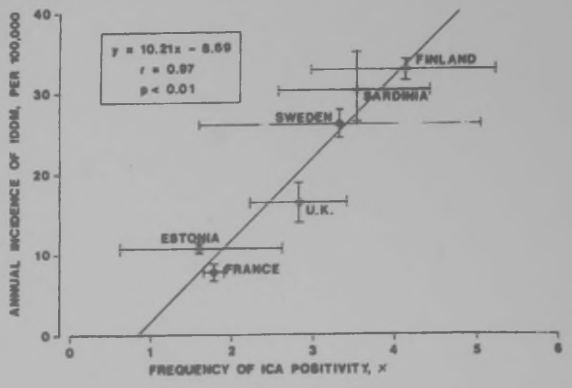


Figure 1—*Correlation between the annual incidence of childhood IDDM and the prevalence of ICAs in nondiabetic children in six countries. The error bars represent 95% CIs. The ICA frequency data are based on the present study and those of Levy-Marchal et al. (4), Landin-Olsson et al. (5), Bingley et al. (8), and Muntoni et al. (9), while the incidence data were derived from Tuomilehto et al. (1), Green et al. (10), and Nyström et al. (11).*

BELA ADOJAAN, MD
MIKAEL KNIP, MD
PAULA VÄHÄSALO, MD
JUKKA KARJALAINEN, MD
IRINA KALITS, MD
HANS K. ÅKERBLOM, MD

From the Hospital of Endocrinology, Department of Internal Medicine (B.A., I.K.), University of Tartu, Estonia; the Department of Pediatrics (M.K., P.V., J.K.), University of Oulu, Oulu; the School of Medicine (M.K.), University of Tampere, Tampere; the Department of Pediatrics (M.K.), Tampere University Hospital, Tampere; and Children's Hospital, Second Department of Pediatrics (H.K.Å.), University of Helsinki, Helsinki, Finland.

Address correspondence to Mikael Knip, MD, Department of Pediatrics, University of Oulu, SF-90220 Oulu, Finland.

References

1. Tuomilehto J, Podar T, Reunanen A, Kalits I, Lounamaa R, Tuomilehto-Wolf E, Adojaan B, Neff B, LaPorte RE: Comparison of incidence of IDDM in childhood between Estonia and Finland, 1980–1988. *Diabetes Care* 14:982–988, 1991
2. Skyler JS, Rabinovitch A: Etiology and pathogenesis of insulin-dependent diabetes mellitus. *Pediatr Ann* 16:682–692, 1987
3. Karjalainen J: Islet cell antibodies as predictive markers for IDDM in children with high background incidence of disease. *Diabetes* 39:1144–1150, 1990
4. Levy-Marchal C, Tichet J, Fajardy I, Gu XF, Dubois F, Czernichow P: Islet cell antibodies in normal French school children. *Diabetologia* 35:577–582, 1992
5. Landin-Olsson M, Palmer JP, Lernmark Å, Blom L, Sundquist G, Nyström L, Dahlquist G: Predictive value of islet cell and insulin autoantibodies for type 1 (insulin-dependent) diabetes mellitus in a population-based study of newly diagnosed diabetic and matched control children. *Diabetologia* 35:1068–1073, 1992
6. Bottazzo GF, Florin-Christensen A, Doniach D: Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* ii:1279–1282, 1974
7. Lernmark Å, Molenaar JL, van Beers VAM, Yamaguchi Y, Nagataki S, Ludvigsson J, Maclaren NK: The Fourth International Serum Exchange Workshop to Standardize Cytoplasmic Islet Cell Antibodies. *Diabetologia* 34:534–535, 1991
8. Bingley PJ, Bonifazio E, Shattock M, Gillmor HA, Sawtell PA, Dunger DB, Scott RDM, Bottazzo GF, Gale EAM: Can islet

cell antibodies predict IDDM in the general population? *Diabetes Care* 16:45–50, 1993
9. Muntoni SA, Loviselli A, Martino E, Velluzzi F, Shattock M, Balestrieri A, Songini M, Muntoni S, Bottazzo G: High prevalence of islet cell antibodies (ICA) in healthy school children in Sardinia (Abstract). *Diabetologia* 35 (Suppl. 1):A32, 1992
10. Green A, Gale EAM, Patterson CC, the European ACE Study Group: Incidence of childhood-onset insulin-dependent diabetes mellitus: the EURODIAB ACE study. *Lancet* 339:905–909, 1992
11. Nyström L, Dahlquist G, Rewers M, Wall S: The Swedish childhood diabetes study: an analysis of the temporal trend in diabetes incidence 1978–87. *Int J Epidemiol* 19:141–146, 1990

II

Ilonen J, Koskinen S, Nejentsev S, Sjöroos M, Knip M,
Schwartz EI, Adojaan B, Kovalchuk L, Sochnevs A.
HLA-DQB1*0304-DRB1*0408 haplotype associated with insulin-dependent
diabetes mellitus in populations in the Eastern Baltic region.
Tissue Antigens 1997; 49: 532–534.

Tissue Antigens 1997: 49: 532–534
Printed in Denmark · All rights reserved

TISSUE ANTIGENS
ISSN 0001-2815

Brief communication

HLA-DQB1*0304-DRB1*0408 haplotype associated with insulin-dependent diabetes mellitus in populations in the eastern Baltic region

J. Ilonen, S. Koskinen, S. Nejentsev, M. Sjöroos, M. Knip, E.I. Schwartz, B. Adojaan, L. Kovalchuk, A. Sochnevs. HLA-DQB1*0304-DRB1*0408 haplotype associated with insulin-dependent diabetes mellitus in populations in the eastern Baltic region.
Tissue Antigens 1997: 49: 532–534. © Munksgaard, 1997

J. Ilonen[1], S. Koskinen[1], S. Nejentsev[3], M. Sjöroos[2], M. Knip[4], E. I. Schwartz[3], B. Adojaan[5], L. Kovalchuk[6] and A. Sochnevs[6]

[1]Turku Immunology Centre and Departments of Virology and [2]Biotechnology, University of Turku, Finland, [3]Department of Medical Genetics, Paediatric Medical Institute, St. Petersburg, Russian Federation, [4]Department of Paediatrics, University of Oulu, Finland, [5]Department of Endocrinology, Tartu University Hospital, Estonia, [6]Institute of Immunology, Medical Academy of Latvia, Riga, Latvia

The rare HLA-DQB1*0304 allele was found increased among IDDM patients in the populations of the eastern Baltic region. Its frequency among IDDM patients was 4.5% (20/443) compared to 1.1% (9/853) in healthy controls in the combined series of Estonian, Latvian and St. Petersburg Russian populations (P=0.0001). HLA-DQB1*0304 in these populations was associated with DRB1*0408, and the haplotype was further characterized by a B35 allele and a typical combination of microsatellite markers from the TNF gene region. The result is compatible with the significance of the 57th amino acid in the DQ β-chain but also emphasizes the importance of alleles in other HLA loci adjacent to DQ in the determination of IDDM susceptibility.

Key words: insulin-dependent diabetes mellitus – HLA-DR4 haplotype – HLA-DQB1*0304

Received 29 October 1996, revised, accepted for publication 2 January 1997

HLA-DQB1*0304 is a rare DQB1*03 subtype combining features of both DQB1*0301 and DQB1*0302 alleles within the hypervariable region of the second exon of DQB1 gene (1, 2). Similar to DQB1*0302 the amino acid 57 of the β-chain of DQB1*0304 is a non-Asp amino acid (alanine) suggested to be crucial in susceptibility to insulin-dependent diabetes mellitus (IDDM), whereas other polymorphic amino acids are identical with the DQB1*0301 allele associated with protection against IDDM (3).

The occurrence of this allele in the region of the eastern Baltic became evident during the validation of a rapid screening method for genetic IDDM susceptibility. The method is based on detection of DQB1 alleles significantly associated with susceptibility to (*0302 and *02) or protection against IDDM (*0602/*0603 and *0301) (4). The sequence-specific oligonucleotide probes hybridizing with *0301 and *0302 were from regions encoding the amino acids 43–47 and 54–58, respectively, and the presence of *0304 would thus produce a positive reaction with both probes. The samples giving three positive reactions including *0301 and *0302 together with *02 or *0602/*0603 were rare during the validation of the method in Finnish IDDM and control subjects (5) but were more common when we analyzed the frequency of susceptible and protective DQ alleles in neighboring Baltic regions. This encouraged us to conduct a systematic study of the frequency of DQB1*0304 in these populations.

Table 1.
The frequency of DQB1*0304 in studied populations. OR = odds ratio

Population	IDDM patients	Controls	P[a]	OR
Estonians	5/97 (5.1%)	2/269 (0.7%)	0.022	7.3
Latvians	6/136 (4.4%)	2/182 (1.1%)	NS	4.2
Russians	9/210 (4.3%)	5/402 (1.2%)	0.035	3.6
Total	20/443 (4.5%)	9/853 (1.1%)	0.0001	4.4
Finns	0/117 (0%)	1/200 (0.5%)	NS	0.6

[a]P value was calculated using chi-square test with continuity correction.

All samples which were found positive for both *0301 and *0302 in the screening test were reanalyzed for the presence of the DQB1*0304 allele using sequence specific primers described by Olerup et al. (6). The results of DQB1*0304 typing in various population samples are shown in Table 1. Although being a rare allele, DQB1*0304 was actually increased among IDDM patients from St. Petersburg, Estonia and Latvia, and the difference between diabetic patients and controls was highly significant in the combined series (Table 1). The patients were all diagnosed to have IDDM before the age of 15 years and control subjects were healthy blood donors. Ethnic Estonians and Latvians were selected for the study from these two countries with sizeable Russian minorities. In a further series of 117 IDDM patients and 200 healthy controls from northern Finland only one DQB1*0304 positive sample was found in the control group. In eight positive samples the polymorphic segment of the HLA-DQB1 second exon was specifically amplified using *0301/*0304 specific primers and sequenced by an automated sequencer (PE Applied Biosystems, Norwalk, CT, USA). In all cases the *0304 specific sequence was confirmed.

The haplotypic combinations of DQB1*0304 were further studied by performing DR4 subtyping using a combination of subgroup-specific amplification (7) and subsequent hybridization with digoxigenin-labeled sequence-specific oligonucleotide probes (8). This demonstrated the presence of the DRB1*0408 allele in all 20 DQB1*0304-positive patients with IDDM and in all but one of the healthy controls (8/9). Sequencing of the amplified DRB1 gene segment in two cases confirmed the presence of the DRB1*0408 specific nucleotide sequence. When HLA-B alleles known to be in linkage with DR4 were studied using sequence-specific amplification (9) in Estonian and Russian samples, HLA-B35 (B3501–3508, 5301) was found in 10 of 14 patients with IDDM (71%) and in all six control samples (100%) positive for DQB1*0304-DRB1*0408, whereas it was present in only 49 of 325 (15%) of DQB1*0302-DRB1*04–positive samples ($P < 0.0001$, $P_c < 0.0001$). Further analysis of TNF gene region microsatellite markers (10) using fluorescence labelled primers to amplify and automated sequencer to measure the size of amplified microsatellites revealed the 10–4–1 allele combination of TNF a-b-c markers in all but one of the 15 studied samples with DQB1*0304-DRB1*0408-B35 compared to a frequency of five in another 49 (10%) analyzed samples with various DQB1*0302-DRB1*04 combinations ($P<0.0001$, $P_c<0.0001$). The TNF 10-4-1 combination was also present in one IDDM patient positive for DQB1*0304-DRB1*0408 but negative for B35 allele.

We have accordingly demonstrated the occurrence of a new "extended" haplotype in the eastern Baltic region combining distinctive features within the class I and TNF gene region together with DRB1*0408-DQB1*0304 in the class II region. In the report of six samples positive for DQB1*0304 in North America, half of them were associated with DRB1*0403 and half with DRB1*0408 (1). The latter one was detected also in three DQB1*0304 positive Czech gypsies (11) similarly to the observation in the present study whereas the Sardinian haplotype combines DQB1*0304 with DRB1*0403 (12). The geographic distribution of the DQB1*0304-DQB1*0408 haplotype remains to be defined by further studies, but this haplotype may apparently serve as a marker in anthropological and disease association studies.

Although detected in only 4–5% of patients with IDDM from this region its definitive association with disease susceptibility provides some insight into the determination of genetic IDDM risk. The Sardinian haplotype linked the DRB1*0403 allele with DQB1*0304, and the association of this particular haplotype with decreased IDDM risk is in line with the hypothesis that the combined effect of DR and DQ molecules plays a crucial role in determining genetic susceptibility to IDDM (13). It has been observed in several studies that DQB1*0302 is not a risk marker when found together with the DRB1*0403 allele or the very similar DRB1*0406 common in Oriental populations (13–16). Our data on DRB1*0408-DQB1*0304 are compatible with this idea as well as the hypothesis on the importance of amino acid 57 in the DQ β-chain. The change at this position from aspartic acid to alanine is enough to convert protection into susceptibility, if this effect is not blocked by the presence of DRB1*0403. However, even the DRB1*0408-DQB1*0302 haplotypes have been found to be strongly decreased among Mexican-American subjects with IDDM (17). An alternative explanation might be that the effect of DRB alleles

in modifying IDDM susceptibility associated with the DQ molecule in fact reflects the impact of more distant, so far, uncharacterized genes in haplotypic linkage.

Acknowledgments

This study was supported by the Novo Nordisk Fond and the Foundation for Paediatric Research in Finland (Ulla Hjelt Fund). We thank Ms Anne Peippo for skillful technical assistance.

References

1. Fenske TS, Baxter-Lowe LA. Characterization of a novel DQB1 allele associated with HLA-DQw3: implications for oligotyping. *Hum Immunol* 1992: **33**: 224–227.
2. Tautz C, Zwollo P, Marsh DG, Baur X. Sequence of a novel HLA-DQB1 allele. *Immunogenetics* 1992: **35**: 421–424.
3. Thorsby E, Rønmngen KS. Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type-1 (insulin-dependent) diabetes-mellitus. *Diabetologia* 1993: **36**: 371–377.
4. Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T. Triple-label hybridization assay for type-1 diabetes-related HLA alleles. *BioTechniques* 1995: **18**: 870–877.
5. Ilonen J, Reijonen H, Herva E et al. Rapid HLA-DQB1 genotyping for four alleles in the assesment of risk for IDDM in the Finnish population. *Diabetes Care* 1996: **19**: 795–800.
6. Olerup O, Aldener A, Fogdell A. HLA-DQB1 and HLA-DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. *Tissue Antigens* 1993: **41**: 119–134.
7. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens* 1992: **39**: 225–235.
8. Victoria-Gutierrez M, Martinez-Naves E, Coto E et al. DNA analysis of HLA-DR4B1 subtypes in multiple sclerosis by specific oligonucleotide probes. *J Neuroimmunol* 1991: **32**: 279–283.
9. Bunce M, Fanning GC, Welsh KI. Comprehensive, serologically equivalent DNA typing for HLA-B by PCR using sequence-specific primers (PCR-SSP). *Tissue Antigens* 1995: **45**: 81–90.
10. Nedospadov SA, Udalova IA, Kuprash DV, Turetskaya RL. DNA sequence polymorphism at the human tumor necrosis factor (TNF) locus. Numerous TNF/lymphotoxin alleles tagged by two closely linked microsatellites in the upstream region of the lymphotoxin (TNF-β) gene. *J Immunol* 1991: **147**: 1053–1059.
11. Cerna M, Fernandez-Viña M, Ivaskova E, Stastny P. Comparison of HLA class II alleles in Gypsy and Czech populations by DNA typing with oligonucleotide probes. *Tissue Antigens* 1992: **39**: 111–116.
12. Cucca F, Muntoni F, Lampis R et al. Combinations of specific DRB1, DQA1, DQB1 haplotypes are associated with insulin-dependent diabetes-mellitus in Sardinia. *Hum Immunol* 1993: **37**: 85–94.
13. Sheehy MJ, Scharf SJ, Rowe JR et al. A diabetes susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. *J Clin Invest* 1989: **83**: 830–835.
14. Awata T, Kuzuya T, Matsuda A, Iwamoto Y, Kanazawa Y. Genetic analysis of HLA class-II alleles and susceptibility to type-1 (insulin-dependent) diabetes-mellitus in Japanese subjects. *Diabetologia* 1992: **35**: 419–424.
15. Rønningen KS, Spurkland A, Tait BD et al. HLA class II associations in insulin-dependent diabetes mellitus among Blacks, Caucasoids, and Japanese. In: Tsuji K, Aizawa M, Sasazuki T, ed. *HLA 1991*. Oxford: Oxford University Press, 1992: Vol. 1: 713–722.
16. VanderAuwera B, VanWaeyenberge C, Schuit F et al. DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. *Diabetes* 1995: **44**: 527–530.
17. Erlich HA, Zeidler A, Chang J et al. HLA class-II alleles and susceptibility and resistance to insulin dependent diabetes-mellitus in Mexican-American families. *Nature Genet* 1993: **3**: 358–364.

Address:
Jorma Ilonen
University of Turku
Department of Virology
Kiinamyllynkatu 13
FIN-20520 Turku
Finland
Fax +358 2-251-3303

III

Nejentsev S, Reijonen H, Adojaan B, Kovalchuk L, Sochnevs A, Schwartz E, Åkerblom HK, Ilonen J. The effects of HLA-B allele on the IDDM risk defined by DRB1*04 subtypes and DQB1*0302. Diabetes 1997; 46: 1888–1892.

The Effect of HLA-B Allele on the IDDM Risk Defined by DRB1*04 Subtypes and DQB1*0302

Sergei Nejentsev, Helena Reijonen, Bela Adojaan, Liliya Kovalchuk, Arthur Sochnevs, Eugene I. Schwartz, Hans K. Åkerblom, and Jorma Ilonen

The genes encoding the HLA-DQ heterodimer molecules, DQB1 and DQA1, have been found to have the strongest association with IDDM risk, although there is cumulative evidence for the effect of other gene loci within the major histocompatibility complex gene region. After the HLA-DQ locus, the HLA-DR locus has been suggested most often as contributing to the disease susceptibility. In this study we analyzed at the population level the effect of DR4 subtypes and class I, HLA-B alleles, on IDDM risk when the influence of the DQ locus was stratified. In all three populations studied (Estonian, Latvian, and Russian), DQB1*0302 haplotypes most frequently carried DRB1*0401 or DRB1*0404. DRB1*0401 was the most prevalent subtype in IDDM patients, whereas DRB1*0404 was decreased in frequency. DRB1*0402 was also prevalent among Russian haplotypes, but was not associated with IDDM risk. When HLA-B alleles were analyzed, strong associations between the presence of specific B alleles and DRB1*04 subtypes were detected. The HLA-B39 allele was found significantly more often in DRB1*0404-DQB1*0302–positive patients than in healthy control subjects positive for this haplotype: 27 of 54 (50%) vs. 4 of 49 (8.2%) ($P < 0.0001$). The results demonstrate that DQ and DR genes cannot explain all of the HLA-linked susceptibility to IDDM, and that the existence of a susceptibility locus telomeric to DR is probable. *Diabetes* 46:1888–1892, 1997

The genetic component in the susceptibility to IDDM is well recognized, as is the localization of the major susceptibility loci within the HLA gene region (1). Recent genome-wide studies have confirmed the importance of HLA, but have also brought evidence for more than 10 other chromosomal loci affecting disease risk (2). The genes coding for HLA-DQ heterodimer molecules have been found to be most strongly associated with IDDM susceptibility or protection against it, suggesting a direct role of these molecules in disease pathogenesis (3).

From the Turku Immunology Centre and Department of Virology (S.N., H.R., J.I.), University of Turku, Turku, Finland; Department of Medical Genetics (S.N., E.I.S.), Pediatric Medical Academy, St. Petersburg, Russia; Department of Endocrinology (B.A.), Tartu University Hospital, Tartu, Estonia; Laboratory of Immunogenetics and Immunology (L.K., A.S.), Latvian Medical Academy, Riga, Latvia; and the Children's Hospital (H.K.Å.), University of Helsinki, Helsinki, Finland.

Address correspondence and reprint requests to Jorma Ilonen, Department of Virology, University of Turku, Kiinamyllynkatu 13, FIN-20520 Turku, Finland. E-mail: jorma.ilonen@utu.fi.

Received for publication 3 March 1997 and accepted in revised form 23 June 1997.

PCR, polymerase chain reaction.

IDDM risk can be defined by the presence of particular DQ alleles. In most of the North European populations, DQB1*0302, which is known to carry the strongest susceptibility to IDDM, is highly prevalent, whereas DQB1*0201 is more prevalent in South European populations. DQB1*0602, *0603, and *0301 have been shown to be protective alleles in most populations (4). However, several studies have suggested participation of other HLA gene loci in IDDM pathogenesis, although, because of the strong linkage disequilibrium, it is difficult to estimate their separate role. The DRB1 gene is the main candidate for an additional susceptibility locus, because DQB1*0302 haplotypes with different DR4 subtypes confer an unequal risk for the disease (5).

To clarify the question of susceptibility loci in addition to DQ, we investigated DR4 subtypes in patients and control subjects matched for the DQB1*0302 allele in Russian, Estonian, and Latvian populations, which so far are poorly defined for DR4 subtypes. Further, we studied the effect of class I HLA B gene alleles on the IDDM risk defined by combinations of specific DRB1 subtypes with DQB1*0302.

RESEARCH DESIGN AND METHODS

Subjects. Individuals from three Baltic populations—Estonians, Latvians, and Russians from St. Petersburg—were studied. Ethnic Estonians and Latvians from these two countries with sizable Russian minorities were selected for the study. The IDDM patients were unrelated subjects; in the Estonian and Latvian populations, age at diagnosis of disease was <15 years; the St. Petersburg population included some young adults. The mean age (± SD) at diagnosis of Estonian patients was 7.4 ± 3.8; of Latvian patients, 8.2 ± 3.8; and of Russian patients, 11.4 ± 6.2 years. Female patients made up 54, 51, and 60% of the Estonian, Latvian, and Russian patient populations, respectively. Control samples were obtained from healthy blood donors or university students. EDTA blood was collected, and DNA was extracted using a standard salting out method.

Study design. In the first phase, 210 IDDM and 402 control subjects from the Russian population, 97 IDDM and 269 control subjects from the Estonian population, and 136 IDDM and 182 control subjects from the Latvian population were studied for HLA-DQB1 markers associated with IDDM risk using a rapid screening method based on lanthanide-labeled probes and time-resolved fluorometry (6). All subjects positive for DQB1*0302 were selected for the further DR4 subtyping, which was carried out by genomic amplification in two polymerase chain reactions (PCRs), with the primer sequences as defined by Olerup and Zetterquist (7). The following primers were used:

5′04 5′-GTT TCT TGG AGC AGG TTA AAC A-3′,
3′047 5′-CTG CAC TGT GAA GCT CTC AC-3′,
and 3′048 5′-CTG CAC TGT GAA GCT CTC CA-3′.

Primers were specific for DRB1*0401,*0405,*0407,*0408,*0409 (5′04–3′047) and *0402,*0403,*0404,*0406,*0410,*0411 (5′04–3′048). The alleles were further distinguished by dot blot hybridization of the amplification product using the following ^{32}P-or digoxygenin-labeled sequence specific oligonucleotides:

5′-GGT GTC CAC CTC GGC CCG CC-3′	DRB1*0403, *0406, *0407, *0411
5′-GCA GAG GCG GGC CGC GGT-3′	DRB1*0404, *0405, *0408, *0410
5′-CGG CCT AGC GCC GAG TAC-3′	DRB1*0405, *0409, *0410, *0411
5′-GAA GAC GAG CGG GCC GCG-3′	DRB1*0402
5′-GAG CAG AAG CGG GCC GCG-3′	DRB1*0401, *0409

TABLE 1
Frequencies (%) of HLA-DRB1*04 subtypes in DQB1*0302-positive patients and control subjects

	Estonians			Latvians		Russians			Combined		
Allele	IDDM (n = 62)	Control (n = 47)	P	IDDM (n = 76)	Control (n = 13)	IDDM (n = 148)	Control (n = 75)	P	IDDM (n = 286)	Control (n = 135)	P
0401	72.6	25.5	<0.0001	60.5	38.5	57.4	37.3	0.007	61.5	33.3	<0.0001
0402	1.6	8.5		11.8	0	18.9	25.3		13.3	17.0	
0403/6	0	6.4		0	0	0	1.3		0	3.0	0.017
0404	29.0	53.2	0.018	26.3	53.8	24.3	32.0		25.9	41.5	0.0018
0405	3.2	2.1		0	0	2.7	1.3		2.1	1.5	
0408	0	6.4		2.6	0	2.0	0		1.7	2.2	
x*	0	4.3		6.6	7.7	2.0	6.7		2.8	5.9	

*Nontypable.

The DRB1*04 alleles were distinguished by combining results of specific amplification and dot blot hybridization. DQB1*0302–positive samples were also analyzed for the presence of nine HLA-B alleles (B*07, 62, 60, 35, 56, 51, 27, 39, and 44). These alleles were chosen for the study because they are known to be in linkage disequilibrium with DR4 in other Caucasian populations (8); or in particular, they are known to characterize IDDM-associated DR4 haplotypes in the neighboring Finnish population (9,10). HLA-B typing was done by means of PCR with sequence-specific primers according to the method described by Bunce et al. (11).

Statistical analysis. The level of significance was assessed by χ^2 test in comparisons between different groups. Odd ratios were calculated according to the formula $(a \times d)/(b \times c)$, where a and b are the numbers of the IDDM patients that were positive and negative for the marker, respectively, and c and d, the respective numbers of control subjects.

RESULTS

The frequency of HLA DQB1*0302 was highly increased in IDDM patients in all populations, but DRB1*04 subtyping revealed an unequal risk of IDDM when DQB1*0302 was found in context of distinct DRB1-DQB1 haplotypes (Table 1). DRB1*0401 was associated with IDDM in all populations, whereas DQB1*0404 was decreased among IDDM patients, although this effect was not significant among the Russian patients. These two alleles were the most prevalent subtypes in all three populations. Only in the Russian patients was DQB1*0402 found in a considerable proportion of DQB1*0302 haplotypes, but without any association with IDDM. DQB1*0403/6 alleles were not found in any of the patients, and although also rare in control samples, the decrease among IDDM patients was significant in the combined data.

HLA-B typing of selected alleles was performed only in Estonian and Russian subjects because of the small number of Latvian control subjects with various DR4 subtypes. The analysis of all DQB1*0302–positive subjects without taking DR4 subtypes into account showed an association of B*39 with the disease. B*7 and B*51 were significantly decreased in the patients in the combined series (Table 2).

Analysis of B alleles associated with different DR4-DQB1*0302 haplotypes revealed ordinary allelic associations as well as some differences between IDDM and control subjects (Tables 3–5). The B*62 allele was significantly more common in DRB1*0401-DQB1*0302–positive than in DRB1*0404-DQB1*0302–positive subjects among both IDDM (P = 0.002) and control subjects (P = 0.018). DRB1*0402-DQB1*0302, which is prevalent in Russian subjects, was frequently associated with B*51, and this allele was significantly more common in DRB1*0402-DQB1*0302–positive than in DRB1*0401-DQB1*0302–positive patients (P = 0.0035).

The most conspicuous difference between IDDM patients and control subjects was found among DRB1*0404-DQB1*0302–positive subjects. B*39 was found in half of both Estonian and Russian IDDM patients, compared with only a few cases among control subjects. The difference was significant

TABLE 2
Frequencies (%) of HLA-B alleles in HLA DQB1*0302–positive IDDM patients and healthy control subjects

	Estonians			Russians		Combined		
Allele	IDDM (n = 58)	Control (n = 46)	P	IDDM (n = 146)	Control (n = 75)	IDDM (n = 204)	Control (n = 121)	P
62	34.5	28.3		24.7	17.3	27.5	21.5	
39	25.9	2.2	0.0023	14.4	6.7	17.6	5.0	0.0018
35	15.5	19.6		13.0	16.0	13.7	17.4	
44	5.2	10.9		16.4	18.7	13.2	15.7	
7	12.1	21.7		11.6	20.0	11.8	20.7	0.045
27	13.8	19.6		10.3	13.3	11.3	15.7	
51	3.4	10.9		10.3	20.0	8.3	16.5	0.039
60	6.9	4.3		5.5	14.7	5.9	10.7	
56	5.2	4.3		0.7	0	2.0	1.7	
xx*	8.6	13.0		17.8	12.0	15.2	12.4	

*None of the above studied alleles.

TABLE 3
Frequencies (%) of HLA-B alleles in HLA-DRB1*0401-DQB1*0302–positive IDDM patients and healthy control subjects

	Estonians			Russians			Combined		
Allele	IDDM (n = 44)	Control (n = 14)	IDDM (n = 85)	Control (n = 28)	P	IDDM (n = 129)	Control (n = 42)	P
62	43.2	35.7	37.6	39.3		39.5	38.1	
39	15.9	0	5.9	3.6		9.3	2.4	
35	18.2	14.3	23.5	10.7		21.7	11.9	
44	6.8	21.4	16.5	21.5		13.2	21.4	
7	6.8	14.3	11.8	14.3		10.1	14.3	
27	13.6	14.3	9.4	10.7		10.9	11.9	
51	0	14.3	5.9	14.3		3.9	14.3	0.043
60	4.5	0	2.4	21.4	0.0028	3.1	14.3	0.021
56	6.7	7.1	1.2	0		3.1	2.4	
xx*	9.1	14.3	14.1	10.7		12.4	11.9	

*None of the above studied alleles.

in both populations, and highly significant in the combined series at the level of $P < 0.0001$, even after multiplication by the number of tested HLA-B alleles (Table 4). Calculations of relative risk estimates or odds ratios for DRB1*0404-DQB1*0302 with and without B*39 allele demonstrated that the combination with B*39 is a marker associated with a higher risk than the DRB1*0401-DQB1*0302 haplotype, whereas without B*39, this haplotype carried no susceptibility (Table 6).

The decrease of B*51 among IDDM patients that was observed when comparing all DQB1*0302–positive subjects was further found in those with the DRB1*0401-DQB1*0302 haplotype (Table 3). B*60 was decreased in Russian and combined IDDM patients with DRB1*0401-DQB1*0302, whereas B*35 was decreased in DRB1*0402-DQB1*0302–positive Russian IDDM patients compared with healthy subjects with this haplotype. On the other hand, the number of subjects without any of the tested B alleles was increased among DRB1*0402-DQB1*0302 patients (Table 5).

The age at diagnosis or proportion of male to female patients did not differ between patients with various HLA-DR4 subtypes. Neither were differences found when those with the combination of HLA-DQB1*0404 and B*39 allele were compared with other patients (data not shown).

DISCUSSION

The results described in this study confirm the presence of several gene loci within the HLA region responsible for the susceptibility to IDDM. The risk defined by the strongest known single risk allele, DQB1*0302, is dependent on the DR4 subtype. Amino acid residues differing between DR4 alleles participate in the formation of the peptide binding groove and have functional effects on peptide binding and antigen presentation (12). If the disease susceptibility and protection associated with DQ molecules is mediated by their role in antigen presentation, this might in fact be more precisely defined by the combination of different class II alleles, DQ and DR molecules together. However, some findings contradict this hypothesis. The hierarchy of DR4 alleles in the definition of susceptibility that has been built in accordance with several studies (5) is not a common rule. In the present study, DRB1*0402, which has been found in some populations to be the strongest DR4 risk factor, did not differ in frequency between Russian patients and control subjects. Also, the populations with the highest IDDM incidence (13)—Finns and Scandinavians—practically lack those DR4 subtypes reported to be associated with the highest risk, 0402 and 0405 (14–16). The effect of

TABLE 4
Frequencies (%) of HLA-B alleles in HLA-DRB1*0404-DQB1*0302–positive IDDM patients and healthy control subjects

	Estonians			Russians			Combined		
Allele	IDDM (n = 17)	Control (n = 25)	P	IDDM (n = 37)	Control (n = 24)	P	IDDM (n = 54)	Control (n = 49)	P
62	11.7	28.0		16.2	0		14.8	14.3	
39	52.9	4.0	0.001	48.6	12.5	0.0086	50.0	8.2	<0.0001
35	11.7	16.0		5.4	16.7		7.4	16.3	
44	0	4.0		13.5	16.7		9.3	10.2	
7	23.5	24.0		16.2	29.2		18.5	26.5	
27	17.6	28.0		10.8	12.5		13.0	20.4	
51	5.9	0		5.4	16.7		5.6	8.2	
60	5.9	8.0		13.5	16.7		11.1	12.2	
56	0	4.0		0	0		0	2.0	
xx*	5.9	12.0		2.7	12.5		3.7	12.2	

*None of the above studied alleles.

TABLE 5
Frequencies (%) of HLA-B alleles in Russian HLA-DRB1*0402-DQB1*0302–positive IDDM patients and healthy control subjects

Allele	IDDM (n = 28)	Control (n = 19)	P
62	3.6	10.5	
39	3.6	5.3	
35	0	21.4	0.045
44	21.4	15.8	
7	0	10.5	
27	10.7	15.8	
51	28.6	31.6	
60	3.6	5.3	
56	0	0	
xx*	42.9	10.5	0.04

*None of the above studied alleles.

DRB1*0404 varies in its protective effect in different populations, from strong to weak or lacking (14,15,17,18), as in the present report.

Our study also suggested a strong specific heterogeneity in the effect of DRB1*0404-DQB1*0302 haplotype, depending on the presence or absence of B*39 allele, with a strong disposition being conferred in the former and no disposition being conferred in the latter. This result might be attributable to a haplotypic effect, but this cannot be confirmed in a population study. In a Finnish family study, B39 divides DRB1*0404-DQB1*0302 haplotypes similarly in terms of IDDM risk (19). In the Finnish population, B39 is also strongly associated with A24, with A24 (9), B39 (16), DR4 being one of the most common DR4 haplotypes found in IDDM patients (9,10,20). A24, B39, and DR4 haplotypes have not been reported as susceptibility haplotypes elsewhere, but A24 has been recently found to be associated with an earlier age at diagnosis and with a more rapid progression to IDDM in an Australian family series (21). A recent study from St. Petersburg also reported the increase of both A24 and B16 alleles among Russian IDDM patients (22).

Other deviations in B allele frequencies between DR-DQ–matched IDDM and control subjects found in the present study also support the role of class I genes. The decreased B7 frequency among IDDM patients was shown only when all DQB1*0302–positive subjects were compared. This decrease may be secondary to the decrease of linked DRB1*15-DQB1*0602 alleles protecting against IDDM, and may reflect the genotype effect of the other chromosome. The decreased frequency of B51 and B60, instead, was seen only in DRB1*0401–positive subjects, and that of B35, only in DRB1*0402–positive subjects. However, these associations were detected for the first time and were of low statistical significance, thus requiring further confirmation.

The results of the present study suggest that class I alleles have a separate role in IDDM in addition to DR and DQ. Another possibility is that the association of IDDM with certain DR4 subtypes appears to be attributable to the linkage disequilibrium with an unidentified gene in the HLA region. The hypothesis of an additional susceptibility locus within the major histocompatibility complex region was supported by recent molecular findings (23,24), as well as by analogy with the NOD mouse model (25). The role and localization of these genetic elements remain to be clarified.

ACKNOWLEDGMENTS

This study was supported by grants from the Academy of Finland, the Novo Nordisk Foundation, the Foundation for Paediatric Research in Finland (Ulla Hjelt Fund), and the Nordic Council Scholarship Program.

We thank Terttu Laurén and Ritva Suominen for their skillful technical assistance.

REFERENCES

1. Wassmuth R, Lernmark Å: The genetics of susceptibility to diabetes. *Clin Immunol Immunopathol* 63:358–399, 1989
2. Todd JA: Genetic analysis of type 1 diabetes using whole genome approaches. *Proc Nat Acad Sci USA* 92:8560–8565, 1995
3. Thorsby E, Rønningen KS: Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type-1 (insulin-dependent) diabetes-mellitus. *Diabetologia* 36:371–377, 1993
4. Ilonen J, Reijonen H: Special genetic features of the populations in Nordic countries as related to risk of IDDM. *Diabetes in the Young* 29:10–16, 1993
5. She J-X: Susceptibility to type I diabetes: HLA-DQ and DR revisited. *Immunol Today* 17:323–329, 1996
6. Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T: Triple-label hybridization assay for type-1 diabetes-related HLA alleles. *Biotechniques* 18:870–877, 1995
7. Olerup O, Zetterquist H: HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 h: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens* 39:225–235, 1992
8. Baur MP, Neugebauer M, Albert E: Reference tables of two-locus haplotype frequencies for all MHC marker loci. In *Histocompatibility Testing 1984*. Albert ED, Baur MP, Mayr WR, Eds. Berlin, Heidelberg, Germany, Springer-Verlag, 1984, p. 677–755
9. Mustonen A, Ilonen J, Surcel H-M, Åkerblom HK: A "new" DR4 associated D specificity "JA" in type 1 (insulin-dependent) diabetes: A9,Bw16,DJA,DR4 haplotype. *Diabetologia* 27:126–128, 1984
10. Tienari PJ, Tuomilehto-Wolf E, Tuomilehto J, Peltonen L: HLA haplotypes in type-1 (insulin-dependent) diabetes-mellitus: molecular analysis of the HLA-DQ locus. *Diabetologia* 35:254–260, 1992
11. Bunce M, Fanning GC, Welsh KI: Comprehensive, serologically equivalent DNA

TABLE 6
Combinations of HLA-B, DRB1, and DQB1 as risk markers for IDDM among Estonians and Russians

Marker combination	Odds ratio (95% CI) Estonians	Russians	Combined
DQB1*0302	8.9 (5.1–15.5)	12.0 (7.9–18.2)	10.9 (7.8–15.1)
DQB1*0302-DRB1*0401	15.8 (7.7–32.7)	9.1 (5.5–15.0)	11.0 (7.4–15.6)
DQB1*0302-DRB1*0404	2.2 (1.1–4.5)	3.4 (1.9–6.0)	2.8 (1.8–4.3)
DQB1*0302-DRB1*0404-B*39	27.4 (3.5–126.2)	12.5 (3.4–34.8)	16.1 (5.3–36.7)
DQB1*0302-DRB1*0404-X†	0.92 (0.36–2.2)	1.8 (0.90–3.6)	1.3 (0.79–2.3)

†B*39 lacking.

typing for HLA-B by PCR using sequence-specific primers (PCR-SSP). *Tissue Antigens* 45:81–90, 1995
12. Hammer J, Gallazzi F, Bono E, Karr RW, Guenot J, Valsasnini P, Nagy ZA, Sinigaglia F: Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association. *J Exp Med* 181:1847–1855, 1995
13. Karvonen M, Tuomilehto J, Libman I, Laporte R: A review of the recent epidemiological data on the worldwide incidence of type-1 (insulin-dependent) diabetes-mellitus. *Diabetologia* 36:883–892, 1993
14. Undlien DE, Friede T, Rammensee H-G, Joner G, Dahl-Jørgensen K, Søvik O, Akselsen HE, Knutsen I, Rønningen KS, Thorsby E: HLA-encoded genetic predisposition in IDDM: DR4 subtypes may be associated with different degrees of protection. *Diabetes* 46:143–149, 1997
16. Sanjeevi CB, Hook P, Landin-Olsson M, Kockum I, Dahlquist G, Lybrand TP, Lernmark Å: DR4 subtypes and their molecular properties in a population-based study of Swedish childhood diabetes. *Tissue Antigens* 47:275–283, 1996
16. Tuokko J, Reijonen H, Ilonen J, Anttila K, Nikkari S, Möttönen T, Yli-Kerttula U, Toivanen A: Increase of HLA-DRB1*0408 and -DQB1*0301 in HLA-B27 positive reactive arthritis. *Ann Rheum Dis* 56:37-40, 1997
17. Caillat-Zucman S, Garchon HJ, Timsit J, Assan R, Boitard C, Djilalisaiah I, Bougneres P, Bach JF: Age-dependent HLA genetic heterogeneity of type-1 insulin-dependent diabetes mellitus. *J Clin Invest* 90:2242–2250, 1992
18. VanderAuwera B, VanWaeyenberge C, Schuit F, Heimberg H, Vandewalle C, Gorus F, Flament J, Belgian Diabetes Registry: DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. *Diabetes* 44:527–530, 1995
19. Reijonen H, Nejentsev S, Tuokko J, Koskinen S, Tuomilehto-Wolf E, Åkerblom HK, Ilonen J, the Childhood Diabetes in Finland Study Group: HLA-DR4 subtype and -B alleles in DQB1*0302 positive haplotypes associated with IDDM. *Eur J Immunogen.* In press
20. Ilonen J, Åkerblom HK, Tiilikainen A, Mustonen A, Herva E, Koskimies S, Kouvalainen K: Increase of HLA haplotype A9-Bw16 in familial insulin-dependent diabetes mellitus in Northern Finland. *Acta Diabetol* 21:167–174, 1982
21. Honeyman MC, Harrison LC, Drummond B, Colman PG, Tait BD: Analysis of families at risk for insulin-dependent diabetes mellitus reveals that HLA antigens influence progression to clinical disease. *Mol Med* 1:576–582, 1995
22. Berkos AS, Bubnova LN, Belianeva EV, Nikolenko VN, Erochina LV: The association of HLA-antigens with insulin-dependent diabetes mellitus (IDDM) in Saint-Petersburg (Russia) (Abstract). *Hum Immunol* 47:157, 1996
23. Pociot F, Briant L, Jongeneel CV, Molvig J, Worsaae H, Abbal M, Thomsen M, Nerup J, Cambon-Thomsen A: Association of tumor necrosis factor (TNF) and class-II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin-dependent diabetes-mellitus. *Eur J Immunol* 23:224–231, 1993
24. Demaine AG, Hibberd ML, Mangles D, Millward BA: A new marker in the HLA class I region is associated with the age at onset of IDDM. *Diabetologia* 38:623–628, 1995
25. Ikegami H, Makino S, Yamato E, Kawaguchi Y, Ueda H, Sakamoto T, Takekawa K, Ogihara T: Identification of a new susceptibility locus for insulin-dependent diabetes mellitus by ancestral haplotype congenic mapping. *J Clin Invest* 96:1936–1942, 1995

IV

Nejentsev S, Koskinen S, Sjöroos M, Reijonen H, Schwartz EI, Kovalchuk L, Sochnev A, Adojaan B, Podar T, Knip M, Simell O, Koskenvuo M, Åkerblom HK, Ilonen J. Distribution of insulin-dependent diabetes (IDDM)-related HLA alleles correlates with the difference in IDDM incidence in four populations of the Eastern Baltic Region. Tissue Antigens 1998; 52: 473–477.

S. Nejentsev
S. Koskinen
M. Sjöroos
H. Reijonen
E.I. Schwartz
L. Kovalchuk
A. Sochnev
B. Adojaan
T. Podar
M. Knip
O. Simell
M. Koskenvuo
H.K. Åkerblom
J. Ilonen

Distribution of insulin-dependent diabetes mellitus (IDDM)-related HLA alleles correlates with the difference in IDDM incidence in four populations of the Eastern Baltic region

Key words:
genetics; HLA alleles; incidence; insulin-dependent diabetes mellitus

Acknowledgments:
The study was supported by the Academy of Finland, the Foundation for Paediatric Research in Finland (Ulla Hjelt Fund) and the Novo Nordisk Foundation. We are grateful for the skilful technical assistance of Mrs. Terttu Laurén and Mrs. Ritva Suominen.

Abstract: The high incidence of insulin-dependent diabetes mellitus (IDDM) in Finland contrasts strikingly with the low rates in the neighbouring populations of countries in the Eastern Baltic region: Estonia, Latvia and Russia. To evaluate the possible contribution of genetic factors to these differences, the frequencies of HLA-DQB1 alleles and relevant DQB1-DQA1 or DQB1-DRB1 haplotypes associated with IDDM risk or protection were analysed among IDDM patients and control subjects from these four populations. An increased frequency of HLA DQB1*0302, DQB1*02-DQA1*05 and DQB1*0302-DRB1*0401 was observed in subjects with IDDM in all studied populations, whereas the prevalence of DQB1*0301 and DQB1*0602 and/or *0603 was decreased among patients. The degree of IDDM risk associated with HLA alleles analysed here did not differ significantly between the populations. Comparisons of the distribution of IDDM-related HLA alleles and haplotypes in the background populations revealed its consonance with IDDM incidence. The combined frequency of high risk genotypes was significantly higher among Finns than in other populations studied. Our data support the hypothesis that variance in the dispersion of HLA alleles is the genetic basis of variation of IDDM incidence observed in the Eastern Baltic region.

Insulin-dependent diabetes mellitus (IDDM) is common in populations of European ancestry, the highest incidence rates being observed in Finland and Sardinia, the disease being rare among Asian Orientals (1, 2). Interestingly, major gradients in IDDM incidence are frequently found also between neighbouring populations which are considered as being genetically relatively close to each other. The Eastern Baltic region is a conspicuous example of a such remarkable small-area variation. The incidence of IDDM is extremely high in Finland – 35.3 per 100,000 children under the age 15 annually (95% confidence interval (CI): 33.1–37.5) – whereas, in contrast, the incidence in the neighbouring Baltic countries and Russia is low: in Estonia 10.3 (8.9–11.7), in Latvia 6.5 (5.6–7.4) and in Russia 5.6 (no data for 95% CI available) (1). To clarify the reasons behind

Authors' affiliations:
S. Nejentsev[1,2], S. Koskinen[1,2], M. Sjöroos[1,3], H. Reijonen[1,2], E.I. Schwartz[4], L. Kovalchuk[5], A. Sochnev[6], B. Adojaan[7], T. Podar[8], M. Knip[9], O. Simell[1,9], M. Koskenvuo[1,9], H.K. Åkerblom[10], J. Ilonen[1,2]

[1]Turku Immunology Centre, University of Turku, Turku, Finland,
[2]Department of Virology, University of Turku, Turku, Finland,
[3]Department of Biotechnology, University of Turku, Turku, Finland,
[4]Department of Medical Genetics, St. Petersburg Paediatric Medical Academy, St. Petersburg, Russia,
[5]Department of Immunology, Medical Academy of Latvia, Riga, Latvia,
[6]Hospital of Endocrinology, University of Tartu, Tartu, Estonia,
[7]Department of Paediatrics, University of Oulu, Oulu, Finland,
[8]Department of Pediatrics, University of Turku, Turku, Finland,
[9]Department of Public Health, University of Turku, Turku, Finland,
[10]Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland

Correspondence to:
Dr. Sergei Nejentsev
Department of Virology
University of Turku
Kiinamyllynkatu 13
FIN-20520 Turku, Finland
Tel: 358-2-3337023
Fax: 358-2-2513303

Received 29 June, revised, accepted for publication 21 August 1998

Tissue Antigens. ISSN 0001-2815

Tissue Antigens 1998: **52**: 473–477
Printed in Denmark.

these differences is an intriguing challenge. Although we know that both environmental and genetic factors are involved in the pathogenesis of IDDM, most of these factors remain poorly characterised. HLA genes are the best defined and, obviously, the major genetic component in the disease aetiology (3). While the DQB1 and DQA1 genes are considered to be the primary IDDM genes, it is generally accepted that alleles of the DRB1 gene also affect the disease susceptibility (4).

In the present study we tested the hypothesis that differences in the distribution of IDDM-related HLA alleles could explain or, at least, would be in accordance with the observations of IDDM incidence. To explore this issue we first studied whether the same alleles of the HLA DQB1 genes and relevant combinations of DQB1 and DQA1 or DRB1 genes conferred the same degree of susceptibility or protection to IDDM in four neighbouring populations of the Eastern Baltic region. Subsequently, the distribution of IDDM-related alleles and the proportion of individuals at high risk were compared between the background populations.

Patients and methods

Samples from patients with newly diagnosed IDDM were collected from the region of Oulu in Northern Finland and the region of Turku in Southwestern Finland. The patients had been diagnosed before the age of 16 at the Departments of Paediatrics, Oulu or Turku University Hospitals, after the year 1989, and had thus not been included in the earlier series of the "Childhood Diabetes in Finland" study. Reference samples were cord bloods of consecutive births at the same hospitals. The patients from Estonia and Latvia had also been diagnosed before the age of 16 but the series from St. Petersburg, Russia, included young adults. The patients and control subjects in Estonia and Latvia were ethnic Estonians and Latvians, respectively, since all subjects representing the sizeable Russian minorities in these countries were excluded from the study. Blood samples were collected from 97 Estonian, 136 Latvian, 211 Russian and 316 Finnish (163 from Oulu and 153 from Turku) IDDM patients and from 269 healthy Estonian, 173 Latvian, 413 Russian and 1,000 Finnish (499 from Oulu and 501 from Turku) reference subjects.

The gene alleles defining the presence of the HLA-DQ heterodimer molecules known to be associated with IDDM risk or protection were determined using two steps. The samples were first studied for the presence of the DQB1*02,*0301,*0302 and *0602 or *0603 alleles using a method based on binding of the biotinylated amplification product onto streptavidin-coated microtitre plate wells, followed by hybridisation with lanthanide-labelled oligonucleotide probes. The fluorescence properties of the different lantha-

Frequency (%) of DQB1 alleles and of DQB1-DQA1 and DQB1-DRB1 haplotypes in IDDM patients and controls in the four populations in the Eastern Baltic region

	Finland			Estonia			Latvia			Russia		
	IDDM	Control	P cor	IDDM	Control	P cor	IDDM	Control	P cor	IDDM	Control	P cor
DQB1*0301	7.9	21.6	<0.00004	10.3	33.5	<0.002	13.2	39.9	<0.00004	7.8	39.0	<0.00004
DQB1*0602/3	7.3	41.2	<0.00004	2.1	44.2	<0.00004	2.2	47.4	<0.00004	4.7	35.6	<0.00004
DQB1*02	48.1	25.5	<0.00004	54.6	31.2	<0.004	60.3	29.5	<0.00004	59.2	28.1	<0.00004
DQB1*02-DQA1*05	42.4	20.2	<0.00004	50.5	22.3	<0.00004	48.5	17.9	<0.00004	45.5	15.7	<0.00004
DQB1*02-DQA1*0201	5.7	6.3	NS	3.1	14.9	NS	13.2	17.9	NS	14.2	15.5	NS
DQB1*0302	70.6	19.3	<0.00004	66.0	17.8	<0.00004	61.0	9.2	<0.00004	71.6	20.6	<0.00004
DQB1*0302-DRB1*0401	54.4	12.4	<0.00004	46.4	5.8	<0.00004	34.6	2.9	<0.00004	40.8	7.5	<0.00004
DQB1*0302-DRB1*0402	0	0	NS	1.0	1.5	NS	6.6	0	NS	13.3	4.6	<0.008
DQB1*0302-DRB1*0403	0.6	1.3	NS	0	1.1	NS	0	0	NS	0	0.5	NS
DQB1*0302-DRB1*0404	19.3	6.2	<0.00004	18.6	8.9	NS	16.7	4.0	<0.003	17.1	6.8	<0.008
Number of subjects	316	1,000		97	269		136	173		211	413	

P cor=Pc×40

Table 1

nides (europium, samarium and terbium) allowed simultaneous detection of three different reaction products in a single microtitre plate well with time-resolved fluorometry (5). The samples positive for DQB1*02 were further studied for DQA1*05 and *0201 alleles. This assay was based on a modified method in which biotinylated sequence-specific probes and a europium-labelled detection probe were utilised (6). The samples positive for DQB1*0302 were further studied for the presence of DR4 subtypes, i.e. alleles of the DRB1 gene, known to be in linkage disequilibrium with DQB1*0302 and modifying the risk conferred by this allele (7). We used either a high-resolution technique of DR4 subtyping, which was designed according to the same principles as the assay for the DQB1 gene (S. Nejentsev, M. Sjöroos, T. Soukka, M. Knip, O. Simell, T. Lövgren, J. Ilonen, manuscript in preparation) or conventional dot-blot hybridisation (8). In the remaining cases the DQB1 alleles alone were regarded sufficient for the final analysis of the risk, as the amount of information increases minimally by the determination of the tightly linked DQA1 and DRB1 alleles.

The chi-squared test with continuity correction was used for the analysis of statistical significance. Odds ratios (OR) were calculated according to the formula $(a \times d)/(b \times c)$, where a and b are the frequencies of patients positive or negative for the given marker, respectively, and c and d are the corresponding numbers for the control subjects.

Results and discussion

HLA-DQ allele associations which have consistently been found to be positively or inversely related to IDDM susceptibility in Caucasi-

Relative risk (OR) and 95% confidence intervals (95% CI) conferred by DQB1 alleles and of DQB1-DQA1 and DQB1-DRB1 haplotypes in the four populations in the Eastern Baltic region

	Finland		Estonia		Latvia		Russia	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
DQB1*0301	0.31	0.20–0.49	0.2	0.1–0.5	0.2	0.1–0.4	0.1	0.07–0.23
DQB1*0602-3	0.11	0.07–0.18	0.03	0.01–0.1	0.03	0.01–0.09	0.09	0.04–0.2
DQB1*02	2.7	2.1–3.6	2.7	1.8–4.4	3.6	2.2–6.0	3.7	2.6–5.4
DQB1*02-DQA1*05	2.9	2.2-3.9	3.6	2.1–6.0	4.3	2.5–7.5	4.7	3.0–6.6
DQB1*0302	10.0	7.4–13.5	8.9	5.1–15.6	15.4	8.0–30.0	9.7	6.5–14.5
DQB1*0302-DRB1*0401	8.4	6.4–11.4	14.7	7.2–39.9	17.7	6.5–52.7	8.5	5.2–13.8
DQB1*0302-DRB1*0404	3.6	2.4–5.4	2.3	1.1–4.7	4.6	1.8–12.2	2.8	1.6–4.9

Table 2

Comparison of frequencies (%) of DQB1 alleles and of DQB1-DQA1 and DQB1-DRB1 haplotypes in the four populations in the Eastern Baltic region

	Allele or haplotype associated with	Finland	Estonia	Latvia	Russia
DQB1*0301	protection	21.6[a]	33.5	39.9	39.0
DQB1*0602-3	protection	41.2	44.2	47.4	35.6[b]
DQB1*02	risk	25.5	31.2	29.5	28.1
DQB1*02-DQA1*05	risk	20.2	22.3	17.9	15.7[c]
DQB1*0302	high risk	19.3	17.8	9.2[d]	20.6
DQB1*0302-DRB1*0401	high risk	12.4[e]	5.8	2.9	7.5
DQB1*0302-DRB1*0404	risk	6.2	8.9	4.0	6.8

P (P cor = P x 42):

[a] Finland vs Estonia<0.00008 (<0.004), Finland vs Latvia<0.000001 (<0.00005), Finland vs Russia<0.000001 (<0.00005)

[b] Russia vs Finland = 0.057 (NS), Russia vs Estonia<0.03 (NS), Russia vs Latvia<0.01 (NS)

[c] Russia vs Estonia<0.04 (NS), Russia vs Finland = 0.08 (NS)

[d] Latvia vs Finland<0.0002 (<0.009), Latvia vs Estonia<0.02 (NS), Latvia vs Russia<0.002 (NS)

[e] Finland vs Estonia<0.003 (NS),Finland vs Latvia<0.0004 (<0.02), Finland vs Russia<0.01(NS)

Table 3

ans were confirmed also here (Table 1). While no previous data on molecular typing of IDDM patients in Estonia or Latvia exist, in a recent study of patients with juvenile rheumatoid arthritis in Latvia the control population had very similar allele frequencies in the control population to those seen here. Typically, DQB1*0301 was found at high frequency and DQB1*0302 relatively seldom (9). The frequency of DQB1*0302 in the Latvian population is also in concordance with earlier data on a low prevalence of the serologically defined DR4 antigen (10). Russian IDDM patients and controls from Moscow (11) showed similar frequencies of DQB1*0302, DQB1*0301 and DQB1*0602 or *0603 alleles as the subjects from St. Petersburg in this study.

Results of DR4 subtyping in Finns and, as we have shown earlier (8), in Estonians, Latvians and Russians, revealed that DRB1*0401 and *0404 were the only alleles found at significant frequencies and able to modify IDDM risk associated with the DQB1*0302 allele. In Russians *0402 was also present in a reasonable number of patients and controls but was a neutral marker in relation to IDDM risk. In Russian patients and controls matched for DQB1*0302, DRB1*0402 was found in 18.5% and 22.4%, respectively, with an OR of 0.8 (95% CI=0.4–1.6). The DRB1*0403, known to be a protective allele (12–14), was rare in all four studied populations and could not significantly modify the risk conferred by DQB1*0302 (Table 1).

Although the relative risks of IDDM (OR) associated with DQB1 alleles, DQB1-DQA1 and DQB1-DRB1 haplotypes were not exactly the same between the populations studied, the 95% CI were overlapping in all comparisons (Table 2). This indicates that the same alleles and haplotypes generally confer a similar degree of risk and the comparisons of their frequency in the background population will be valid in estimating the relative level of genetic susceptibility to IDDM in a particular population.

The comparisons of the frequency of HLA DQB1 alleles, DQB1-DQA1 and DQB1-DRB1 haplotypes between the four populations are shown in Table 3. Each population could be characterised in terms of the prevalence of IDDM-predisposing and IDDM-protective HLA alleles and/or haplotypes. Finland was shown to have a decreased frequency (relative to other studied populations) of the protective DQB1*0301 allele and an increased frequency of the highly susceptible DQB1*0302-DRB1*0401 haplotype. In contrast, the Latvian population had a low prevalence of the highly susceptible DQB1*0302 allele and a tendency to an increased frequency of the protective DQB1*0301 allele. A population was considered to show a "tendency" of being different when the contrast in allele frequency was notable, but P-values became insignificant after correction for the number of comparisons done. Thus the Russian population could be characterised by the tendencies to an increased frequency of DQB1*0301 and a low prevalence of DQB1*0602-3, which both

Estimation of the degree of risk conferred by high-risk HLA genotypes, and comparison of their combined frequencies in the four populations in the Eastern Baltic region

	Finland			Estonia			Latvia			Russia		
	OR	95% CI	Frequency	OR	95% CI	Frequency	OR	95% CI	Frequency	OR	95% CI	Frequency
DQB1*02/0302	15.1	9.1–25.3	2.3%	8.6	4.0–19.2	4.5%	13.5	4.9–40.4	2.9%	18.5	9.8–35.3	3.4%
DQB1*0302/xDRB1*0401/v	5.5	3.8–7.9	6.4%	19.4	6.1–46.1	1.5%	17.4	3.9–55.4	1.2%	6.6	3.2–13.9	2.9%
DQB1*02/yDQA1*05/z	2.1	1.4–3.1	8.8%	6.1	2.6–14.3	4.1%	4.6	1.9–11.7	4.6%	3.4	1.7–4.7	4.1%
combined	11.1	8.2–15.1	17.5%*	23.2	12.3–44.3	10.0%	18.7	9.5–37.2	8.7%	8.9	12.0–29.8	10.4%

* P (P cor=P×3): Finland vs Estonia<0.005 (<0.015), Finland vs Latvia<0.006 (<0.02), Finland vs Russia<0.0011 (<0.004)

v: any DRB1 allele except *0403, 0406

x: any DQB1 allele except *02, 0301, 0602 or 0603

y: any DQB1 allele except *0302, 0301, 0602 or 0603

z: any DQA1 allele except *0201

Table 4

confer protection, and a tendency to carry the susceptible haplotype DQB1*02-DQA1*05 at a low frequency. In the Estonian population all protective and susceptible alleles and haplotypes were found to be distributed at intermediate frequencies. These data are mostly in accordance with the differences in the disease incidence observed between the populations studied.

Another approach for comparing the level of genetic susceptibility between populations is to compare the combined proportion of all individuals at risk. While representing a more direct estimate than the comparisons of allele and haplotype frequencies, it is hampered by the large number of genotypes, some of which being present at very low population frequencies and often conferring different degrees of IDDM risk in various populations. In Table 4 we compared combined population frequencies of all individuals at high risk, i.e. those carrying the DQB1*02/0302 or the DQB1*0302/x-DRB1*0401/v or the DQB1*02/y-DQA1*05/z genotype. The degree of risk conferred by each genotype was similar in the studied populations, as the 95% confidence intervals are overlapping in all comparisons. When combined, these genotypes comprise 64.0% of all Latvian IDDM patients, 70.3% of Finnish, 72.2% of Estonian and 68.7% of Russian IDDM patients. Although a substantial proportion of individuals at low risk was not included in the analysis, the difference in the combined frequency of these genotypes in background populations was rather demonstrative: Finland has the highest proportion of susceptible individuals – 17.5%. Therefore, we can assume that, as far as HLA is the primary IDDM locus (3), the differences in the distribution of its alleles are the genetic basis of the variation of IDDM incidence. Our data support this hypothesis.

In conclusion, we found that differences in the frequencies of IDDM-related, susceptible and protective HLA alleles and haplotypes in the background population are in concordance with the variance in IDDM incidence observed in the Eastern Baltic region.

References

1. Karvonen M, Tuomilehto J, Libman I, LaPorte R. A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. *Diabetologia* 1993: **36**: 883–92.
2. Shen SX, Wang HB, Chen ZW et al. The incidence of insulin-dependent diabetes mellitus in urban districts of Shanghai (1989–1993). *J Pediatr Endocrinol Metab* 1996: **9**: 469–73.
3. Tood JA. Genetic analysis of type 1 diabetes using whole genome approaches. *Proc Natl Acad Sci U S A* 1995: **92**: 8560–5.
4. Cucca F, Todd JA. HLA susceptibility to type 1 diabetes: methods and mechanisms. In: Browning MJ, McMichael AJ, ed. *HLA and MHC: genes, molecules and functions*. Oxford: BIOS Scientific Publishers, 1996: 383–406.
5. Sjoroos M, Iitia A, Ilonen J, Reijonen H, Lovgren T. Triple-label hybridization assay for type-1 diabetes-related HLA alleles. *Biotechniques* 1995: **18**: 870–7.
6. Sjoroos M, Ilonen J, Reijonen H, Lovgren T. Time-resolved fluorometry based sandwich hybridization assay for HLA-DQA1 typing. *Dis Markers* 1998: **14**: 9–19.
7. She JX. Susceptibility to type I diabetes: HLA-DQ and DR revisited. *Immunol Today* 1996: **17**: 323–9.
8. Nejentsev S, Reijonen H, Adojaan B et al. The effect of HLA-B allele on the IDDM risk defined by DRB1*04 subtypes and DQB1*0302. *Diabetes* 1997: **46**: 1888–92.
9. Rumba I, Denisova A, Sochnev A, Nilsson B, Sanjeevi CB. HLA class II genes in Latvian patients with juvenile rheumatoid arthritis. *Tissue Antigens* 1997: **49**: 56–60.
10. Belchenko I, Sochnew A, Denisova A. An immunogenetic study of native Latvians. *Proc Latvian Acad Sci B* 1994: **558**: 54–6.
11. Gavrilov DK, Kuraeva TL, Dedov II, Sergeev AS, Nosikov VV. Frequency analysis of HLA-DQA1 and HLA-DQB1 gene alleles and suceptibility to type 1 diabetes mellitus in Russian patients. *Acta Diabetol* 1994: **31**: 82–6.
12. Van der Auwera B, Van Waeyenberge C, Schuit F et al. DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous DQA1-0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. Belgian Diabetes Registry. *Diabetes* 1995: **44**: 527–30.
13. Huang HS, Peng JT, She HY et al. HLA-encoded susceptibility to insulin-dependent diabetes mellitus is determined by DR and DQ genes as well as their linkage disequilibria in a Chinese population. *Hum Immunol* 1995: **44**: 210–9.
14. Awata T, Kuzuya T, Matsuda A, Iwamoto Y, Kanazawa Y. Genetic analysis of HLA class II alleles and susceptibility to type 1 (insulin-dependent) diabetes mellitus in Japanese subjects. *Diabetologia* 1992: **35**: 419–24.

V

Adojaan B, Podar T.
Lapseeas alanud insuliinisõltuva suhkurtõve immunoloogilised ja geneetilised tegurid Eestis.
Eesti Arst 1998; N. 6: 488–491.

Eesti Arst, 1998, 6, 488–491

Lapseeas alanud insuliinisõltuva suhkurtõve immunoloogilised ja geneetilised tegurid Eestis

Bela Adojaan Toomas Podar

insuliinisõltuv suhkurtõbi, epidemioloogia, levimus, immunoloogia, geneetika, Eesti

Insuliinisõltuv suhkurtõbi on sageli lapseeas algav raske krooniline haigus. Haiguse etioloogia ei ole siiani veel teada. Seetõttu puudub insuliinisõltuva suhkurtõve profülaktika võimalus. Viimase 20 aasta jooksul kogunenud andmed lubavad järeldada, et insuliinisõltuv suhkurtõbi tekib geneetiliselt vastuvõtlikel indiviididel autoimmuunse mehhanismiga (2,4). Paraku on immunoloogiliste ja geneetiliste tegurite seos insuliinisõltuva suhkurtõvega komplitseeritud. Seetõttu on selle haiguse etioloogia ja patogeneesi paremaks mõistmiseks vaja uurida erineva geneetilise ja keskkonna taustaga populatsioone (5, 9). Praeguseks on informatsioon insuliinisõltuva suhkurtõvega seotud immunoloogiliste ja geneetiliste tegurite leviku kohta Eestis puudulik.

Eeltoodust lähtudes oli käesoleva uuringu peaeesmärgiks teha kindlaks mitme teadaoleva insuliinisõltuva suhkurtõvega seotud immunoloogilise ja geneetilise teguri levik kolmes Eesti elanikkonna rühmas: 1) vastavastatud lapseeas alanud insuliinisõltuva suhkurtõvega patsiendid; 2) lapseeas alanud insuliinisõltuva suhkurtõvega patsientide terved lähisugulased ja 3) üldpopulatsiooni valim.

Eesmärgid: 1) leida uuringurühmades Langerhansi saarekeste vastaste (ICA), glutamaadi dekarboksülaasivastaste (GADA) ja türosiini fosfataasi vastaste antikehade (IA-2A) levik; 2) teha kindlaks HLA-DQBI lookuse insuliinisõltuva suhkurtõve riskiga seotud alleelide ning mõnede teiste HLA alleelide kombinatsioonide esinemissagedus Eesti üldpopulatsioonis ja insuliinisõltuvat suhkurtõbe põdejatel, samuti kaasnevad riskimäärad; 3) jälgida insuliinisõltuvat suhkurtõbe põdejate terveid õdesid-vendi haiguse tekke suhtes kolm aastat; 4) kõrvutada Eesti andmeid teistes riikides saadutega.

Uurimismaterjal ja -meetodid. Esimese uuringurühma moodustasid lapseeas alanud insuliinisõltuvat suhkurtõbe põdejad. Aastail 1993–1994 vastavastatud 29-l alla 15 aasta vanusel haigel määrati antikehi. 97-l insuliinisõltuvat suhkurtõbe põdejal määrati HLA-DR ja HLA-DQ alleele. Teise rühma kuulus 71 lapseeas alanud insuliinisõltuvat suhkurtõbe põdeja tervet õde-venda, keda uuriti nii antikehade kui ka HLA regiooni alleelide esinemise osas ja jälgiti kolm aastat insuliinisõltuva suhkurtõve tekke suhtes. Tervete kontrollrühma kuulus 614 lasteaia- ja koolilast vanuses 3–18 aastat, kellel määrati ICA ja GADA, ning 269 arstiteaduskonna üliõpilast ja doonorit, keda uuriti HLA-DR ja HLA-DQ alleelide suhtes.

ICA määrati immunofluorestsentsmeetodil (6), GADA kvantitatiivse immunopretsipitatsiooni radioligandsel meetodil (8) ja IA-2A radioimmunoloogilisel meetodil (13). ICA tulemuste võrreldavus teiste töödega tagati ICA standardiseerimise referents-standardi kasutamisega (7), GADA ja IA-2A võrreldavus aga positiivse standardseerumi kasutamisega (10, 13). HLA-DR ja HLA-DQ alleele määrati originaalmeetodil Sjöroosi järgi (*simultaneus triple-label hybridization assay for* HLA *alleles*) (12). HLA alleele uuriti kahes etapis. Esmalt uuriti DQB1*0201,

Bela Adojaan, Toomas Podar — Tartu Ülikooli Kliinikumi Endokrinoloogiahaigla

*0301, *0302, *0602/0603 suhtes. Neil, kel esines DQB1*0201, määrati ka DQA1 *0201 ja *0501. Neil, kel esines DQB1*0302, uuriti DRB1*0401, *0402, *0403, *0404, *0405, *0408. DQB1 *0301 ja *0302 positiivseid indiviide uuriti DQB1*0304 suhtes.

Alleelide ja antikehade leviku erinevuse hindamiseks kasutati χ^2- ja Fisheri testi. Normaalse jaotumusega muutujate kirjeldamisel kasutati keskmist ja standardhälvet. Korrelatsioonanalüüs tehti Spearmani meetodil. Statistiliselt oluliseks peeti $P<0,05$. Iga uuritud alleeli jaoks leiti šansside suhe (*odds ratio*) (SS). Vajaduse korral kasutati Haldane'i korrektsiooni. Multiiblite võrdluste korral kasutati P väärtuse korrelatsiooni Bonferroni järgi (3).

Tulemused. Kõige sagedam antikeha vastavastatud lapseeas alanud insuliinisõltuvat suhkurtõbe põdejail oli ICA (79%-l) (vt. joonis 1.) Järgnesid GADA 38%-l ja IA-2A 35%-l juhtudest. Vähemalt üks mõõdetud antikehadest oli leitav 86%-l vastavastatud insuliinisõltuvat suhkurtõbe põdejaist, kõik kolm uuritud antikeha ainult 17%-l indiviididest.

Üks ja rohkem uuritud antikehadest oli leitav 13-l (18%) insuliinisõltuva suhkurtõvega patsientide õel-vennal. Kõige sagedamini leitavaks antikehaks oli GADA — 17%-l. Järgnesid ICA (7%) ja IA-2A (6%). Enamikul sellest rühmast (82%-l) ei leitud ühtegi antikeha.

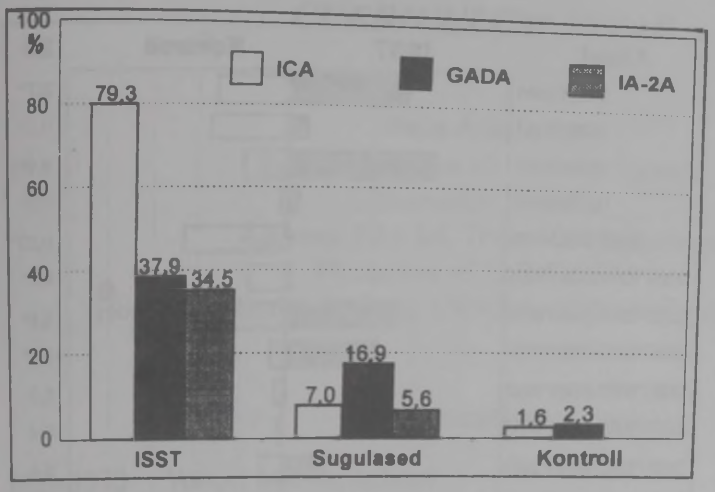
Tervete lasteaia- ja koolilaste seas leiti ICA-d 10-l (1,6%) ja GADA-d 14-l (2,3%). Kaks ICA- ja GADA-positiivset isikut haigestus edaspidi insuliinisõltuvasse suhkurtõvesse. Samu antikehi uuriti 1994. aastal korduvalt neil kaheksal, kellel 1991. aastal leiti seerumist ICA ning kes polnud haigestunud. Selgus, et pooltel neist oli ICA kadunud, GADA tiiter aga vähenenud.

Uuritud alleelidest oli Eestis insuliinisõltuvat suhkurtõbe põdejail kõige sagedamini HLA-DQB1*0302 — 66%-l (vt. joonis 2). Järgnes HLA-DQB1*0201 (55%). Mõlemad alleelid esinesid neil haigetel statistiliselt oluliselt sagedamini kui üldpopulatsioonis. Meie uuritud HLA-DQB1 alleelidest kandis suuremat riski insuliinisõltuva suhkurtõve suhtes HLA-DQB1*0302 (SS 8,9), mis ületas HLA-DQB1*0304 poolt edastatava haigestumisriski (SS 7,3). Viimati nimetatud alleeli esines haigete hulgas küll harva, ainult 5%-l juhtudest. Statistiliselt olulise suurenenud insuliinisõltuva suhkurtõve riskiga oli seotud HLA-DQB1*0201 (SS 2,7; $P<0,05$).

Uuritud HLA-DQB1 alleelidest madalaima insuliinisõltuva suhkurtõve riskiga oli Eestis *0602/0603. See alleel oli 44%-l Eesti populatsioonist ja ainult 2%-l insuliinisõltuvat suhkurtõbe põdejaist. DQB1*0602/0603 vähendas haiguse riski 38 korda võrreldes nendega, kellel seda alleeli ei olnud. Samuti oli madala insuliinisõltuva suhkurtõve riskiga DQB1*0301 (SS 0,2; $P<0,05$).

Neil haigeil kõige sagedamini esinev kahe alleeli kombinatsioon oli HLA-DQB1*0201-DQA1*0501, mida leiti pooltel uurituist. Tervete kontrollrühmas oli seda kombinatsiooni samuti märkimisväärselt — 22%-l. Insuliinisõltuvat suhkurtõbe põdejate hulgas oli sageduselt järgmine kombinatsioon HLA-DQB1*0302-DRB1*0401, mida leiti 43%-l uurituist, tervete seas aga ainult 5%-l. Nende kahe alleelikombinatsiooniga kaasnenud insuliinisõltuva suhkurtõve risk oli vastavalt 3,6 ja 13,9.

71 õdede-vendade rühma liikmest haigestus kolmeaastase jälgimise vältel kolm. Neist kahel oli HLA-DQB1*0201-DQA1*0501 alleelide kombinatsioon, Eesti insuliinisõltuvat suhkurtõbe põdejate sagedaim, ja ühel HLA-DQB1*0302-DRB1*0401. Kõik kolm lähisugulast kuulusid nende 13 hulka, kellel leiti antikehad. Neil kõigil oli leitud vähemalt kahte



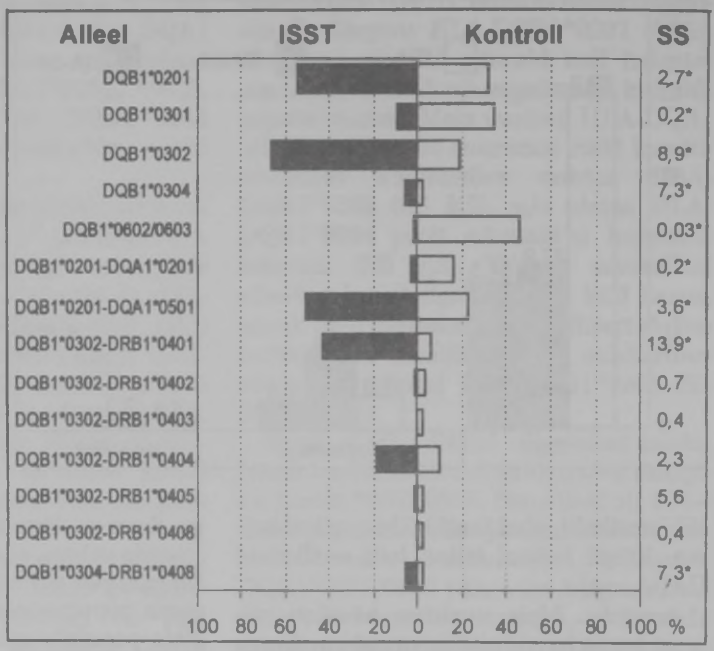
Joonis 1. Langerhansi saarekeste (ICA), glutamaadi dekarboksülaasi (GADA) ja türosiini fosfataasi vastaste (IA-2A) antikehade levimus (%) vastavastatud insuliinisõltuvat suhkurtõbe põdejate (ISST), nende tervete õdede-vendade (sugulased) ja üldpopulatsiooni (kontroll) seas.

liiki antikehi, ühel isegi kõiki uuritud kolme. Kõigil kolmel isikul leiti antikehad GADA vastu.

Arutelu. Meie uurimus käsitleb esimest korda insuliinisõltuva suhkurtõvega seotud antikehade ICA, GADA ja IA-2A levikut Eestis vastavastatud insuliinisõltuva suhkurtõvega laste, nende laste tervete õdede-vendade hulgas ja üldpopulatsiooni valimis. Nimetatud antikehade levik Eestis ei erine märkimisväärselt teistest populatsioonidest (13). Kõige kõrgem kolme antikeha levimus oli ootuspäraselt vastavastatud insuliinisõltuva suhkurtõvega laste seas, mis kinnitab aktiivselt toimivat autoimmuunset protsessi. Antikehade levimusmääralt järgnes õdede-vendade rühm, kelle seas omakorda oli antikehade esinemissagedus suurem kui tervete populatsioonis. Insuliinisõltuvat suhkurtõbe põdejate esimese astme sugulaste suurem risk haigestuda kinnitus, 4% neist haigestus kolme järgneva aasta jooksul.

On leitud, et ICA levimus populatsioonides on umbes 10 korda suurem insuliinisõltuva suhkurtõve levimusest, s.t. enamikul ICA suhtes positiivsetel isikutel ei arene nimetatud haigus mitte kunagi. Üldpopulatsiooni ICA levimuse kõrvutamisel meiega sarnaselt tehtud uurimustega Soomes, Prantsusmaal, Sardiinias ja Rootsis selgus, et esineb tugev seos insuliinisõltuva suhkurtõve haigestumuse ja sama populatsiooni ICA levimuse vahel. Neis populatsioonides, kus haigestumus oli kõrgem, oli suurem ka ICA levimusmäär ($r=0,97$; $P<0,05$) (1).

Meie uurimusega täienes teave mitme HLA regiooni alleelide leviku kohta Eestis nii üldpopulatsioonis kui insuliinisõltuvat suhkurtõbe põdejate seas. Eesti haigete vastuvõtlikkuse ja kaitsva iseloomuga alleelid ei erine teiste Euroopa populatsioonide omast (9, 11). Insuliinisõltuva suhkurtõve suhtes riski vähendavateks alleelideks on Eestis DQB1*0301 ja DQB1*0602/0603, sarnaselt mitme teise populatsiooniga (9). Märkimisväärne on väikese riski alleeli DQB1*0602/0603 suur levimus Eesti populatsioonis, eriti aga tema väga tugev haigestumisriski vähendav toime (neli korda kõrgem kui Soomes). See võib olla üks põhjusi, miks Eestis on haigestumus üle kolme korra väiksem kui Soomes. Uuringu käigus leidsime senini haruldasena kirjeldatud alleelide kombinatsiooni DQB1*0304-DRB1*0408, mida esines Eesti üldpopulatsioonis väga harva (0,7%), ent oluliselt sagedamini insuliinisõltuvat suhkurtõbe põdejate rühmas (5%).



Joonis 2. HLA-DQB1 alleelide ning HLA-DQB1-DQA1 ja HLA-DQB1-DRB1 alleelikombinatsioonide levimus (%) insuliinisõltuvat suhkurtõbe põdejatel (ISST) ja üldpopulatsiooni (kontroll) seas ning äärmiste suhe (SS). *SS statistiliselt oluline; P<0,05.

KIRJANDUS: 1. *Adojaan, B., Knip, M., Vähäsalo, P. a.o.* Diabetes Care, 1996, 19, 1452–1453. — 2. *Adojaan, B.* Eesti Arst, 1996, 4, 322–324. — 3. *Armitage, P., Berry, G.* In: Statistical methods in medical research. 3rd ed. Oxford, 1994, 331. — 4. *Bottazzo, G. F., Florin-Christensen, A., Doniach, D.* Lancet, 1974, II, 1279–1283. — 5. *Green, A., Gale, E. A. M., Patterson, C. C.* Lancet, 1992, 339, 905–909. — 6. *Karjalainen, J. K.* Diabetes, 1990, 39, 1144–1150. — 7. *Lernmark, Å., Molenaar, J. L., van Beers, V. A. M. a.o.* Diabetologia, 1991, 34, 534–535. — 8. *Petersen, J. S., Kim, R. H., Moody, A. a.o.* Diabetes, 1994, 43, 459–467. — 9. *Rønningen, K. S., Spurkland, A., Tait, B. D. a.o.* In: HLA, 1991, Oxford, 12–15. — 10. *Schmidli, R. S., Colman, P. G., Bonifacio, E.* Diabetes, 1995, 44, 636–640. — 11. *She, J. X.* Immunol. Today, 1996, 17, 323–329. — 12. *Sjöroos, M., Iitiä, A., Ilonen, J. a.o.* Biotechniques, 1995, 18, 870–877. — 13. *Wiest-Landenburger, U., Hartmann, R., Berling, K. a.o.* Diabetes, 1997, 46, 565–571.

Summary

Immune and genetic factors of childhood onset IDDM in Estonia. Newly diagnosed childhood onset patients with insulin-dependent diabetes mellitus (IDDM), their healthy siblings and background population were investigated for the prevalence of IDDM-associated antibodies and HLA region alleles. Islet cell (ICA), glutamic acid decarboxylase (GADA) and tyrosine phosphatase (IA-2A) antibodies and selected HLA-DRB1, DQA1 and DQB1 alleles were detected. The most frequent IDDM associated antibody in newly diagnosed childhood onset IDDM patients in Estonia is ICA (79%) followed by GADA (38%) and IA-2A (35%). Data from several countries indicates that the incidence of IDDM is closely associated with the frequency of ICA in the background population. The most frequent of the studied HLA alleles among Estonian IDDM patients is HLA-DQB1*0302 (present in 66%) followed by HLA-DQB1*0201 (55%). The single HLA-DQB1 allele carrying the highest risk for IDDM is HLA-DQB1*0302 (OR 8.9) in Estonia followed by the novel HLA-DQB1*0304 allele (OR 7.3), although it was present only in 5% of IDDM cases. The allele carrying the highest protection against IDDM in Estonia is HLA-DQB1*0602/0603. This allele was present in 44% of the Estonian population and reduced the risk of IDDM by factor of 38 compared to those lacking this allele.

CURRICULUM VITAE

Bela Adojaan
Born September 10, 1954 in Vormsi
Citizenship: Estonian
Address: Pikk 64, Tartu 50603, Estonia
Phone/fax +372 7 436 361
Hospital of Endocrinology, Clinicum of the University of Tartu

Education

1963–1970	Vormsi Middle School
1970–1973	Haapsalu 1-st Secondary School
1973–1979	Faculty of Medicine, University of Tartu
1993–1997	Postgraduate study at the Department of Internal Medicine, University of Tartu
	Estonian, Russian, English (specialty related)

Professional employment

1979–1980	Laboratory of hormonal regulation, University of Tartu
1980–1998	Hospital of Endocrinology, Clinicum of the University of Tartu

Scientific work

Epidemiology, immunology and genetics of insulin-dependent diabetes mellitus
Growth disorders
Eight papers on these topics have been published in international peer-reviewed Current Contents listed journals and four in Estonian Doctor, during the past five years, five and three, respectively

Social activities

1997–	Estonian Osteoporosis Club, Chairwoman,
1995–1998	Estonian Endocrine Society, Secretary,
1998	Organizing committee of the Baltic Conference of Endocrinology, Member

Special courses

1992	Pediatric Clinic of the University of Helsinki, one month
1993	Pediatric Clinic and immunology lab of the University of Oulu, two months
1995	Endocrinology lab of the University of Ulm, three months
1992–1998	Specialized endocrinology courses in Poland, Finland, Sweden, Italy, Switzerland, USA, Spain

Teaching

Lectures to doctors in training in endocrinology, pediatricians
Co-author, "Physical development of children. Growth and growth disorders", Tartu, 1998

ELULOOKIRJELDUS

Bela Adojaan
Sündinud 10. septembril 1954 Vormsis
Kodakondsus: Eesti
Aadress: Pikk 64, Tartu 50603, Eesti
Tel/Faks (27) 436 361
Tartu Ülikooli Kliinikumi Endokrinoloogiahaigla

Haridus

1963–1970	Vormsi Põhikool
1970–1973	Haapsalu 1. Keskkool
1973–1979	Tartu Ülikooli arstiteaduskond
1993–1997	Tartu Ülikooli sisekliinik, doktorantuur
	Eesti, vene, inglise keel (eriala piires)

Erialane teenistuskäik

1979–1980	Tartu Ülikooli hormonaalregulatsiooni labor
1980–1998	Tartu Ülikooli kliinikumi endokrinoloogiahaigla

Teadustegevus

Insuliinisõltuva suhkurtõve epidemioloogia, immunoloogia, geneetika
Kasvuhäired
Nendel teemadel on ilmunud kaheksa artiklit rahvusvahelistes eelrefereeritavates ja Current Contentsis tsiteeritavates ajakirjades ja neli Eesti Arstis Viimasel viiel aastal on ilmunud viis artiklit rahvusvahelistes eelrefereeritavates ja Current Contentsis tsiteeritavates ajakirjades ning kolm Eesti Arstis

Erialane täiendus

1992	Helsingi Ülikooli lastekliinik, ühekuune täiendus laste-endokrinoloogias
1993	Oulu Ülikooli lastekliinik ja immunoloogia laboratoorium, kahekuune täiendus

1995	Ulmi Ülikooli endokrinoloogia laboratoorium, kolmekuune täiendus
1992–1998	Endokrinoloogiaalased täienduskursused Poolas, Soomes, Rootsis, Itaalias, Šveitsis, USA-s, Hispaanias

Õppetöö

Loengud täiendusarstidele endokrinoloogiast.
Õppevahendi "Lapse füüsiline areng. Kasvamine ja kasvuhäired", Tartu, 1998 kaasautor

Ühiskondlik tegevus

Alates 1997–	Eesti osteoporoosi klubi, juhataja
1995–1998	Eesti Endokrinoloogide Seltsi sekretär
1998	Balti Endokrinoloogiakonverentsi korralduskomitee liige

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos.** The natural course of gastric ulcer in connection with chronic gastritis and Helicobacter pylori. Tartu, 1991.
2. **Mihkel Zilmer.** Na-pump in normal and tumorous brain tissues: Structural functional a. tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar.** Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik.** Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation) Tartu, 1992.
5. **Ants Peetsalu.** Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and Helicobacter pylori. Tartu, 1992.
6. **Marika Mikelsaar.** Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus.** Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar.** Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm.** On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross.** Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo.** Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik.** The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa.** Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk.** Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar.** Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi.** The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar.** Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang.** The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak.** Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.

20. **Tõnis Karki.** Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.
21. **Reet Mändar.** Vaginal microflora during pregnancy and its transmission to newborn. Tartu 1996.
22. **Triin Remmel.** Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu 1996.
23. **Toomas Kivastik.** Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk.** Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets.** Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik.** Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi.** Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo.** Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber.** *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla.** Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk.** Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf.** Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv.** Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi.** Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva.** Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin.** Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev.** Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in udmurtia. Tartu, 1998.
38. **Allen Kaasik.** Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto.** Pharmacological studies on anxiogenic and antiaggressive propreties of antidepressants. Tartu, 1998.
40. **Maire Vasar.** Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge.** Humoral immune responses to allergens in early childhood. Tartu, 1998.

42. **Heli Grünberg.** The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.
43. **Epp Sepp.** Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots.** Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe.** Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv.** Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.

ISSN 1024–395X
ISBN 9985–56–383–2