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RESEARCH ARTICLE

Genetic screening for individuals at high risk for type 1 diabetes in the general population using HLA Class II alleles as disease markers. A comparison between three European populations with variable rates of disease incidence

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Abstract

Background To develop screening strategies for identification of individuals at increased genetic risk for type 1 diabetes in three populations with variable disease incidence rates and distinct ethnic origin.

Methods A stepwise HLA DQB1-DQA1-DRB1-based screening approach was evaluated. Patients with childhood-onset type 1 diabetes were recruited from Finland ($n = 1739$), Hungary ($n = 149$), and Greece ($n = 119$). Consecutive newborns (2568 from Finland and 1047 from Greece) or healthy schoolchildren ($n = 177$ from Hungary) served as controls.

Results The DQB1*02/0302 genotype conferred the highest disease risk in all populations. The DQB1*02/y (y $\neq$ DQB1*0301,*0302,*0602,*0603, *0604) genotypes were more common and conferred a higher disease risk in the Greek population (OR 4.9) compared to the Finns (OR 1.2). DQB1*0302/x (x $\neq$ DQB1*02, *0301, *0602, *0603, *0604) genotypes were, in contrast, more prevalent among Finnish cases (32.7%) as compared to Hungarians (18.1%) or Greeks (13.5%). The protective DQB1*0602 or *0603 positive genotypes were most common in the Finns, while DQB1*0301 was more common in Hungarians and Greeks. In all groups, DQA1 and DRB1*04 typing considerably increased the sensitivity of the DQB1-based screening. The different high-risk genotype combinations present in about 10% of the background population had a diagnostic sensitivity of 60% in Finland and 80% in Hungary and Greece.

Conclusions HLA DR-DQ-based screening is a feasible tool for the identification of individuals at increased genetic risk for type 1 diabetes in populations with diverse genetic background. The risk markers should, however, be individually selected for the target population since the screening efficiency of various markers is highly dependent on the ethnic group studied.

Keywords type 1 diabetes mellitus; genetic susceptibility; HLA DQ; genetic screening; incidence

Introduction

Immune-mediated destruction of the pancreatic β-cells develops in individuals carrying disease-predisposing gene variants. Since the initiation of this process may occur early in life, follow-up observations on infants at increased genetic risk are critical for understanding type 1 diabetes etiology [1,2]. To adequately address the question of recruitment of study subjects for such trials, strategies using various approaches for the identification of individuals at increased genetic risk need to be explored.

Ongoing follow-up studies focusing on type 1 diabetes etiology and prevention target high-risk cohorts recruited using genetic screening. In the DAISY (Diabetes Autoimmunity Study in the Young) program, newborn infants carrying diabetes-associated HLA DRB1 and DQB1 alleles are selected for surveillance of diabetes-related autoimmunity [3]. Similarly, in the staging of diabetes risk of antibody-positive first-degree relatives of affected individuals, protective genetic markers are used as an exclusion criterion in The Diabetes Prevention Trial-Type 1 (DPT-1) [4]. The international nutritional primary prevention study, Trial to Reduce IDDM in the Genetically at Risk (TRIGR), is being performed in newborn infants with at least one first-degree relative affected by T1D (type 1 diabetes) who carry high-risk HLA genotypes [5]. In addition, a secondary prevention trial using intranasal insulin is underway in autoantibody-positive young children genetically at risk and recruited from the general population in the framework of the Type 1 Diabetes Prediction and Prevention (DIPP) project [6]. In the DIPP study, genetic testing is performed from cord blood samples and the screening approach currently used selects children with DQB1*02/*0302 and DQB1*0302/x genotypes where x is any allele except *02 or a protective one (*0301 or *0602) [7]. In one of the three participating regions also, boys with the DQA1*05/c-DQB1*02/y risk genotype (c $\neq$ DQA1*0201 and y $\neq$ DQB1*0301 or *0302 or *0602 or *0603) are included in the follow-up. This approach was chosen on the basis of a case–control series, which implicated a sensitivity of 64.2% and a specificity of 86.3% for the basic genotyping criteria [8]. Improvements in the sensitivity and specificity of the genetic screening strategy would enable a higher number of future affected cases to be identified and would facilitate the observation of individuals with more diverse risk genotypes. In addition, a higher specificity would substantially reduce the number of low-risk individuals followed and would consequently lead to lower costs [9].

The genetic background of type 1 diabetes is polygenic where the major disease locus is located in the HLA complex [10]. Most of the disease risk conferred by the HLA complex originates from the DQ genotype; whereas certain DRB1 variants modify the effect of specific DQ haplotypes [11,12]. Owing to the strong linkage disequilibrium between the HLA genes, the DQA1 alleles can, in most cases, be deduced from the DQB1 variant. Therefore, DQA1 typing is informative only when a DQB1 allele is present in haplotypes carrying different DQA1 alleles. The genetic risk assessment is further complicated by the fact that the frequencies of various susceptible and protective alleles show substantial ethnic variation [12].

In this study, we compared the feasibility of various combinations of disease markers for genetic screening between the high-incidence Finnish and the low-incidence Hungarian and Greek populations. In addition, we explored the options for improvements in the screening strategy currently used in Finland by evaluating the effect of genotyping for informative DQA1 and DRB1 alleles on diagnostic sensitivity and specificity of the DQB1-based screening.

Subjects and methods

All patients presented with classical T1D and were diagnosed according to the WHO criteria. All cases in the Finnish and Hungarian, and 95% in the Greek cohort were diagnosed before the age of 15 years and were of Caucasian origin. The local Ethics Committees had approved the study and informed consent was obtained from the participating subjects and/or from their parents.

Finnish patients and controls

The Finnish patients ($n = 1739$) were derived from four university departments [Turku ($n = 385$), Tampere ($n = 219$), Oulu ($n = 322$), Helsinki ($n = 428$)], and several other hospitals located all over Finland also contributed to the study population ($n = 385$). The patients comprised 788 boys (45.3%) and 951 girls (54.7%). Mean age at diagnosis was 8.49 ± 4.36 years. A cohort of infants born consecutively in the regions of Turku, Oulu, and Tampere were used as controls and genotyped for selected DQB1 alleles and further genotyped for specific DQA1 and DRB1*04 alleles ($n = 2568$, 52.7% boys and 47.3% girls).

Greek patients and controls

Blood samples of Greek patients ($n = 119$) were collected at the Diabetes Center of the Department of Pediatrics, University of Athens, Faculty of Nursing (62 boys – 52.1% – and 57 girls – 47.9% – ; mean age at diagnosis 9.3 ± 5.2 years). Control samples ($n = 1047$) were collected from a cohort of newborns delivered in three maternity hospitals in the Athens region (518 boys – 49.5% – and 529 – girls – 50.5% –).

Hungarian patients and controls

Newly diagnosed cases ($n = 149$) registered between January 1, 1980 and December 31, 1996 in the Baranya County (southern Hungary) were sampled. Patients were

identified through the Hungarian Childhood Diabetes Registry that ascertains at least 96% of the Hungarian children developing type 1 diabetes before the age of 15 years [13]. The patient series comprised 71 boys (47.7%) and 78 girls (52.3%) with a mean age at diagnosis of 8.8 ± 4.2 years. Healthy, racially matched schoolchildren randomly selected from the same region were used as controls ($n = 177$; 86 boys – 48.6% – and 91 girls with a mean age of 10.8 ± 2.6 years).

HLA genotyping

In the Finnish and Greek cohorts, HLA typing was performed using a high-throughput fluorescence-based oligonucleotide hybridization method [7] [8,14]. This enabled identification of DQB1*02, *0301, *0302, *0602, *0603, *0604, DQA1*0201, *03, *05 and DRB1*0401, *0402, *0403/06, *0404, *0405, *0407, and *0408 alleles. DQB1 typing was performed as an initial step in all samples, then DQA1 typing was done in subjects positive for DQB1*02, while DRB1*04 alleles were analyzed in subjects positive for DQB1*0302. In cases with protective combinations comprising DQB1*06 alleles or additional neutral alleles, the differentiation between DQB1*0602/*0603, *0603/*0603, *0603/*0604, *0602/*0604, and *0603/x genotypes was not possible and they are displayed as DQB1*0602/3/4 in Table 1. In the Hungarian material, HLA DRB1, DQA1, and DQB1 alleles were identified using the phototyping method [15] and confirmed by direct sequencing where necessary.

Statistical analysis

Chi-square statistics or the Fisher's exact test were used for comparisons of the frequencies of analyzed genotypes. A p-value of 0.05 or less was considered significant. Diagnostic sensitivity, specificity, and positive predictive values were calculated with standard methods. The study had an 80% power to detect a significant difference of 4% in genotype frequencies in the Finnish, 12% in the Greek, and 15% in the Hungarian sample.

Results

HLA DQB1 genotype frequencies in the three populations are shown in Table 1. The earlier estimated power of the screening strategy currently used for the Finnish population was confirmed by the results. The combined sensitivity for the three risk genotypes originally selected for screening in Finns (DQB1*02/*0302, OR (odds ratio) 12.2; DQB1*0302/x, x ≠ DQB1*02, *0301, *0602, *0603, *0604, OR 4.5; DQB1*0302/*0604, OR 5.7) was 63.14%, and specificity was 86.72%. In addition, DQB1*02/y (y ≠ DQB1*0301, *0302, *0602, *0603, *0604), *02/*0604,

Table 1. Disease risk conferred by HLA DQB1 genotypes in three ethnic groups with high and low incidence rate of type 1 diabetes

	Finland						Hungary						Greece					
	Diabetes		Control				Diabetes		Controls				Diabetes		Controls			
	$n = 1739$		$n = 2568$				$n = 149$		$n = 177$				$n = 119$		$n = 1047$			
DQB1	n	%	n	%	OR	p	n	%	n	%	OR	p	n	%	n	%	OR	p
02/y[a]	248	14.26	310	12.07	1.21	0.04	39	26.17	31	17.51	1.67		49	41.18	131	12.51	4.89	$<10^{-6}$
02,0301	40	2.30	92	3.58	0.63	0.02	2	1.34	19	10.73	0.11	3×10^{-4}	3	2.52	95	9.07	0.26	0.02
02,0302	464	26.68	74	2.88	12.27	$<10^{-6}$	48	32.21	3	1.69	27.56	$<10^{-6}$	26	21.85	21	2.01	13.66	$<10^{-6}$
02,0304	5	0.29	0		16.29	0.01	1	0.67	0		3.59		0		2	0.19	1.75	
02,0602	9	0.52	100	3.89	0.13	$<10^{-6}$	0		3	1.69	0.17		0		15	1.43	0.28	
02,0603	10	0.58	57	2.22	0.25	3.2×10^{-5}	2	1.34	5	2.82	0.47		1	0.84	16	1.53	0.55	
02,0604	26	1.50	21	0.82	1.84	0.05	5	3.36	4	2.26	1.50		2	1.68	13	1.24	1.36	
0301/z[b]	30	1.73	226	8.80	0.18	$<10^{-6}$	7	4.70	41	23.16	0.16	5×10^{-6}	3	2.52	350	33.43	0.05	$<10^{-6}$
0301,0302	68	3.91	70	2.73	1.45	0.038	4	2.68	2	1.13	2.41		5	4.20	44	4.20	1.00	
0301,0602	2	0.12	89	3.47	0.03	$<10^{-6}$	0		4	2.26	0.13		0		23	2.20	0.18	
0301,0603	4	0.23	45	1.75	0.13	8×10^{-6}	1	0.67	4	2.26	0.29		0		31	2.96	0.14	0.03
0301,0604	4	0.23	19	0.74	0.31	0.04	0		1	0.56	0.39		0		14	1.34	0.30	
0302/x[c]	569	32.72	250	9.74	4.51	$<10^{-6}$	27	18.12	10	5.65	3.70	8×10^{-4}	16	13.45	39	3.72	4.01	7×10^{-6}
0302,0602	20	1.15	79	3.08	0.37	5.4×10^{-5}	0		0				0		4	0.38	0.97	
0302,0603	57	3.28	50	1.95	1.71	8×10^{-3}	1	0.67	2	1.13	0.59		0		4	0.38	0.97	
0302,0604	65	3.74	17	0.66	5.83	$<10^{-6}$	4	2.68	0		10.98	0.04	1	0.84	1	0.10	8.86	
0304,0604	3	0.17	0		10.35		0		0				0		0			
0602/q[d]	9	0.52	341	13.28	0.03	$<10^{-6}$	1	0.67	11	6.21	0.10	6×10^{-3}	0		39	3.72	0.11	0.01
0602/3/4	7	0.40	326	12.69	0.03	$<10^{-6}$	0		8	4.52	0.07	7×10^{-3}	0		57	5.44	0.07	2×10^{-3}
0604/p[e]	21	1.21	76	2.96	0.40	2.2×10^{-4}	0		4	2.26	0.13		7	5.88	72	6.88	0.85	
x/x[a]	78	4.49	326	12.69	0.32	$<10^{-6}$	7	4.70	25	14.12	0.30	8×10^{-3}	6	5.04	76	7.26	0.68	

[a] y ≠ DQB1*0301, *0302, *0602, *0603, *0604.
[b] z ≠ DQB1*02, *0302, *0602, *0603, *0604.
[c] x ≠ DQB1*02, *0301, *0602, *0603, *0604.
[d] q ≠ DQB1*02, *0301, *0302, *0603, *0604.
[e] p ≠ DQB1*02, *0301, *0302, *0602, *0603.

*0301/*0302, and *0302/*0603 genotypes were also found to confer significant disease risk in the Finnish population. As a new finding in Finns, we observed that the highest risk was conferred by the DQB1*02/*0304 genotype (OR 16.3, $p = 0.01$), and that the *0304/*0604 genotype was present only in affected cases, although at a low frequency.

In the Hungarian and Greek populations, the highest disease risk was conferred by the *02/*0302 genotype, when DQB1 genotypes were considered. Interestingly, this genotype was more common and conferred a higher disease risk in Hungarians than in the other two ethnic groups. The DQB1*0302/x genotype conferred disease risk in all three populations; however, it was more common in the Finnish population as compared to Hungarians and Greeks (9.7% vs 5.7 and 4.0%, $p < 10^{-6}$). In Greeks, the 02/y genotype conferred a high disease risk (OR 4.9, $p < 10^{-6}$), while a considerably weaker effect was detected in Finns (OR 1.2, $p = 0.04$). In the smaller Hungarian series, the difference between patients and controls did not reach statistical significance (OR 1.67). A protective effect of the DQB1*0301/z (z ≠ DQB1*02, *0302, *0602, *0603, *0604) genotype was seen in all three populations, this genotype being more common in Hungarians and Greeks (23.2 and 33.4% vs 8.8% in Finns) with the most pronounced effect in Greeks (OR 0.05, $p < 10^{-6}$). Both DQB1*0602/q and *0602/03/04 genotypes were protective in all three series. They were most common among Finns (13.3%, 12.7%) and were substantially less prevalent in the Greek population (3.7%, 5.4%) and in Hungarians (6.2%, 4.5%).

When DQA1 alleles were analyzed in the Finnish DQB1*02/y genotypes, individuals carrying DQA1*03/*05 alleles had significantly higher risk than other subgroups (Table 2). In Greeks, a high disease risk was seen in association with DQB1*02/y-DQA1*05 or *03 or *03/*05 genotypes, whereas a significant protection was conferred by the DQB1*02/y-DQA1*02 combination, which effect was not as prominent in the other ethnic groups.

The predisposing effect of DQB1*02/*0302 genotypes was higher in all three populations, when DQA1*03/*05 alleles were present. The DQA1 alleles did not significantly change the protective effect of DQB1*02/*0301, DQB1*02/*0602, and DQB1*02/*0603 genotypes (data not shown). However, when the DQB1*02/*0604 genotype was analyzed in the Finnish population, only those positive for the DQA1*05 allele conferred significant disease risk (OR 2.3, $p = 0.015$). A similar tendency was seen in the other two ethnic groups, although the differences remained nonsignificant because of the small numbers.

When DRB1*04 alleles were analyzed (Table 3.) in the DQB1*02/*0302- DQA1*0201/*03 genotypes, *0402 allele was associated with significant disease risk in the Greeks, while *0401 and *0404 alleles conferred susceptibility in Finns. The *0403 allele was increased in this set of Greek controls as compared to cases, but the difference was not significant. In the DQB1*02/0302-DQA1*03/*05 group, an increased disease risk was detected in association with the *0401 and *0404 alleles in the Finns, with the *0401 and *0402 alleles in Hungarians, and with the *0401, *0402, and *0405 alleles in the Greek population. A differential effect of DRB1*04 alleles was also detectable in DQB1*0301/*0302 genotypes in the Finnish population. The *0401 allele was associated with susceptibility, while *0403 conferred resistance to T1D in the Finnish series. The strongest effect of the DRB1*04 alleles on diabetes susceptibility was detected among those with the DQB1*0302/x genotype in all three populations. DRB1*0401 and 0404 alleles conferred susceptibility in the Finnish group, while 0403 conferred

Table 2. The effect of HLA DQA1 alleles on disease risk conferred by selected DQB1*02 positive genotypes in three ethnic groups with high and low incidence rates of type 1 diabetes

		Finland						Hungary						Greece					
		Diabetes		Control				Diabetes		Controls				Diabetes		Controls			
		$n = 1739$		$n = 2568$				$n = 149$		$n = 177$				$n = 119$		$n = 1047$			
DQB1	DQA1	*n*	%	*n*	%	OR	*p*	*n*	%	*n*	%	OR	*p*	*n*	%	*n*	%	OR	*p*
02/y[a]		248	14.26	310	12.07	1.21	0.04	39	26.17	31	17.51	1.67		49	41.18	131	12.51	4.89	<10−6
02/y	05/a[b]	156	9.11	192	7.49	1.24		32	21.48	16	9.04	2.75	2.7×10^{-3}	37	31.09	58	5.53	7.69	$<10^{-6}$
02/y	03,05	47	2.74	21	0.82	3.42	$<10^{-6}$							5	4.20	4	0.38	11.44	9×10^{-4}
02/y	03/b[c]	0		2	0.08	0.30		2	1.34	1	0.56	2.39		6	5.04	4	0.38	13.85	1×10^{-4}
02/y	0201,05	8	0.47	27	1.05	0.44		2	1.34	4	2.26	0.59		1	0.84	15	1.43	0.58	
02/y	0201/c[d]	27	1.58	63	2.46	0.64		3	2.01	10	5.65	0.34		0		48	4.58	0.09	5×10^{-3}
02,0302		464	26.68	74	2.88	12.27	$<10^{-6}$	48	32.21	3	1.69	27.56	<10−6	26	21.85	21	2.01	13.66	$<10^{-6}$
02,0302	0201,03	49	2.86	27	1.05	2.77	2×10^{-5}	2	1.34	2	1.13	1.19		3	2.52	10	0.95	2.68	
02,0302	03,05	397	23.18	47	1.83	16.17	$<10^{-6}$	46	30.87	1	0.56	78.60	$<10^{-8}$	22	18.49	9	0.86	26.16	$<10^{-6}$
02,0604		26	1.50	21	0.82	1.84	0.05	5	3.36	4	2.26	1.50		2	1.68	13	1.24	1.36	
02,0604	0201/c	2	0.12	5	0.19	0.60		0		2	1.13	0.23		0		6	0.57	0.67	
02,0604	05/a	24	1.40	16	0.62	2.27	0.015	5	3.36	2	1.13	3.04		1	0.84	7	0.67	1.26	

[a] y ≠ DQB1*0301, *0302, *0602, *0603, *0604
[b] a ≠ DQA1*0201, *03
[c] b ≠ DQA1*0201, *05
[d] c ≠ DQB1*03, *05

Table 3. The effect of HLA DRB1*04 alleles on disease risk conferred by DQB1*0302 positive genotypes in three ethnic groups with high and low incidence rates of type 1 diabetes

Genotype			Finland						Hungary						Greece					
			Diabetes		Control				Diabetes		Control				Diabetes		Control			
			$n = 1712$		$n = 2563$				$n = 149$		$n = 177$				$n = 119$		$n = 1047$			
DQB1	DQA1	DRB1*04	*n*	%	*n*	%	OR	*p*	*n*	%	*n*	%	OR	*p*	*n*	%	*n*	%	OR	*p*
02,0302	0201,03		49	2.86	27	1.05	2.77	2×10^{-5}	2	1.34	2	1.13	1.19		3	2.52	10	0.95	2.68	
02,0302	0201,03	0401	32	1.87	16	0.62	3.03	3×10^{-4}	2	1.34	1	0.56	2.39		0		2	0.19	1.75	
02,0302	0201,03	0402													2	1.68	0		44.57	0.01
02,0302	0201,03	0403	0		1	0.04	0.50								0		6	0.57	0.67	
02,0302	0201,03	0404	17	0.99	9	0.35	2.85	0.01	0		1	0.56	0.39		0		1	0.09	2.92	
02,0302	0201,03	0405	0		1	0.04	0.50								1	0.84	0		26.52	
02,0302	03,05		397	23.18	47	1.83	16.17	$<10^{-6}$	46	30.87	1	0.56	78.60	$<10^{-8}$	22	18.49	9	0.86	26.16	$<10^{-6}$
02,0302	03,05	0401	294	17.17	28	1.09	18.78	$<10^{-6}$	29	19.46	0		86.91	$<10^{-8}$	5	4.20	2	0.19	22.92	1.8×10^{-4}
02,0302	03,05	0402	1	0.06	1	0.04	1.50		11	7.38	0		29.48	1×10^{-4}	9	7.56	0		180.11	$<10^{-8}$
02,0302	03,05	0403	5	0.29	3	0.12	2.50								2	1.68	3	0.28	5.95	
02,0302	03,05	0404	94	5.49	15	0.59	9.87	$<10^{-6}$	1	0.67	0		3.59		2	1.68	2	0.19	8.93	
02,0302	03,05	0405	2	0.12	0		7.50		3	2.01	0		8.48		4	3.36	1	0.09	36.38	4.8×10^{-4}
02,0302	03,05	0408	1	0.06	0		4.50		2	1.34	1	0.56	2.39		0		1	0.09	2.92	
0301,0302			68	3.91	70	2.73	1.45	0.038	4	2.68	2	1.13	2.41		5	4.20	44	4.20	1.00	
0301,0302		0401/k[b]	55	3.21	40	1.56	2.09	5×10^{-4}	1	0.67	0		3.59		1	0.84	2	0.19	4.43	
0301,0302		0402/l[c]	0		1	0.04	0.50		0		0				2	1.68	7	0.66	2.54	
0301,0302		0403/m[d]	0		8	0.31	0.09	0.017	0		0				1	0.84	17	1.61	0.51	
0301,0302		0404/n[e]	12	0.70	21	0.82	0.85		2	1.34	0		6.02		1	0.84	7	0.66	1.26	
0301,0302		0405/o[f]	1	0.06	0		4.50								0		5	0.47	0.79	
0302/x[a]			569	32.72	250	9.74	4.51	$<10^{-6}$	27	18.12	10	5.65	3.70	8×10^{-4}	16	13.45	39	3.72	4.01	7×10^{-6}
0302/x		0401/k	424	24.77	124	4.84	6.48	$<10^{-6}$	16	10.74	2	1.13	10.53	1×10^{-4}	5	4.20	0		100.63	1×10^{-5}
0302/x		0402/l	0		2	0.08	0.30		7	4.70	1	0.56	8.68	0.019	5	4.20	10	0.95	4.55	0.01
0302/x		0401,0404	45	2.63	18	0.70	3.82	$<10^{-6}$	1	0.67	2	1.13	0.59		1	0.84	0		26.52	
0302/x		0401,0402	1	0.06	0		4.50		0		1	0.56	0.39		2	1.68	0		44.57	0.01
0302/x		0404/n	93	5.43	82	3.20	1.74	4×10^{-4}	0		3	1.69	0.17		0		2	0.19	1.75	
0302/x		0403/m	0		21	0.82	0.03	2×10^{-5}							0		20	1.89	0.21	
0302/x		0402,0405													2	1.68	0		44.57	0.01
0302,0603			57	3.28	50	1.95	1.71	8×10^{-3}	1	0.67	2	1.13	0.59		0		4	0.38	0.97	
0302,0603		0401	44	2.57	28	1.09	2.39	3.7×10^{-4}	1	0.67	1	0.56	1,19							
0302,0603		0403	0		5	0.20	0.14		0		1	0.56	0.39							
0302,0603		0404	11	0.64	17	0.66	0.97													
0302,0603		0405	2	0.12	0		7.50								0		1	0.09	2.92	

[a] x≠DQB1*02, *0301, *0602, *0603, *0604.
[b] k≠DRB1*0402, *0403, *0404, *0405.
[c] l≠DRB1*0401, *0403, *0404, *0405.
[d] m≠DRB1*0401, *0402, *0404, *0405.
[e] n≠DRB1*0401, *0402, *0403, *0405.
[f] o≠DRB1*0401, *0402, *0403, *0404.

significant protection. The DRB1*0401 and 0402 alleles were associated with increased risk in Hungarians and Greeks, similar to the *0402/*0405 combination in the latter series. Among those with the DQB1*0302/*0602 and *0302/*0604 genotypes, the DRB1 alleles did not modify the disease associations (data not shown). However, the DRB1*0401 allele was associated with disease in those with the DQB1*0302/*0603 genotype among Finns (OR 2.4, $p = 3.7 \times 10^{-4}$), but not in the other two cohorts.

The relation between HLA markers and gender was also analyzed. HLA DQA1*05/a-DQB1*02/y (where a $\neq$ DQA1*0201 or *03 and y $\neq$ DQB1*0301, *0302, *0602, or *0603) genotypes were significantly more common among cases in boys (15.5 vs 7.9%, OR 2.1, 95% CI: 1.6–2.8; $p < 10^{-6}$) in the Finnish population, while no difference was seen in girls (10.6% vs 8.9%, $p =$ ns). The risk conferred by this particular combination in boys was even higher in the Hungarian population (32.9% in cases vs 8.4% in controls, OR 5.3, 95% CI:2.0–14.7; $p = 2.7 \times 10^{-4}$) and in the Greek (31.1% vs 5.5%, OR 7.7, 95%CI: 4.7–12.8; $p < 10^{-6}$).

We analyzed the diagnostic sensitivity and specificity of various DQB1, DQB1-DQA1, and DQB1-DQA1-DRB1 genotype combinations. The relation of sensitivity to the background frequency of various markers in the three populations (100-specificity) is depicted in Figure 1. In all three ethnic groups, DQB1 screening gave the lowest sensitivity values, and additional DQA1 and DRB1*04 typing increased the sensitivity of various combinations. Interestingly, the difference between the three typing approaches was smallest for the Finnish population.

All DRB1-DQA1-DQB1 genotypes obtained in the three ethnic groups were sorted according to odds ratio, and the combinations present in about 10% of the background population were selected and compared in Table 4. The diagnostic sensitivity value of the risk genotypes was 63.1% for the Finnish population, while it was 79.2% in Hungarians and 81.5% in Greeks (Table 4). The positive predictive value (PPV) was 4.2% in the Finnish population, while it was lower in Hungarians and Greeks (1.1 and 1.2 respectively).

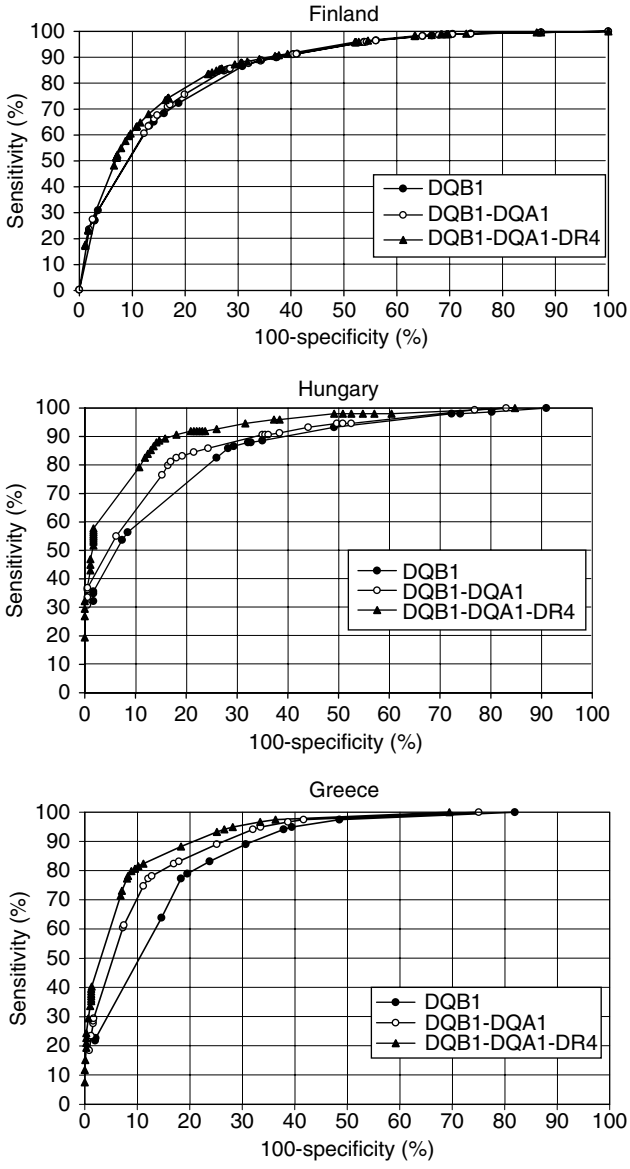

Figure 1. Sensitivity of various HLA screening approaches for type 1 diabetes in populations with high and low disease incidence

Discussion

In the present study, we compared the diagnostic value of HLA DQB1-DQA1-DRB1 risk markers as tools for the identification of individuals at increased risk of type 1 diabetes in three European populations with different disease incidence. Finland has the highest incidence of type 1 diabetes in the world (36.5 cases/10^5 persons/year), while Hungary and Greece have considerably lower incidence rates (9.1 and 9.7 cases/10^5 persons/year respectively) [16]. Finns and Greeks represent extreme northern and southern European populations with a relatively wide genetic distance [17]. There were clear differences in the major DQB1 risk genotype combinations between the three ethnic groups. The DQB1*02/y conferred a high disease risk in the Greek population, a finding that has also been reported in other Mediterranean ethnic groups [18] [19]. In contrast, this combination had a much weaker effect among Hungarians and Finns. Importantly, DQA1 typing revealed that in the Greek population, DQB1*02/y positive individuals with DQA1*0201 allele had a decreased disease risk; however, this effect was not present in the Finns where the risk associated with DQB1*02/y was low anyway.

The strong predisposing effect of DQB1*02/0302 was universally present in the three ethnic groups; interestingly, its prevalence was highest among patients in Hungarians. This genotype conferred a higher disease risk in all three groups in the presence of the DQA1*05 allele. In addition, the risk was further increased by the

Table 4. A comparison of sensitivity of risk genotype combinations at a predetermined level of background frequency (app. 10%) in high and low incidence populations

DQB1	DQA1	DRB1*04	Sensitivity (%)	Background frequency (%)	OR	95%CI	PPV %
Finland							
0304/any			0.5	0	25.2	3.2–199.2	–
02,0302		s[e]	25.7	2.7	12.4	9.5–16.1	6.6
0302/x[a]		s	27.7	5.5	6.4	5.2–7.8	3.5
0302,0604		s	3.8	0.7	5.7	3.4–9.6	4.0
02/y[b]	03,05		2.7	0.8	3.3	2.0–5.5	2.4
0302,0603		s	2.7	1.1	2.5	1.5–3.9	1.8
Total			63.14	10.77	14.2	12.1–16.4	4.2
Hungary							
02,0302	03,05		30.9	0.6	52.9	12.6–222.2	8.1
0304/any			4.7	–	18.7	2.3–151.1	–
0301,0302		s	4.0	–	16.1	2.0–132.2	–
0302,0604			2.7	–	11.0	1.3–95.0	–
0302/x		s	15.4	1.7	9.3	3.1–27.4	1.4
02/y	05/a[c]		21.5	9.0	2.7	1.4–5.1	0.4
Total			79.19	11.3	28.9	15.9–52.7	1.1
Greece							
02,0302	0201,03 or 03	s	2.5	0	62.9	7.0–567.8	–
02,0302	03,05		18.5	0.8	28.2	12.7–62.7	3.5
02,0302	03/b[d]	s	0.8	0	26.5	2.4–294.7	–
0302/x		s	13.5	1.0	15.8	7.2–34.5	2.1
02/y	03 or 05		40.3	6.2	10.0	6.5–15.5	1.0
0302,0604		s	0.8	0.1	8.8	1.2–63.3	1.3
02,0604	03 or 05		1.7	0.7	3.0	0.8–11.3	0.4
0301,0302		s	3.4	1.5	2.4	0.9–6.7	0.3
Total			81.51	10.22	38.3	22.6–65.6	1.2

PPV, positive predictive value.
[a]x≠DQB1*02, *0301, *0602, *0603, *0604.
[b]y ≠ DQB1*0301, *0302, *0602, *0603, *0604.
[c]a ≠ DQA1*0201, *03.
[d]b ≠ DQA1*0201, *05.
[e]s = DRB1*0401, *0402, *0404, *0405.

DRB1*0401 allele in Finns and Hungarians and by the *0402 in the Greek population.

The DRB1*04 subtyping gave informative differences also for the DQB1*0302 positive genotypes. DQB1*0403 was strongly protective in Finns and a similar tendency was seen in the Greek cohort. In the Finnish population, DRB1*0401 and *0404 were significantly associated with disease, while *0401 and *0402 showed significant disease association among Greeks and Hungarians. An interesting phenomenon was observed in relation to DQB1*0302/*0603 genotypes in the Finnish series. Only the DRB1*0401-DQB1*0302/*0603 combination conferred significant disease risk, suggesting that the DRB1*0401 allele is critical for the predisposing effect of this genotype. This finding confirms our previous results indicating that this genotype is associated with diabetes among Finns, but not in populations with lower disease incidence rates [20].

We compared the sensitivity and population frequency of the three screening approaches. In the first one, only DQB1 alleles were considered, DQB1 typing with additional DQA1 typing of DQB1*02 positive genotypes was analyzed in the second alternative, and, in the third option, DRB1*04 typing was also taken into account in individuals carrying DQB1*0302 (see Figure 1).

In all ethnic groups, the informative value of typing DQA1 and DRB1*04 alleles was clear; however, the effect was more pronounced in populations with low disease incidence. These findings are in line with our earlier observations that relative risk for susceptibility genotypes is higher in low-incidence countries and vice versa; consequently, additional risk markers provide more information in these populations than in the high-incidence area that Finland represents [21].

Follow-up studies focus on a well-defined cohort at increased genetic risk, the size of which needs to be limited. For example, in the DIPP study, 13 to 15% of the screened newborn population is eligible for the observational study [6]. For comparison, we have chosen risk marker combinations from the three studied populations at a background frequency of approximately 10% and displayed the genotypes involved and the corresponding sensitivity values (Table 4). As expected, the diagnostic sensitivity and the odds ratio for the marker combination were higher in Hungarians and Greeks as compared to those in Finns. However, owing to the higher disease incidence, the positive predictive value for the risk genotype combination was highest in the Finnish population.

It is important to emphasize that screening for a disease with polygenic etiology is hampered by the high frequency

of risk markers in the general population. The power of predictive screening strategies is always a compromise between specificity and sensitivity (see Figure 1). Even a slight increase in sensitivity could result in a substantial increase in costs due to the increased size of the cohort to be followed [9]. By introducing additional genetic screening steps, although the costs of the screening are higher, the sensitivity could be increased without considerably changing the size of the follow-up cohort.

The effect of gender in individuals with the DQB1*02/y-DQA1*05/a genotype on disease susceptibility was confirmed here in the three populations with variable disease incidence rates and different ethnic origin. The finding is in agreement with earlier studies that indicated a specific DR3-associated etiological pathway for type 1 diabetes in male patients [8,22,23]. Interestingly, the strength of this effect seems to be stronger in low-incidence ethnic populations.

The genetic screening method used here was based on the PCR amplification of the gene region of interest directly from a dried blood spot, followed by hybridization of lanthanide-labeled allele-specific probes and time-resolved fluorimetry [7,8] [14]. This process is robust and suitable for automation and high-throughput sample analysis.

In conclusion, we demonstrated that HLA DR-DQ-based screening is a suitable tool for identifying individuals at high genetic risk for type 1 diabetes in populations with diverse genetic background. However, risk markers should be individually selected for the target population since the screening efficiency of various markers is highly dependent on the ethnic group studied. The selection criteria – number of alleles and loci – need to be established on a representative sample of affected cases and randomly selected nondiabetic individuals from the background population.

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