nature genetics

Widespread non-additive and interaction effects within HLA loci modulate the risk of autoimmune diseases

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Human leukocyte antigen (HLA) genes confer substantial risk for autoimmune diseases on a log-additive scale. Here we speculated that differences in autoantigen-binding repertoires between a heterozygote's two expressed HLA variants might result in additional non-additive risk effects. We tested the non-additive disease contributions of classical HLA alleles in patients and matched controls for five common autoimmune diseases: rheumatoid arthritis (n_{cases} = 5,337), type 1 diabetes (T1D; n_{cases} = 5,567), psoriasis vulgaris (n_{cases} = 3,089), idiopathic achalasia (n_{cases} = 727) and celiac disease (n_{cases} = 11,115). In four of the five diseases, we observed highly significant, non-additive dominance effects (rheumatoid arthritis, $P = 2.5 \times 10^{-12}$; T1D, $P = 2.4 \times 10^{-10}$; psoriasis, $P = 5.9 \times 10^{-6}$; celiac disease, $P = 1.2 \times 10^{-87}$). In three of these diseases, the non-additive dominance effects were explained by interactions between specific classical HLA alleles (rheumatoid arthritis, $P = 1.8 \times 10^{-3}$; T1D, $P = 8.6 \times 10^{-27}$; celiac disease, $P = 6.0 \times 10^{-100}$). These interactions generally increased disease risk and explained moderate but significant fractions of phenotypic variance (rheumatoid arthritis, 1.4%; T1D, 4.0%; celiac disease, 4.1%) beyond a simple additive model.

Genetic variation in HLA genes, within the major histocompatibility complex (MHC) locus, is associated with many autoimmune diseases[1–3]. For most of these diseases, the MHC region explains more disease risk than any other locus. Previous research has shown non-additive effects (disease contributions beyond the cumulative effects of individual alleles) at classical MHC genes in resistance to infectious diseases[4–7], and researchers have proposed that non-additive effects may also occur in autoimmunity[8–12]. Indeed, some studies have reported synergistic interactions between specific HLA haplotypes[13–15], but non-additive effects and interactions have not been systematically characterized in large population cohorts and across multiple diseases.

In an additive model, the effects on disease of the two alleles for a gene are independent and combine linearly (on a log-odds scale); that is, the first and second copies of an allele multiplicatively increase risk by the same amount. Non-additive effects describe deviations from this linear relationship and may arise from interactions between two alleles or from the intrinsic effects of individual alleles (for example, haploinsufficiency)[16,17]. Because both alleles at a given HLA locus are expressed, heterozygous genotypes might confer expanded antigen-binding properties and elevated autoantigen presentation, depending on the degree of complementarity between the two alleles[18].

To test for the presence of non-additive effects, we used SNP2HLA[19] to impute HLA alleles from dense Immunochip-based SNP genotype data in 5 autoimmune diseases: seropositive rheumatoid arthritis (n = 5,337 cases and 11,049 controls)[20,21], T1D (n = 5,567 cases and 6,265 controls)[22], psoriasis vulgaris (n = 3,089 cases and 5,964 controls)[23], idiopathic achalasia (n = 727 cases and 2,911 controls)[24] and celiac disease (n = 11,115 cases and 9,042 controls)[25] (**Supplementary Table 1**). We demonstrated accurate HLA imputation elsewhere[19] using the Immunochip platform and the same reference panel (Type 1 Diabetes Genetics Consortium (T1DGC), n = 5,225)[26].

For each of these five diseases, we focused our analyses on the HLA loci with the strongest effects (rheumatoid arthritis, *HLA-DRB1* (refs. 20,21); T1D, *HLA-DRB1–HLA-DQA1–HLA-DQB1* (ref. 22); psoriasis, *HLA-C*[23]; achalasia, *HLA-DQA1–HLA-DQB1* (ref. 24); celiac disease, *HLA-DQA1–HLA-DQB1* (ref. 25)). We focused on four-digit classical alleles, which distinguish HLA gene variants at the amino acid sequence level[27]. For rheumatoid arthritis and psoriasis, we analyzed haplotypes defined by classical alleles at a single HLA gene. Because T1D, achalasia and celiac disease each have independent associations with multiple, linked HLA genes[22,24,25], we combined

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Received 12 February; accepted 16 July; published online 10 August 2015; doi:10.1038/ng.3379

the phased four-digit classical alleles from separate genes into multi-locus haplotypes for these diseases. For our primary test, we restricted our analysis to common alleles at each locus (reference panel allele frequency > 5%; **Supplementary Table 2**) and to individuals carrying only these common alleles ('common allele data set'; **Supplementary Table 1**). This approach ensured the highest imputation accuracy and increased the statistical power to estimate the true additive component of each haplotype by providing a sufficient number of homozygote events. As a secondary test, we analyzed both rare and common haplotypes that were present in at least ten homozygous individuals ('full data set'; **Supplementary Table 1**).

To assess for non-additive associations, we first examined the disease risk of homozygotes and heterozygotes for each haplotype. Under additivity, for a given allele, the log odds of heterozygotes in comparison to non-carriers of the allele should be half that of homozygotes. However, we found that many haplotypes deviated from this linear relationship (**Fig. 1a** and **Supplementary Table 3**). We also observed an excess of heterozygous genotypes deviating from Hardy-Weinberg equilibrium in cases (**Fig. 1b** and **Supplementary Table 4**) but not controls (**Fig. 1c** and **Supplementary Table 4**). In contrast, 43 rheumatoid arthritis–associated non-MHC SNPs[28] followed an additive relationship perfectly (**Fig. 1d** and **Supplementary Table 5**) and also followed Hardy-Weinberg equilibrium (**Fig. 1e** and **Supplementary Table 6**). We note that the lower effect sizes of non-MHC variants may limit our ability to detect non-additive effects.

To test for the general presence of non-additive effects, we constructed one global logistic regression model for each disease that included additive and dominance terms for all common haplotypes simultaneously (**Fig. 2a**). The additive term captures the dosage effect (0, 1 or 2 copies) of a given haplotype, and the dominance term captures any deviations from the additive scenario[29]. Strikingly, for four of the five diseases, the inclusion of dominance terms improved the fit of the models (rheumatoid arthritis, $P_{df=5} = 2.5 \times 10^{-12}$; T1D, $P_{df=5} = 2.4 \times 10^{-10}$; psoriasis, $P_{df=7} = 5.9 \times 10^{-6}$; celiac disease, $P_{df=6} = 1.2 \times 10^{-87}$; where $P_{df=n}$ is the P value assuming n degrees of freedom). In the achalasia data set, we observed a non-significant trend ($P_{df=5} = 0.066$); power may have been limited owing to the relatively small sample size of this data set. These results were consistent in the full data sets, including a larger set of common and rare alleles (**Supplementary Table 7**), and also when using regression on a probit scale[30] instead of logistic regression (**Supplementary Table 8**). In a purely additive model, common HLA haplotypes explained 8.1% of phenotypic variance for rheumatoid arthritis, 13.3% of phenotypic variance for T1D, 5.9% of phenotypic variance for psoriasis and 21.1% of phenotypic variance for celiac disease. The addition of dominance terms explained an additional 0.9%, 1.1%, 0.9% and 1.9% of phenotypic variance, respectively (**Fig. 2b**). These values are comparable to that for the largest known non-MHC rheumatoid arthritis risk effect; the rs2476601 *PTPN22* risk allele explains 0.8% of the total phenotypic variance in rheumatoid arthritis[31].

When we examined the non-additive effects of individual HLA haplotypes separately, we observed that most haplotypes showed significant non-additive contributions in rheumatoid arthritis, T1D and celiac disease (**Table 1** and **Supplementary Table 3**). In contrast, of the seven common haplotypes tested in psoriasis, only HLA-C*06:02 showed a non-additive effect. Across all 4 diseases, 14 of 23 HLA haplotypes showed non-additivity and 12 had a positive dominance component; thus, for most alleles, heterozygosity confers a higher risk of autoimmunity than expected from homozygote disease risk (**Fig. 2c** and **Supplementary Table 9**).

We considered that these non-additive effects might originate from imputation artifacts. To ensure high-quality imputation,

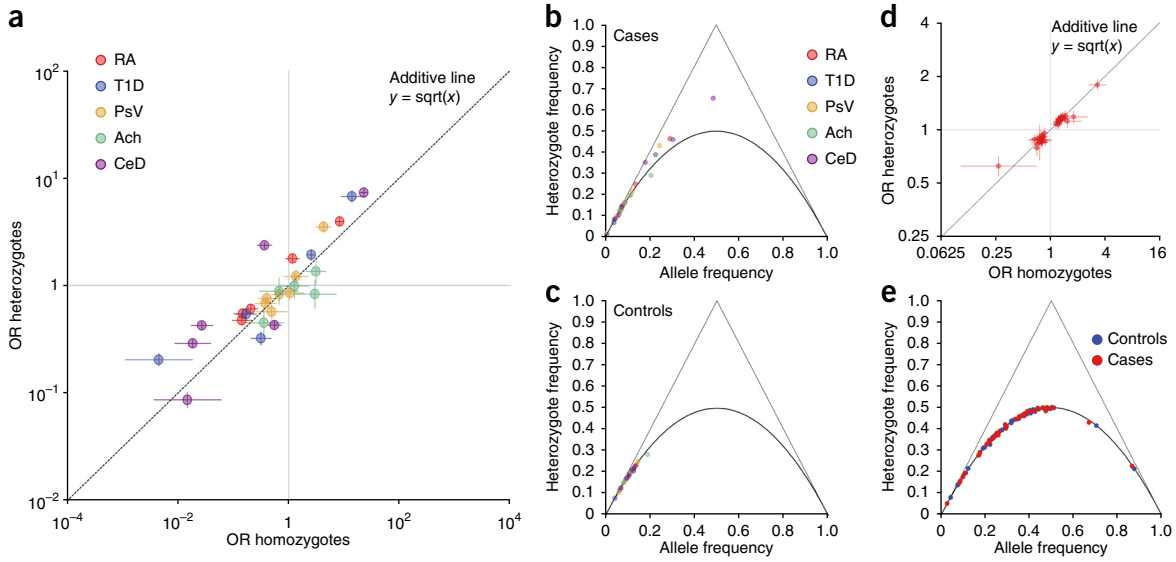

Figure 1 Disease associations of HLA and non-HLA variants. (**a**) Disease associations of HLA haplotypes with rheumatoid arthritis (RA), type 1 diabetes (T1D), psoriasis vulgaris (PsV), idiopathic achalasia (Ach) and celiac disease (CeD). For each common haplotype, the odds ratio (OR) for heterozygotes (versus non-carriers) is plotted against the odds ratio for homozygotes (versus non-carriers). The dashed line represents a purely log-additive relationship in which heterozygotes have exactly half the risk of homozygotes (on a log-odds scale). Data points above the dashed line represent haplotypes with a positive dominance component, and those below the dashed line represent haplotypes with a negative dominance component. Error bars represent 95% confidence intervals. (**b**,**c**) De Finetti diagrams of the proportion of heterozygotes in relation to the frequency of each HLA haplotype in the indicated disease, shown separately for cases (**b**) and controls (**c**). The solid line represents the expected proportion of heterozygotes under Hardy-Weinberg equilibrium. (**d**) Disease association of 43 known genome-wide rheumatoid arthritis–associated SNPs located outside the MHC region, using the same plotting scheme as for **a**. No single SNP shows significant deviation from the dashed line (representing a purely additive disease contribution). (**e**) De Finetti diagram of heterozygote frequency for the 43 non-MHC SNPs in **d**, given separately for controls and cases.

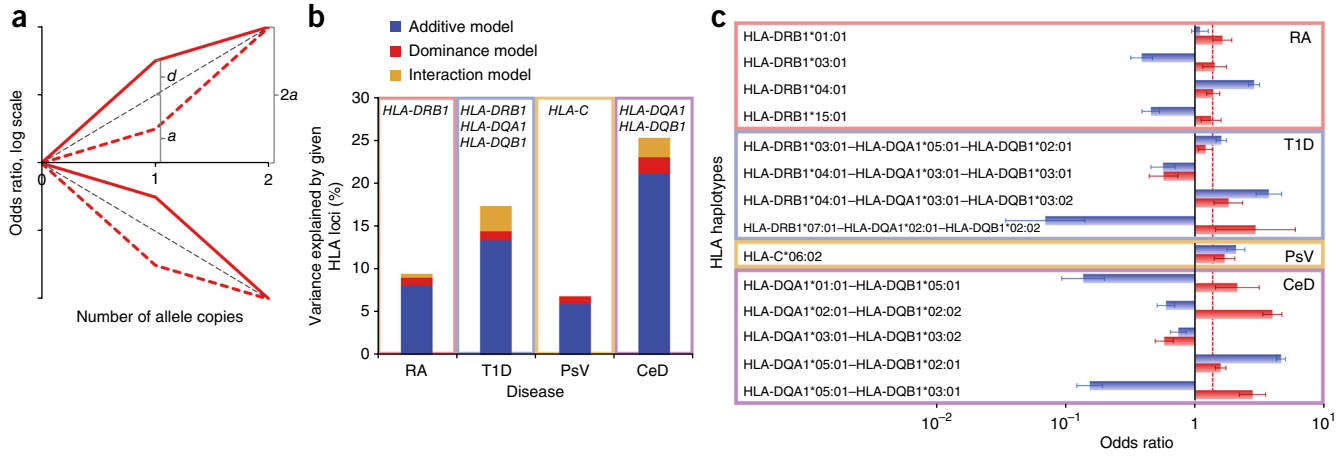

Figure 2 Non-additive contribution of the HLA region to autoimmune disease risk. (**a**) Schematic overview of possible non-additive scenarios. The log odds for heterozygote genotypes can be divided into an additive effect (*a*) and a dominance component (*d*), which represents the departure from additivity. Depending on the signs of *a* and *d*, there are four possible scenarios, represented by red lines. Dashed black lines represent the expected log odds under a purely additive model ($d = 0$). As an example, the values of *a* and *d* are given for the solid red line (corresponding to a risk variant with a positive dominance component). (**b**) Phenotypic variance explained by the additive, dominance and interaction effects of HLA haplotypes for each disease with a significant non-additive HLA contribution: rheumatoid arthritis, T1D, psoriasis vulgaris and celiac disease. (**c**) For each common HLA haplotype with a significant non-additive effect in rheumatoid arthritis, T1D, psoriasis vulgaris or celiac disease, we calculated the additive (blue bars) and dominance (red bars) components of the log odds for heterozygotes. The dashed line indicates the median of the dominance components depicted in the figure. Error bars represent 95% confidence intervals.

we only used samples genotyped on the Immunochip, containing dense SNP coverage (>5,000 SNPs within the MHC region)[19]. Additionally, our primary analyses focused on common alleles that were well represented in the reference panel and had high imputation confidence (INFO) scores[32] (>0.973, median of 1.003; **Supplementary Table 2**). Also, the significance of non-additive effects was unrelated to INFO scores (Kendall's $\tau = -0.08$, $P = 0.56$; **Supplementary Fig. 1** and **Supplementary Table 10**). Finally, we conducted a stringent permutation analysis where we reassigned case-control status on the basis of an additive risk model for HLA haplotypes; this approach conserved the additive effect of each haplotype (**Supplementary Fig. 2**) and simultaneously maintained any imputation inaccuracies within the data set. In celiac disease, psoriasis, T1D and rheumatoid arthritis, the significance of non-additive effects in 10,000 trials never exceeded that of the actual data (**Supplementary Fig. 3**). These results argue that our findings cannot be explained by imputation artifacts.

In rheumatoid arthritis, T1D and achalasia, the strongest additive disease associations point to individual amino acids (rather than four-digit classical alleles)[20,22,24], and we indeed found non-additive disease associations also at the amino acid level (**Supplementary Fig. 4** and **Supplementary Table 11**). We did observe residual non-additive haplotype effects after adjusting for the amino acid residue with the most significant non-additive effect (**Supplementary Note**).

Table 1 Effect sizes of common HLA haplotypes with significant non-additive effects

	Locus				Additive model		Non-additive model (additive + non-additive components)		
								Heterozygote effect	Homozygote effect
Disease	*HLA-C*	*HLA-DRB1*	*HLA-DQA1*	*HLA-DQB1*	*P*	OR (95% CI)	*P*	OR (95% CI)	OR (95% CI)
Rheumatoid arthritis		01:01			1.6×10^{-22}	1.54 (1.41–1.68)	1.3×10^{-8}	1.77 (1.61–1.96)	1.17 (0.87–1.58)
		04:01			2.0×10^{-206}	3.31 (3.05–3.59)	1.4×10^{-7}	3.93 (3.54–4.36)	8.34 (6.87–10.12)
		03:01			1.2×10^{-51}	0.50 (0.45–0.55)	8.6×10^{-4}	0.54 (0.49–0.61)	0.15 (0.10–0.22)
		15:01			7.7×10^{-43}	0.55 (0.51–0.60)	1.2×10^{-3}	0.60 (0.54–0.67)	0.21 (0.15–0.28)
T1D		07:01	02:01	02:02	4.2×10^{-50}	0.38 (0.33–0.43)	1.9×10^{-5}	0.32 (0.28–0.37)	0.31 (0.20–0.48)
		04:01	03:01	03:01	2.2×10^{-167}	0.18 (0.16–0.21)	3.4×10^{-5}	0.20 (0.18–0.23)	0.005 (0.001–0.018)
		04:01	03:01	03:02	5.4×10^{-224}	6.09 (5.38–6.90)	3.5×10^{-5}	6.72 (5.88–7.68)	13.86 (8.79–21.86)
		03:01	05:01	02:01	5.2×10^{-35}	1.70 (1.56–1.85)	3.5×10^{-3}	1.92 (1.71–2.16)	2.55 (2.12–3.08)
Psoriasis vulgaris	06:02				1.5×10^{-92}	2.94 (2.65–3.28)	4.9×10^{-8}	3.49 (3.09–3.95)	4.28 (3.11–5.87)
Celiac disease			02:01	02:02	1.3×10^{-67}	1.86 (1.73–1.99)	6.7×10^{-62}	2.36 (2.18–2.55)	0.36 (0.27–0.50)
			05:01	03:01	5.3×10^{-156}	0.35 (0.32–0.38)	3.0×10^{-25}	0.42 (0.39–0.46)	0.03 (0.02–0.04)
			05:01	02:01	4.3×10^{-675}	5.78 (5.38–6.20)	4.7×10^{-19}	7.28 (6.67–7.95)	22.80 (19.43–26.75)
			03:01	03:02	8.5×10^{-67}	0.50 (0.46–0.54)	3.5×10^{-11}	0.43 (0.39–0.47)	0.55 (0.42–0.73)
			01:01	05:01	4.0×10^{-216}	0.27 (0.25–0.29)	3.2×10^{-6}	0.29 (0.26–0.32)	0.02 (0.01–0.04)

Additive and non-additive effect sizes are shown for all haplotypes with significant non-additive disease contribution in rheumatoid arthritis (*HLA-DRB1*), T1D (*HLA-DRB1–HLA-DQA1–HLA-DQB1*), psoriasis vulgaris (*HLA-C*) or celiac disease (*HLA-DQA1–HLA-DQB1*). For diseases associated with multiple HLA loci, linked classical alleles across those loci were analyzed as haplotypes. *P* values indicate the significance of improvement in fit of separate haplotype-specific models after sequentially including the additive and non-additive terms for a given haplotype. Odds ratios (ORs) and 95% confidence intervals (CIs) are given for a purely additive scenario (equivalent to *a* in **Fig. 2a**) and for a non-additive scenario in which heterozygotes and homozygotes have separate effects (equivalent to $a + d$ and $2a$ in **Fig. 2a**, respectively). Haplotypes are ordered by the significance of the non-additive effect in each data set.

In most cases, we did not have sufficient power to differentiate the non-additive interaction effects of key amino acid positions from those of classical alleles.

We then investigated whether interactions between specific haplotypes might explain the observed dominance effects. If the disease risk of a specific genotype combination of two alleles deviates from the disease risk expected from both alleles alone, then there is an interaction between the two alleles. Notably, such interactions may give rise to apparent dominance effects at individual alleles. For each disease, we defined a global logistic regression model that simultaneously included interaction terms between all common haplotypes within a given locus. For three diseases (rheumatoid arthritis, T1D and celiac disease), including additive and interaction terms resulted in a significant improvement in fit in comparison to a model with additive and dominance terms (rheumatoid arthritis, $P_{df=5} = 1.8 \times 10^{-3}$; T1D, $P_{df=5} = 8.6 \times 10^{-27}$; celiac disease, $P_{df=9} = 6.0 \times 10^{-100}$). Hence, the observed non-additive effects for rheumatoid arthritis, T1D and celiac disease are at least partially explained by interactions between HLA haplotypes. The models with additive and interaction terms explained 9.5%, 17.3% and 25.2% of phenotypic variance for rheumatoid arthritis, T1D and celiac disease, respectively; interactions yielded an additional 0.5%, 2.9% and 2.3% of phenotypic variance over a model with additive and dominance terms (**Fig. 2b**).

In contrast, psoriasis showed no evidence of interactions ($P_{df=14} = 0.92$). To further identify interactions, we imputed an additional 5,294 psoriasis cases and 10,295 controls genotyped on platforms other than the Immunochip (**Supplementary Table 1c**). Although this increased our sample size dramatically, we still observed no evidence of interactions ($P_{df=14} = 0.87$; **Supplementary Table 12**). The contrast between psoriasis and the other diseases may be related to recent suggestions that the psoriasis association with HLA-C*06:02 is caused by variation in an enhancer element[33], rather than antigenic binding properties. Achalasia showed no evidence for interaction effects in our primary analysis ($P_{df=5} = 0.15$) and only nominal evidence when testing the full data set. All other diseases yielded qualitatively identical results when we tested the full data sets with both rare and common haplotypes (**Supplementary Table 7**). Again, probit regression analysis showed qualitatively identical results (**Supplementary Table 8**).

We then identified the specific HLA haplotypes contributing to the interaction effects in rheumatoid arthritis, T1D and celiac disease. For rheumatoid arthritis, seven of the ten possible interactions were significant ($P < 0.005 = 0.05/10$), all of which increased disease risk beyond the separate additive contribution of each haplotype (**Fig. 3a**,**c**). For T1D, seven of the ten interactions were significant ($P < 0.005 = 0.05/10$), with five increasing risk and two reducing risk (**Supplementary Fig. 5**); these interactions are detailed elsewhere[22]. For celiac disease, among the 15 possible haplotype pairs, there were 4 significant pairwise interactions ($P < 0.003 = 0.05/15$), each increasing risk (**Fig. 3b**,**d**). The identified interactions refined our previous findings of non-additive disease contributions. For instance, in celiac disease, HLA-DQA1*05:01–HLA-DQB1*02:01 had a significant non-additive component ($P = 4.7 \times 10^{-19}$) with $d > 0$, indicating an elevated disease risk in heterozygotes. This elevated disease risk is explained by the interaction model, in which we observed significant risk-increasing interactions between HLA-DQA1*05:01–HLA-DQB1*02:01 and three other haplotypes (**Fig. 3d**).

a Rheumatoid arthritis

	HLA-DRB1	*01:01	*03:01	*04:01	*07:01	*15:01
HLA-DRB1	Additive OR	1.96	0.84	3.84	0.82	1.00 (ref)
*15:01	1.00 (ref)	**2.06 (1.6×10^{-7})**	1.50 (0.02)	**1.43 (1.8×10^{-3})**	1.27 (0.20)	
*07:01	0.82	**2.02 (8.9×10^{-6})**	1.03 (0.89)	**1.49 (3.6×10^{-3})**		
*04:01	3.84	**1.87 (2.9×10^{-8})**	**1.92 (5.2×10^{-7})**			
*03:01	0.84	**1.63 (2.2×10^{-3})**				
*01:01	1.96					

b Celiac disease

	HLA-DQA1–HLA-DQB1	*01:01 *05:01	*02:01 *02:02	*03:01 *03:01	*03:01 *03:02	*05:01 *02:01	*05:01 *03:01
HLA-DQA1–HLA-DQB1	Additive OR	0.86	2.76	0.79	4.53	13.03	1.00 (ref)
*05:01 *03:01	1.00 (ref)	1.14 (0.67)	**16.85 (7.0×10^{-74})**	2.04 (0.12)	0.98 (0.91)	**2.05 (2.2×10^{-7})**	
*05:01 *02:01	13.03	**3.74 (1.9×10^{-10})**	**4.36 (2.4×10^{-44})**	0.71 (0.37)	0.79 (0.03)		
*03:01 *03:02	4.53	1.08 (0.75)	1.23 (0.15)	1.05 (0.90)			
*03:01 *03:01	0.79	1.42 (0.51)	1.53 (0.32)				
*02:01 *02:02	2.76	1.08 (0.77)					
*01:01 *05:01	0.86						

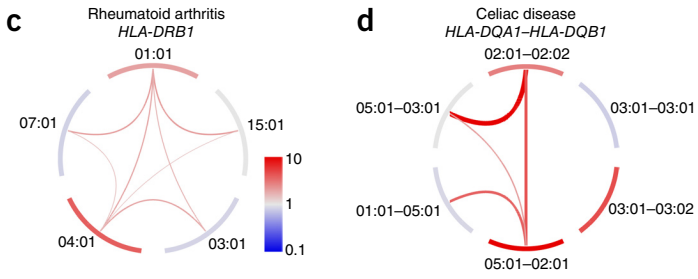

Figure 3 Interaction effects among HLA haplotypes. Pairs of common haplotypes with significant interaction effects (beyond the combined additive effect) are shown. (**a**,**b**) For rheumatoid arthritis (*HLA-DRB1*) (**a**) and celiac disease (*HLA-DQA1–HLA-DQB1*) (**b**), we ran a global regression model that included additive terms for each haplotype and interaction terms for each pair of haplotypes. The fold change in odds ratio due to the inclusion of interaction effects is displayed for each haplotype pair, and the *P* value associated with each interaction odds ratio is shown in parentheses. Additive odds ratios are also displayed for each haplotype, shaded in light gray. The total odds ratio of a haplotype pair is the product of the two haplotypic additive odds ratios and one interaction odds ratio. "Ref" indicates the reference haplotype for each regression model. Bolded values indicate interactions that were significant after multiple-test correction ($P < 0.05/10 = 0.005$ for rheumatoid arthritis, $P < 0.05/15 = 0.003$ for celiac disease). (**c**,**d**) Significant interactions are visualized for *HLA-DRB1* (rheumatoid arthritis) (**c**) and *HLA-DQA1–HLA-DQB1* (celiac disease) (**d**). Outer node segments represent haplotypes, with color indicating additive disease contribution, while internal arcs represent significant interaction effects. For both nodes and arcs, red color indicates disease risk and blue color indicates protection, with effect sizes following the scale in **c**. The effect sizes of the interactions are also represented by the widths of the arcs.

Our results build on previous studies that proposed specific non-additive associations in different autoimmune diseases. While previous studies of heterozygote risk in rheumatoid arthritis highlighted haplotypes with a common 'shared epitope' at HLA-DRβ1 positions 70–74 (refs. 34,35), we discovered significant interactions between shared epitope haplotypes and non–shared epitope haplotypes. There was no evidence for a previously reported interaction between HLA-DRB1*04:01 and HLA-DRB1*04:04 (ref. 36; **Supplementary Table 13** and **Supplementary Note**). Some specific interactions in T1D have been described previously, such as an elevated disease risk for HLA-DRB1*03:01–HLA-DQB1*02:01/HLA-DRB1*04:01–HLA-DQB1*03:02 genotypes[15]. Our recent comprehensive investigation of T1D also confirmed this interaction and found additional interactions with both increasing and decreasing effects on risk[22].

In celiac disease, the DQ2.5 haplotype, composed of HLA-DQA1*05:01 and HLA-DQB1*02:01, is the primary contributor to disease susceptibility[37,38]. Here we confirmed that HLA-DQA1*05:01–HLA-DQB1*02:01 has the strongest association in an additive model ($P = 4.3 \times 10^{-675}$), and we also found significant interactions between HLA-DQA1*05:01–HLA-DQB1*02:01 and other haplotypes. Some of these combinations contained HLA-DQA1*05:01 and HLA-DQB1*02:01 in *trans*, but we also observed haplotype combinations that have not previously been implicated (for example, HLA-DQA1*05:01–HLA-DQB1*02:01/HLA-DQA1*01:01–HLA-DQB1*05:01; interaction odds ratio (OR) = 3.74, $P = 1.9 \times 10^{-10}$). Interestingly, the interaction with the strongest risk effect in our data (HLA-DQA1*02:01–HLA-DQB1*02:02/HLA-DQA1*05:01–HLA-DQB1*03:01; interaction OR = 16.85, $P = 7.0 \times 10^{-74}$), identified in previous studies[39], contained the protective DQ7 haplotype (comprising HLA-DQA1*05:01–HLA-DQB1*03:01; homozygote OR = 0.03), highlighting the complexity of interactions in the HLA region.

Some previously reported interacting variants within a single haplotype have been disputed for only tagging hidden causal variants[40]. However, here we show interactions between homologous haplotypes, which are unaffected by any linked variation. Interestingly, there was little overlap between diseases in the interacting pairs of haplotypes, suggesting that the precise interactions are disease specific. These interactions may depend on the exact autoantigens driving disease susceptibility[2]. Such a scenario would be consistent with previous observations, for example, in rheumatoid arthritis, where immune reactions against different citrullinated autoantigens seem to be restricted by specific HLA-DR variants[41,42]. Additional complexity may arise from parent-of-origin effects in T1D (and potentially other diseases)[43–45]. One possible mechanistic explanation for the interactions between HLA class II haplotypes is the formation of αβ heterodimers in *trans*. In celiac disease, where the major disease antigen is known, expression of the gliadin peptide-presenting HLA-DQ2.5 molecule is the primary determinant of disease risk. Genotypes carrying HLA-DQA1*05:01 and HLA-DQB1*02:01 in *trans* can form this heterodimer, explaining the observed interaction effect between these haplotypes[38]. The differential intrinsic stability of certain *trans* heterodimers may also affect disease risk, as suggested for HLA-DQ in T1D[46]. Further independent replication, for instance in other ancestry groups, and experimental investigations of causal mechanisms are needed to generalize the findings and precisely understand how interacting alleles confer genetic predisposition for these complex diseases.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

This project was supported by grants from the German Research Foundation (DFG; LE 2593/1-1 and LE 2593/2-1 (T.L.L.), GO 1795/1-1 (I.G.), KN 378/2-1 (M.K.) and SCHU 1596/5-1 (J.S.)), by grants from the US National Institutes of Health (1R01AR062886 (P.I.W.d.B.), R01AR065183 (J.T.E.), 1R01AR063759-01A1 (S.R.), 5U01GM092691 (S.R.) and 1UH2AR067677-01 (S.R.)), by the IMI (European Union)–funded program BTCure (L.K.) and by the Netherlands Organization for Scientific Research (Vernieuwingsimpuls VIDI Award NWO project 016.126.354 (P.I.W.d.B.)). Sample collection for J.M. was supported by a grant from the Instituto de Salud Carlos III (RD12/0009). M.M.N. received support for this work from the Alfried Krupp von Bohlen und Halbach-Stiftung and is a member of the DFG-funded Excellence Cluster ImmunoSensation.

AUTHOR CONTRIBUTIONS

T.L.L., A.J.D., S.R., P.I.W.d.B. and S.R.S. conceived the study, coordinated the study and wrote the initial version of the manuscript. T.L.L., A.J.D., S.R., B.H., X.H., Y.O., P.I.W.d.B. and S.R.S. contributed to the study design and analysis strategy. T.L.L., A.J.D. and S.R. conducted all analyses. The following authors organized and contributed subject samples and provided SNP genotype data: S.E., T.W.J.H., L.K., J.M., S.R.-D., J.W. and P.K.G. (rheumatoid arthritis); W.-M.C., S.O.-G. and S.S.R. (T1D); G.A., A.F., D.D.G., R.P.N., P.R., P.E.S., L.C.T. and J.T.E. (psoriasis); J.G.-A., D.A.v.H., A.Z. and C.W. (celiac disease); and J.B., G.E.B., I.G., M.K., M.M.N., M.M.W. and J.S. (achalasia). The following authors contributed to critical writing and review of the manuscript: X.H., D.A.v.H., M.K., S.E., S.S.R., L.K., A.Z., C.W., Y.O. and T.W.J.H. All authors contributed to the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Samples. We analyzed genotype data from previously published studies of HLA association in anti-citrullinated protein antibody–positive ($ACPA^+$) rheumatoid arthritis (n = 16,386)[21], T1D (n = 11,832)[22], psoriasis vulgaris (n = 9,053)[23], idiopathic achalasia (n = 3,638)[24] and celiac disease (n = 20,157)[25]. Each data set contained samples from multiple case-control genome-wide association study (GWAS) cohorts, and all individuals had European ancestry (**Supplementary Table 1**). Each genotype data set has undergone stringent quality control for the original studies above; here only post–quality control data were used. Similarly, case-control matching was performed as in the original studies. Generally, cases and controls were from the same patient collection and were matched for country or region of origin. In most (but not all) of the studies, principal components were additionally used to adjust for any residual stratification. For details on data availability and specific quality control, see **Supplementary Table 14**. All samples were collected from individuals after consent.

HLA genotypes and imputation quality. The SNP genotype data for the MHC region, obtained from previous disease-specific studies, were generated on the Illumina Immunochip platform[47]. Following previous studies, we defined the MHC region as the region on chromosome 6 from 29 Mb to 34 Mb. We imputed 4-digit classical HLA alleles with SNP2HLA[19], using dense SNP data across the MHC region for each disease data set (number of SNPs used for imputation: rheumatoid arthritis, 4,499; T1D, 4,604; psoriasis, 4,030; achalasia, 3,773; celiac disease, 3,249) and a reference panel of 5,225 individuals of European ancestry from T1DGC[26]. Cases and controls were imputed together, to ensure consistent imputation quality across all samples. We have separately demonstrated high imputation accuracy using genotype data from the Immunochip platform[19]. Furthermore, we found no evidence for a potential bias in imputation accuracy due to using a disease-specific reference panel (**Supplementary Table 15**).

For each allele, the INFO score was calculated from the ratio of the observed variance in dosage to the expected variance under Hardy-Weinberg equilibrium[32]

$$\mathrm{INFO} = \frac{\mathrm{var}(x)}{2(p)(1-p)} \quad (1)$$

where x is the imputed dosage and p is the frequency of the allele. An INFO score close to 0 indicates poor imputation quality, whereas a score closer to 1 indicates higher quality; a value greater than 1 is also possible. Because of the presence of non-additive effects that inflated the disease risk in heterozygotes, the allele distribution in disease cases deviated from Hardy-Weinberg equilibrium. Therefore, we calculated INFO scores using the variance and allele frequency in controls only (**Supplementary Table 2**). However, because the imputation algorithm does not take case-control status into account, we expected that imputation quality should be similar in cases and controls. For rheumatoid arthritis, we also calculated INFO scores within each cohort to test whether lower INFO scores (i.e., lower quality of imputed genotypes) were associated with a higher likelihood of detecting non-additive effects, which could suggest that non-additive effects are an artifact of imputation errors.

Selection of genes for analysis. For each disease, we selected the HLA genes that were most significantly associated with disease risk in previous studies (rheumatoid arthritis, *HLA-DRB1* (ref. 21); T1D, *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* (ref. 22); psoriasis, *HLA-C*[23]; achalasia, *HLA-DQA1* and *HLA-DQB1* (ref. 24); celiac disease, *HLA-DQA1* and *HLA-DQB1* (ref. 25)). For diseases in which more than one HLA gene was implicated as conferring major independent risk contribution, we defined haplotypes according to unique combinations of four-digit classical alleles at each relevant gene. We used phased best-guess genotypes from SNP2HLA to ensure that each haplotype contained classical alleles on the same chromosome.

For rheumatoid arthritis and psoriasis, we repeated the analysis using imputed dosages (which range on a continuous scale from 0 to 2) rather than best-guess genotypes (which are restricted to the integer values 0, 1 and 2; see **Supplementary Table 16**). Because imputed dosages do not contain phasing information, we did not perform this analysis for the diseases involving multiple genes.

Selection of alleles for analysis. We performed all association tests with two data sets: the common allele data set and the full data set. In the common allele data set, we restricted our analysis to classical alleles with a frequency greater than or equal to 5% in the T1DGC reference panel or haplotypes comprising these classical alleles (rheumatoid arthritis, m = 5; T1D, m = 5; psoriasis, m = 7; achalasia, m = 5; celiac disease, m = 6; where m indicates the number of included HLA alleles or haplotypes). This cutoff ensured very high imputation quality, and the INFO scores for all alleles in the common allele subset exceeded 0.97 (**Supplementary Table 2**). We also ensured that all haplotypes in the common subset had at least ten homozygous individuals. (If fewer than ten homozygotes are present, the additive and non-additive terms are statistically indistinguishable.) In the full data set, we included all m variants (4-digit classical alleles or haplotypes) with at least 10 homozygous individuals (rheumatoid arthritis, m = 11; T1D, m = 11; psoriasis, m = 13; achalasia, m = 9; celiac disease, m = 10).

We ensured complete data in both data sets by excluding all individuals who lacked exactly two best-guess alleles at a given locus. For analyses that used imputed dosages, we excluded all individuals whose total dosage across the relevant alleles was less than 1.95 or greater than 2.05.

Statistical framework for association testing. To analyze the effects of HLA haplotypes on disease risk, we used a logistic regression framework (probit regressions yielded qualitatively identical results; **Supplementary Table 8**). We began by presenting a baseline model, consistent with the models used to fine map HLA effects in recent publications for rheumatoid arthritis[21], T1D[22], psoriasis[23], achalasia[24] and celiac disease[25]. These models assume a purely additive contribution from each haplotype. To control for cohort-specific effects and population stratification, we included an indicator variable for each cohort, the first L principal components (for rheumatoid arthritis, psoriasis and achalasia) and a sex term (for T1D and celiac disease) as covariates. This resulted in the following logistic regression model:

$$\log(\mathrm{odds}_i) = \theta + \sum_{j=1}^{m-1} a_j x_{i,j} + \sum_{k=1}^{K} \delta_{i,k}\left(\lambda_k + \sum_{l=1}^{L} \pi_{k,l} p_{i,k,l}\right) + \gamma g_i \quad (2)$$

where θ is the logistic regression intercept, a_j is the additive effect of allele j and $x_{i,j}$ is the allelic dosage (using the best-guess genotype or imputed dosage) of allele j in individual i. For a multiallelic locus with m possible alleles, we included $m-1$ a parameters, and we set the final a parameter to 0 to denote the reference allele. We arbitrarily selected the most common allele in the controls as the reference allele. The parameter $\delta_{i,k}$ is a binary indicator variable that equals 1 if and only if individual i is in cohort k, and λ_k is the effect for the kth cohort. Among a total of K cohorts, we arbitrarily selected the largest cohort as the reference cohort and set its λ parameter to 0.

For rheumatoid arthritis, psoriasis and achalasia, we also included the first L principal components, where $p_{i,k,l}$ is the value of principal component l in cohort k for individual i and $\pi_{k,l}$ is the corresponding effect size. To be consistent with HLA fine-mapping studies on other diseases, we used L = 10 for rheumatoid arthritis[21] and psoriasis[23], L = 5 for achalasia[24] and no principal components for T1D[22] or celiac disease[25].

We included an additional covariate to account for sex differences in T1D and celiac disease, once again to conform to previous HLA fine-mapping analyses[22,25]; however, the sex covariate had no significant effect on our results (**Supplementary Table 17**). Here γ is the effect of sex, and g_i is a binary indicator variable that equals 1 if and only if individual i is female. We did not include the g_i parameter for rheumatoid arthritis, psoriasis or achalasia, according to refs. 21, 23 and 24, respectively.

We tested the significance in fit for each model by calculating the change in deviance (defined as −2 times the difference in log likelihood) from the original model to the revised model. This value follows a χ^2 distribution with n degrees of freedom, where n is the number of new parameters introduced in the revised model. For the additive model, n is one less than the total number of tested haplotypes (to account for a reference haplotype).

Analysis of dominance and interaction effects. For each disease, we tested for non-additive effects by including a dominance term d_j for each represented haplotype in the relevant data set (the common allele subset or the full data set)

$$\log(\text{odds}_i) = \theta + \sum_{j=1}^{m-1} a_j x_{i,j} + \sum_{j=1}^{m} d_j \delta_{x_{i,j}} + \sum_{k=1}^{K} \delta_{i,k} \left(\lambda_k + \sum_{l=1}^{L} \pi_{k,l} p_{i,k,l} \right) + \gamma g_i \quad (3)$$

where d_j represents the dominance effect of allele j and $\delta_{x_{i,j}}$ denotes that individual i is heterozygous for haplotype j. For analyses involving best-guess genotypes, $\delta_{x_{i,j}} = 1$ if and only if $x_{i,j} = 1$. For dosage-based analyses, we used the formula $\delta_{x_{i,j}} = 1 - \text{abs}(1 - x_{i,j})$. We assessed the change in deviance between the additive model and the dominance model, which follows a χ^2 distribution with m degrees of freedom (one for each haplotype). To determine the relative non-additive effect of a specific haplotype, we constructed a separate model for each haplotype by repeating the model in equation (3) for a single value of j (1 degree of freedom). For the single-haplotype models, we used a significance threshold of $P < 0.05/m$ to correct for multiple tests.

We also constructed an interaction model, which contains an additive term for each haplotype and an interaction term between each pair of haplotypes

$$\log(\text{odds}_i) = \theta + \sum_{j=1}^{m-1} a_j x_{i,j} + \sum_{j=1}^{m} \sum_{h=j+1}^{m} \phi_{j,h} x_{i,j} x_{i,h} + \sum_{k=1}^{K} \delta_{i,k} \left(\lambda_k + \sum_{l=1}^{L} \pi_{k,l} p_{i,k,l} \right) + \gamma g_i \quad (4)$$

where $\phi_{j,h}$ is the effect size of the interaction between alleles j and h. We did not include dominance terms in this model, owing to partial redundancy between dominance and interaction terms. The interaction model contains an additional $m(m-1)/2$ degrees of freedom (one for each pairwise interaction), in comparison to the additive model. We assessed the change in deviance between the dominance model and the interaction model, which follows a χ^2 distribution with $m(m-1)/2 - m$ degrees of freedom. To determine the relative significance of individual interaction terms, we compared the P values associated with each ϕ parameter, and we used a significance threshold of $P < 0.05/(m(m-1)/2)$.

To compare the disease risk in homozygotes and heterozygotes, we constructed additive models after excluding all homozygous individuals (to estimate the heterozygous effect size) or excluding all heterozygous individuals (to estimate the homozygous effect size). This separation of genotype groups was necessary to estimate the true additive component for each HLA haplotype's disease risk (from homozygotes only) and to subsequently allow for the calculation of the dominance component (on the basis of heterozygotes only). For dosage-based analyses, we defined heterozygous individuals as those with a dosage greater than 0.95 and less than or equal to 1.05, whereas homozygous individuals were those with a dosage greater than 1.05.

Calculation of phenotypic variance explained. We calculated the proportion of phenotypic variance explained by a given locus using the liability threshold model[48,49]. We assumed that disease risk is the consequence of an underlying liability score. Each individual with a score above a prespecified threshold gets the disease[50]. Each genotype has the same threshold, but the distribution of liability can differ among genotypes. The variance between the genotype-specific liability functions is a measure of the variance explained by the locus. For a more detailed description, including equations, see the **Supplementary Note**.

Analysis of amino acid–level non-additive effects. For rheumatoid arthritis, we also analyzed the non-additive effects of individual amino acid positions encoded within specific HLA genes. We used imputed amino acid genotypes at positions 13, 71 and 74 of HLA-DRβ1, and we analyzed all residues at these positions with a frequency greater than or equal to 5% in the T1DGC reference panel. We used the previously described allele-level models (equations (2) and (3)) to assess the non-additive effects of amino acid variants.

Because the residues at amino acid positions within a given locus are in strong linkage disequilibrium, we used a stepwise conditioning approach to test the effects at successive positions on rheumatoid arthritis risk, following refs. 20 and 51. We analyzed positions in the order of significance of their additive contributions to disease risk. First, we analyzed non-additive effects at HLA-DRβ1 position 13. Then, we analyzed HLA-DRβ1 position 71 while conditioning on HLA-DRβ1 position 13, and we analyzed HLA-DRβ1 position 74 while conditioning on HLA-DRβ1 positions 13 and 71. To condition on a specific amino acid position, we included all possible amino acid variants at that position as covariates, but we excluded any variant that had strong correlations to other variants in the data set ($R^2 > 0.97$)[23].

Permutation of imputed HLA genotypes. To verify that the observed non-additive effects were not a subtle artifact of imputation inaccuracies, we permuted SNP–imputed HLA genotypes across cases and controls, on the basis of the case probability predicted by a purely additive model (equation (2)). This approach conserves allele frequencies (which confer additive disease associations) within cases and controls and also conserves individual genotypes. However, it randomizes the distribution of homozygote and heterozygote genotypes among cases and controls. We performed 10,000 permutations, and for each permutation we recorded the deviance of a non-additive model with dominance terms for all relevant HLA haplotypes (equation (3)). To validate the permuted cohorts, we also compared the distribution of additive effects for each relevant HLA haplotype with the observed values in the actual data set (**Supplementary Fig. 2**).

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doi:10.1038/ng.3379