# Important denominator between autoimmune comorbidities: a review of class II HLA, autoimmune disease, and the gut

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Human leukocyte antigen (HLA) genes are associated with more diseases than any other region of the genome. Highly polymorphic HLA genes produce variable haplotypes that are specifically correlated with pathogenically different autoimmunities. Despite differing etiologies, however, many autoimmune disorders share the same risk-associated HLA haplotypes often resulting in comorbidity. This shared risk remains an unanswered question in the field. Yet, several groups have revealed links between gut microbial community composition and autoimmune diseases. Autoimmunity is frequently associated with dysbiosis, resulting in loss of barrier function and permeability of tight junctions, which increases HLA class II expression levels and thus further influences the composition of the gut microbiome. However, autoimmune-risk-associated HLA haplotypes are connected to gut dysbiosis long before autoimmunity even begins. This review evaluates current research on the HLA-microbiome-autoimmunity triplex and proposes that pre-autoimmune bacterial dysbiosis in the gut is an important determinant between autoimmune comorbidities with systemic inflammation as a common denominator.

**GRAPHICAL ABSTRACT**
Graphical representation of central hypothesis.
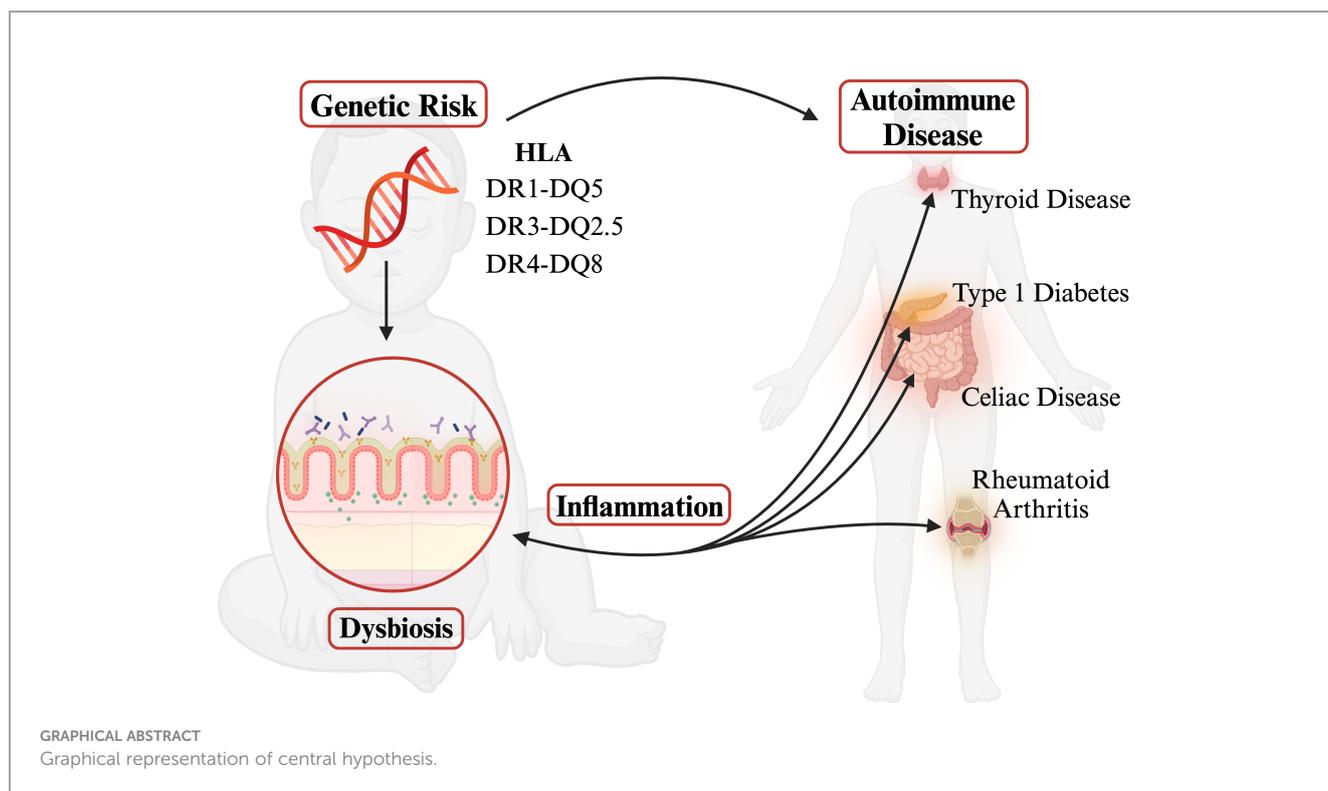
# 1 Introduction

The major histocompatibility complex (MHC) has long been established as the human genetic region associated with the greatest number of autoimmune diseases (1, 2). The MHC is broadly categorized into three classes: class I, which encodes for *HLA-A, HLA-B, HLA-C, HLA-E, HLA-F,* and *HLA-G* genes; class II, the focus of this review, which encodes for *HLA-DR, HLA-DQ,* and *HLA-DP* genes; and class III, which includes components of the complement system, immune regulators, and non-immune associated genes (2–4). Classically, class I HLA are present on all cells, while class II HLA are expressed on the surface of antigen presenting cells (APC) like dendritic cells and macrophages. Class I HLA-peptide combinations bind CD8$^+$ T cell αβ T cell receptors (TCRs) for inspection of internally found antigens, like signals of viral infection and cancer. Class II HLA present externally found peptides to CD4$^+$ T cell TCRs, such as bacteria and other foreign pathogens. However, cross-class presentation has been observed to bypass MHC restriction (4–6). The presentation of externally found antigens to T cells instigates a cascade leading to destruction of the perceived pathogen. HLA-peptide-TCR interaction specificity is fundamental to an effective cell-mediated adaptive immune response (7). The peptide repertoire available for presentation by class II HLA largely depends on the structure of the binding pocket.

HLA DR and DQ loci are highly polymorphic and exhibit an elevated amount of linkage disequilibrium. The combination of these features contribute to creating distinctive and behaviorally differential HLA haplotypes (8). This review will cover autoimmune-risk-associated class II HLA haplotypes DR4-DQ8, DR3-DQ2, and DR1-DQ5. The most polymorphic regions of the DR and DQ molecules are located within extracellular regions making up the peptide-binding cleft, which cause structure-altering changes at the amino acid level (9–12). These structural variations alter peptide-binding and thus antigen-presenting capabilities (7, 13, 14). The structural differences between haplotype molecules result in unique sensitivities and can be the determining factor for many autoimmune diseases, such as type 1 diabetes (T1D), celiac disease (CD), rheumatoid arthritis (RA), and autoimmune thyroid disease (AITD), including Grave's disease (GD) and Hashimoto's disease (HD) (9, 15–18).

Epidemiological data show an increase in the frequency of autoimmune diseases over the past few decades that cannot be explained by genetics alone (19–21). Many autoimmune disorders share the same risk-associated HLA haplotypes often resulting in comorbidity despite having differing etiologies (22–24). The combination of high polymorphism and linkage disequilibrium within the gene dense MHC region leads to difficulty in determining the mechanism for the autoimmune associations observed (1, 2). This gap is where the role of the gut microbiome has become increasingly essential in defining the pathogenesis of these autoimmune diseases (25–30). It has been theorized that the dysbiosis seen in autoimmune diseases is associated with systemic inflammation, resulting in loss of barrier function and permeability of tight junctions, allowing for possible increased exposure of HLA

proteins to bacterial antigens (31–33). HLA class II proteins are expressed in the upper villi of small intestinal enterocytes at a steady state in the presence of a healthy gut microbiome and are an integral part of maintaining homeostasis; however, dysbiosis and inflammation cause an increase in HLA class II expression in small intestinal crypts and the colonic epithelium, which can in turn influence the composition of the gut microbiome (32, 34–39). Notably, the increase in HLA class II expression levels is active-disease dependent; for example, celiac patients with exposure to gliadin show HLA upregulation whereas celiac patients in remission have HLA class II levels of controls (40). However, certain HLA haplotypes, specifically the known risk HLA discussed here, are associated with gut dysbiosis before autoimmunity occurs (36, 39, 41, 42). Such evidence suggests that certain HLA may be predisposing an individual to systemic inflammation originating from the gut microbiome by clearing beneficial microbes and creating the potential for dysbiosis early in life. The tripartite HLA-microbiome-autoimmunity link is not trivial. This review summarizes current research on the impact class II HLA haplotypes have on the microbiome and its correlation to autoimmune disease onset. Our hypothesis is that bacterial dysbiosis in the gut leads to systemic inflammation which leads to autoimmunity (Graphical Abstract). The sources and types of inflammation can vary, causing different autoimmune disease outcomes.

# 2 DR-DQ haplotype structure and nomenclature

Class II HLA DR and DQ loci represent the greatest genetic determinants of multiple autoimmune diseases. HLA-DR is a heterodimer consisting of an α (DRA) and β (DRB) chain, each of which have two extracellular domains, an intramembranous domain, and a cytoplasmic tail (Figure 1). DRA has two potential α polypeptide chains for the HLA-DR heterodimer, but the allelic differences do not result in function-altering polymorphisms (43, 44). The HLA-DR β chain can be encoded by DRB1, DRB2 (pseudogene), DRB3, DRB4, and DRB5 genes (43). Many DRB1 allelic variations are associated with multiple autoimmune diseases and are the basis for the HLA-DR naming system. For example, HLA-DR4 is the name for the DRB1*04 allele group. HLA-DQ is also a highly variable αβ heterodimer forming a type 1 membrane protein. DQA and DQB can both be encoded by two paralogs: DQA1, DQA2, DQB1, DQB2, respectively. Both DQA1 and DQB1 are highly polymorphic resulting in hundreds of possible combinations (43).

HLA DR4-DQ8 is the nomenclature used to represent that an individual has the gene products of HLA DRA1-DRB1*04:01/02/04/05/08 and DQA1*03:01-DQB1*03:02/04 (11). DR3-DQ2.5 represents gene products of DRB1*03:01/02/03/04-DQA1*05:01-DQB1*02:01. DR1-DQ5 represents DRB1*01:01/02-DQA1*01:01-

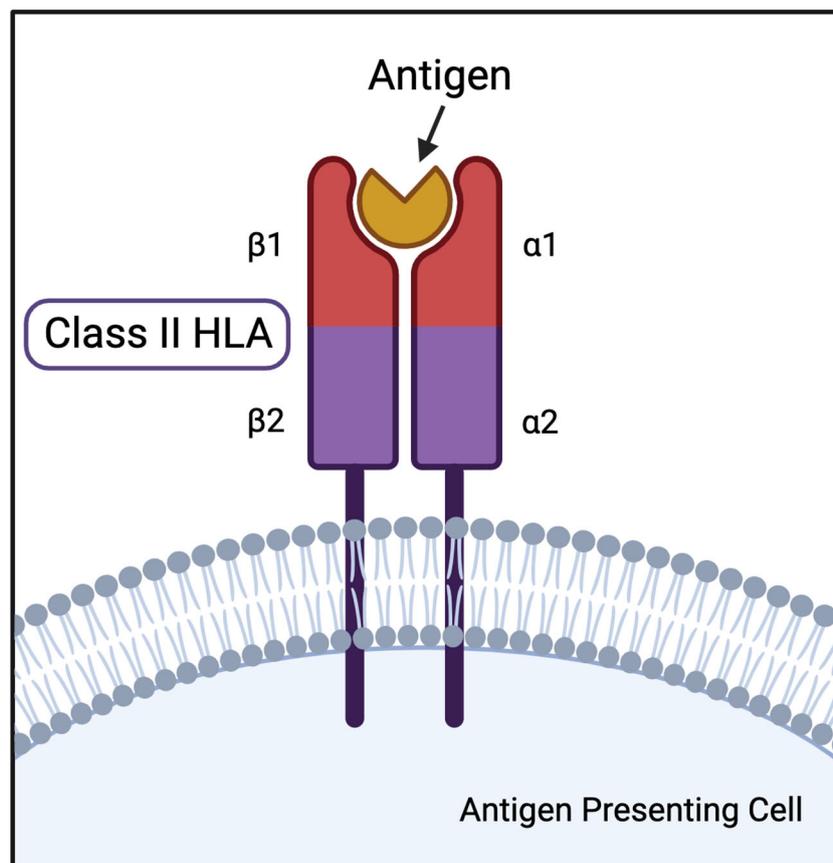

FIGURE 1
Illustration of class II HLA protein structure with antigen in peptide binding pocket.

DQB1*05:01. DR5-DQ7.5 represents DRB1*05:01-DQA1*05:05-DQB1*03:01. For clarification, the HLA naming system (e.g., DRB1*04:01) is the gene locus name (e.g., DRB1), followed by an asterisk, the serologic designation of the allelic group (e.g., 04), a colon, and then the numeric designation of the specific HLA protein (e.g., 01). The naming system can be further expanded to a six-digit identifier that includes another colon followed by a two-digit number that represents a synonymous DNA substitution in the coding region (e.g., DRB1*04:01:01). For this review, the four-digit naming system will be sufficient.

Because DQA1 and DQB1 can both have polymorphisms, unique DQ heterodimers can be formed by pairing α and β chains from the same chromosome (cis) or opposite chromosomes (trans). While the cis form of DQ has been studied predominantly, trans variants are functional and surface expressed (45). This trans molecular formation means that a person heterozygous for DR4-DQ8 and DR3-DQ2.5 can produce a DQ2.3 (DQA1*03:01-DQB1*02:01) molecules from the α chain of DQ8 and the β chain of DQ2.5 (46). For this review, all DQ can be assumed to be cis unless specifically reported as trans.

# 3 Class II HLA autoimmunity association

## 3.1 Type 1 diabetes

Type 1 diabetes (T1D) is characterized by autoimmune destruction of pancreatic β-cells, resulting in a lifelong exogenous insulin dependency that affects millions of people worldwide (47). While there are over 50 known regions of the genome to show association with genetic risk for T1D, the greatest genetic determinants are MHC class II DRB1, DQA1, and DQB1 (48). The haplotypes most strongly associated with T1D susceptibility in those with European ancestry are DRB1*04:01-DQA1*03:01-DQB*03:02, DRB1*04:05-DQA1*03:01-DQB1*03:02, and DRB1*04:02-DQA1*03:01-DQB1*03:02, and then DRB1*04:04-DQA1*03:01-DQB1*03:02 conferring weaker disease susceptibility (9, 49). These well-known associations have predominantly been studied in European-centric populations and they may not be translatable to other ethnic backgrounds. For example in the Japanese population DR4-DQ8 is not prevalent; DRB1*04:05-DQA1*03:03-DQB1*04:01 (DR4-DQ4) and DRB1*09:01-DQA1*03:02-DQB1*03:03 (DR9-DQ9) are the most susceptible haplotypes for T1D (50). Three haplotypes, DR4-DQ8, DR3-DQ2.5, and DR4-DQ4, which combine the risk haplotypes for Caucasian and Japanese populations are the most highly associated with T1D in the Taiwanese population (51). For the Caucasian population, the development of diabetes-associated autoantibodies and T1D is significantly more common in children with heterozygous HLA DR3-DQ2/DR4-DQ8 compared to homozygous DR4-DQ8/DR4-DQ8 and DR3-DQ2.5/DR3-DQ2.5 (11, 12, 52). In Finland, children with high-risk HLA DR3-DQ2/DR4-DQ8 genotypes have a 45-fold increased risk for T1D compared to those with neutral or protective genotypes (17). The age of T1D diagnosis and type of autoantibody first observed are

also strongly associated with HLA genotypes (53, 54). Insulin autoantibodies (IAA) and insulinoma-associated-2 autoantibodies (IA2A) are strongly correlated with DR4-DQ8 (12, 55). Approximately 1 in 20 heterozygous for DR3-DQ2/DR4-DQ8 will be diagnosed with T1D by the age of 15 (11). The proportion of HLA DR3-DQ2/DR4-DQ8 heterozygous T1D subjects decreases with increasing age at diagnosis (12). Seroconversion and development of T1D is associated with specific residues at both the DR and the DQ loci, the motif lysine, alanine, glycine at DRB1 β71, β74, β86 residues, which corresponds with DRB1*04:01, and glutamine, alanine, and aspartic acid at DQ α44, β57, and β135 residues, respectively, which correspond to DQA1*03:01-DQB1*03:02 (56, 57). An alanine at DQ β57 is most strongly associated with T1D (58).

In addition to DR4-DQ8, among the highest risk HLA haplotypes for T1D is DRB1*0301-DQA1*05:01-DQB1*02:01 (DR3-DQ2.5) (9). For the Caucasian population, the development of diabetes-associated autoantibodies is significantly associated with both the homozygous DR3-DQ2.5 and heterozygous DR3-DQ2.5/DR4-DQ8 (11, 12, 52). As previously discussed, the age of T1D diagnosis and type of autoantibody first observed are also strongly associated HLA haplotype; autoantibodies, IAA and IA2A, are associated with DR4-DQ8 and early seroconversion, but glutamic acid decarboxylase autoantibodies (GADA) are found in individuals have later seroconversion and are strongly associated with DR3-DQ2.5 (12, 52–55). Recent evidence suggests that GAD peptides bind to DR3-DQ2.5 molecules and in turn induce CD4+ T cell cytokine expression (59). In addition to the cis DR3-DQ2.5/DR4-DQ8 heterodimer, the heterozygote DR3-DQ2.5/DR4-DQ8 in trans heterodimer form encoded by DQA1*05:01-DQB1*03:02 is also very high risk for T1D (9). At a young age the risk for T1D is highest with heterozygous HLA-DR3-DQ2.5/DR4-DQ8, approximately 1 in 20 with this HLA haplotype pairing will be diagnosed with T1D by the age of 15 (11). The strongest amino acid association with T1D onset is an alanine at residue DQ β57, which is seen in both DQ2 and DQ8 (58, 60).

## 3.2 Celiac disease

Celiac disease (CD) is autoimmune enteropathy characterized by the immunogenicity of gliadin peptides derived from dietary gluten. The chief HLA determinant of CD development is HLA-DQ (61). While HLA-DQ2.5 discussed in the next section has the highest risk association, DQ8 specifically, DQA1*03:01-DQB1*03:02, represents about 2–10% of the Caucasian CD population (62). The determining factor for HLA-associated CD susceptibility is the preferential binding of negatively charged gliadin-derived glutamate residues to certain binding pockets of DQ molecules, specifically P1 and P9 for DQ8 and P4, P6 and P7 for DQ2.5 and DQ2.2 (61, 63).

Globally, CD seroprevalence is 1.4% and 1.8% in North America. However, a study recently found that the highest prevalence is 3.1% within those from northwest India (64, 65). Within the Caucasian population, DQ2.5 is the predominant HLA predictor of CD susceptibility, approximately 95% of those with CD

are positive for *DQA1\*05:01-DQB1\*02:01* (DQ2.5); the remaining CD population has either *DQA1\*03:01-DQB1\*03:02* (DQ8) or *DQA1\*02:01-DQB1\*02:02* (DQ2.2) (66). DQ2.5 also has the strongest risk association with CD within the Indian population (67). DQ2.5 was the greatest CD-associated HLA in Moroccan, Libyan, Greek, and Italian CD populations; however, approximately 73.9%, 80.7%, 81.3%, and 78.2% of the populations, respectively, were DQ2.5 positive (68). The determining factor for HLA-associated CD susceptibility is the preferential binding of negatively charged gliadin-derived glutamate residues to certain binding pockets of DQ molecules, specifically P4, P6 and P7 for DQ2.5 and DQ2.2 (63). While DQ2.5 is the predominant isoform seen in association with CD susceptibility and the majority of the remaining CD population carry DQ8, *DQA1\*02:01-DQB1\*02:02* (DQ2.2), linked to *DRB1\*07:01* (DR7), represents about 3.4% of the CD population (69). Generally, DQ2.2 is associated with very low CD risk; however, when heterozygous with DQ2.5 or *trans* configuration creates DQ2.5, a high risk association is observed (69, 70). Risk for CD is associated with DQ2.2 predominantly when individuals also carry DR3-DQ2.5 or DR5-DQ7.5 because the *DQA1\*05:05* of DQ7.5 is nearly identical to the *DQA1\*05:01* of DQ2.5 which means the *DQB1\*02:02* of DQ2.2 and *DQA1\*05:05* of DQ7.5 can make a *DQA1\*05:05-DQB1\*02:02* (DQ2.5) heterodimer (70).

## 3.3 Autoimmune thyroid diseases

Autoimmune thyroid diseases (AITD) include both Graves' disease (GD), which is an autoimmune hyperthyroidism, and Hashimoto's disease (HD), which is an autoimmune hypothyroidism. HD is characterized by positive autoantibodies to thyroglobulin and thyroid peroxidase, whereas GD is characterized by autoantibodies against the thyroid stimulating hormone receptor, thyroglobulin, and thyroid peroxidase (71). The chief genetic determinant of GD susceptibility is *DRB1\*03:04-DQA1\*05:01-DQB1\*02:01* (DR3-DQ2.5); however, the *DQB1\*02:01* locus appears to be associated through linkage disequilibrium as opposed to actual influence on susceptibility (18, 72, 73). The strongest amino acid association with GD is an arginine at residue DR β74, which is integral to the binding and presentation of thyroglobulin (18, 72, 74). The positive charge provided by arginine in this positive likely facilitates auto-antigen presentation (75). Though less convincingly, DR3 is also associated risk of HD susceptibility and, in those with HD and T1D, DR3 is responsible for joint susceptibility (18, 75).

## 3.4 Autoimmune arthritis

The association of *DRB1\*04* and genetic predisposition for rheumatoid arthritis (RA) has been observed since the late 1980s (76, 77). RA is the autoimmune destruction of the synovium in the small joints characterized by the presence of autoantibodies: rheumatoid factor, anti-cyclic citrullinated peptide-2, and anti-

carbamylated protein (78, 79). Within the Caucasian population of RA patients, significant associations are seen with *DRB1\*04:01*, *DRB1\*04:04*, *DRB1\*04:05* and 95% of those with severe arthritis expressed *DRB1\*04:01* (80, 81). Those homozygous for DR4 have the highest risk association for RA (82). While allotypes of DR4 are high risk for RA, *DRB1\*04:02* is not associated with the disease (80). The determining factor for HLA-associated RA susceptibility is a positive charge at the DRβ71 amino acid residue—*DRB1\*04:01* and *DRB1\*04:04* have a positively charged lysine or arginine, respectively, at this position whereas *DRB1\*04:02* has a negatively charged glutamic acid (80). It is important to note that while certain DR4 alleles have long been observed in Caucasian RA studies, there is no statistical significance in the prevalence of DR4 in RA patients versus controls within the Iranian population and no association between RA-associated autoantibodies and risk HLA in the Japanese population (79, 83).

For RA patients who lack DR4, *DRB1\*01:01/02* (DR1) is notably associated with RA susceptibility (77). Like *DRB1\*04:01/04*, *DRB1\*0101* carries a positively charged arginine at the DRβ71 amino acid residue, which is a determining factor for HLA-associated RA susceptibility (80). A small study shows that the majority multi-drug resistant RA patients have *DRB1\*01:01/02* (84). HLA-*DRB1\*04:01*, *DRB1\*04:05*, and *DRB1\*01:01* share a common motif at residues β11, β13, β71, and β74, specifically an alanine at position 74 and a positively charged lysine or arginine at position 71, influencing the DRB1 P4 binding pocket (85). Also, homozygote DR1-DQ5 is also strongly associated with juvenile idiopathic arthritis (JIA) with an odds ratio of 3.6, which increases to 6.4 when individual was breastfed for fewer than 8 months (86). JIA was also associated with DR5-DQ7 in individuals who breastfed under 8 months (86).

# 4 Autoimmune comorbidity

Despite differing etiologies, as discussed, many autoimmune disorders share the same risk-associated HLA haplotypes often resulting in comorbidity. Individuals with T1D are 4.9 times more likely to have RA as adults than the general population (87). A 2011 study suggests that 12.3% of the T1D population assessed have AITD and 24.6% have CD (88). However, that number was an overestimation. A 2023 study found that while 18.6% of the T1D population tests positive for CD, 12.6% were serologically false positive and only 6% are actually confirmed CD patients (89), which is in agreement with prevalence found in many other studies (90). Globally, biopsy-confirmed CD prevalence is 0.7%; however, biopsy-confirmed CD prevalence is 1.6% in the general AITD population and 2.6% in the hyperthyroid community (64, 91). For those with CD, 26% of the population have AITD compared to 2–5% of the general population; individuals with CD are 2.4 times more likely to develop an AITD and 5.9 times more likely if they are female (92, 93). The odds of having RA is also higher in CD, occurring nearly 2 times as often compared to the general population (94).

# 5 Evidence for autoimmunity-associated dysbiosis

## 5.1 Type 1 diabetes

While T1D is caused by autoreactive T cells, a link between T1D and notable microbial patterns and intestinal inflammation is evident (36, 95, 96). The microbiome of T1D children lack diversity and have higher levels of butyrate-producing and mucin-degrading bacteria than healthy children (38, 97). Microbiome differences of those with future T1D diagnosis can be seen as early as one year of age (42). Research from the Finnish Type 1 Diabetes Prediction and Prevention Study (DIPP), a prospective, general-population cohort, shows high abundance of *Bacteroides dorei* and *Bacteroides vulgatus* between 12 and 15 months before seroconversion (37). Active T1D cases are associated with higher relative abundance of *Ruminococcus* and *Prevotella copri* and lower relative abundance of *Bifidobacterium, Lactobacillus, Roseburia*, and *Faecalibacterium* (Table 1) (98–100). The microbial composition observed in T1D patients likely leads to intestinal permeability, causing intestinal inflammation (101). Intestinal permeability results in increased exposure of intestinal immune cells to bacteria antigens. Intestinal biopsies from T1D children showed an increase in class II HLA molecule expression, and high levels of CD25+ cells (102). Increased exposure to commensal bacteria and excessive immune response over time could result in aberrant self-tolerance mechanisms. T1D patients exhibit immune dysregulation with higher percentages of Th1, Th17, and TNFa+ T cells (103).

## 5.2 Celiac disease

CD has obvious connections to gastrointestinal distress, gliadin peptides induce upregulation of zonulin and the shielding of gliadin peptides from destruction by lysosomes increases peptide secretion into the intestinal lamina propria, perpetuating inflammation and intestinal permeability (29, 63). Studies focusing on fecal microbiota show lower abundance of *Bifidobacterium* and *Lactobacillus* species in CD patients than healthy controls, both of which are considered to have anti-inflammatory effects (29, 104–106). Research from the Celiac Disease Genomic Environmental Microbiome and Metabolomic (CDGEMM) study, a prospective cohort of healthy infants with a first-degree relative who has CD, shows microbiome composition patterns up to 15 months before disease onset—increased abundance of *Dialister invisus, Parabacteroides* sp., *Porphyromonas* sp., *Ruminococus bicirculans*, Lachnospiraceae and decreased abundance of *Streptococcus thermophilus, Faecalibacterium prausnitzii*, and *Clostridium clostridioforme* (107). Microbiome differences between those with a future diagnosis of CD and healthy matched controls can be seen as early as one year of age (41). Children progressed to CD diagnosis not only have a distinct microbiome composition compared to healthy controls but also have an increased IgA response, resulting in more IgA-coated bacteria, suggesting altered bacterial clearance (108). A recent study shows that changes in the gut microbiome, specifically abundance of Veillonellaceae, may have causal effects on CD development, while Pasteurellaceae abundance differences may be caused by the disease itself (109). Since CD occurs in the small intestine, intestinal location specific studies reveal the importance of location in microbial composition—higher abundance of *Escherichia coli, Prevotella salivae*, and *Neisseria* are associated with CD when sampling the duodenum (110).

## 5.3 Autoimmune thyroid disease

The gut-thyroid axis is a relatively new discussion point in study of autoimmune thyroid disease (AITD). Evidence that suggests dysbiosis is seen across AITD patients and the disruption of the gut microbial composition affects thyroid hormone metabolism (111, 112). Serum lipopolysaccharide and zonulin are significantly higher in GD patients than healthy controls and fecal transplant from GD patients into a mouse model significantly increases the incidence of GD (113, 114). However, specific microbiome community dynamics and potential for microbial biomarkers remains conflicted. The ratio of Firmicutes to

TABLE 1 Autoimmune disease association with HLA genetics, bacteria, and each other.

| Disease | Genetic Risk (HLA) | Comorbidity | Positive Association with Disease | Negative Association with Disease |
|---|---|---|---|---|
| Autoimmune Arthritis | DR4-DQ8 DR1-DQ5 DR5-DQ7 (JIA specific) | T1D, CD | *Collinsella, Eggerthella, Faecalibacterium, Prevotella copri* | *Bifidobacterium, Bacteroides* |
| Autoimmune Thyroid Disease | DR3-DQ2.5 | CD, T1D | *Lactobacillus, Bacteroides fragilis* | *Lactobacillus, Bacteroides fragilis* |
| Celiac Disease | DR3-DQ2.5 DR4-DQ8 DR7-DQ2.2 (with DR3-DQ2.5 or DR5-DQ7) | AITD, RA, T1D | *Dialister invisus, Parabacteroides* sp., *Porphyromonas* sp., *Ruminococus bicirculans*, Lachnospiraceae, Veillonellaceae, Pasteurellaceae | *Bifidobacterium, Lactobacillus* |
| Type 1 Diabetes | DR4-DQ8 DR3-DQ2.5 | RA, AITD, CD | *Ruminococcus, Prevotella copri, Bacteroides dorei, Bacteroides vulgatus* | *Bifidobacterium, Lactobacillus, Roseburia, Faecalibacterium* |

AITD, Autoimmune thyroid disease; CD, Celiac disease; JIA, Juvenile idiopathic arthritis; RA, Rheumatoid arthritis; T1D, Type 1 diabetes.

Bacteroidetes is seen elevated in one study but significantly decreased in the AITD patients of a different study (115). Specifically, *Bacteroides fragilis* is observed as both higher and lower in abundance in AITD patients compared to healthy controls depending on the study (Table 1) (113, 115). *Lactobacillus* is proposed as both a potential probiotic for AITD amelioration and a potential major player in AITD pathogenesis (115, 116). Larger and more extensive microbiome studies may be required if a potential microbial biomarker for the gut-thyroid axis is determined.

## 5.4 Autoimmune arthritis

Despite emphasis on joint inflammation, the majority of RA patients also exhibit gastrointestinal disorders and significant gut microbiome differences are observed in RA patients versus controls (Table 1) (117–119). Patients with RA have a decreased microbial diversity and distinct microbial patterns (120). *Collinsella*, *Eggerthella*, and *Faecalibacterium* segregate with RA in random forest modeling (121) *Collinsella* and *Eggerthella* correlate with increased intestinal permeability, mucosal inflammation, and immune response, and *Collinsella* is confirmed to correlate with increased proinflammatory cytokine IL-17A, gut permeability, and RA disease severity (119, 121). Specifically, *Prevotella copri* is more abundant in new-onset RA patients, while *Bacteroides* and *Bifidobacterium* are decreased in the same population (120). *Prevotella* is also substantially more prevalent in children with a future diagnosis of JIA, while *Bifidobacterium* and *Bacteroides* species are reduced in the same cohort (86). A recent study in mice and colonic tissue shows increased intestinal permeability and zonulin-1 expression upon exposure to fecal bacteria from pre-RA human individuals (122). A recent small human RA study following bowel cleansing and fasting found a link between intestinal microbes and inflammation specific to RA, suggesting dysbiosis as a primary player in disease activity (123).

## 6 Evidence for HLA-associated dysbiosis

The fundamental role of class II HLA is to bind to foreign peptides and present them on the plasma membrane for recognition by CD4+ T helper cells. Within the gut, this antigen presentation leads to B cell production of secretory IgA. IgA mediates microbial composition by inhibiting bacterial adhesion to epithelial cells, regulating bacterial epitope expression, and facilitating the elimination of bacteria from the gut *via* peristaltic and mucociliary actions (124, 125). Structural variety associated with HLA allelic polymorphisms alters microbiome composition by linking MHC-peptide binding affinity differences to which bacteria get eliminated by IgA (36, 126, 127). With regard to gut microbiome composition, HLA polymorphisms significantly alter biases in antibody-mediated selection against microbiota and in turn correlate to unique microbial communities (14). Evidence shows that populations with functionally similar HLA also feature similar microbial patterns (128). Many studies group HLA

haplotypes by autoimmune risk group. Therefore, most analysis available look at both DR3-DQ2 and DR4-DQ8 as either pooled homozygotes and/or heterozygotes. Future research could benefit from analyzing risk haplotypes against each other to verify their similarities and differences in influence over the microbiome. Compared to low-risk or neutral haplotypes, high-risk HLA DR3-DQ2 and DR4-DQ8 are associated with higher abundance of *Prevotella copri* at the species level, *Agathobacter, Bacteroides, Blautia, Dorea, Enterococcus, Intestinimonas, Klebsiella, Veillonella* at the genus level, and Enterobacteriaceae, which includes *Klebsiella*, Lachnospiraceae, which includes *Agathobacter, Blautia*, and *Dorea*, and Ruminococcaceae, which includes *Intestinimonas*, at the family level (36, 129–132). *Bifidobacterium* and *Lactobacillus* stand out as either negatively associated or in lower abundance in DR3-DQ2 and DR4-DQ8 compared to protective or neutral alleles (36, 39, 133, 134). Of note, the large general population cohort, All Babies in Southeat Sweden (ABIS), found that when controlling for breastfeeding, DR5-DQ7 is a significant factor in an infant's likelihood to be colonized by *Lactobacillus* at all (135). This correlation may be associated with DQ7.5 *trans* configuration with DQ2.2 to create DQ2.5, the primary risk allele for CD. Aside from the association of increased relative abundance of *Bifidobacterium* in homozygous the DR1-DQ5 population (39), there has been limited examination into the role of DR1-DQ5 in microbiome community constructs to date.

## 7 Discussion

Many autoimmune disorders share the same risk-associated HLA haplotypes often resulting in comorbidity despite differing etiologies (22–24). The role of the gut microbiome has become increasingly essential in defining the pathogenesis of these autoimmune diseases (25–30). It has been theorized that dysbiosis seen in autoimmune diseases is associated with systemic inflammation, resulting in loss of barrier function and permeability of tight junctions, allowing for possible increased exposure of HLA proteins to bacterial antigens (31–33). HLA class II proteins are expressed in the upper villi of small intestinal enterocytes at a steady state in the presence of a healthy gut microbiome and are an integral part of maintaining homeostasis. However, dysbiosis and inflammation cause an increase in HLA class II expression in small intestinal crypts and the colonic epithelium, which can in turn influence the composition of the gut microbiome (32, 34–39). The fundamental role of class II HLA is to bind to foreign peptides and present them on the plasma membrane for recognition by CD4+ T helper cells. Within the gut, this antigen presentation leads to B cell production of secretory IgA. IgA mediates microbial composition by inhibiting bacterial adhesion to epithelial cells, regulating bacterial epitope expression, and facilitating the elimination of bacteria from the gut *via* peristaltic and mucociliary actions (124, 125). Structural differences associated with HLA allelic polymorphisms alter microbiome composition by linking HLA-peptide binding affinity differences to which bacteria get eliminated by IgA (36, 126, 127). The precedence for HLA molecular "preference" for specific peptides can be seen in celiac disease, where HLA DQ2 and DQ8 affinity for negatively charged residues results in class II MHC

molecules binding and presenting gliadin peptides, leading to autoimmunity. However, certain HLA haplotypes, specifically the known risk HLA alleles discussed here, are associated with microbiome community dynamics that implicate dysbiosis before autoimmunity occurs (36, 39, 41, 42). Such evidence suggests that certain HLA may be predisposing an individual to systemic inflammation originating from the gut microbiome by clearing beneficial microbes and creating the potential for dysbiosis early in life. Increased exposure to commensal bacteria and excessive immune response over time could result in aberrant self-tolerance mechanisms.

Patterns emerge when investigating the overlap between HLA-associated and autoimmune-associated microbiomes. These relationships are unsurprising when considering the common risk-associated HLA haplotypes by autoimmunities. For example, *Prevotella copri* is more abundant in RA, JIA, and T1D patients compared to controls and is also associated HLA DR3-DQ2 and DR4-DQ8 (98, 99, 120, 131). This pattern makes sense when considering that HLA DR4-DQ8 is a risk-associated genotype for both RA and T1D. Higher abundances of inflammatory microbes, like *Klebsiella* and *Veillonella*, are associated with autoimmunity and risk; while conversely, lower abundances of known anti-inflammatory microbes like *Bifidobacterium* and *Lactobacillus* are associated with both autoimmune disorders and risk HLA (29, 104–106, 135–137). Causal relationships between microbiome composition and autoimmune onset are starting to be investigated. A recent study shows that abundance of Veillonellaceae may have causal effects on CD development (109). Prior to seroconversion, significantly higher abundance of *Bacteroides* species are observed in children with future T1D and JIA autoantibody seroconversion (37, 86). Both of these bacteria are associated with risk HLA (36, 138) Microbiome community differences can be seen as early as one year of age between those who go on to acquire an autoimmune disease versus those who do not (37, 41, 42). It is possible that the common denominator here is the introduction of early-life inflammation caused by HLA-specific dysbiosis.

It is important to note that all these studies focus on fecal microbiota, meaning the microbial composition is likely exclusively colonic and does not represent the small intestines. The field would benefit from microbial sampling from within a variety of locations in the gut. Also, there is limited current research into the gut-thyroid axis. Larger and more extensive microbiome studies may be required if a potential microbial biomarker for the gut-thyroid axis is to be determined. Many of the large cohort studies in this review focus on high-genetic-risk communities. To truly determine the impact of genetics on the gut, the field would benefit from general population studies that can compare risk vs. non-risk groups.

To validate the hypothesis that gut dysbiosis leads to early-life inflammation and elevate the link between gut microbiome composition and autoimmune disease onset, we propose an organ-on-a-chip model of human intestines. Within this model system, HLA-specific intestinal cultures could be generated to establish phenotypic differences between risk, neutral, and protection-associated tissue. To investigate innate immune response, we suggest quantifying cytokine secretion and examining zonulin, mucin, and permeability levels at baseline and following co-culture with either specific microbes of interest or a bacterial community culture (139, 140). It would be of interest to explore an adaptive immune response, as well. It is possible to characterize HLA-specific T cell response to commensal gut bacterial peptides through the presentation of secreted bacterial peptides to T cell stimulation assays measured with flow cytometry and ELISpot (141). An HLA-specific intestinal organ-on-a-chip model could also be used to measure T cell response by co-culturing peripheral blood mononuclear cells within the bottom chamber of the microfluidic chip and assessing T cell stimulation from the peptides that make it through the epithelial barrier in the microfluidic system.

The tripartite HLA-microbiome-autoimmunity link is not trivial. Risk HLA may be predisposing an individual early in life to dysbiosis originating in the clearance of beneficial microbes and/or promotion of inflammatory microbes, creating the potential systemic inflammation later in life. While it may be enticing to put emphasis on the dysbiosis and inflammation seen after autoimmune onset because of the clear evidence that autoimmune-induced permeability of tight junctions allows for increased exposure of HLA proteins to bacterial antigens, it is important to consider genetics and the initial role haplotype-specific peptide binding affinities may play in defining an individual's microbiome.

## Author contributions

MB: Conceptualization, Data curation, Writing – original draft. ET: Conceptualization, Supervision, Writing – review & editing. JI: Supervision, Validation, Writing – review & editing. JL: Conceptualization, Project administration, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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