Published in final edited form as:

J Med Genet. 2009 July ; 46(7): 469–471. doi:10.1136/jmg.2009.066647.

The effect of the *MHC* locus on autoantibodies in type 1 diabetes

Hui-Qi Qu¹ and Constantin Polychronakos^{1,*}

¹Departments of Pediatrics and Human Genetics, McGill University, Montreal H4H 2P4, Québec, Canada

Abstract

OBJECTIVE—This study aimed to investigate whether the presence of autoantibodies specific for type 1 diabetes (T1D) is determined by the major genetic susceptibility locus for the disease at the *HLA* genes, using the T1D Genetics Consortium data.

METHODS—We analyzed anti-IA-2 and anti-GAD 65 autoantibody data from 2,282 T1D patients from 1117 multiplex families. *HLA* genotyping was available for all cases and their parents and association with autoantibody positivity was tested by the transmission disequilibrium test.

RESULTS—Association of anti-IA-2 with the *HLA* genes was detected at high statistical significance. *HLA-DRB1*0401* confers both the strongest type 1 diabetes risk, and positive association of anti-IA-2, whereas the *DRB1*03-DQA1*0501-DQB1*0201* haplotype, associated less strongly with T1D, showed a significant negative association with anti-IA-2 positivity. Interestingly, *HLA-A*24* is also negatively associated with anti-IA-2, independently of the *DRB1*03-DQA1*0501-DQB1*0201* haplotype. No statistically significant association was identified between anti-GAD65 and *HLA*.

CONCLUSIONS—This study highlights that IA-2 as an autoantigen depends on *HLA* genotype and suggests new insights into the mechanism of loss of immune tolerance.

Keywords

autoantibody; GAD65; *HLA*; IA-2; Type 1 diabetes

Introduction

Type 1 diabetes (T1D) is due to T-cell-mediated autoimmune destruction of the pancreatic β -cells. The role of humoral autoimmunity in this destruction is less clear but antibodies against a number of T1D-specific autoantigens are detectable in most cases. The protein tyrosine phosphatase-2 (also known as islet antigen-2, IA-2, encoded by *PTPRN*) and the 65-kD glutamate decarboxylase 2 (GAD65) are the most highly predictive¹. However, a significant proportion of patients diagnosed with T1D show absence of either or both autoantibodies². To which extent this represents heterogeneity in the causative cellular autoantigens is an unanswered question. Such heterogeneity may be due to the effect of genotype at the major T1D susceptibility locus that involves the highly polymorphic antigen-presenting proteins of the Human Leukocyte Antigen (*HLA*) genes.

*Correspondence: Dr. Constantin Polychronakos, Departments of Pediatrics and Human Genetics Director, Pediatric Endocrinology McGill University Health Center (Children's Hospital), 2300 Tupper, Montréal, QC Canada H3H 1P3. Tel.: 514 412 4315; Fax: 514 412 4264. constantin.polychronakos@mcgill.ca.

Conflict of Interest statement: None declared.

The association between the *HLA* genes and autoantibodies in T1D has been reported by previous studies. A consistent finding was the association between anti-IA-2 and the *HLA* class II genes, e.g. the positive association between anti-IA-2 and *HLA DR4*³⁻⁵, and the negative association between anti-IA-2 and *HLA DQ2*⁶. In this study, we aimed to examine systematically the association of anti-IA-2 and anti-GAD65 positivity with both *HLA* class I and class II genes in a large family cohort.

RESEARCH DESIGN AND METHODS

Subjects

This study was based on the December 2007 data release by the T1D Genetics Consortium (T1DGC) (www.t1dgc.org)⁷. The T1DGC genotyped HLA-A, HLA-B, HLA-Cw, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and *HLA-DRB1*, in 10,072 individual from 2,315 families with at least two children diagnosed with diabetes (4,951 T1D cases). Criteria of T1D diagnosis were age at diagnosis below 35 years and uninterrupted treatment with insulin within six months of diagnosis. For siblings of probands diagnosed under the age of 35, the age-at-diagnosis limit was extended to 45 if they were lean and had positive antibodies and/or low C-peptide levels at diagnosis. *HLA* genotypes and results of anti-IA-2 and anti-GAD65 testing were available in 2,282 T1D patients from 1,117 multiplex families with *HLA* genotyping data. The patients were recruited world-wide but the majority of the patients (>90%) are of European descent. We compared auto-antibody(−) *versus* auto-antibody(+) patients in terms of the transmission ratio of the eight *HLA* genes by the transmission disequilibrium test (TDT), an approach immune to population stratification.

Statistics

For the transmission disequilibrium test (TDT) we used the TDTPHASE program in the UNPHASED software (<http://www.hgmp.mrc.ac.uk/~fdudbrid/software/unphased/>)⁸. A total of 130 comparisons were performed (the eight *HLA* genes have a total of 130 alleles) tested for association with positivity for two autoantibodies. We used the Bonferroni-corrected significance threshold $\alpha = 0.05 / 260 = 1.92 \times 10^{-4}$. This is a conservative approach since, because of linkage disequilibrium (LD), the *HLA* loci examined are associated with each other.

RESULTS AND DISCUSSION

Among 2,282 T1D patients, 696 (30.5%) patients are auto-antibody(−), and the remaining 1586 (69.5%) patients have at least one auto-antibody(+). Summary statistics are shown in Table 1. The distribution of anti-IA-2 has no obvious gender difference, whereas anti-GAD65 has lower prevalence in male patients. For either anti-IA-2 or anti-GAD65, the antibody(−) group had younger age-of-onset of T1D and longer disease duration at the time of sampling.

Since our study was performed on cases only, auto-antibody association with the *HLA* genes is independent of the effect of *HLA* on T1D risk. No statistically significant difference was observed the transmission ratio of each *HLA* allele in the anti-GAD65 (−) group *versus* the anti-GAD65(+) group. The lowest *P* value of 8.72×10^{-3} is larger than the corrected significant level $\alpha = 1.9 \times 10^{-4}$. By contrast, differences in transmission on the basis of anti-IA-2 reactivity met the Bonferroni-corrected significance threshold ($P < 1.92 \times 10^{-4}$) in five *HLA* alleles (Table 2). As shown by previous studies, auto- antibodies may disappear years after the disease onset⁹, which can explain the phenomenon that the antibody(−) group had longer disease duration at the time of sampling, as shown in Table 1. Therefore, the antibody(−) cases with long disease duration may have been antibody(+) previously. To

address this issue, we compared the transmission ratio of the *HLA* alleles between the antibody(–) cases with disease duration ≤ 10 years and the antibody(–) cases with disease duration >10 years. We found no statistical difference of the transmission ratio of the five anti-IA-2-associated alleles in terms of disease duration. The anti-IA-2 associations of these *HLA* alleles remain valid if we look at short disease duration and long disease duration separately (Table 3).

*HLA-DRB1*0401* entails both the strongest T1D risk and positive association of anti-IA-2 (Table 2). The T1D cases with at least one *HLA-DRB1*0401* allele have higher prevalence of anti-IA-2 (57.7%) than the T1D cases without the *HLA-DRB1*0401* allele (36.3%). This result confirms a recent report of higher anti-IA-2 prevalence in patients carrying *HLA-DRB1*0401*⁵. In addition, we found that *HLA* class II alleles were negatively associated with anti-IA-2. *HLA-DQB1*0201*, *HLA-DRB1*03*, and *HLA-DQA1*0501*, entail medium T1D risk, and are negatively associated with anti-IA-2 (Table 2). *DRB1*03*, *DQA1*0501*, and *DQB1*0201*, are in tight LD and compose a remarkably conserved, T1D-predisposing haplotype¹⁰. The T1D association of the *DRB1*03-DQA1*0501-DQB1*0201* haplotype has OR (95% CI)= 2.06 (1.91-2.22), while the association with anti-IA-2 has OR (95% CI)=0.63 (0.51- 0.77), $P=6.06 \times 10^{-6}$. The T1D cases with at least one *DRB1*03-DQA1*0501-DQB1*0201* haplotype have lower prevalence of anti-IA-2 (41.2%), than the T1D cases without the *DRB1*03-DQA1*0501-DQB1*0201* haplotype (55.6%). Therefore, in this large cohort of T1D cases, we validated a previous report that anti-IA-2 is less common in patients with *DQ2* haplotype⁶.

Interestingly, we found that *HLA* class I allele *HLA-A*24* was also negatively associated with anti-IA-2. *HLA-A*24* is in very low LD with the *HLA* class II alleles *DRB1*03*, *DQA1*0501*, and *DQB1*0201* (Supplementary Fig.1). The negative association can not be explained by the LD. In the T1D cases without any of the *DRB1*03*, *DQA1*0501*, and *DQB1*0201* alleles, *HLA-A*24* is negatively associated with anti-IA-2 with OR (95% CI)=0.57 (0.38, 0.84), $P=0.004$.

IA-2 is a major islet cell autoantigen¹¹. The auto-antigenicity is from the cytoplasmic domain of IA-2¹². IA-2 is involved in glucose-stimulated insulin secretion and its targeted disruption can result in alterations of insulin secretion¹³. Our findings show that the relative importance of IA-2 as an autoantigen depends on *HLA* genotype and permit some insight into the mechanism of loss of tolerance. As shown by Sidney et al.¹⁴, the IA-2 epitopes GVAGLLVALAV (586-596) and MSSGSFINISV (499-509) can bind with *DQB1*0201*, but not *DRB1*0401*. Our data are consistent with the conclusion that efficient presentation of IA-2 induces immune tolerance and inhibits anti-IA-2 reactivity. This may, at least partially, explain the somewhat lower predisposition conferred by *DQB1*0201* than by *DRB1*0401*. The role of *HLA* class I genes in T1D was highlighted by recent genetic study¹⁵. Our study suggests that anti-IA-2 may be involved in the *HLA* class I genetic effect in T1D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research utilizes resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. H.Q.Q. is supported by a fellowship from the Canadian Institutes of Health Research.

REFERENCES

1. Atkinson MA, Maclaren NK. Islet cell autoantigens in insulin-dependent diabetes. *J Clin Invest*. 1993; 92:1608–16. [PubMed: 8408615]
2. Verge, CF.; Stenger, D.; Bonifacio, E.; Colman, PG.; Pilcher, C.; Bingley, PJ.; Eisenbarth, GS. Diabetes; Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop; 1998; p. 1857-1866.
3. Genovese S, Bonfanti R, Bazzigaluppi E, Lampasona V, Benazzi E, Bosi E, Chiumello G, Bonifacio E. Association of IA-2 autoantibodies with HLA DR4 phenotypes in IDDM. *Diabetologia*. 1996; 39:1223–6. [PubMed: 8897011]
4. Sanjeevi CB, Hagopian WA, Landin-Olsson M, Kockum I, Woo W, Palmer JP, Lernmark A, Dahlquist G. Association between autoantibody markers and subtypes of DR4 and DR4-DQ in Swedish children with insulin-dependent diabetes reveals closer association of tyrosine pyrophosphatase autoimmunity with DR4 than DQ8. *Tissue Antigens*. 1998; 51:281–6. [PubMed: 9550329]
5. Williams AJ, Aitken RJ, Chandler MA, Gillespie KM, Lampasona V, Bingley PJ. Autoantibodies to islet antigen-2 are associated with HLA-DRB1*07 and DRB1*09 haplotypes as well as DRB1*04 at onset of type 1 diabetes: the possible role of HLA-DQA in autoimmunity to IA-2. *Diabetologia*. 2008; 51:1444–8. [PubMed: 18504544]
6. Graham J, Hagopian WA, Kockum I, Li LS, Sanjeevi CB, Lowe RM, Schaefer JB, Zarghami M, Day HL, Landin-Olsson M, Palmer JP, Janer-Villanueva M, Hood L, Sundkvist G, Lernmark A, Breslow N, Dahlquist G, Blohme G. Genetic Effects on Age-Dependent Onset and Islet Cell Autoantibody Markers in Type 1 Diabetes. *Diabetes*. 2002; 51:1346–1355. [PubMed: 11978629]
7. Rich SS, Concannon P, Erlich H, Julier C, Morahan G, Nerup J, Pociot F, Todd JA. The Type 1 Diabetes Genetics Consortium. *Ann N Y Acad Sci*. 2006; 1079:1–8. [PubMed: 17130525]
8. Frank D. Pedigree disequilibrium tests for multilocus haplotypes. *Genetic Epidemiology*. 2003; 25:115–121. [PubMed: 12916020]
9. Taplin CE, Barker JM. Autoantibodies in type 1 diabetes. *Autoimmunity*. 2008; 41:11–8. [PubMed: 18176860]
10. Stewart CA, Horton R, Allcock RJN, Ashurst JL, Atrazhev AM, Coggill P, Dunham I, Forbes S, Halls K, Howson JMM, Humphray SJ, Hunt S, Mungall AJ, Osoegawa K, Palmer S, Roberts AN, Rogers J, Sims S, Wang Y, Wilming LG, Elliott JF, de Jong PJ, Sawcer S, Todd JA, Trowsdale J, Beck S. Complete MHC Haplotype Sequencing for Common Disease Gene Mapping. *Genome Res*. 2004; 14:1176–1187. [PubMed: 15140828]
11. Lan MS, Wasserfall C, Maclaren NK, Notkins AL. IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:6367–6370. [PubMed: 8692821]
12. Lampasona V, Bearzatto M, Genovese S, Bosi E, Ferrari M, Bonifacio E. Autoantibodies in insulin-dependent diabetes recognize distinct cytoplasmic domains of the protein tyrosine phosphatase-like IA-2 autoantigen. *J Immunol*. 1996; 157:2707–2711. [PubMed: 8805677]
13. Saeki K, Zhu M, Kubosaki A, Xie J, Lan MS, Notkins AL. Targeted Disruption of the Protein Tyrosine Phosphatase-Like Molecule IA-2 Results in Alterations in Glucose Tolerance Tests and Insulin Secretion. *Diabetes*. 2002; 51:1842–1850. [PubMed: 12031972]
14. Sidney J, del Guercio MF, Southwood S, Sette A. The HLA molecules DQA1*0501/B1*0201 and DQA1*0301/B1*0302 share an extensive overlap in peptide binding specificity. *J Immunol*. 2002; 169:5098–108. [PubMed: 12391226]
15. Nejentsev S, Howson JMM, Walker NM, Szeszeko J, Field SF, Stevens HE, Reynolds P, Hardy M, King E, Masters J, Hulme J, Maier LM, Smyth D, Bailey R, Cooper JD, Ribas G, Campbell RD, Clayton DG, Todd JA. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature*. 2007; 450:887–892. [PubMed: 18004301]

Table 1

The general information of the T1D patients

	<i>n</i> =2282	χ^2 test by gender (<i>P</i>)	Median age-of-onset (25% ~ 75% quartiles, year)	Mann-Whitney test of age-of-onset <i>Z</i> (<i>P</i>)	Median duration at the time of sampling (25% ~ 75% quartiles, year)	Mann-Whitney test of duration <i>Z</i> (<i>P</i>)
anti-IA-2 (-)	Male 607 (50.3%); Female 600 (49.7%)	1.0 (<i>P</i> =0.321)	8 (4 ~ 14)	5.0 (<i>P</i> = 6.02 × 10 ⁻⁷)	10 (5 ~ 18)	11.8 (<i>P</i> =6.35 × 10 ⁻³²)
anti-IA-2 (+)	Male 563 (52.4%); Female 512 (47.6%)		10 (6 ~ 13)		6 (2 ~ 11)	
anti-GAD65 (-)	Male 634 (53.9%); Female 543 (46.1%)	6.6 (<i>P</i> =0.010)	7 (4 ~ 11)	11.9 (<i>P</i> =1.14 × 10 ⁻³²)	10 (5 ~ 16)	8.9 (<i>P</i> = 8.41 × 10 ⁻¹⁹)
anti-GAD65 (+)	Male 536 (48.5%); Female 569 (51.5%)		11 (6 ~ 16)		6 (3 ~ 12)	

Table 2
The anti-IA-2 association of the *HLA* genes in T1D cases

Allele	Frequency	T1D association OR (95% CI)	Anti- IA-2 association OR (95% CI)	Anti- IA-2 association <i>P</i> value
<i>HLA-DQB1*0201</i>	0.328	1.48 (1.40, 1.57)	0.66 (0.56, 0.78)	1.72×10^{-6}
	0.198	2.69 (2.46, 2.94)	1.75 (1.37, 2.23)	6.93×10^{-6}
<i>HLA-DRB1*0401</i>				
	0.261	2.04 (1.91, 2.18)	0.64 (0.52, 0.78)	1.03×10^{-5}
<i>HLA-DRB1*03</i>				
<i>HLA-DQA1*0501</i>	0.321	1.30 (1.22, 1.39)	0.71 (0.60, 0.84)	8.19×10^{-5}
	0.104	1.39 (1.25, 1.54)	0.56 (0.42, 0.75)	9.45×10^{-5}
<i>HLA-A*24</i>				

Table 3
The T1D disease duration and the anti-IA-2 association of the *HLA* genes

Allele	Anti- IA-2 (–) with T1D duration ≤ 10 years vs. >10 years OR (95% CI)	Anti- IA-2 (+) vs. Anti- IA-2 (–) with T1D duration ≤10 years OR (95% CI)	Anti- IA-2 (+) vs. Anti- IA-2 (–) with T1D duration >10 years OR (95% CI)
<i>HLA-DQB1*0201</i>	0.98 (0.77, 1.24)	0.67 (0.55, 0.82)	0.65 (0.53, 0.81)
<i>HLA-DRB1*0401</i>	0.81 (0.57, 1.15)	1.94 (1.44, 2.60)	1.57 (1.15, 2.14)
<i>HLA-DRB1*03</i>	0.96 (0.73, 1.27)	0.65 (0.51, 0.82)	0.62 (0.48, 0.80)
<i>HLA-DQA1*0501</i>	1.03 (0.81, 1.29)	0.70 (0.58, 0.86)	0.72 (0.58, 0.89)
<i>HLA-A*24</i>	1.06 (0.71, 1.58)	0.53 (0.37, 0.77)	0.57 (0.40, 0.80)