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HLA Homozygosity and Likelihood of Sensitization in Kidney Transplant Candidates

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Background. Homozygosity for HLAs has been associated with adverse outcomes after viral infection as well as pregnancy-induced HLA sensitization. We sought to assess the relationship between HLA locus homozygosity and the level of HLA antibody sensitization. **Methods.** We measured sensitization using the calculated panel reactive antibody value for a large cohort of 147 461 patients added to the US OPTN/United Network for Organ Sharing kidney transplant waitlist between December 2014 and December 2019. We used multinomial logistic modeling to compare 62 510 sensitized patients to 84 955 unsensitized controls. **Results.** We found that the number of homozygous HLA loci was strongly associated with the level of sensitization. Within mildly, highly, or extremely sensitized candidates, women displayed a higher relative abundance of HLA homozygosity at multiple HLA loci as compared with men, with attenuation of this effect in Black candidates. In a multivariable logistic model, the number of homozygous HLA loci interacted with female sex but not with other factors associated with sensitization, including recipient ethnicity and a history of prior kidney transplant. **Conclusions.** This study shows that HLA homozygosity is an innate genetic factor that affects the likelihood of HLA sensitization. Further research is needed to identify the immunologic mechanisms that underlie this observation.

(Transplantation Direct 2022;8: e1312; doi: 10.1097/TXD.0000000000001312).

HLA sensitization remains an important disparity, which limits access to deceased and living donor kidney transplant.[1,2] Sensitization results from exposure to nonself HLA antigens, most commonly through blood transfusions,[3,4] pregnancy,[5,6] and previous organ transplantation.[7,8] Patient characterics such as ethnicity[7,9] are also associated with the level of sensitization, although the biological mechanisms of this remain unclear.

Several aspects of the epidemiology of sensitization remain enigmatic. First, sensitization is a pervasive problem, affecting 25%–40% of candidates for solid organ[10,11] and stem cell transplants.[12] Next, HLA antibodies can be identified in a small percentage of women and men apheresis donors without a history of pregnancy or transfusion[5,13-15] suggesting that additional factors, such as crossreactivity/heterologous immunity, may contribute to sensitization.[16,17]

An individual's HLA genotype is comprised of 2 haplotypes, one inherited from each parent.[18] Compared with other human genes, HLA loci display higher levels of heterozygosity, driven by balancing selection.[19] HLA heterozygosity has been found to be a marker of improved outcomes in a number of viral infections, including human immunodeficiency virus[20] and hepatitis C,[21] as well as in the response to cancer immunotherapy.[22] Conversely, HLA homozygosity has been associated with adverse outcomes in coronavirus disease 2019[23] and an increased risk of lymphoma development.[24] HLA homozygosity has been identified as a risk factor for pregnancy-induced HLA sensitization.[6]

In this study, we analyze the relationship between HLA homozygosity and sensitization as measured using the calculated panel reactive antibody (CPRA) value for candidates on the kidney transplant waitlist in the United States. We

hypothesized that HLA homozygosity may increase the likelihood of sensitization and sought to evaluate this relationship across patient characteristics including sex and ethnicity.

MATERIALS AND METHODS

This study was performed under an approved protocol of the Cedars-Sinai Institutional Review Board (Pro00049901).

Study Cohort

We identified 184 828 kidney transplant candidates added to the United Network for Organ Sharing (UNOS) waitlist between December 4, 2014 (the day the Kidney Allocation System took effect) and December 31, 2019 (**Figure 1, SDC,** http://links.lww.com/TXD/A415). Because CPRA is only defined for 4 ethnic groups (White, Black, Hispanic/Latinx, and Asian), individuals of other ethnicities (n = 3459) were excluded. There were 33 904 candidates with incomplete HLA typing (of which 0.02% were missing HLA-A, 90.30% HLA-C, 0.02% HLA-B, 0.02% HLA-DR, and 79.20% HLA-DQ) that were also excluded. The final study cohort comprised a nonsensitized group of 84 955 candidates and a sensitized group of 62 510 candidates.

CPRA Calculation

CPRA was calculated for each unique set of HLA-A, -C, -B, -DR, and -DQ unacceptable HLA antigens (UA-HLA), for each candidate. The National Marrow Donor Program (NMDP)-CPRA was used given its greater accuracy for sensitized candidates as compared with UNOS-CPRA.[25] For candidates with >1 CPRA value, the peak CPRA value prior to transplant was utilized. The CPRA calculator was developed in R[26] and previously published.[10]

HLA Typing and Assignment of Homozygosity

When present, we converted HLA alleles into UNOS antigen categories using a previously described mapping table.[27] We found that the frequency of homozygosity for HLA split antigens was more prevalent in each successively more sensitized group (**Table 1, SDC,** http://links.lww.com/TXD/A415), suggesting that homozygosity for split antigens is functionally similar to homozygosity for broad antigens. As such, split antigens were mapped to the corresponding broad antigen using a mapping table (**Table 2, SDC,** http://links.lww.com/TXD/A415). HLA homozygosity was determined based on the broad HLA antigen assignments for the HLA-A, -C, -B, -DR, and -DQ loci and candidates were designated as homozygotes if both antigens at a locus were identical. To assess if HLA antigen frequencies matched a reference population dataset, homozygosity for each antigen was calculated for each ethnic group in the study cohort and compared with the NMDP registry (**Table 3, SDC,** http://links.lww.com/TXD/A415). Correlation was 0.995 for White, 0.994 for Black, 0.991 for Hispanic/Latinx, and 0.979 for Asian candidates. Frequencies of HLA homozygosity for each locus in the study cohort were also compared with the NMDP registry and differed by ≤2.5% for White, Black, and Hispanic/Latinx groups, but differed by 3.7%–7.2% for Asian candidates (**Table 4, SDC,** http://links.lww.com/TXD/A415).

Statistical Analysis

The relationship between the number of homozygous HLA loci and CPRA for each candidate was nonlinear (**Figure 2, SDC,** http://links.lww.com/TXD/A415). Thus, multinomial logistic modeling was used for analysis, with sensitization stratified into 4 groups based on the peak CPRA during listing/prior to transplant: (1) nonsensitized (no UA-HLA or CPRA = 0); (2) mildly sensitized (CPRA 1–69); (3) highly sensitized (CPRA 70–94); and (4) extremely sensitized (CPRA 95–100). CPRA thresholds for each sensitization group were based on assigning the 17 CPRA categories for candidates in the kidney allocation system into 3 groups of successively higher levels of sensitization composed of 6, 5, and 6 categories, respectively.

Single and multivariable multinomial modeling was performed to determine the relationship between the number of homozygous HLA loci (assessed on an ordinal scale with values of 0, 1, 2, 3, 4, and 5), covariates, and sensitization. Covariates included recipient sex, age, ethnicity, diabetes, blood type, and prior kidney transplant. Interaction terms between each covariate and the number of homozygous HLA loci were also included. For the single and multivariable models, we only considered a covariate significantly associated with sensitization if the $P < 0.05$ for all sensitization groups (ie, mildly, highly, and extremely sensitized groups).

All analyses were performed with R version 4.0.5.[26]

RESULTS

Characteristics of the Study Cohort

We identified 147 465 kidney transplant candidates between 2014 and 2019 that met the inclusion criteria. This included 84 955 (57.6%) in the nonsensitized group (CPRA 0), 39 203 (26.6%) in the mildly sensitized group (CPRA 1–69), 10 995 (7.5%) in the highly sensitized group (CPRA 70–94), and 12 311 (8.3%) in the extremely sensitized group (CPRA 95–100). As expected, women were present at higher percentages in the highly and extremely sensitized groups (Table 1). The percentage of Black candidates, the number of previous kidney transplants, and time on the waitlist increased progressively in each successively more sensitized group ($P < 0.001$).

Single Locus HLA Homozygosity and Sensitization

Given homozygosity at a single HLA locus (HLA-A, -C, -B, -DR, or -DQ), we calculated the odds of presence in the mildly, highly, or extremely sensitized groups (as compared with the nonsensitized group) using multinomial logistic modeling. For most HLA loci and within most ethnic groups, we found that homozygotes had significantly higher odds of being present in the mildly sensitized, highly sensitized, and extremely sensitized groups (Figure 1; **Table 5, SDC,** http://links.lww.com/TXD/A415). The magnitude of this association increased for each successively more sensitized group and tended to be stronger for homozygosity at HLA-B and -DR than at HLA-A, -C, and -DQ.

Multiple Homozygous HLA Loci and Sensitization

The median number of homozygous HLA loci in the study cohort was 1. Most candidates in the study cohort (83 814 or 56.8%) exhibited homozygosity at 1 or more HLA loci, and homozygosity for 2 or more loci was frequent (35 312 or 23.9%) (Table 2). The percentage of candidates in the highly sensitized and extremely sensitized groups generally increased with a progressively higher number of homozygous HLA loci. The percentage of Black candidates decreased from the 0 homozygous loci group to the 5 homozygous loci group, whereas the percentage of White and Asian candidates increased. The percentage of candidates with type 1

Study cohort characteristics by sensitization group

	Nonsensitized N = 84 955	Mildly sensitized (CPRA 1–69) N = 39 203	Highly sensitized (CPRA 70–94) N = 10 995	Extremely sensitized (CPRA 95–100) N = 12 311
Women	30.0%	39.8%	67.1%	63.6%
Age	51.8 (15.0)	51.3 (14.4)	50.7 (13.5)	48.1 (13.4)
Ethnicity				
Caucasian	45.9%	42.2%	40.8%	34.9%
Black	27.4%	34.4%	36.5%	42.8%
Hispanic/Latinx	19.9%	17.0%	16.7%	16.7%
Asian	6.8%	6.5%	6.1%	5.6%
BMI	31.2 (47.9)	31.8 (52.1)	30.8 (49.3)	29.4 (39.6)
Diabetes				
Type 1	3.4%	3.3%	3.7%	4.8%
Type 2	39.2%	38.0%	32.7%	25.8%
Unknown type	0.8%	0.8%	0.8%	1.2%
Blood type				
A	33.0%	31.2%	31.7%	32.0%
AB	3.87%	3.54%	3.91%	3.87%
B	14.5%	15.7%	15.0%	15.7%
O	48.6%	49.6%	49.4%	48.4%
Prior kidney Txp	4.6%	8.6%	29.4%	55.2%
Days on waitlist	1169 (898)	1272 (857)	1288 (950)	1726 (1395)

BMI, body mass index; CPRA, calculated panel reactive antibody; Txp, transplant.
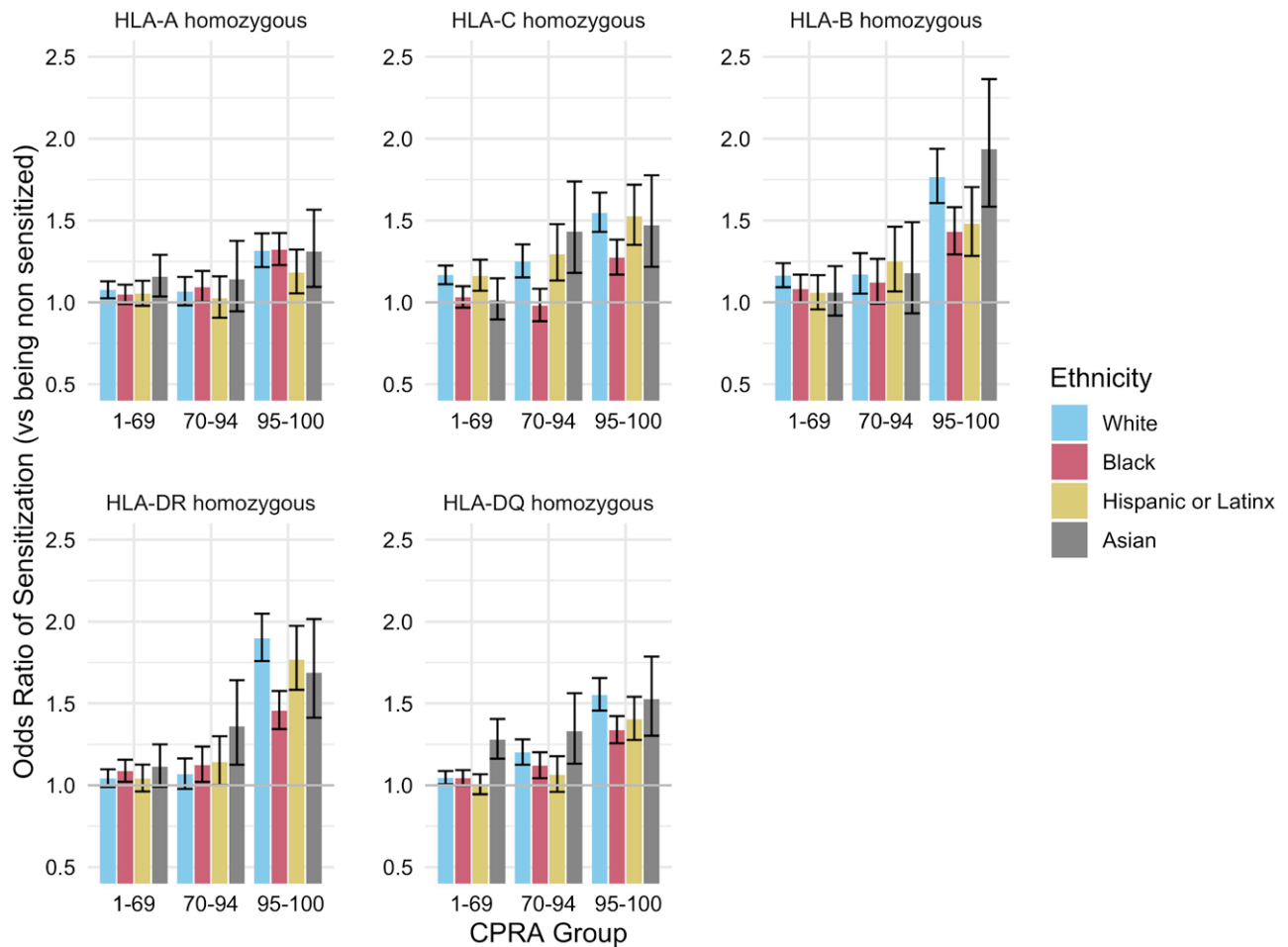
FIGURE 1. Single locus HLA homozygosity and HLA sensitization. Using multinomial logistic modeling, we calculated the odds of presence in the mildly (CPRA 1–69), highly (CPRA 70–95), or extremely sensitized groups (CPRA 95–100) as compared with the nonsensitized group (no unacceptable HLA antigens of CPRA 0) given homozygosity at a single HLA locus (HLA-A, -C, -B, -DR, or -DQ). We found that homozygotes had significantly higher odds of being present in the mildly sensitized, highly sensitized, and extremely sensitized groups for most HLA loci and ethnic groups. CPRA, calculated panel reactive antibody.

diabetes mellitus or a previous kidney transplant increased with a progressively higher number of homozygous HLA loci. Other demographic factors were similar between candidates grouped by the number of homozygous HLA loci.

We further hypothesized that candidates having multiple homozygous HLA loci would have an increased likelihood of sensitization. Given the number of homozygous HLA loci, we calculated the odds of presence in the mildly, highly, or extremely sensitized groups (as compared with the nonsensitized group) using multinomial logistic modeling. Generally we found that homozygotes at multiple HLA loci had significantly higher odds of being present in the mildly sensitized, highly sensitized, and extremely sensitized groups (Figure 2; **Table 6, SDC,** http://links.lww.com/TXD/A415) and that the magnitude of the effect tended to increase with the number of homozygous loci. There were several exceptions: (1) Black candidates with 1, 4, or 5 homozygous HLA loci did not have a higher odds of being present in the mildly or highly sensitized groups and (2) Hispanic/Latinx candidates with 1 or 5 homozygous HLA loci did not have a higher odds of being present in the mildly or highly sensitized groups. For the extremely sensitized group, there was a trend toward a decreased magnitude of the effect of homozygosity at multiple HLA loci on sensitization in Black candidates.

Multiple Homozygous HLA Loci and Sensitization by Sex and Ethnicity

Women comprised a minority of the candidates in the study cohort (56 291 women, 38.2%) but a majority of the candidates in the highly and extremely sensitized groups (15 211 women, 65.2%). When assessed by ethnicity, Black women (Figure 3A) and Black men (Figure 3B) showed a higher percentage of candidates in the highly and extremely sensitized groups as compared with the other ethnic groups.

The study cohort was further divided into 3 groups by the number of homozygous HLA loci: candidates with 0 or 1 homozygous HLA loci (112 152 or 76.1%), candidates with 2 or 3 homozygous HLA loci (31 761 or 21.5%), and candidates with 4 or 5 homozygous HLA loci (3641 or 2.5%). We crosstabulated the proportion of individuals in each homozygosity group (ie, 0 of 1, 2 of 3, or 4 of 5 loci) and sensitization group (non, mildly, highly, and extremely sensitized) by sex and ethnicity. For both women and men of all ethnic groups (Figure 4), there was a higher frequency of candidates with 2 of 3 and 4 of 5 homozygous loci in the mildly, highly, and extremely sensitized groups as compared with candidates with 0 of 1 homozygous HLA loci ($P < 0.001$). Women displayed a higher relative abundance of homozygotes at multiple HLA loci in sensitized groups as compared with men, with attenuation of this effect in Black candidates. For women, the relative abundance of candidates with 4 of 5 homozygous loci in the mildly, highly, and extremely sensitized groups (as compared with the nonsensitized group) was higher for Asian (1.49-fold) and White candidates (1.28-fold) than Hispanic/Latinx candidates (1.19-fold), and lowest for Black candidates (1.15-fold). Men displayed an overall lower relative abundance of candidates with 4 of 5 homozygous loci in sensitized groups, but relative abundance was higher for Hispanic/Latinx (1.32-fold) and White candidates (1.24-fold) than Asian candidates (1.16-fold), and again lowest for Black candidates (1.04-fold).

TABLE 2.

Study cohort characteristics by the total number of homozygous HLA loci

| | Total number of homozygous HLA loci | | | | | | |
| | 0 | 1 | 2 | 3 | 4 | 5 | |
Variable	**N = 63 650**	**N = 48 503**	**N = 24 331**	**N = 7340**	**N = 2341**	**N = 1300**	**P**
Sensitization group							<0.001
Nonsensitized	59.8%	57.4%	54.9%	52.8%	50.4%	51.5%	
Mildly sensitized	26.3%	26.8%	26.6%	27.4%	26.1%	25.1%	
Highly sensitized	7.13%	7.52%	7.90%	8.07%	8.54%	7.38%	
Extremely sensitized	6.75%	8.28%	10.6%	11.7%	15.0%	16.1%	
Women	38.5%	37.9%	37.8%	38.2%	38.4%	40.5%	0.092
Age	51.3 (14.7)	51.3 (14.6)	51.1 (14.6)	51.3 (14.8)	51.4 (15.2)	50.7 (15.1)	0.398
Ethnicity							<0.001
White	44.8%	42.0%	42.0%	43.7%	52.5%	55.5%	
Black	30.7%	33.8%	31.2%	26.8%	18.1%	12.6%	
Hispanic/Latinx	19.1%	17.9%	18.8%	18.8%	18.4%	18.8%	
Asian	5.4%	6.3%	8.0%	10.7%	11.0%	13.1%	
BMI	31.2 (48.7)	31.1 (47.2)	31.2 (48.9)	31.4 (53.4)	30.0 (40.6)	32.5 (66.1)	0.759
Diabetes							<0.001
Type 1	3.9%	2.9%	3.3%	3.9%	5.04%	6.38%	
Type 2	37.3%	37.6%	37.4%	35.6%	36.0%	31.8%	
Unknown type	0.8%	0.8%	0.9%	0.9%	1.24%	0.69%	
Blood type							0.169
A	32.6%	32.2%	31.9%	32.0%	32.6%	34.2%	
AB	3.74%	3.83%	3.78%	4.05%	3.72%	3.00%	
B	14.7%	15.0%	15.5%	14.4%	15.2%	14.3%	
O	48.9%	49.0%	48.7%	49.5%	48.5%	48.5%	
Prior kidney Txp	11.7%	11.5%	11.8%	11.6%	14.5%	13.1%	<0.001

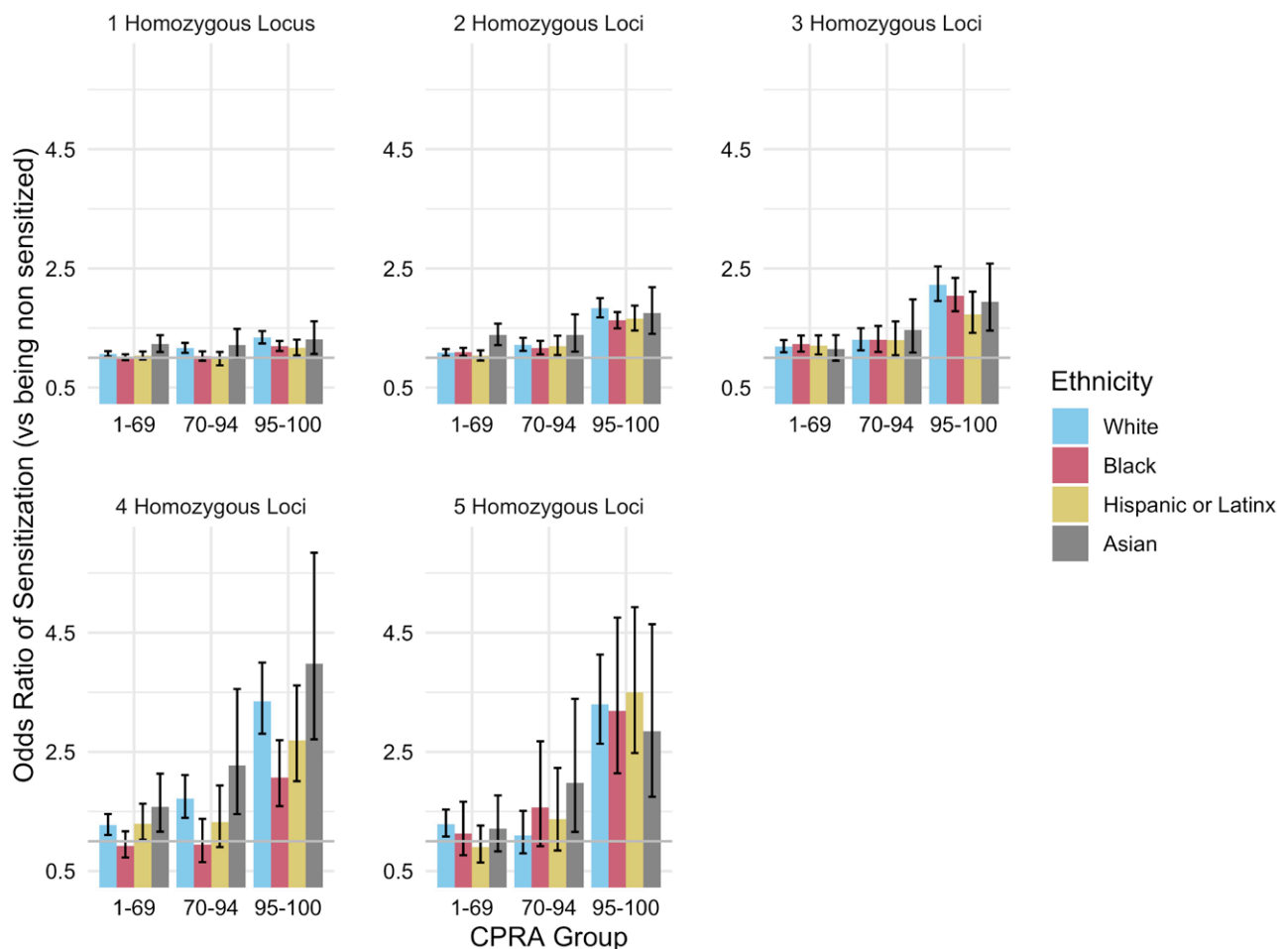BMI, body mass index; Txp, transplant.

FIGURE 2. Multiple homozygous HLA Loci and HLA sensitization. Using multinomial logistic modeling, we calculated the odds of presence in the mildly (CPRA 1–69), highly (CPRA 70–95), or extremely sensitized groups (CPRA 95–100) as compared with the nonsensitized group (no unacceptable HLA antigens of CPRA 0) given homozygosity at multiple HLA loci. Generally, we found that homozygotes at multiple HLA loci had significantly higher odds of being present in the mildly sensitized, highly sensitized, and extremely sensitized groups, with the magnitude of the effect tending to increase with the number of homozygous loci. CPRA, calculated panel reactive antibody.

Factors Associated with Sensitization

Based on the preceding analyses, we sought to identify factors independently associated with sensitization. Covariates were analyzed in single variable multinomial models. We also included an interaction between each covariate and the number of homozygous HLA loci (assessed on an ordinal scale with values of 0 to 5). We found that the number of homozygous HLA loci, female sex, Black ethnicity, and a history of prior kidney transplant were all associated with an increased odds of presence in the mildly, highly, and extremely sensitized groups as compared with the nonsensitized group (**Table 7, SDC,** http://links.lww.com/TXD/A415). Female sex, Black ethnicity, and a history of prior kidney transplant also displayed a significant positive interaction with the number of homozygous HLA loci. In contrast, increasing age and diabetes type 2 were associated with a decreased odds of presence in the mildly, highly, and extremely sensitized groups. Increasing age displayed a significant positive interaction with the number of homozygous HLA loci.

A multivariable model was then constructed using these factors, including their interaction, with the number of homozygous HLA loci (Table 3). In this model, we found that the number of homozygous HLA loci remained significantly associated with an increased odds of presence in the mildly

sensitized (OR 1.08/homozygous locus, 95% CI 1.03-1.14, P = 0.002) and extremely sensitized groups (OR 1.10/homozygous locus, 95% CI 1.00-1.20, P = 0.044), but not in the highly sensitized group (OR 0.96/homozygous locus, 95% CI 0.87-1.05, P = 0.383). Female sex, Black ethnicity, and a history of prior kidney transplant remained associated with an increased odds of presence in each progressively more sensitized group. Of these, female sex was the only factor displaying an significant interaction with HLA homozygosity, such that increasing number of homozygous HLA loci was associated with an increased odds of presence in the highly (OR 1.07/homozygous locus, 95% CI 1.02-1.11, P = 0.004) and extremely sensitized groups (OR 1.15/homozygous locus, 95% CI 1.11-1.21, P < 0.001).

DISCUSSION

In this study, we assessed the association between HLA homozygosity and sensitization. We found that homozygosity at HLA-A, -C, -B, -DR, and -DQ was associated with sensitization, and the magnitude of this effect varied by the specific locus. More significantly, we found that as the number of homozygous loci increased, the odds of being sensitized, especially highly sensitized or extremely sensitized, increased. The magnitude of this effect varied by ethnicity, with a lower
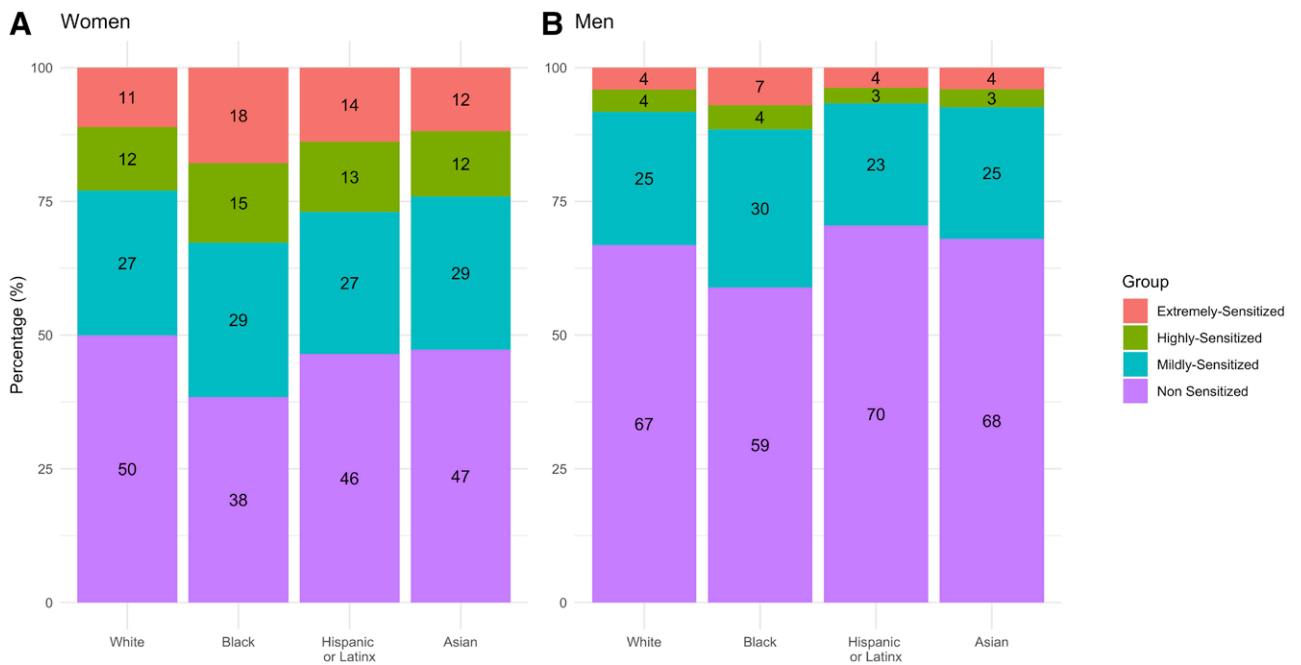
FIGURE 3. Sensitization group by sex and ethnicity. Percentage of women (A) and men (B) in each sensitization group by ethnic group. There were more Black women and men in the highly and extremely sensitized groups as compared with the other ethnic groups.

FIGURE 4. Sensitization group by HLA homozygosity and ethnicity for women and men. The cohort was divided into 3 groups by the number of homozygous HLA loci: candidates with 0 or 1 homozygous HLA loci (dark blue), candidates with 2 or 3 homozygous HLA loci (light blue), and candidates with 4 or 5 homozygous HLA loci (red). There was a higher frequency of candidates with 2 of 3 and 4 of 5 homozygous loci in the mildly, highly, and extremely sensitized groups as compared with candidates with 0 of 1 homozygous HLA loci ($P < 0.001$).

magnitude of this effect in Black candidates. Women comprised a majority of the highly and extremely sensitized candidates and we observed a higher relative abundance of women candidates with homozygosity at multiple HLA loci as compared with men. In a multivariable model, the number of homozygous HLA loci was associated with an increased odds of presence in the mildly and extremely sensitized groups, but not in the highly sensitized group. These differences are likely attributable to the significant interaction between female sex and HLA homozygosity. In contrast, other factors associated with sensitization, including recipient ethnicity and a history of prior kidney transplant did not interact with HLA homozygosity.

In studies of apheresis donors, only a small percentage (<10%) of women without a history of pregnancy are sensitized.[5,13,14] End-stage renal disease has also not been associated with sensitization in the absence of other sensitizing events.[28] In this study, we have used female sex as a surrogate marker of previous pregnancy, as parity is not available for transplant candidates in the UNOS dataset. We found that the number of homozygous HLA loci interacted only with female sex to increase the likelihood of sensitization, suggesting that HLA homozygosity is an innate factor that seems to enhance the effect of pregnancy-related sensitization.

We found that mildly, highly, and extremely sensitized Black candidates, who comprised a larger percentage of these groups compared with other ethnicities, were enriched for candidates possessing multiple homozygous HLA loci at a lower magnitude compared with White, Hispanic/Latinx, and Asian candidates. HLA haplotypes are strongly related to ethnicity and the Black population is well known to have higher HLA heterozygosity.[29] Although we observed a strong relationship between Black ethnicity and an increased odds of presence in each progressively more sensitized group, ethnicity did not interact with the number of homozygous HLA loci. These findings suggest that HLA homozygosity is not a mediator of the increased burden of sensitization faced by Black candidates.

The biological mechanisms responsible for the higher burden of HLA sensitization in Black kidney transplant candidates remain unclear. Differences in B cell subsets and B cell receptor (BCR) signaling have been demonstrated in healthy lymphocyte donors as well as patients with systemic lupus erythematosus of African American ancestry.[30,31] Importantly, polymorphism at the immunoglobulin heavy chain locus is extreme[32] and may also contribute to ethnicity-specific differences in humoral response, as has been noted after influenza vaccination.[33] Given that highly and extremely sensitized Black candidates have diminished access to kidney transplant as compared with similarly sensitized White candidates,[34] further research is urgently needed to define the biologic mechanisms of sensitization in Black candidates.

The mechanism by which HLA homozygosity promotes sensitization is not established. During B cell development,
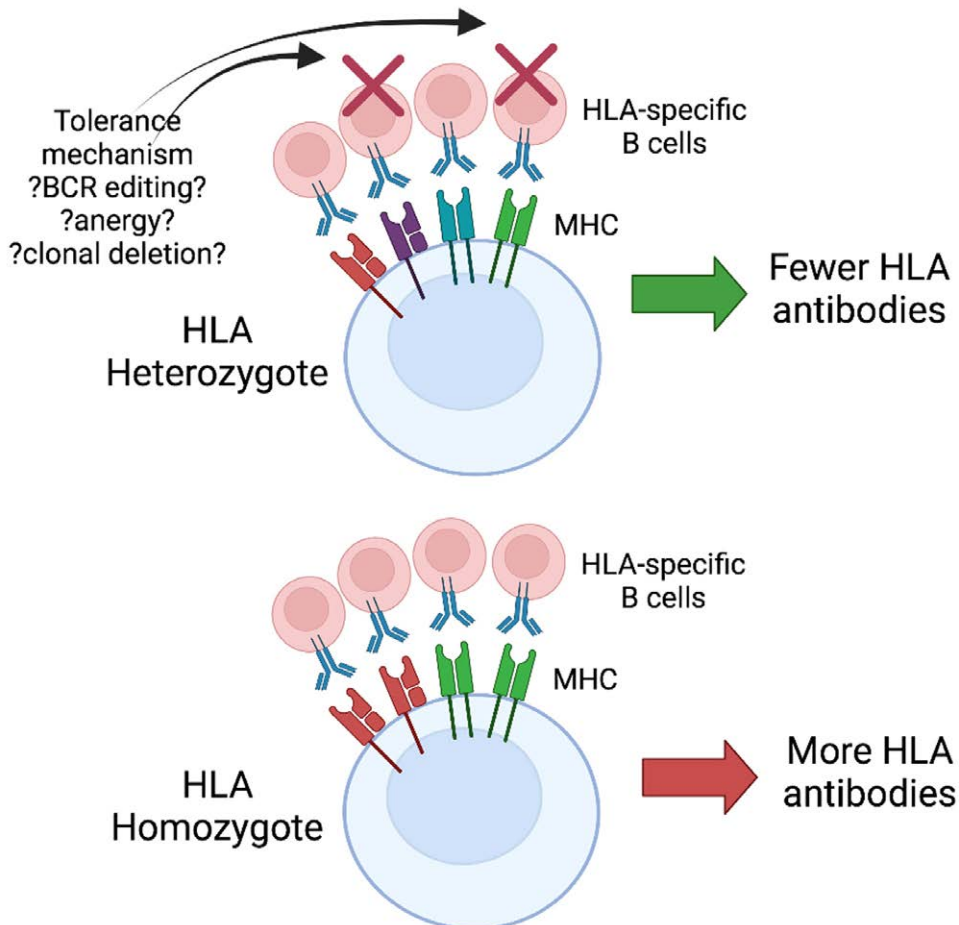

FIGURE 5. Proposed model for the mechanism of HLA homozygosity mediated sensitization. We hypothesize that increased HLA homozygosity leads to a less diverse repertoire of self-HLA on the cell surface, which in turn leads to less "pruning" of B cells with HLA-specific receptors, ultimately resulting in a larger number or breadth of HLA antibodies. BCR, B cell receptor.

V/D/J and V/J genes are randomly recombined to generate the heavy and light chains of the BCR, respectively.[35] As a result of this stochastic process, autoreactivity of the BCR is common.[36] Mechanisms for tolerizing autoreactive B cells include receptor editing, induction of anergy, and clonal deletion.[37] Receptor editing,[38] the process by which the light chain gene undergoes a secondary V/J recombination and the heavy chain gene undergoes replacement, seems to be the major mechanism of tolerance for membrane proteins such as HLA.[39,40]

We hypothesize that during B cell development in the bone marrow, increased HLA homozygosity leads to a less diverse repertoire of self-HLA on the cell surface, which in turn leads to less "pruning" of B cells with HLA-specific B cell receptors. Pruning of B cells is likely to occur via receptor editing. This results in a larger number and breadth of HLA-specific B cells, and a downstream larger number and breadth of HLA antibodies (Figure 5). In this way, sensitization related to HLA homozygosity is not due to a defect in tolerance per se, but a "loophole" in tolerance attributable to a limited repertoire of self-HLA epitopes. In support of this hypothesis, patients with systemic lupus erythematosus, which is characterized by pathologically decreased BCR editing,[41] have been found to have elevated levels of HLA antibodies.[42,43] Further studies are needed to verify this hypothesis.

Interestingly, although we observed that a history of kidney transplant was strongly associated with sensitization, HLA homozygosity did not enhance its effect. We postulate that the mechanisms of transplant-related sensitization may be distinct from those of pregnancy-related sensitization. More specifically,

TABLE 3.

Multivariable model of factors associated with sensitization including interactions with HLA homozygosity

	Sensitization group					
	Mildly sensitized (CPRA 1–69)		Highly sensitized (CPRA 70–94)		Extremely sensitized (CPRA 95–100)	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Number of homozygous HLA loci	1.08 (1.03-1.14)	0.002	0.96 (0.87-1.05)	0.383	1.10 (1.00-1.20)	0.044
Sex						
Male	Reference		Reference		Reference	
Female	1.61 (1.56-1.67)	<0.001	5.55 (5.22-5.90)	<0.001	5.36 (5.02-5.72)	<0.001
Sex × HLA homozygosity						
Female	0.98 (0.96-1.01)	0.149	1.07 (1.02-1.11)	0.004	1.15 (1.11-1.21)	<0.001
Age (per decade)	0.99 (0.98-1.00)	0.147	1.01 (0.99-1.03)	0.286	0.93 (0.91-0.95)	<0.001
Age × HLA homozygosity	1.00 (0.99-1.01)	0.897	1.02 (1.01-1.04)	0.002	1.03 (1.02-1.05)	<0.001
Ethnicity						
White	Reference		Reference		Reference	
Black	1.38 (1.33-1.44)	<0.001	1.75 (1.64-1.87)	<0.001	2.96 (2.76-3.19)	<0.001
Hispanic/Latinx	0.95 (0.91-1.00)	0.050	1.12 (1.03-1.21)	0.009	1.56 (1.42-1.70)	<0.001
Asian	0.98 (0.91-1.05)	0.582	0.93 (0.82-1.06)	0.308	1.14 (0.99-1.32)	0.066
Ethnicity × HLA homozygosity						
Black	0.99 (0.96-1.02)	0.450	0.98 (0.94-1.03)	0.524	0.97 (0.92-1.02)	0.204
Hispanic/Latinx	0.98 (0.94-1.01)	0.179	1.01 (0.95-1.07)	0.807	0.98 (0.93-1.04)	0.549
Asian	1.03 (0.98-1.08)	0.197	1.09 (1.01-1.178)	0.030	1.05 (0.97-1.13)	0.260
Diabetes						
None	Reference		Reference		Reference	
Type 1	0.95 (0.87-1.04)	0.283	0.74 (0.64-0.86)	<0.001	0.75 (0.64-0.87)	<0.001
Type 2	1.01 (0.98-1.05)	0.450	0.97 (0.97-1.04)	0.400	0.92 (0.86-0.99)	0.031
Unknown type	0.95 (0.79-1.15)	0.618	0.84 (0.61-1.16)	0.293	0.98 (0.72-1.32)	0.871
Diabetes × HLA homozygosity						
Type 1	0.98 (0.92-1.05)	0.577	1.03 (0.93-1.13)	0.589	1.07 (0.98-1.17)	0.147
Type 2	1.00 (0.98-1.03)	0.885	1.01 (0.96-1.05)	0.768	0.98 (0.93-1.02)	0.328
Unknown type	0.90 (0.79-1.03)	0.138	0.89 (0.71-1.12)	0.316	0.94 (0.77-1.14)	0.543
Blood type						
O	Reference		Reference		Reference	
A	0.94 (0.91-0.98)	0.001	0.93 (0.88-1.00)	0.036	0.95 (0.89-1.02)	0.157
B	1.04 (0.99-1.09)	0.145	1.02 (0.94-1.11)	0.652	1.12 (1.03-1.23)	0.010
AB	0.88 (0.81-0.96)	0.004	0.87 (0.75-1.01)	0.066	0.85 (0.72-1.00)	0.055
Blood type × HLA homozygosity						
A	1.00 (0.97-1.03)	0.868	1.01 (0.97-1.06)	0.574	1.03 (0.98-1.07)	0.242
B	0.99 (0.96-1.03)	0.625	0.99 (0.93-1.05)	0.685	0.96 (0.91-1.02)	0.173
AB	0.98 (0.92-1.05)	0.564	1.05 (0.94-1.16)	0.380	1.02 (0.92-1.14)	0.712
Prior kidney Txp						
No	Reference		Reference		Reference	
Yes	2.30 (2.16-2.45)	<0.001	12.64 (11.72-13.63)	<0.001	40.88 (37.95-44.03)	<0.001
Prior kidney Txp × HLA homozygosity						
Yes	0.93 (0.89-0.98)	0.007	1.01 (0.96-1.07)	0.682	0.98 (0.93-1.03)	0.459

CPRA, calculated panel reactive antibody; Txp, transplant.

we postulate that the proinflammatory cytokine milieu of transplant rejection may lead to bypass of normal tolerance mechanisms, making the recipient's HLA genotype irrelevant in this context. In support of this hypothesis, the ratio of interleukin-10 to tumor necrosis factor-α expression by transitional B cells is a powerful marker of cellular rejection,[44] and autoantibodies have been found to be increased during acute humoral/mixed rejection after kidney transplant.[45] In contrast, pregnancy is a protolerogenic state, characterized by a decrease in the number of circulating transitional B cells,[46] presumably leading to preservation of normal tolerance mechanisms and antibody development in relation to the recipient's HLA genotype.

To the best of our knowledge, HLA homozygosity has not previously been identified as a factor contributing to sensitization in solid organ transplant candidates. Because HLA genes are highly polymorphic,[18] homozygosity occurs at a frequency of 10%–30% for each locus. Thus, a large cohort is needed to observe the relationship between HLA homozygosity and sensitization, especially when considering candidates who have homozygosity at multiple HLA loci. Our use of a cohort of 147 461 patients facilitated our ability to identify the associations reported here.

This study has several important limitations that merit discussion. First, there were a significant number of candidates that were excluded due to missing typing at the HLA-C and -DQ loci. Omission of these candidates could lead to skew in the cohort. Reassuringly, we found that the frequency of HLA homozygosity was similar in our cohort as compared with NMDP for Whites, Blacks, and Hispanics. We did find a higher percentage of homozygotes at all HLA loci in Asian candidates in UNOS as compared with NMDP. This could be due to differences in split versus broad antigen reporting, as Asian candidates displayed a higher percentage of homozygosity related to split antigens (**Table 1, SDC,** http://links. lww.com/TXD/A415), or could be due to differences between these databases in population substructure.

Next, there are several factors that can affect the accuracy of CPRA as the metric of a candidate's level of sensitization. The criteria utilized for designating UA-HLA vary between HLA laboratories,[47] and as such CPRA values could underestimate a candidate's level of sensitization at transplant centers with high thresholds. Likewise, we focused on HLA-A, -C, -B, -DR, and -DQ, and did not investigate the role of the HLA-DP locus. Inclusion of HLA-DP UA-HLA has been shown to increase CPRA values, especially in highly sensitized candidates.[48] Additionally, single antigen bead assays may miss rare specificities that are not present on the beads.[49]

Our analysis was based on low-resolution (antigen level) typing available in the OPTN database. Assessing candidate HLA typing at the allelic level could allow for characterization of the effect of interallelic distance on sensitization as a continuous rather than dichotomized (eg, homozygous versus heterozygous) quantity. Granular data on the stimuli for sensitization, for example, parity and history of transfusion, were not available in the database. Such data could lead to an improved understanding of how alloimmune stimuli lead to the development of HLA antibodies in the context of HLA homozygosity. Last, HLA antibody profiles change over time and our use of maximum CPRA during listing may introduce bias.

In summary, we have found that HLA homozygosity increases the likelihood of sensitization in kidney transplant candidates. These results are particularly relevant to women as

they comprise the majority of highly and extremely sensitized kidney transplant candidates and there is a specific interaction between the number of homozygous HLA loci and female sex, presumably via pregnancy. Thus, HLA homozygosity is an innate factor that seems to enhance the effect of pregnancy-related sensitization. Further research is needed to identify the immunologic mechanisms that underlie this observation.

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