Georgopoulos AP, James LM. Immunogenetic Association of 127 Human Leukocyte Antigen (HLA) Alleles with 30 Cancers in Continental Western European Countries. J Immunological Sci. (2022); 6(2): 6-17

Journal of Immunological Sciences

Original Research Article Open Access

Immunogenetic Association of 127 Human Leukocyte Antigen (HLA) Alleles with 30 Cancers in Continental Western European Countries

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Article Info

Article Notes
Received: February 09, 2022
Accepted: May 05, 2022

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Keywords
Cancer
Human leukocyte antigen
Epidemiology
Immunity
genetics

ABSTRACT

Human leukocyte antigen (HLA) genes have been associated with susceptibility and protection against a number of cancers. Here we used an immunogenetic epidemiological approach to evaluate the overall influence of 127 HLA Class I and II alleles on 30 types of cancer. We found a preponderance of protective alleles (negatively correlated with cancer prevalences), especially for HLA Class I. Of the 30 cancers investigated, 13 were associated with mostly protective HLA effects whereas only 2 were associated with mostly susceptibility HLA alleles. Taken together, these findings highlight the broad influence of HLA on cancer and the complexity of HLA-cancer associations.

Introduction

Cancer is a leading cause of death worldwide and represents a major global burden with an estimated 19 million new cases annually, 8% of which are in Western Europe[1]. Consequently, identification of factors that promote cancer susceptibility and protection are of paramount importance. To that end, growing research has documented the influence of human leukocyte antigen (HLA) on various types of cancer[2-5].

Two main classes of HLA, which are cell-surface proteins encoded by genes on chromosome 6, are instrumental in immune system surveillance and T-cell activation aimed at elimination of non-self (e.g., viral, bacterial) and mutated antigens including those produced by tumors[4,6]. HLA Class I molecules (HLA-A, B, C genes), expressed on nucleated cells, bind small peptides (8-10 amino acid residues) from proteolytically degraded cytosolic viruses, bacteria, and tumors to export them to the cell surface for presentation to CD8+ cytotoxic T cells, thereby signaling cell destruction. HLA Class II molecules (HLA-DPB1, DQB1, DRB1 genes), expressed on lymphocytes and professional antigen presenting cells (e.g., macrophages, dendritic cells, and monocytes), present larger peptides (12-22 amino acid residues) derived from endocytosed exogenous antigens to CD4+ T cells to initiate antibody production and adaptive immunity. HLA is the most highly polymorphic region of the human genome; that variability gives rise to alterations of the binding groove which impact binding affinity with and elimination of foreign antigens,[7] and ultimately influence disease susceptibility[8-11].

Variations in HLA have been found to influence cancer susceptibility[2,11] and treatment response[4,5]. A recent study of HLA gene expression in 33 cancers documented variability in HLA gene

expression across cancer types and demonstrated that HLA gene expression was significantly associated with survival and response to immune checkpoint blockade (ICB) therapy[12]. In addition, a recent population-genome-wide association study (GWAS) across 18 cancer types identified shared genetic basis of several cancer types including several single nucleotide polymorphisms (SNPs) in the HLA region[13]. Furthermore, HLA SNPs that are associated with increases in certain cancers were associated with decreases in other cancer types[13], highlighting the complex associations between HLA and cancer. HLA genes are the most highly polymorphic of the human genome and, to our knowledge, the association of a large number of HLA alleles with several types of cancer have not been investigated. Here we adopted an immunogenetic epidemiological approach to evaluate the overall influence of 127 high-resolution HLA lass I and II alleles on the population prevalence of 30 cancers in 14 Continental Western European countries. For that purpose, we computed estimates of HLA protection on cancer and relied on their 95% confidence intervals[14-18] to quantify outcomes of protection for individual alleles, allele groups (HLA Class I and II, genes, HLA supertypes), and individual cancers.

Materials and Methods

Prevalence of 30 cancers

The population prevalence of the 30 cancers named in Table 1 in 2016 was computed for each of the following 14 countries in Continental Western Europe (CWE): Austria, Belgium, Denmark, Finland, France, Germany, Greece, Italy, Netherlands, Portugal, Norway, Spain, Sweden, and Switzerland. Specifically, the total number of people with each cancer in each of the 14 CWE countries was identified from the Global Health Data Exchange[19], a publicly available catalog of data from the Global Burden of Disease study, the most comprehensive worldwide epidemiological study of more than 350 diseases. The number of people with each cancer in each country was divided by the total population of each country in 2016 (Population Reference Bureau[20]) and expressed as a percentage (data given in the Appendix). We have previously shown that life expectancy for these countries are virtually identical[21]; therefore, life expectancy was not included in the current analyses.

HLA

The frequencies of all reported HLA alleles of classical genes of Class I (A, B, C) and Class II (DPB1, DQB1, DRB1) for each of the 14 CWE countries were retrieved from the website allelefrequencies.net (Estimation of Global Allele Frequencies)[22,23] on October 20, 2020. As we reported previously[24], there was a total of 2746 entries of alleles from the 14 CWE countries, of which 844 alleles occurred in at least one country ("distinct" alleles). Of those, 127 alleles occurred in 9 or more countries and were used in further analyses. This criterion is somewhat arbitrary but reasonable; it was partially validated in a previous study[24]. The distribution of those alleles to the HLA classes and their genes is given in Table 2.

Table 1: The 30 cancers studied. "Full name" is the name as it appears in the database[8] from which the data were obtained. "Abbreviated name" is a shorter name used in this paper.

	Full name of cancer[8]	Abbreviated name
1	Bladder cancer	Bladder
2	Brain and central nervous system cancer	Brain
3	Breast cancer	Breast
4	Cervical cancer	Cervical
5	Colon and rectum cancer	Colorectal
6	Esophageal cancer	Esophageal
7	Gallbladder and biliary tract cancer	Gallbladder
8	Hodgkin lymphoma	Hodgkin
9	Kidney cancer	Kidney
10	Larynx cancer	Larynx
11	Lip and oral cavity cancer	Oral
12	Liver cancer	Liver
13	Malignant skin melanoma	Malignant melanoma
14	Mesothelioma	Mesothelioma
15	Multiple myeloma	Multiple myeloma
16	Nasopharynx cancer	Nasopharynx
17	Neoplasms	Neoplasms
18	Non-Hodgkin lymphoma	Non Hodgkin
19	Non-melanoma skin cancer	Non melanoma skin
20	Non-melanoma skin cancer (basal-cell carcinoma)	Non melanoma basal cell
21	Non-melanoma skin cancer (squamous-cell carcinoma)	Non melanoma squamous cell
22	Other pharynx cancer	Other Pharynx
23	Ovarian cancer	Ovarian
24	Pancreatic cancer	Pancreatic
25	Prostate cancer	Prostate
26	Stomach cancer	Stomach
27	Testicular cancer	Testicular
28	Thyroid cancer	Thyroid
29	Tracheal, bronchus, and lung cancer	Tracheal, bronchus and lung
30	Uterine cancer	Uterine

In addition, alleles of Class I A and B genes were assigned to a supertype[25]. (Supertypes for gene C of Class I or any gene of Class II have not been described.) Of a total of 56 alleles of Class I A and B genes, 53 alleles could be assigned to supertypes based on the assignments provided by Sidney et al.[25], namely all 20 A gene alleles and 33/36 B gene alleles; B*13:02, B*47:01 and B*49:01 were unassigned (Fig. 2 in ref[25]). The distribution of alleles into supertypes is given in Table 3, and individual details are given in Table 4.

Table 2: Distribution of 127 HLA alleles analyzed to Class and Genes.

	Class I (N = 69 alleles)			Class II (N = 58 alleles)		
Gene	A	B	C	DPB1	DQB1	DRB1
Count	20	36	13	15	14	29

Table 3: Distribution of 56 Class I A and B alleles in supertypes

Supertype	Count
A01	6
A02	3
A03	6
A24	3
A103	1
A124	1
B07	8
B08	1
B27	8
B44	11
B58	3
B62	2
Unassigned	3
Total	56

Table 4: The 127 HLA alleles used and their Class, gene and supertype assignments.

Index	Allele	Class	Gene	Supertype
1	A*01:01	I	A	A01
2	A*02:01	I	A	A02
3	A*02:05	I	A	A02
4	A*03:01	I	A	A03
5	A*11:01	I	A	A03
6	A*23:01	I	A	A24
7	A*24:02	I	A	A24
8	A*25:01	I	A	A01
9	A*26:01	I	A	A01
10	A*29:01	I	A	A24
11	A*29:02	I	A	A01 A24
12	A*30:01	I	A	A01 A03
13	A*30:02	I	A	A01
14	A*31:01	I	A	A03
15	A*32:01	I	A	A01
16	A*33:01	I	A	A03
17	A*33:03	I	A	A03
18	A*36:01	I	A	A01
19	A*68:01	I	A	A03
20	A*68:02	I	A	A02
21	B*07:02	I	B	B07
22	B*08:01	I	B	B08
23	B*13:02	I	B	Unassigned
24	B*14:01	I	B	B27
25	B*14:02	I	B	B27
26	B*15:01	I	B	B62
27	B*15:17	I	B	B58
28	B*15:18	I	B	B27
29	B*18:01	I	B	B44
30	B*27:02	I	B	B27
31	B*27:05	I	B	B27
32	B*35:01	I	B	B07
33	B*35:02	I	B	B07
34	B*35:03	I	B	B07
35	B*35:08	I	B	B07
36	B*37:01	I	B	B44
37	B*38:01	I	B	B27
38	B*39:01	I	B	B27
39	B*39:06	I	B	B27
40	B*40:01	I	B	B44
41	B*40:02	I	B	B44
42	B*41:01	I	B	B44
43	B*41:02	I	B	B44
44	B*44:02	I	B	B44
45	B*44:03	I	B	B44
46	B*44:05	I	B	B44
47	B*45:01	I	B	B44
48	B*47:01	I	B	Unassigned
49	B*49:01	I	B	Unassigned
50	B*50:01	I	B	B44
51	B*51:01	I	B	B07
52	B*52:01	I	B	B62
53	B*55:01	I	B	B07
54	B*56:01	I	B	B07
55	B*57:01	I	B	B58
56	B*58:01	I	B	B58
57	C*01:02	I	C	
58	C*03:03	I	C	
59	C*04:01	I	C	
60	C*05:01	I	C	
61	C*06:02	I	C	
62	C*07:01	I	C	
63	C*07:02	I	C	
64	C*07:04	I	C	
65	C*12:02	I	C	
66	C*12:03	I	C	
67	C*14:02	I	C	
68	C*15:02	I	C	
69	C*16:01	I	C	
70	DPB1*01:01	II	DPB1	
71	DPB1*02:01	II	DPB1	
72	DPB1*02:02	II	DPB1	
73	DPB1*03:01	II	DPB1	
74	DPB1*04:01	II	DPB1	
75	DPB1*04:02	II	DPB1	
76	DPB1*05:01	II	DPB1	
77	DPB1*06:01	II	DPB1	
78	DPB1*09:01	II	DPB1	
79	DPB1*10:01	II	DPB1	
80	DPB1*11:01	II	DPB1	
81	DPB1*13:01	II	DPB1	
82	DPB1*14:01	II	DPB1	
83	DPB1*17:01	II	DPB1	
84	DPB1*19:01	II	DPB1	
85	DQB1*02:01	II	DQB1	

86	DQB1*02:02	II	DQB1	
87	DQB1*03:01	II	DQB1	
88	DQB1*03:02	II	DQB1	
89	DQB1*03:03	II	DQB1	
90	DQB1*04:02	II	DQB1	
91	DQB1*05:01	II	DQB1	
92	DQB1*05:02	II	DQB1	
93	DQB1*05:03	II	DQB1	
94	DQB1*06:01	II	DQB1	
95	DQB1*06:02	II	DQB1	
96	DQB1*06:03	II	DQB1	
97	DQB1*06:04	II	DQB1	
98	DQB1*06:09	II	DQB1	
99	DRB1*01:01	II	DRB1	
100	DRB1*01:02	II	DRB1	
101	DRB1*01:03	II	DRB1	
102	DRB1*03:01	II	DRB1	
103	DRB1*04:01	II	DRB1	
104	DRB1*04:02	II	DRB1	
105	DRB1*04:03	II	DRB1	
106	DRB1*04:04	II	DRB1	
107	DRB1*04:05	II	DRB1	
108	DRB1*04:07	II	DRB1	
109	DRB1*04:08	II	DRB1	
110	DRB1*07:01	II	DRB1	
111	DRB1*08:01	II	DRB1	
112	DRB1*08:03	II	DRB1	
113	DRB1*09:01	II	DRB1	
114	DRB1*10:01	II	DRB1	
115	DRB1*11:01	II	DRB1	
116	DRB1*11:02	II	DRB1	
117	DRB1*11:03	II	DRB1	
118	DRB1*11:04	II	DRB1	
119	DRB1*12:01	II	DRB1	
120	DRB1*13:01	II	DRB1	
121	DRB1*13:02	II	DRB1	
122	DRB1*13:03	II	DRB1	
123	DRB1*13:05	II	DRB1	
124	DRB1*14:01	II	DRB1	
125	DRB1*15:01	II	DRB1	
126	DRB1*15:02	II	DRB1	
127	DRB1*16:01	II	DRB1	

Data analysis

HLA profiles for each cancer were derived by computing, first, the Pearson correlation coefficient, r, between the prevalence of a cancer and the population frequency of an allele, and then Fisher z-transform it, r', to normalize its distribution:

$$\text{HLA-cancer Protection/Susceptibility (P/S) estimate } r' = \operatorname{atanh}(r) \quad (1)$$

Negative P/S estimates indicate a protective association ("protective" alleles), whereas positive P/S estimates indicate a susceptibility association ("susceptibility" alleles). Thus 30 Cancer-HLA profiles were computed, each consisting of 127 values of r'. These data were tabulated in a 127 allele (rows) x 30 cancers (columns) matrix ("Cancer-HLA" matrix). We then derived estimates of HLA-Cancer protection and susceptibility by transforming the values of r' into binary values, by assigning the values of 1 and zero to negative (protective) and positive (susceptibility) r' values, respectively. Therefore, each row (Allele) comprised 30 such binary values (one per Cancer), and each column (Cancer) comprised 127 binary values (one per allele). From these data, we derived the following estimates of HLA-Cancer associations: (a) the proportion of alleles protective for a cancer (Protective Proportion, PP), (b) its 95% Confidence Intervals (CI), and (c) the upper-to-lower Confidence Limit Ratio (CLR)[17]:

$$\text{CLR} = \frac{\text{Upper 95\% CI}}{\text{Lower 95\% CI}} \quad (2)$$

The PP provided an overall estimate, from the current sample, of the HLA-Cancer association but its assessment in the context of HLA-Cancer protection rests with the values of its 95% CI and corresponding CLR. More specifically, the values of 95% CI demarcate the range within which the "true" PP in a large sample (the population) would be found 95% of times (in repeated measurements)[14,16,18]. Finally, the upper-to-lower CLR assesses the stability of the PP estimate, with lower CLR indicating a more stable estimate. Since this study is not about testing an overarching hypothesis, we did not report P-values nor refer to significance testing. Instead, we applied the 0.5 cutoff point to the estimate of PP and its 95% CI to assign a specific HLA-Cancer effect to one of 3 groups: (a) "Mostly Protective", if PP > 0.5 *and* its lower 95% CI > 0.5; (b) "Mostly Susceptibility", if PP < 0.5 *and* its upper 95% CI < 0.5; and (c) "Mixed", if lower 95% CI < 0.5 *and* upper 95% CI > 0.5, where the PP estimate can take any value from 0 to 1. Since the focus of this study was the overall assessment of HLA-Cancer relations, no analyses of HLA relations to individual cancer types were performed. The Wilson score[26] was used to derive the asymptotic standard error and 95% CI. Statistical analyses were performed using the IBM–SPSS package (IBM SPSS Statistics for Windows, Version 27.0, 64–bit edition. Armonk, NY: IBM Corp; 2019) and Intel FORTRAN (Microsoft Visual Studio Community 2019, Version 16.7.5; Intel FORTRAN Compiler 2021).

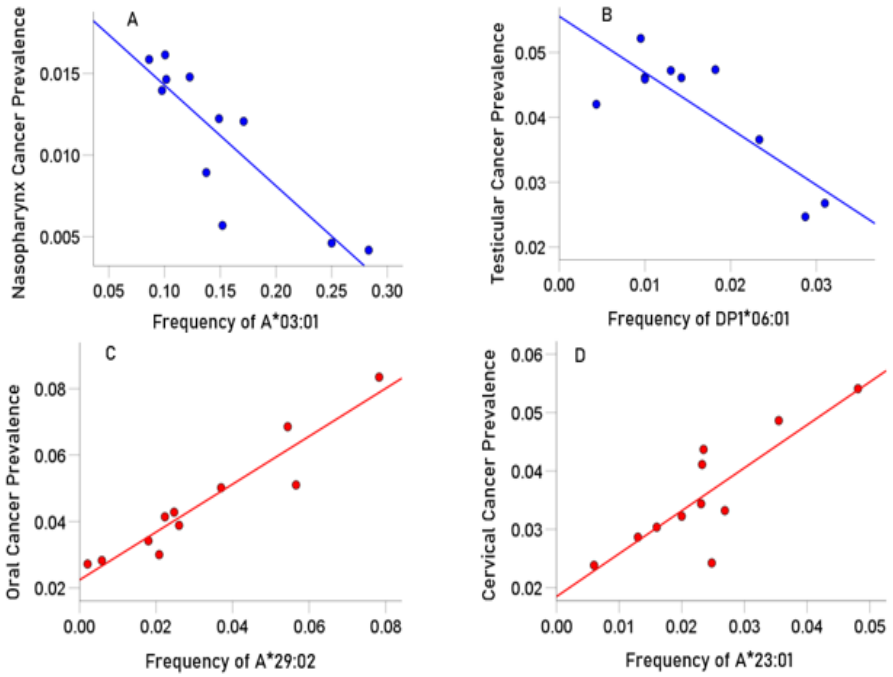
Results

The immunogenetic protective and susceptibility influence of four HLA alleles on various types of cancer are illustrated in Fig. 1A, B and Fig. 1C,D, respectively.

Effects of individual alleles on cancer

The effect of individual alleles on cancer are given

in Table 5. The basic finding is that all alleles had both protective and susceptibility effects on various cancers. Most of the alleles (99/127 = 78%) were in the "Mixed" group, whereas 20/127 (16%) and 8/127 (6%) were in the "Mostly Protective" and "Mostly Susceptibility" groups. The PP estimates were relatively stable; the mean ± SEM of CLR was 2.021 ± 0.039 (range 1.35 – 3.47).



Figures 1: Examples of two HLA cancer protective alleles illustrating the negative influence of HLA allele frequency on cancer prevalence.
A, the prevalence (%) of cancer of nasopharynx in 11 CWE countries is plotted against the frequency of A*03:01: r = −0.862, r' = −1.301, P = 0.001.
B, the prevalence of testicular cancer in 9 CWE countries is plotted against the frequency of DPB1*06:02: r = −0.832, r' = −1.195, P = 0.003.
C and D: Examples of two HLA cancer susceptibility alleles illustrating the positive influence of HLA allele frequency on cancer prevalence.
C, the prevalence (%) of oral cancer in 11 CWE countries is plotted against the frequency of A*29:02: r = 0.949, r' = 1.822, P < 0.001.
D, the prevalence of cervical cancer in 11 CWE countries is plotted against the frequency of A*23:01: r = 0.834, r' = 1.201, P = 0.001.

Table 5: Proportion protective and associated statistics for the 127 individual alleles tested. Blue: Protective; Red: Susceptibility; Black: Mixed.

Index	Allele	N protective (out of 30 cancers total)	Proportion protective	Asymptotic standard error	Lower 95% CI	Upper 95% CI	Confidence Limit Ratio
1	A*01:01	19	0.633	0.088	0.455	0.781	1.716
2	A*02:01	13	0.433	0.09	0.274	0.608	2.219
3	A*02:05	12	0.4	0.089	0.246	0.577	2.346
4	A*03:01	18	0.6	0.089	0.423	0.754	1.783
5	A*11:01	12	0.4	0.089	0.246	0.577	2.346
6	A*23:01	16	0.533	0.091	0.361	0.698	1.934
7	A*24:02	20	0.667	0.086	0.488	0.808	1.656
8	A*25:01	9	0.3	0.084	0.167	0.479	2.868
9	A*26:01	17	0.567	0.09	0.392	0.726	1.852
10	A*29:01	21	0.7	0.084	0.521	0.833	1.599
11	A*29:02	11	0.367	0.088	0.219	0.545	2.489
12	A*30:01	12	0.4	0.089	0.246	0.577	2.346
13	A*30:02	10	0.333	0.086	0.192	0.512	2.667
14	A*31:01	21	0.7	0.084	0.521	0.833	1.599
15	A*32:01	18	0.6	0.089	0.423	0.754	1.783
16	A*33:01	14	0.467	0.091	0.302	0.639	2.116
17	A*33:03	24	0.8	0.073	0.627	0.905	1.443
18	A*36:01	16	0.533	0.091	0.361	0.698	1.934

19	A*68:01	19	0.633	0.088	0.455	0.781	1.716
20	A*68:02	22	0.733	0.081	0.556	0.858	1.543
21	B*07:02	21	0.7	0.084	0.521	0.833	1.599
22	B*08:01	15	0.5	0.091	0.332	0.668	2.012
23	B*13:02	16	0.533	0.091	0.361	0.698	1.934
24	B*14:01	14	0.467	0.091	0.302	0.639	2.116
25	B*14:02	13	0.433	0.09	0.274	0.608	2.219
26	B*15:01	18	0.6	0.089	0.423	0.754	1.783
27	B*15:17	10	0.333	0.086	0.192	0.512	2.667
28	B*15:18	18	0.6	0.089	0.423	0.754	1.783
29	B*18:01	14	0.467	0.091	0.302	0.639	2.116
30	B*27:02	19	0.633	0.088	0.455	0.781	1.716
31	B*27:05	24	0.8	0.073	0.627	0.905	1.443
32	B*35:01	14	0.467	0.091	0.302	0.639	2.116
33	B*35:02	21	0.7	0.084	0.521	0.833	1.599
34	B*35:03	10	0.333	0.086	0.192	0.512	2.667
35	B*35:08	16	0.533	0.091	0.361	0.698	1.934
36	B*37:01	18	0.6	0.089	0.423	0.754	1.783
37	B*38:01	17	0.567	0.09	0.392	0.726	1.852
38	B*39:01	18	0.6	0.089	0.423	0.754	1.783
39	B*39:06	21	0.7	0.084	0.521	0.833	1.599
40	B*40:01	19	0.633	0.088	0.455	0.781	1.716
41	B*40:02	26	0.867	0.062	0.703	0.947	1.347
42	B*41:01	15	0.5	0.091	0.332	0.668	2.012
43	B*41:02	18	0.6	0.089	0.423	0.754	1.783
44	B*44:02	19	0.633	0.088	0.455	0.781	1.716
45	B*44:03	13	0.433	0.09	0.274	0.608	2.219
46	B*44:05	18	0.6	0.089	0.423	0.754	1.783
47	B*45:01	17	0.567	0.09	0.392	0.726	1.852
48	B*47:01	20	0.667	0.086	0.488	0.808	1.656
49	B*49:01	14	0.467	0.091	0.302	0.639	2.116
50	B*50:01	17	0.567	0.09	0.392	0.726	1.852
51	B*51:01	18	0.6	0.089	0.423	0.754	1.783
52	B*52:01	20	0.667	0.086	0.488	0.808	1.656
53	B*55:01	7	0.233	0.077	0.118	0.409	3.466
54	B*56:01	17	0.567	0.09	0.392	0.726	1.852
55	B*57:01	14	0.467	0.091	0.302	0.639	2.116
56	B*58:01	15	0.5	0.091	0.332	0.668	2.012
57	C*01:02	17	0.567	0.09	0.392	0.726	1.852
58	C*03:03	15	0.5	0.091	0.332	0.668	2.012
59	C*04:01	17	0.567	0.09	0.392	0.726	1.852
60	C*05:01	15	0.5	0.091	0.332	0.668	2.012
61	C*06:02	10	0.333	0.086	0.192	0.512	2.667
62	C*07:01	16	0.533	0.091	0.361	0.698	1.934
63	C*07:02	13	0.433	0.09	0.274	0.608	2.219
64	C*07:04	20	0.667	0.086	0.488	0.808	1.656
65	C*12:02	16	0.533	0.091	0.361	0.698	1.934
66	C*12:03	13	0.433	0.09	0.274	0.608	2.219
67	C*14:02	18	0.6	0.089	0.423	0.754	1.783
68	C*15:02	18	0.6	0.089	0.423	0.754	1.783
69	C*16:01	12	0.4	0.089	0.246	0.577	2.346
70	DPB1*01:01	16	0.533	0.091	0.361	0.698	1.934
71	DPB1*02:01	12	0.4	0.089	0.246	0.577	2.346
72	DPB1*02:02	16	0.533	0.091	0.361	0.698	1.934
73	DPB1*03:01	7	0.233	0.077	0.118	0.409	3.466

74	DPB1*04:01	22	0.733	0.081	0.556	0.858	1.543
75	DPB1*04:02	24	0.8	0.073	0.627	0.905	1.443
76	DPB1*05:01	16	0.533	0.091	0.361	0.698	1.934
77	DPB1*06:01	19	0.633	0.088	0.455	0.781	1.716
78	DPB1*09:01	9	0.3	0.084	0.167	0.479	2.868
79	DPB1*10:01	13	0.433	0.09	0.274	0.608	2.219
80	DPB1*11:01	10	0.333	0.086	0.192	0.512	2.667
81	DPB1*13:01	15	0.5	0.091	0.332	0.668	2.012
82	DPB1*14:01	17	0.567	0.09	0.392	0.726	1.852
83	DPB1*17:01	12	0.4	0.089	0.246	0.577	2.346
84	DPB1*19:01	14	0.467	0.091	0.302	0.639	2.116
85	DQB1*02:01	12	0.4	0.089	0.246	0.577	2.346
86	DQB1*02:02	14	0.467	0.091	0.302	0.639	2.116
87	DQB1*03:01	10	0.333	0.086	0.192	0.512	2.667
88	DQB1*03:02	10	0.333	0.086	0.192	0.512	2.667
89	DQB1*03:03	23	0.767	0.077	0.591	0.882	1.492
90	DQB1*04:02	24	0.8	0.073	0.627	0.905	1.443
91	DQB1*05:01	23	0.767	0.077	0.591	0.882	1.492
92	DQB1*05:02	10	0.333	0.086	0.192	0.512	2.667
93	DQB1*05:03	7	0.233	0.077	0.118	0.409	3.466
94	DQB1*06:01	22	0.733	0.081	0.556	0.858	1.543
95	DQB1*06:02	19	0.633	0.088	0.455	0.781	1.716
96	DQB1*06:03	19	0.633	0.088	0.455	0.781	1.716
97	DQB1*06:04	21	0.7	0.084	0.521	0.833	1.599
98	DQB1*06:09	16	0.533	0.091	0.361	0.698	1.934
99	DRB1*01:01	15	0.5	0.091	0.332	0.668	2.012
100	DRB1*01:02	17	0.567	0.09	0.392	0.726	1.852
101	DRB1*01:03	18	0.6	0.089	0.423	0.754	1.783
102	DRB1*03:01	9	0.3	0.084	0.167	0.479	2.868
103	DRB1*04:01	17	0.567	0.09	0.392	0.726	1.852
104	DRB1*04:02	14	0.467	0.091	0.302	0.639	2.116
105	DRB1*04:03	13	0.433	0.09	0.274	0.608	2.219
106	DRB1*04:04	21	0.7	0.084	0.521	0.833	1.599
107	DRB1*04:05	7	0.233	0.077	0.118	0.409	3.466
108	DRB1*04:07	16	0.533	0.091	0.361	0.698	1.934
109	DRB1*04:08	24	0.8	0.073	0.627	0.905	1.443
110	DRB1*07:01	14	0.467	0.091	0.302	0.639	2.116
111	DRB1*08:01	24	0.8	0.073	0.627	0.905	1.443
112	DRB1*08:03	18	0.6	0.089	0.423	0.754	1.783
113	DRB1*09:01	25	0.833	0.068	0.664	0.927	1.396
114	DRB1*10:01	19	0.633	0.088	0.455	0.781	1.716
115	DRB1*11:01	10	0.333	0.086	0.192	0.512	2.667
116	DRB1*11:02	16	0.533	0.091	0.361	0.698	1.934
117	DRB1*11:03	9	0.3	0.084	0.167	0.479	2.868
118	DRB1*11:04	10	0.333	0.086	0.192	0.512	2.667
119	DRB1*12:01	14	0.467	0.091	0.302	0.639	2.116
120	DRB1*13:01	15	0.5	0.091	0.332	0.668	2.012
121	DRB1*13:02	14	0.467	0.091	0.302	0.639	2.116
122	DRB1*13:03	16	0.533	0.091	0.361	0.698	1.934
123	DRB1*13:05	19	0.633	0.088	0.455	0.781	1.716
124	DRB1*14:01	12	0.4	0.089	0.246	0.577	2.346
125	DRB1*15:01	16	0.533	0.091	0.361	0.698	1.934
126	DRB1*15:02	20	0.667	0.086	0.488	0.808	1.656
127	DRB1*16:01	16	0.533	0.091	0.361	0.698	1.934

Table 6: Proportion Protective and associated statistics: Overall, HLA Class I and II, and their genes. N total is the number of alleles in the group specified x 30 cancers. Blue: Protective; Black: Mixed.

	N protective	N total	Proportion protective	Asymptotic standard error	95% Confidence Interval		Confidence Limit Ratio
					Lower	Upper	
Overall	2038	3810	0.535	0.008	0.519	0.551	1.062
Class I	1128	2070	0.545	0.011	0.523	0.566	1.082
A	324	600	0.540	0.02	0.500	0.579	1.158
B	604	1080	0.559	0.015	0.529	0.589	1.113
C	200	390	0.513	0.025	0.463	0.562	1.214
Class II	910	1740	0.523	0.012	0.499	0.546	1.033
DPB1	222	450	0.493	0.024	0.447	0.539	1.206
DQB1	230	420	0.548	0.024	0.500	0.595	1.190
DRB1	458	870	0.526	0.017	0.493	0.559	1.134

Table 7: Proportion protective and associated statistics for HLA Class I genes A and B supertypes. N total is number of alleles in a supertype (Table 3) x 30 cancers. Blue: Protective; Black: Mixed.

Supertype	N protective	N total	Proportion protective	Asymptotic standard error	95% Confidence Interval		Confidence Limit Ratio
					Lower	Upper	
A01	89	180	0.494	0.037	0.422	0.567	1.344
A02	47	90	0.522	0.053	0.420	0.622	1.481
A03	108	180	0.600	0.037	0.527	0.669	1.269
A24	57	90	0.633	0.051	0.530	0.726	1.370
A01 A03	12	30	0.400	0.089	0.246	0.577	2.346
A01 A24	11	30	0.367	0.088	0.219	0.545	2.489
B07	124	240	0.517	0.032	0.454	0.579	1.275
B08	15	30	0.500	0.091	0.332	0.668	2.012
B27	144	240	0.600	0.032	0.537	0.660	1.229
B44	194	330	0.588	0.027	0.534	0.640	1.199
B58	39	90	0.433	0.052	0.336	0.536	1.595
B62	38	60	0.633	0.062	0.507	0.744	1.467

Overall effects of HLA on cancer, including effects of Class and Genes (Table 6)

There was an overall protective effect of HLA on cancer, and similarly for HLA Class I in general and for HLA Class I genes A and B, in particular; all of these groups had "mostly Protective mostly" effects. The remainder (HLA Class II and genes DPB1, DQB1 and DRB1) had "Mixed effects". However, the highest PP estimate was 0.559, which means that at least 40% of the alleles in those groups had a susceptibility effect, although the overall bias was towards a protective effect. As shown in the CLR column of Table 6, the PP estimates were stable, with all CLR values < 2, and all but one < 1.5.

Effects of A and B supertypes on cancer

Of 12 supertypes tested, supertypes A03, A24, B27, B44 and B52 had a statistically significant protective effect both in the test of proportions (Table 7). However, the highest PP estimate was 0.633, which means that at least 35% of the alleles in any supertype had a susceptibility effect on cancer. This can be evaluated by looking up in Table 5 the effects of individual alleles assigned to a specific supertype. Finally, the CLR values ranged from 1.199 to 2.489.

Effects on specific cancer types

The overall effects of HLA on specific cancer types are shown in Table 8. It can be seen that "Mostly Protective" effects predominated, so that cancers with such effects (N = 13) outnumbered cancers with "Mostly Susceptibility" effects (N =2) by more than 6 times.

Discussion

Here we used a population immunogenetic approach to evaluate the association of 127 HLA alleles with 30 cancers in 14 Continental Western European countries. The findings document a preponderance of protective effects of HLA on cancer in general and on specific cancers in particular. Every allele exerted both protective and susceptibility effects for various types of cancer. However, the strength of these effects varied across individual alleles, groups of alleles (HLA Class I and II, genes and supertypes), and across cancer types. We qualified this strength by relying on the 95% confidence intervals of the effect estimate and were thus able to grade the effect as "Mostly Protective", "Mostly Susceptibility", and "Mixed". We found Mostly Protective effects overall and in HLA Class I A and B. Analyses examining the effects of individual alleles on

Table 8: Proportion protective and associated statistics for one-sample proportions for the 30 cancers studied. Blue: Protective; Red: Susceptibility; Black: Mixed.

Index	Cancer	N protective (Out of 127 alleles total)	Proportion protective	Asymptotic standard error	Lower 95% CI	Upper 95% CI	CLR
1	Bladder	55	0.433	0.044	0.35	0.52	1.486
2	Brain	62	0.488	0.044	0.403	0.574	1.424
3	Breast	83	0.654	0.042	0.567	0.731	1.289
4	Cervical	51	0.402	0.043	0.320	0.489	1.528
5	Colorectal	69	0.543	0.044	0.457	0.627	1.372
6	Esophageal	79	0.622	0.043	0.535	0.702	1.312
7	Gallbladder	66	0.52	0.044	0.433	0.605	1.397
8	Hodgkin	63	0.496	0.044	0.411	0.582	1.416
9	Kidney	78	0.614	0.043	0.527	0.694	1.317
10	Larynx	54	0.425	0.044	0.343	0.512	1.493
11	Oral	79	0.622	0.043	0.535	0.702	1.312
12	Liver	60	0.472	0.044	0.388	0.559	1.441
13	Malignant melanoma	79	0.622	0.043	0.535	0.702	1.312
14	Mesothelioma	77	0.606	0.043	0.519	0.687	1.324
15	Multiple myeloma	74	0.583	0.044	0.496	0.665	1.341
16	Nasopharynx	49	0.386	0.043	0.306	0.473	1.546
17	Neoplasms	78	0.614	0.043	0.527	0.694	1.317
18	Non Hodgkin	75	0.591	0.044	0.504	0.672	1.333
19	Non melanoma skin	56	0.441	0.044	0.358	0.528	1.475
20	Non melanoma basal cell	55	0.433	0.044	0.350	0.520	1.486
21	Non melanoma squamous cell	59	0.465	0.044	0.380	0.551	1.450
22	Other Pharynx	80	0.63	0.043	0.543	0.709	1.306
23	Ovarian	75	0.591	0.044	0.504	0.672	1.333
24	Pancreatic	75	0.591	0.044	0.504	0.672	1.333
25	Prostate	76	0.598	0.043	0.511	0.680	1.331
26	Stomach	58	0.457	0.044	0.373	0.543	1.456
27	Testicular	75	0.591	0.044	0.504	0.672	1.333
28	Thyroid	71	0.559	0.044	0.472	0.642	1.360
29	Tracheal, bronchus, lung	67	0.528	0.044	0.441	0.612	1.388
30	Uterine	60	0.472	0.044	0.388	0.559	1.441

cancer documented particularly robust protective effects for HLA-B*40:02, but also protective effects conferred by several other Class I and Class II alleles (Table 5). The protective effects of B*40:02 observed here are consistent with a previous finding of beneficial effects of B*40:02 with regard to melanoma treatment outcomes[5]. Those effects were attributed to amino acid composition in the binding pocket that enhances binding affinity and facilitates immune presentation of neoantigens[5]. The present findings suggest that B*40:02 likely has enhanced capability of binding epitopes from a number of different cancers.

Although fewer in number than protective HLA, several alleles were associated with broad susceptibility to cancer. Specifically, Class I HLA-B*55:01 and three HLA Class II alleles – DPB1*03:01, DQB1*05:03, DRB1*04:05 were associated with significantly fewer protective effects and, accordingly, a higher proportion of susceptibility effects. At least one of these alleles (DPB1*03:01) has previously been associated with cervical cancer risk[27] and hepatocellular carcinoma[28]. In the case of HLA susceptibility to cancer, here too, structural features of the peptide binding groove purportedly impairing the strength of the interaction with the T cell receptor and hindering neoantigen binding have been implicated[5]. It is possible that structural features of these 4 alleles may inhibit binding of several different types of cancer epitopes although the mechanism underlying reduced cancer protection associated with those particularly alleles remain to be investigated. Finally, although the relative proportion of these 4 alleles indicate significantly less protection, each was protective against 7 of the 30 cancers indicating some, albeit less, protection against cancers than other alleles.

Overall, Class II HLA was not significantly associated with cancer protection although several specific Class II alleles were. For example, we found that DRB1*09:01 was protective against 25 of 30 cancers. Previous studies have documented decreased risk of cervical cancer in DRB1*09:01 carriers[29]. Our findings suggest that protective effects of DRB1*09:01 extend well beyond cervical cancer.

In line with the evolutionary role of HLA in host protection, the overall effects of HLA on specific cancer types demonstrated that of the 30 cancers studied, 13 were associated with predominantly protective HLA effects with regard to population prevalence. Breast cancer, in particular, was associated with the greatest proportion of protective HLA effects. In contrast, only nasopharynx and cervical cancer were associated with a statistically significant proportion of HLA alleles that favor susceptibility. Nasopharynx cancer is relatively rare, accounting for only 0.8% of cancers[1]. Cervical cancer, however, is one of the most common types of cancer in women[1]. Findings from the current study suggest that the high prevalence of cervical cancer may be partially associated with the preponderance of HLA-cervical cancer associations favoring susceptibility, presumably due to a relative inability of those alleles to effectively bind and eliminate human papillomavirus (HPV). Indeed, HLA genes have been shown to be important moderators of the persistence of HPV infection and disease progression[30].

Analyses regarding HLA supertype associations with cancer merit comment. Supertypes reflect groups of HLA alleles with similar structural features in the binding groove[25]. The present analyses revealed overall protective effects of 5 HLA supertypes; however, we also found that alleles within a supertype exhibited different associations with cancer. For example, the B*44 supertype includes 11 alleles, of which 10 had Mixed effects and only one was Mostly Protective for cancer. Therefore, analyses aimed at HLA-disease associations need to rely on the association of specific HLA alleles with a given disease in order to avoid misleading conclusions based on HLA supertype association with disease.

The findings of the present study characterize HLA-cancer associations at the population level and highlight the complexity of associations between HLA and cancers. It should be noted that the present study focused exclusively on HLA-cancer associations in Continental Western Europe and may not generalize to other populations where HLA and cancer frequencies vary. Finally, the HLA-cancer associations documented here reflect overall cancer/risk protection conferred by HLA. HLA relations to individual cancer types were not evaluated; however, analyses aimed at determining the HLA protection/susceptibility profile for individual cancers are underway.

Acknowledgements

We dedicate this paper to the memory of Dr. Angeliki 'Lily' Georgopoulos, a sparkling medical scientist and compassionate physician. Partial funding for this study was provided by the University of Minnesota (the Anita Kunin Chair in Women's Healthy Brain Aging, the Brain and Genomics Fund, the McKnight Presidential Chair of Cognitive Neuroscience, and the American Legion Brain Sciences Chair) and the U.S. Department of Veterans Affairs. The sponsors had no role in the current study design, analysis or interpretation, or in the writing of this paper. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Author contributions

A.P.G. conceived the study; L.M.J. and A.P.G. contributed to data retrieval and analysis and writing the paper.

Additional Information

Competing interests: Authors declare no competing interests.

Data and materials availability: Data are publicly available from the websites mentioned in the Materials and Methods section.

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APPENDIX: Cancer prevalences (percent of population) for the 14 CWE countries studied. Numbers in the Cancer column indicate the cancer types in Table 1.

Cancer	Austria	Belgium	Denmark	Finland	France	Germany	Greece	Italy	Netherlands	Norway	Portugal	Spain	Sweden	Switzerland
1	0.160495	0.182679	0.204592	0.103505	0.200569	0.141235	0.243121	0.291993	0.215679	0.148059	0.193791	0.270801	0.163699	0.120562
2	0.028030	0.062458	0.151934	0.107722	0.060662	0.015916	0.086716	0.031262	0.071787	0.133757	0.026247	0.046891	0.090049	0.063021
3	0.716133	0.935909	0.805015	0.913484	0.892634	0.920869	0.846515	1.023279	1.034470	0.646759	0.805729	0.754296	0.886465	0.806802
4	0.032251	0.030363	0.033378	0.023842	0.033218	0.043694	0.034386	0.041112	0.028660	0.032648	0.054106	0.048641	0.024739	0.024246
5	0.368466	0.409325	0.496664	0.372435	0.422199	0.477471	0.363982	0.575149	0.593907	0.512094	0.501416	0.605819	0.411244	0.398248
6	0.011989	0.019247	0.018124	0.015650	0.017067	0.031595	0.005728	0.008267	0.033660	0.010735	0.011906	0.012056	0.010180	0.017204
7	0.007188	0.008467	0.007611	0.007708	0.003941	0.011898	0.005562	0.011008	0.006328	0.006951	0.008284	0.011048	0.005468	0.005031
8	0.017623	0.020869	0.022029	0.028147	0.023858	0.021941	0.051940	0.028149	0.028491	0.023605	0.018375	0.030885	0.016704	0.021425
9	0.059679	0.062317	0.066399	0.101814	0.071965	0.106038	0.059829	0.121483	0.090047	0.090319	0.053740	0.078951	0.048833	0.053492
10	0.024585	0.037191	0.032551	0.020688	0.052793	0.027343	0.052565	0.045469	0.028570	0.019097	0.030387	0.057612	0.016612	0.024627
11	0.034202	0.050173	0.044133	0.035649	0.051026	0.041432	0.027224	0.030036	0.038859	0.027816	0.068549	0.083469	0.028279	0.042820
12	0.016988	0.011094	0.011930	0.021734	0.018315	0.017449	0.009487	0.020614	0.009293	0.007766	0.012081	0.018327	0.006480	0.019508
13	0.155317	0.161624	0.279253	0.207105	0.163535	0.194133	0.098798	0.168792	0.311084	0.293956	0.089723	0.132074	0.277498	0.240250
14	0.003286	0.006594	0.006450	0.005198	0.007471	0.005931	0.001606	0.004186	0.010043	0.004618	0.001839	0.003052	0.004242	0.006598
15	0.020749	0.023311	0.028776	0.029890	0.022798	0.031450	0.019868	0.038271	0.029269	0.033592	0.009948	0.026299	0.022087	0.028934
16	0.005692	0.012067	0.005754	0.004613	0.014783	0.008932	0.015861	0.014637	0.012232	0.005022	0.013960	0.016142	0.004177	0.011754
17	25.402148	12.129196	11.354226	13.008606	11.395796	17.288905	11.427260	14.752724	12.066444	15.936168	9.606932	12.280181	12.896458	11.915964
18	0.095709	0.097870	0.091865	0.129690	0.105514	0.110426	0.060407	0.132533	0.130739	0.097079	0.103953	0.097434	0.112269	0.101248
19	0.009279	0.014538	0.017942	0.017067	0.023406	0.023622	0.020263	0.023146	0.016984	0.016206	0.043869	0.018580	0.021846	0.033401
20	0.005916	0.007883	0.009306	0.008888	0.010043	0.013845	0.009439	0.013819	0.005997	0.008002	0.006177	0.010149	0.012418	0.017836
21	0.009822	0.013370	0.015666	0.015438	0.020469	0.016755	0.018400	0.018113	0.018381	0.015179	0.044831	0.016214	0.015082	0.022747
22	0.014909	0.014070	0.025311	0.009281	0.022968	0.016675	0.002664	0.008592	0.013099	0.013659	0.013263	0.011308	0.008291	0.015608
23	0.035020	0.031801	0.037215	0.043874	0.032210	0.041454	0.035857	0.036949	0.039044	0.039287	0.018979	0.040680	0.028421	0.030699
24	0.019278	0.015058	0.016668	0.025348	0.016098	0.026733	0.016808	0.020455	0.018199	0.016008	0.011273	0.015482	0.011865	0.017970
25	0.639822	0.537096	0.577858	0.860169	0.527890	0.722608	0.483197	0.563873	0.725372	0.722331	0.600947	0.454652	0.783261	0.689391
26	0.039905	0.028387	0.025361	0.033289	0.029546	0.046712	0.045539	0.067790	0.044077	0.028214	0.055033	0.060895	0.020385	0.033607
27	0.046114	0.026762	0.053612	0.028513	0.047357	0.052172	0.042026	0.047221	0.046182	0.045892	0.025076	0.024889	0.056579	0.038460
28	0.056250	0.039798	0.032303	0.050987	0.037893	0.051300	0.031232	0.084169	0.047240	0.045782	0.056324	0.043268	0.028710	0.046703
29	0.103245	0.108026	0.131820	0.090158	0.104387	0.122976	0.111465	0.103634	0.132718	0.107393	0.051975	0.115183	0.049051	0.077115
30	0.123007	0.125776	0.122065	0.142600	0.134496	0.119680	0.146040	0.226512	0.165885	0.122498	0.156584	0.170166	0.128506	0.100809