REFINING HLA-ASSOCIATED TYPE 1 DIABETES RISK ASSESSMENT WITH DATA FROM SIX UNDERSTUDIED COUNTRIES

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Janelle A. Noble[1], Erik H. Rozemuller[2], Stéphane Besançon[3], Assa Sidibé[4], Gunduz Ahmadov[5], Bedowra Zabeen[6], Asher Fawwad[7], Mohammad Yakoob Ahmedani[7], Mohamed Abdullah[8], Eddy Jean-Baptiste[9], Philippe Larco[9], Nancy Larco[9], Julia E. Von Oettingen[10], Maaike Rijkers[11], Fereshte Dadkhodaie[11], Helma De Bruin[11], Jannetje Kooij[11], Harper R.N. Martin[12], Ningyi Song[13], Julie A. Lane[12], Graham D, Ogle[14], Steven J. Mack[1]

[1]UCSF, Berkeley, CA, United States. [2]TxMiller Foundation, Utrecht, Netherlands. [3]ONG Santé Diabète, Bamako, Mali. [4]Hopital du Mali, Bamako, Mali. [5]Azerbaijan Medical University, Baku City, Azerbaijan. [6]Bangladesh Institute of Research and Rehabilitation of Diabetes, Endocrine and Metabolic Disorders, Dhaka, Bangladesh. [7]Baqai Institute of Diabetology and Endocrinology Karachi, Pakistan. [8]Sudanese Children's Diabetes Association, Khartoum, Sudan. [9]Fondation Haitienne de Diabète et de Maladies Cardiovasculaires (FHADIMAC), Port-au-Prince, Haiti. [10]Research Institute of the McGill University Health Centre, Montreal, QC, Canada. [11]GenDx, Utrecht, Netherlands. [12]Children's Hospital Oakland Research Institute, Oakland, CA, United States. [13]Huazhong University of Science and Technology, Wuhan, China. [14]Sydney Medical School, Sydney, NSW, Australia.

UCSF Benioff Children's Hospital Oakland

C·H·O·R·I Children's Hospital Oakland Research Institute

Introduction

The recent approval of the drug teplizumab for intervention in type 1 diabetes (T1D) strengthens the impetus for disease prediction. HLA genes were the first identified as associated with T1D and remain, by far, the strongest contributors to risk. Extreme polymorphism of HLA-encoding genes and strong linkage disequilibrium (LD) among those loci complicate efforts to identify the specific alleles responsible. HLA-associated T1D risk data are presented here for T1D patients and controls from six under-resourced and understudied countries. T1D-associated alleles, haplotypes, and amino acid positions were determined for each data set, and the results were compared among them.

Subjects

Target enrollment was 100 T1D patients, aged 2-22, and 200 unrelated, non-diabetic controls from each country. Samples were collected between 2013 and 2017, facilitated by the "Life for a Child" program. Actual numbers of samples used in these analyses are shown below.

	Patients	Controls
Azerbaijan	103	185
Bangladesh	78	180
Haiti	54	63
Mali	99	200
Pakistan	99	169
Sudan	50	191

Methods

Genotyping:
- GenDx NGSgo®-MX11-3 kit (Azerbaijan, Bangladesh, Mali, and Pakistan)
- GenDx NGSgo®-MX6-1 kit (Haiti controls)
- Omixon Holotype HLA 96/11 kit(Haiti patients)

Genotype calling:
- Omixon HLA Twin™ v. 4.2.0 with IPD-IMGT/HLA Database release version 3.39.0
- GenDx NGSengine 2.23.1.23474 and IPD-IMGT/HLA Database release version 3.45.1
- IPD-IMGT/HLA Database release version 3.53.0 was used to verify novel sequences

Association analyses:
- BIGDAWG version 3.0.6

HLA Association

Locus-level Analysis

Locus-level significance ranged strikingly among populations: all loci were associated in the Pakistani data set, while only DRB1 was associated in Bangladesh.

TABLE 1. Locus-level significance for association of individual HLA loci

	A	B	C	DRB1	DRBX	DQB1	DQA1	DPB1	DPA1
Azerbaijan	ns	1.8e-4	1.5e-3	2.2e-16	1.8e-9	2.2e-16	2.2e-16	ns	ns
Bangladesh	ns	ns	ns	3.4e-4	ns	ns	ns	ns	
Haiti	ns	ns	ns	3.8e-3	2.4e-3	3.9e-4			
Mali	1.9e-4	5.0e-3	1.8e-2	3.5e-11		7.1e-10	3.8e-12	4.1e-3	4.4e-2
Pakistan	8.7e-4	5.7e-10	3.4e-5	2.2e-16	8.8e-5	2.2e-16	2.2e-16	2.5e-2	1.6e-3
Sudan	ns	ns	ns	8.6e-13	3.0e-7	5.1e-14	6.8e-15	ns	ns

Numbers are p values in scientific notation, e.g. 1.8e-4 = 1.8 x 10^{-4}; grey shading = locus not tested; ns = not significant. DRBX = DRB3, DRB4, and DRB5

CONCLUSION: T1D association is most consistently observed and is strongest in the class II loci encoding DR and DQ antigens.

Allele-level Analysis

Class II: DR and DQ

TABLE 2: T1D-associated DRB1~DQA1~DQB1 haplotypes observed in at least two of six data sets.

DRB1~DQA1~DQB1 haplotype	Azerbaijan	Bangladesh	Haiti	Mali	Pakistan	Sudan
03:01~05:01~02:01	3.1(3.6e-12)	ns	4.3(2.0e-3)	1.5(2.0e-4)	4.8(2.2e-16)	3.0(1.9e-10)
04:05~03:03~02:02	1.1(1.2e-2)	ns	ns	2.7(8.1e-5)	ns	1.4(2.0e-3)
13:01~01:03~06:03	0.02(3.0e-3)	ns	ns	0.0(1.6e-2)	0.09(1.3e-2)	ns
15:02~01:03~06:01	0.0(2.0e-3)	ns	ns	ns	0.01(1.0e-3)	0.0(3.4e-2)
15:03~01:02~06:02	ns	ns	0.33(6.0e-3)	ns	ns	0.0(3.7e-2)

Data are presented as Odds Ratio (p value).

CONCLUSION: Associations are consistent with those commonly seen in populations of European ancestry at the serogroup level (DR3, DR4, DR2); however, individual alleles can vary, e.g., *DRB1*04:05* is observed rather than the common European *DRB1*04:01*.

Class II: DP

*DPA1*01:03* was the only allele with significant T1D association in more than one data set (MA and PK). A very strong positive T1D association was observed for *DPA1*01:03-DPB1*04:02* (OR=11.8, p=6.2e-5) in Mali, which was inconsistent with marginally significant data from Azerbaijan (OR=0.63, p=0.065) and with published data suggesting that *DPB1*04:02* is T1D *protective.* The predisposing effect for DPB1*04:02 in Mali was attributable to its presence on the extended haplotype: *A*24:02~B*27:05~C*02:02~DRB1*04:05~DRB4*01:03~DQB1*02:02~DQA1*02:01~DPB1*04:02~DPA1*01:03*

Class I: A, B, C

TABLE 3: T1D-associated class I loci observed in at least two of six data sets.

Allele	Azerbaijan	Bangladesh	Haiti	Mali	Pakistan	Sudan
A*02:05	ns	ns	ns	ns	8.5(8.1e-5)	2.4(4.5e-2)
A*24:02	ns	2.02(7.0e-3)	4.4(1.0e-2)	ns	ns	ns
B*08:01	4.0(1.0e-3)	ns	ns	ns	3.1(5.5e-5)	ns
B*35:03	2.3(2.5e-2)	ns	ns	ns	0.36(2.1e-2)	ns
B*50:01	2.6(4.0e-3)	ns	ns	ns	5.6(1.8e-5)	ns

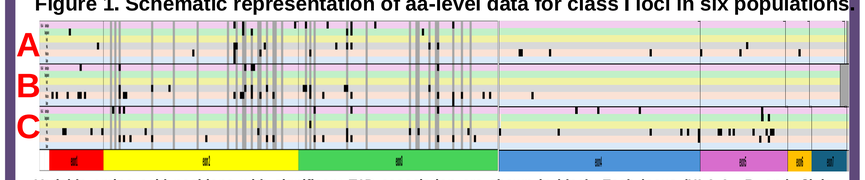
Data are presented as Odds Ratio (p value).

CONCLUSION: A few associations are reproduced in more than one country for HLA-A and HLA-B, including the established risk locus *A*24:02*; however, the most commonly-reported HLA-B association (*B*39:06*) was not seen. No significant associations were seen in more than one country for HLA-C. Four alleles were suggestive of HLA-C association in two countries; however, in three of four of these pairs, effects of the same allele were opposite (*i.e.*, protective in one data set and predisposing in the other) in those populations.

Amino acid Analysis

Association analysis of individual amino acid (aa) positions in a locus is independent of called allele status, allowing inclusion of rare alleles that would be binned in allele-level analyses. Data for aa analyses of class I loci in all six data sets are represented schematically in Figure 1. The position of each amino acid in each encoded antigen was analyzed for T1D association, beginning with the leader peptide and going through the C terminal amino acid position.

Figure 1. Schematic representation of aa-level data for class I loci in six populations.



Variable amino acid positions with significant T1D association are shown in black. Each locus (HLA-A, -B, and -C) is represented by a set of 6 rows representing individual countries. Exons are designated by colors at the bottom of the chart. Positions encoding amino acids participating in the peptide binding pocket are indicated by vertical gray lines in exons 2 and 3.

CONCLUSION: As expected, the highest concentration of significantly variable amino acids is located in exons 2 and 3, which encode the peptide-binding groove, with the majority in positions that encode the peptide binding pockets. Less variability is seen in exons 1 (encoding signal), 4 (encoding Ig-like domain not contributing to peptide binding groove), 5 (encoding transmembrane region), and 6-8 (encoding cytoplasmic portion of the antigen).

HLA-A

Significantly-associated AA positions in the HLA-A locus are rare outside exons 2 and 3 (Figure 1). Table 4 compares effects of significantly T1D-associated HLA-A alleles with effects of aa positions 276 (exon 5) and 321 (exon 6).

TABLE 4: Risk effects of T1D-associated alleles vs. aa positions in HLA-A.

HLA-A allele	Data Set(s)	Allele effect	Position 276	aa effect	Position 321	aa effect
02:05	PK,SU	S	pro	S	ser	S
02:06	PK	S	pro	S	ser	S
02:11	PK	S	pro	S	ser	S
03:01	BA	P	leu	P	thr	P
11:01	PK	P	leu	P	thr	P
24:02	BA,MA	S	pro	S	ser	S
29:02	MA	S	pro	S	ser	S
30:01	MA	P	leu	P	thr	P

BA = Bangladesh; MA = Mali; PK = Pakistan; SU = Sudan.
P = T1D protective; S = T1D susceptible.

CONCLUSION: Effects of positions 276 and 321 track perfectly with effects of the alleles in which they are found, suggesting that those positions might be useful in predicting genetic risk.

HLA-B

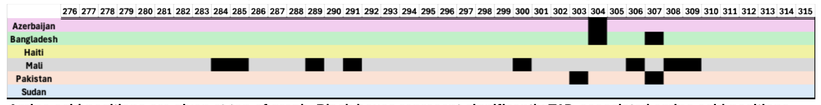
Data for aa-level analysis of HLA-B revealed almost no associated aa positions outside exons 2 and 3, with the notable exception of the positions encoding the leader peptide (exon1) in Pakistan (Figure 1).

CONCLUSION: Little or no additional risk information was seen with aa analysis of the HLA-B loci.

HLA-C

For HLA-C, the T1D-associated polymorphic aa positions are spread throughout the gene (Figure 1). A cluster of T1D associated aa positions was observed in exon 5 between positions 300 and 309. A schematic representation of this region is shown in Figure 2.

FIGURE 2. Schematic representation of T1D-associated aa positions in exon 5.



Amino acid positions are given at top of graph. Black boxes represent significantly T1D-associated amino acid positions.

CONCLUSION: HLA-C has fewer T1D-associated aa in exons 2 and 3 and more associated polymorphisms in other area of the gene, than do HLA-A and -B. especially in exon 5, which encodes the transmembrane region. HLA-C is a dominant ligand for Killer-cell immunoglobulin receptors (KIR) on Natural Killer (NK) cells, which are important in innate immunity. Notably, position 308 of the HLA-C gene has been reported to inhibit NK function (Davis et al., J Exp Med, 189:1265), suggesting that *the role of HLA-C in T1D risk may be due to an innate immunity mechanism rather than traditional peptide-HLA binding*. Possible mechanisms remain to be determined but may include stabilization of the expressed protein or its mobility in the cell membrane.

Summary

- The study of varied populations confirmed HLA class II T1D risk associations but demonstrated variability among populations.
- HLA class I T1D associations were less strong than those for class II.
- Amino acid analysis revealed differences among class I loci and suggested that HLA-C-associated T1D risk may be through innate, rather than adaptive, immune function.
- Expression studies for both HLA class II and class I antigens are sparse in the literature but represent a crucial next step in understanding how HLA affects T1D risk.

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