Supporting Information

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SI Text

Direct Sequencing of Exon 2 of the Human HLA-DRB1 Gene. Genomic DNA was amplified and sequenced using allele specific primers as shown in Table S5. PCRs were performed in a 20-μl reaction mixture containing 50 ng genomic DNA; 5 pmol of each primer; PCR buffer containing 50 mmol/L KCl, 10 mmol/liter Tris-HCl (pH 8.3), 2.25 mmol/liter $MgCl_2$, 200 mmol/liter each of deoxy (d)-ATP, dGTP, dTTP, and dCTP, and 1 unit of AmpliTaq DNA polymerase (PE Applied Biosystems). Reaction mixtures were heated to 94°C for 7 min and then cycled 30 times as follows: 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. The PCR products were sequenced using the same allele-specific primers used for amplification (Table S5). Sequencing was performed using the ABI Big Dye DNA sequencing kit (Applied Biosystems), and the sequencing products were separated on an ABI-3130 automated sequencer (PE Applied Biosystems).

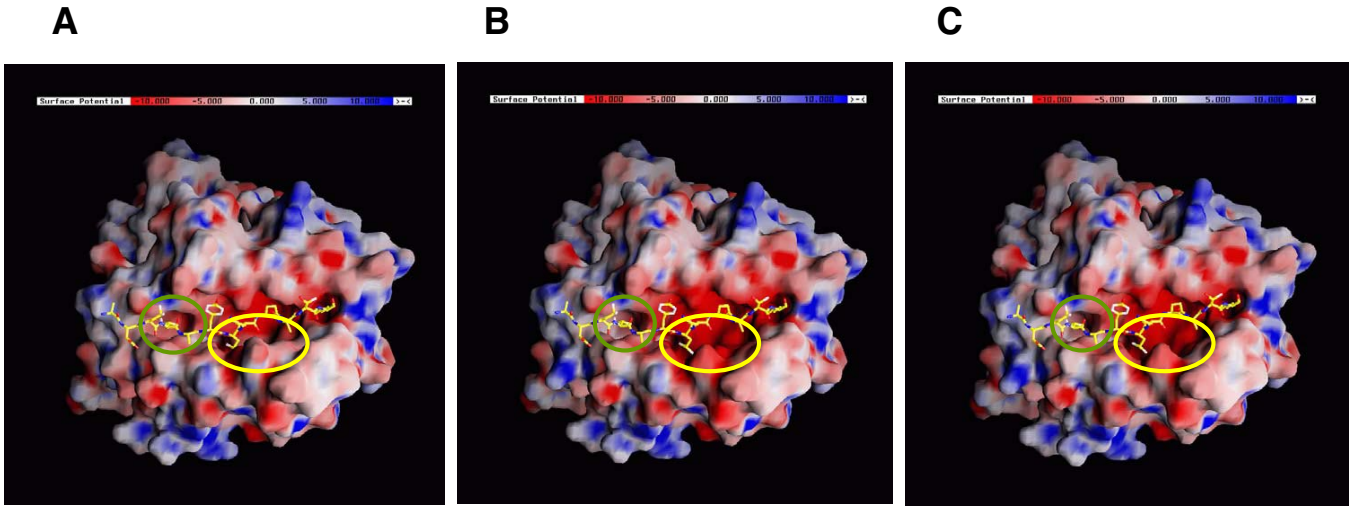

Fig. S1. Structural models of EAT-susceptible and -resistant I-E. (*A*) EAT-susceptible I-E (β71 Lys). (*B*) EAT-resistant I-E (β71 Glu). (*C*) EAT-resistant I-E (β71 Ala). The electrostatic potential was computed in each system to characterize the surface properties of the molecule. The differences are considered to be relevant to the predisposition to EAT. The peptide-binding groove is shown with the moth cytochrome *c* peptide. Significant differences were observed in P1 and P4 pockets (shown in green circles and yellow ellipses, respectively). In EAT-resistant I-E, both the P1 and P4 pockets are larger in size. Thus, EAT-inducing peptides might bind to these larger pockets, but they are more likely to dissociate from MHC.

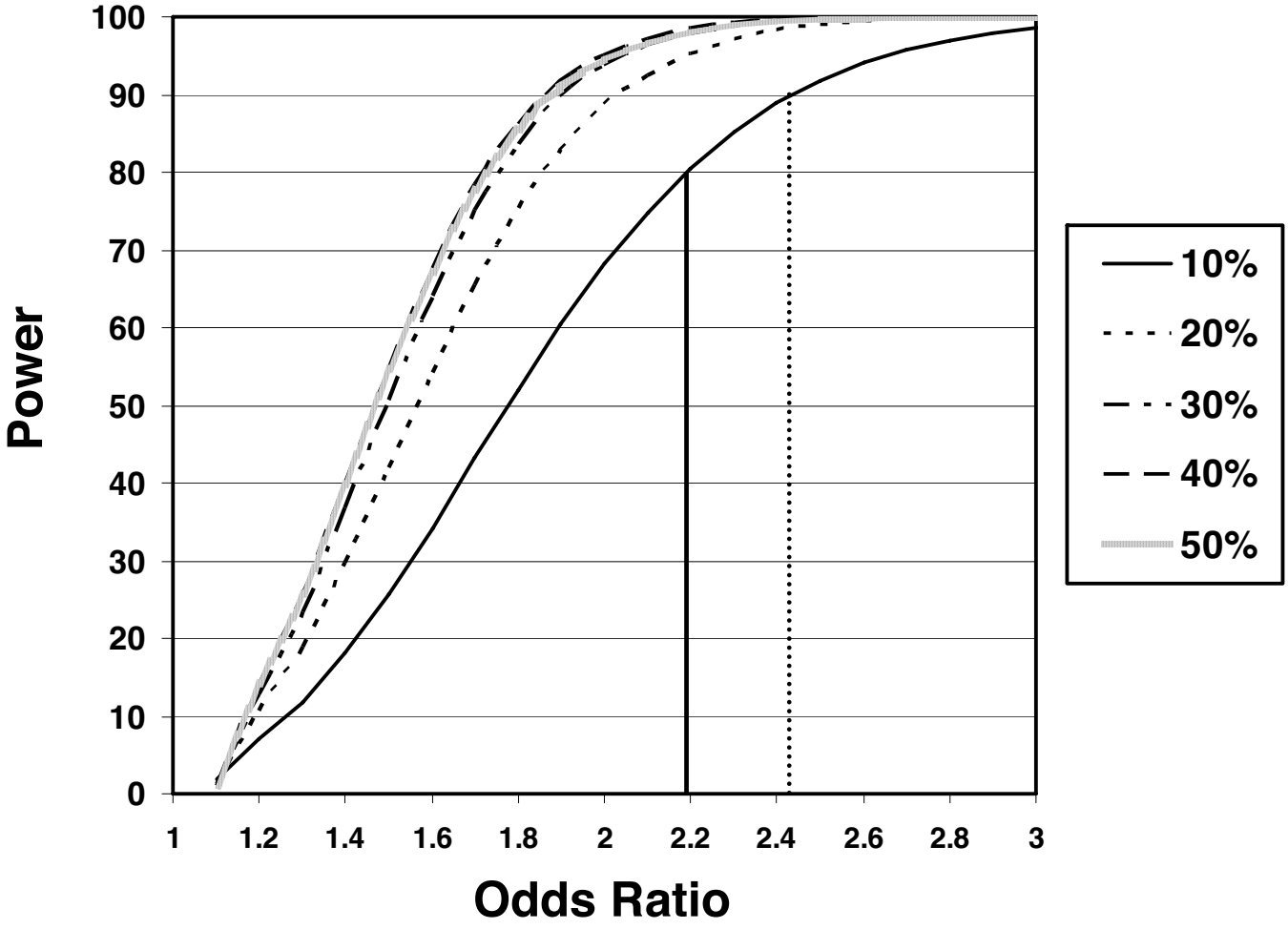

Fig. S2. Power calculation curves calculated for our study dataset of 94 patients and 149 controls. The *x*-axis shows the expected odds ratios (ORs), and the *y*-axis shows the power of the study. Each curve was calculated for a different minor allele frequency of the tested allele in the control population. The power curves show that our study had 80% power to detect a difference between the patients and the controls resulting in OR $\geq$ 2.2 with an alpha of 0.05; our study had 90% power to detect a difference between patients and controls resulting in OR $\geq$ 2.43 with an alpha of 0.05. Therefore, our study was adequately powered to detect significant associations of HLA-DR amino acid variants with HT.

Table S1. HLA-DR typing in HT patients and controls

HLA-DR	HT (%) $n = 94$	Controls (%) $n = 153$	P value
1	15 (8)	44 (14.4)	0.02
2	22 (11.7)	34 (11.1)	NS
3	29 (15.4)	22 (7.2)	2×10^{-3}
4	37 (19.7)	43 (14.1)	0.06
7	17 (9)	28 (9.2)	NS
8	3 (1.6)	17 (5.6)	0.03
9	0 (0)	4 (1.3)	NS
10	4 (2.1)	9 (2.9)	NS
11	38 (20.2)	51 (16.7)	NS
12	1 (0.5)	5 (1.6)	NS
13	18 (9.6)	37 (12.1)	NS
14	4 (2.1)	12 (3.9)	NS

NS, not significant.

Table S2. Polymorphic HLA-DR amino acid residues in HT patients and controls

Amino acid position	Amino acid variants	Patients (%) $n = 188$	Controls (%) $n = 298$	P value
26	Tyrosine	34 (18)	24 (8.1)	6.9×10^{-4}
	Others	154 (82)	274 (91.9)	
28	Aspartic acid	136 (72.3)	191 (64.1)	0.06
	Others	52 (27.7)	107 (35.9)	
30	Tyrosine	149 (79.3)	192 (64.4)	7.5×10^{-4}
	Others	39 (20.7)	106 (35.6)	
32	Tyrosine	120 (63.8)	187 (62.8)	Not significant
	Others	68 (36.2)	111 (37.2)	
37	Tyrosine	81 (43.1)	103 (34.6)	0.06
	Others	107 (56.9)	195 (65.4)	
47	Tyrosine	99 (52.7)	188 (63.1)	0.02
	Others	89 (47.3)	110 (34.6)	
57	Aspartic acid	149 (79.3)	227 (76.2)	Not significant
	Others	39 (20.7)	71 (23.8)	
67	Phenylalanine	32 (17)	39 (13.1)	Not significant
	Others	156 (20.7)	259 (86.9)	
70	Glutamine	124 (66)	161 (54)	4.3×10^{-3}
	Others	64 (34)	137 (46)	
71	Lysine	89 (47.3)	69 (23.2)	1.7×10^{-8}
	Others	99 (52.7)	229 (76.8)	
74	Arginine	27 (14.4)	14 (4.7)	1.5×10^{-4}
	Others	161 (85.6)	284 (95.3)	
77	Threonine	139 (73.9)	218 (73.2)	Not significant
	Others	49 (26.1)	80 (26.8)	
86	Valine	91 (48.4)	141 (47.3)	Not significant
	Others	97 (51.6)	157 (52.7)	

Table S3. Polymorphic I-E amino acid residues in strains of mice susceptible and resistant to EAT

Amino acid position	Amino acid variants	Susceptible mice (%) $n = 12$	Resistant mice (%) $n = 10$
6	Tryptophan	12 (100)	6 (60)
	Arginine		4 (40)
9	Glutamic acid	12 (100)	9 (90)
	Glycine		1 (10)
10	Tyrosine	12 (100)	10 (100)
11	Cysteine	12 (100)	7 (70)
	Valine		3 (30)
12	Lysine	11 (92)	6 (60)
	Threonine	1 (8)	4 (40)
23	Arginine	12 (100)	7 (70)
	Histidine		3 (30)
26	Leucine	11 (92)	5 (50)
	Phenylalanine	1 (8)	5 (50)
28	Glutamic acid	1 (8)	8 (80)
	Valine	11 (92)	
	Lysine		1 (10)
	Aspartic acid		1 (10)
30	Tyrosine	12 (100)	7 (70)
	Phenylalanine		3 (30)
31	Phenylalanine	12 (100)	7 (70)
	Isoleucine		3 (30)
34	Leucine	12 (100)	6 (60)
	Arginine		4 (40)
37	Asparagine	12 (100)	9 (90)
	Tryptophan		1 (10)
38	Leucine	12 (100)	9 (90)
	Valine		1 (10)
47	Phenylalanine	12 (100)	6 (60)
	Tyrosine		4 (40)
51	Threonine	12 (100)	10 (100)
57	Aspartic acid	12 (100)	9 (90)
	Glutamic acid		1 (10)
63	Serine	12 (100)	9 (90)
	Arginine		1 (10)
67	Phenylalanine	11 (92)	5 (50)
	Isoleucine	1 (8)	5 (50)
68	Leucine	12 (100)	10 (100)
70	Glutamine	12 (100)	7 (70)
	Aspartic acid		3 (30)
71	Lysine	11 (92)	6 (60)
	Arginine	1 (8)	1 (10)
	Glutamic acid/Alanine		3 (30)
74	Glutamic acid	10 (83)	5 (50)
	Alanine	2 (17)	2 (20)
	Serine		3 (30)
78	Valine	10 (83)	5 (50)
	Tyrosine	2 (17)	5 (50)
81	Histidine	11 (92)	10 (100)
	Arginine	1 (8)	
86	Serine		9 (100)
	Phenylalanine	10 (83)	1 (10)
	Leucine	2 (17)	
88	Lysine	2 (17)	9 (100)
	Asparagine	10 (83)	1 (10)
92	Arginine	3 (25)	9 (100)
	Proline	9 (75)	1 (10)

Table S4. List of EAT-associated amino acid residues in I-E, their positions in peptide-binding pockets, and changes upon amino acid substitutions

Residue*	MHC pocket†	Susceptible	Resistant	Changes‡
β26	P4 (bottom)	Leu ($P = 0.03$)	Phe ($P = 0.03$)	Larger in size
β28	P4 (bottom) P6 (bottom)‖	Val ($P = 1.8 \times 10^{-5}$)	Glu¶ ($P = 6.6 \times 10^{-4}$)	Negative, polar
β30	P6 (bottom)	Tyr ($P = 0.04$)	Phe	Loss of −OH
β71	P4 (side)	Lys§ ($P = 0.04$)	Glu¶/Ala ($P = 0.04$)	Smaller in size negative/uncharged
β74	P4 (side)	Glu¶ ($P = 0.03$)	Ser ($P = 0.03$)	Smaller in size uncharged
β86	P1 (bottom)	Phe ($P = 6.1 \times 10^{-4}$)	Ser ($P = 1.9 \times 10^{-5}$)	Smaller in size polar
β88	P1 (side)	Asn ($P = 6.1 \times 10^{-4}$)	Lys§ ($P = 6.1 \times 10^{-4}$)	Larger in size positive

*The five residues shown in bold (β28, β30, β71, β86, and β88) interact directly with the antigenic peptide.
†The location of amino acid in MHC pocket is described in parentheses, such as bottom, side, and above.
‡The changes described here reflect the change in the amino acid residue itself (resulting from the amino acid substitution from EAT-susceptible to -resistant residue), not the changes in MHC pocket. Blue, positive charge; red, negative charge.
§Positive charge.
¶Negative charge.
‖Partially.

Table S5. Primers used for direct sequencing of exon 2 of the human *HLA-DRB1* gene

HLA-DR	Forward	Reverse	Size, bp
1	TTCTTGTGGCAGCTTAAGTT	CCGCTGCACTGTGAAGCTCT	266
2	TTCCTGTGGCAGCCTAAGAGG	CCGCTGCACTGTGAAGCTCT	266
3	TGGAGTACTCTACGTCT	CAACCCCGTAGTTGTGTCTG	234
4	GTTTCTTGGAGCAGGTTAAAC	CCGCTGCACTGTGAAGCTCT	266
7	CCTGTGGACAGGGTAAGTATA	CCGTAGTTGTGTCTGCACAC	266
9	AACCACGTTTCTTGAAGCAGGA	CCGTAGTTGTGTCTGCACAC	266
10	CCGTTGCTGGAAAGACGCG	CCGCTGCACTGTGAAGCTCT	266
11, 12, 13, 14	AGTGTCTTCTCAGGAGGCCG	TCACAGGGACTCAGGCCCCG	359