IMMUNOGENETICS AND GENETIC SUSCEPTIBILITY IN THE PATHOGENESIS OF AUTOIMMUNE HEPATITIS

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ABSTRACT

Autoimmune hepatitis is a progressive liver disease. Its pathogenesis is unclear, but needs a ‘trigger’ to initiate the disease in a genetically susceptible person. The susceptibility is partly related to MHCII class genes, and more so with human leukocyte antigen (HLA). Several mechanisms have been proposed which, however, cannot fully explain the immunologic findings in autoimmune hepatitis. The susceptibility to any autoimmune disease is determined by several factors where genetic and immunological alterations, along with, environmental factor are active. MHCII antigens as a marker for AIH, or a predictor of treatment response and prognosis has been investigated. Since MHCII antigens show significant ethnic heterogeneity, mutations in MHCII may merely act as only precursors of the surface markers of immune cells, which can be of significance, because the changes in HLA and MHC are missing in certain populations. One such marker is the CTLA-4 (CD152) gene mutation, reported in the phenotypes representing susceptibility to AIH. Other candidate genes of cytokines, TNF, TGF-beta1 etc, have also been investigated but with unvalidated results. Paediatric AIH show differences in genetic susceptibility. Genetic susceptibility or resistance to AIH may be associated with polypeptides in DRB1 with certain amino-acid sequences. Understanding which genes are implicated in genesis and/or disease progression will obviously help to identify key pathways in AIH and provide better insights into its pathogenesis. But studies to identify responsible genes are complex because of the complex trait of AIH.

Key words: Autoimmune hepatitis, Genetic susceptibility, Genetics, Immunogenetics, Hepatitis, Pathogenesis, genetic studies, Polymorphism

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic hepatitis occurring in children and adults of all ages and can progress to cirrhosis, characterized by autoimmune features, including the presence of circulating auto antibodies and high serum globulin concentrations. There are two major groups Type 1 and Type 2 AIH according to the auto antibodies present. The classical form of AIH, Type 1 AIH, is characterized by circulating antinuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA) alone or in combination, and Type 2 AIH is defined by the presence of anti liver-kidney- microsomes antibodies (ALKM-1) with or without liver cytosol antigen (ALC-1). Variant forms of AIH, called overlap syndromes, also have associated features of other forms of chronic liver disease, particularly primary biliary cirrhosis or primary sclerosing cholangitis. The diagnosis is made in patients with compatible clinical signs, symptoms, and histology and laboratory abnormalities after excluding other causes.
of chronic hepatitis. Laboratory and histological abnormalities include abnormal liver function tests, increased total IgG levels, serologic markers (ANA, SMA, anti-LKM-1, or anti-LC1), and interface hepatitis in liver biopsy. It has issued Diagnosis of AIH includes a) characteristic serologic and histological findings and b) the exclusion of other types of chronic liver disease as per the American Association for the Study of Liver Diseases (AASLD), 2010 guidelines. However, the nonspecific histologic changes in AIH make the measurement of auto antibodies and IgG or gamma globulin important in diagnosing AIH. A scoring system earlier developed and subsequently revised by the International Autoimmune Hepatitis Group to standardize the diagnosis for population studies and clinical trials has shown to be of limited value in individual cases. A less complicated system using simplified criteria was proposed to be used in individual patients; and is based upon titers of auto antibodies, IgG levels, liver histology, and exclusion of viral hepatitis. A probable diagnosis of AIH is made if the total points are six, while a definite diagnosis is made if the total points are ≥7.

**Pathogenesis:** The pathogenesis of AIH is still obscure and complex, but the circulating auto antibodies do not have any role in it. The liver is continuously exposed to a large number of antigens includes food, intestinal organisms, toxins, malignant cells, including self-body antigens. But compared to lymph nodes, the immune responses in liver are not uniformly effective at times. Since the liver has a central role in the induction and maintenance of immune tolerance, AIH occurs primarily due to immune system dysregulation, resulting in progressive liver cell destruction consequent to a loss of immune tolerance towards hepatocytes. The exact origin of the immune dysregulation in AIH is still unknown, but the current hypothesis is that it is the consequence of an environmental triggering event (virus, medications) in a genetically susceptible individual of a particular sex and age (females and young age). Most pathogens have specific pathogen-associated molecular patterns (PAMP) to which Toll-like receptor (TLR) can bind. These TLR can be stimulated by environmental “triggers” to create an environment whereby an auto-antigens may be actively present in the genesis of inflammation in auto-immune diseases. Usually up-regulation of important pro-inflammatory genes like type 1 interferons are then quickly and efficiently induced by these TLRs when there is liver infection. Therefore, TLR stimulation would result in circulating auto or self-antigens creating a pro-inflammatory state which in turn would lead to augmented activation of specific immune-reactive cells. This is evidenced by demonstration of higher levels of several different hepatic TLRs like TLR3, TLR4 and TLR9 in primary biliary cirrhosis.

**Rationale for investigating genes in any complex autoimmune disease are that:** (a) the information will be useful in disease diagnosis and population screening, (b) which will provide useful prognostic indices for disease management, especially the development of individualized treatment regimens in transplantation, but also in autoimmune and viral diseases and, (c) and will lead to a better understanding of the pathogenesis of these diseases.

**The complexities of genetic background and susceptibility in AIH:** Autoimmune liver diseases are not Mendelian autosomal or sex-linked genetic traits. There is no simple pattern of inheritance in AIH that can be explained by a single gene and therefore it is a “genetically complex” disease. It is known that nearly all human genes are polymorphic. “Complex traits” involve one or more genes (alleles) acting alone or in combination and alters (increases or reduce) the risk of a disease and so AIH can be termed as a polygenic disorder. Complex traits were earlier called ‘polygenic’ (involving multiple genes), multifactorial (implying interactions between the host gene and other environmental factors) or oligogenic (mutations in several different genes causing the same disease).

The extent of heritable component in AIH is still debatable and weak. However, HLA-DRB1*03 (DR3) and DRB1*1301 (DR13) and HLA-DQB1*0201 are reported to be transmitted to patients compared to unaffected siblings in type 1 and 2 AIH, respectively in families of AIH. It is important to recognize that in different geographic regions and ethnic groups, different susceptibility alleles may exist which may reflect differing triggering antigens. For example, epidemiologic studies in European and North-American Caucasians characteristically demonstrate a high prevalence of a HLA B8, DR3 and DR4 haplotype.
Naturally occurring genetic polymorphisms generally determine the susceptibility of an individual to autoimmune diseases. Single nucleotide polymorphisms (SNPs) are the commonest polymorphism (90% of all polymorphism) done by scanning the entire candidate gene. SNPs evolves probably due to microbial pressure and as a consequence of natural selection for enhanced resistance/susceptibility to certain pathogens. But identifying disease promoting mutation (DPM) and polymorphism (DPP) in a “genetically complex” disease like AIH is not simple. Because an association does not necessarily imply a primary relationship between the polymorphism and disease, but may be due to linkage disequilibrium between the polymorphism and DPM elsewhere in the same gene or a neighbouring gene. Currently, the best strategy for investigating a gene possibly will be to identify the informant SNPs and investigate SNP- haplotypes using these informative SNPs as tags-termed ‘haplotype tagging’.  

Association analyses usually focus on genes that affect the immune system belonging to both the human leukocyte antigen (HLA) and non-HLA immune modulator genes and is the basis of most of the work in AIH due to paucity of multiple families to study, 10 low levels of penetrance, late onset and a few family members being involved. Besides the MHC susceptibility genes, other non-MHC genes may also be involved in autoimmune processes in AIH, especially those which regulate the immune responses to antigens, like CTLA-4. SNPs within the cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene have been implicated in AIH. 11 The gene encoding CTLA-4 on chromosome 2q33 may influence autoimmunity and is more common in type 1 AIH and may represent a second susceptibility allele. 12 CTLA-4 is an example of genetic variants with risk for developing different human autoimmune diseases and is also associated with an increased risk of infections with parasites, viruses and invasive bacterial infections. 13,14 But, definite evidence of any particular infectious agent or CTLA 4 gene polymorphisms is related to the disease onset or an increased susceptibility to AIH in different regions 15 since there are reports of a number of other SNPs of various other genes also which are associated with increased susceptibility to AIH in different populations/ethnic groups. 16,17,18,19,20 Conversely, CTLA-4 can also prevent the development of autoimmune disease due to the inhibition of T cell responses by negatively regulating peripheral T cell function 21 but with differential expression levels among T cell subsets. CTLA-4 variants may also influence the susceptibility of an individual towards infection. It is, however, unclear whether CTLA-4 acts directly on the T cell that expresses it or acts on the antigen-presenting cell (either by binding of the ligand B7 by CTLA-4 which leads to back-signalling into antigen-presenting cells or by down-regulating B7 expression) 22. To sum up, over expression of CTLA-4 inhibits T cell activation, facilitating infections and subsequent tissue damage due to impaired bacterial clearance; with or without specific activation in T cell subpopulations like T regulatory cells, allowing tissue damage due to auto reactive T cells This needs to be addressed in further studies. Conversely, infection may modulate the surface expression of the CTLA-4 ligands, CD80 and CD86 on APCs. In order to understand these mechanisms, animal models need to be developed which complement clinical and epidemiological studies for AIH. The most accepted consensus is that susceptibility to develop Type 1 AIH is due mainly to genes encoding HLA DBB1 alleles within the MHC class. T cell activation is also likely to be involved in the pathogenesis of AIH because MHC molecules also present antigens to CD4 cells. The reasons for the greater influence of specific HLA haplotypes for example, in AIH compared to other immunoregulatory genes, lies either with the dominant role played by key MHC alleles in the immune response and/or with the potential for each haplotype to encode multiple susceptibility alleles which may have an additive contribution to disease risk. To detect other susceptibility genes, a genome-wide study 23 of 400 Japanese Type 1 autoimmune hepatitis patients reported two microsatellite markers on chromosomes 11 and 18, though no specific or unique proteins were found near in or those markers. The search for more of such markers may be helpful in the future. In addition to MHC I and II classes, SNPs can also exist in the MHC class III region (e.g. TNF IgA, cytokines, vitamin D receptor, CD45 and Fas receptor and CA genes) and have been associated with susceptibility to AIH.
Ethnic variations in susceptibility to AIH: Studies suggest that there are probably three different models in AIH, and different genetic associations exist in different populations as discussed below. Additionally, the peptides presented by HLA molecules to the T cell may be antigenically different. In Japan, Argentina, and Mexico, susceptibility to AIH is attributed to DRB1*0405 and DRB1*0404, alleles with arginine replacing lysine at position 71, the motif LLEQ-R being shared with DRB1*0401 and DRB1*0301.24 In Europe and North America, susceptibility to Type 1 AIH is associated with the presence of HLA DR3 (DRB1*0301) and DR4 (DRB1*0401), both alleles showing a lysine residue at position 71 of the DRB1 polypeptide and the LLEQKR amino acid sequence at positions 67 to 72.10 This implies that the K or R at position 71 in relation to LLEQ-R may be important for developing susceptibility to AIH, auto antigen binding, complementary to this amino acid sequence. Another model based on valine/glycine at position 86 of the DR-β polypeptide has been suggested, signifying an important HLA association in AIH patients from Argentina and Brazil.25 Thirdly, in a study from Japan, patients with AIH type 1 were found to have DRB1 alleles with histidine at position 13.25 Thus, these HLA associations may be different from the geographically unique local environmental triggers that precipitate Type 1 AIH in different environments. However, auto antigenically the target (hepatocytes) remains the same to initiate AIH.

It is noteworthy that presence of the HLA DRB1*1301 allele, which identifies the risk of paediatric Type 1 AIH in South America, is also associated with a chronic hepatitis A viral infection which may be a part of “molecular mimicry”. To summarize, from the discussion above, it appears that genetic susceptibility or resistance to AIH may be related to a specific amino acid sequence within DRB1. Those with the DRB1*0301 are younger patients and treatment failure is frequent in them. A severe disease and higher requirement of liver transplantation characterizes those who are associated with HLA B8. AIH with DRB1*0401-DRB4*0103 also develop additional autoimmune disorders like thyroiditis or diabetes mellitus more commonly. Association of HLA-B8 and HLA DR3 has also been found to be predictive of a lower achieving remission, frequent relapses, end-stage liver diseases and need for liver transplantations.26,27 A linkage disequilibrium study in families of AIH patients has confirmed that, HLA-DRB1*03 (DR3) and DRB1*1301 (DR13) as well as HLA-DQB1*0201 were selectively transmitted to patients compared to unaffected siblings in type 1 and type 2 AIH, respectively.27 Another study has suggested that HLA-DR13 by itself alone could be another risk factor.28 From a small study done in western India, DRB1*14 was the allele associated with AIH in India.29 A point to be noted is that possible differences in the occurrence of HLA alleles between both patients and normal subjects of same populations can affect the frequency of the disease across different regions as well.

Reasons for ethnic variations of genetic susceptibility: When the findings of studies based on different populations vary there occurs a question—should one always expect studies in different populations to concur? It can depend on the degree of racial or ethnic separation or the degree of geographic isolation of the tested populations with different genetic profiles. So, we cannot expect the English and Norwegian populations to vary as much as the English and Japanese. This also explains the differences between MHC-encoded susceptibility to Type 1 AIH in Brazilian versus North American and European whites in a well conducted study.30 Similarly, DRB1*03 alleles were more common in American patients with type 1 AIH than German Type 2 AIH patients (51% vs 17%); but DRB1*0301 was more frequently found in Type 1 AIH (51% vs 17%). The frequency of DRB1*04 alleles was also higher (64%) in the Type 1 patients after exclusion of the DR1*03 alleles. In contrast, patients with Type 2 AIH more frequently had DRB1*07, DRB1*15; and DQB1*06, DRB4*01 and DQB1*06 also occurred more frequently in the Type 2 German patients than in healthy US subjects31 suggesting a distinct variation in ethnicity.

These differences in susceptibility alleles among various ethnic groups can partially be explained by the shared motif hypothesis as discussed above which proposes that multiple alleles can encode for identical motifs within HLA class II. For example, susceptibility alleles reportedly will encode the motifs at position 67-72 of class II HLA in 94% of Type 1 AIH patients. In contrast, HLA-DB1*1501 was found to be predictive of a lower achieving remission, frequent relapses, end-stage liver diseases and need for liver transplantations.26,27 A linkage disequilibrium study in families of AIH patients has confirmed that, HLA-DRB1*03 (DR3) and DRB1*1301 (DR13) as well as HLA-DQB1*0201 were selectively transmitted to patients compared to unaffected siblings in type 1 and type 2 AIH, respectively.27 Another study has suggested that HLA-DR13 by itself alone could be another risk factor.28 From a small study done in western India, DRB1*14 was the allele associated with AIH in India.29 A point to be noted is that possible differences in the occurrence of HLA alleles between both patients and normal subjects of same populations can affect the frequency of the disease across different regions as well.
(associated with a reduced risk to develop type 1 AIH), encodes for the ILEQAR motif, that is distinctly different from LLEQKR or LLEQRR sequences.

The potential role of allelic variations within several genes encoding components of the innate and adaptive immune system suggests some disturbances of host resistance to microbial infection and their implication in the initiation and/or perpetuation of inflammatory processes. Hepatitis viruses (e.g. Hepatitis C) probably triggers a non-specific autoimmune reaction by facilitating activated CD4 cells to interact with autoantigens and thus initiate liver damage. Experimental studies show that these CD4\(^+\) T cells activated by hepatocytes are more likely to transform into Th2 cells and impair the CD8 cell function, hence the innate immune system Over-expression/up-regulation of pro-inflammatory genes may also be involved in the development of autoimmune inflammation in the liver through the innate immune system in the liver as the liver is an organ with special innate immune characteristics. Any hepatitis (viral, toxic) can also trigger AIH by possibly expressing the MHC class II molecule by damaged hepatocytes. The autoimmune “triggering” effects of drugs and chemicals like minocyclin, statins and herbs reported with AIH are also well known but not fully understood at present. Possible explanations may be the hepatotoxic effects releasing autoantigens, followed by an up-regulation of immunoregulatory proteins like P450s, or by hapten production by modifying this abnormal or existing hepatic protein, making them significantly immunogenic to produce AIH. However, this will probably take a long time to manifest as a significant disease clinically.

A peculiar Type 2 AIH is described, affecting ~ 20% of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also called autoimmune polyendocrine syndrome 1, which is an autosomal recessive disorder caused by homozygous mutations in the AIRE\(E\) gene. It is characterized by autoimmune diseases like a primary adrenocortical failure, hypoparathyroidism and chronic candidiasis. The AIRE\(E\) gene sequence consists of 14 exons containing 50 different mutations, with a 13 bp deletion at nucleotide 964 in exon 8 accounting for more than 70% of APECED alleles in the UK. But common mutations in the AIRE gene do not play a major role in AIH.

**Genetic susceptibility in type 2 AIH:** Type 2 AIH patients frequently present a more severe disease course and are usually resistant to treatment. HLA-DQB1*0201 was found to be significantly associated with susceptibility to Type 2 AIH in a recent study. DR3 or DR7, both associated with Type 2 AIH, shows a linkage disequilibrium with DQ2. Interestingly, HLA-DRB1*03 was found associated with Type 2 AIH patients which show both LKM1 and LC1 antibodies in their sera, while HLA-DRB1*07 was predominant amongst Type 2 AIH patients, whose only serological marker was anti-LKM1, signifying HLA alleles can also modulate autoantigenic humoral response in AIH. Other reports show that susceptibility to Type 2 AIH is conferred by HLA DR7 (DRB1*0701) and DR3 (DRB1*0301); and DRB1*0701 predicts a more aggressive disease and poor prognosis.\(^{37}\) Xenoinmunisation with plasmid DNA coding for Type 2 human autoantigens was performed in three mouse strains which differ in their MHC and/or non-MHC genes to study the genetic susceptibility to AIH in an animal study.\(^{38}\) This study showed that both MHC and non-MHC genes may be involved in increased susceptibility for Type2 AIH.

For Type 2 AIH, HLA-DRB1*07 has been observed more in German, Brazilian and British populations while HLA-DRB1*03 was found as a risk factor in Spanish patients.\(^ {39,40,41}\) again confirming an ethnic variability for susceptibility.

**Genetic susceptibility in “overlap syndromes”**: It is known that PBC, AIH and PSC are the three major immune-mediated hepatopathies. Variant forms of these diseases are called overlap syndromes, but no standard definition exists. Patients with overlap syndromes present with both hepatic (AIH) and cholestatic (PBC, PSC) serum liver tests and exhibit histological features of AIH and PBC or PSC. A similar genetic predisposition may play a role in the development of these overlaps syndromes as all three disorders share some common genetic susceptibility factors. It's already known that the MHC-genes tend to play an important role in determining the risk in AIH and PSC, and a lesser role in PBC. Studies have already identified a number of other potential candidate genes for these diseases with weaker effects on disease risk.\(^ {32,44}\)
Genetic differences in pediatric AIH: Evidence in support of pediatric AIH and adult AIH clinically being different with particular genetic associations exist. Brazilian type I AIH children showed a strong association with the HLA-DRB1*1301-DQB1*0603 haplotype. 44 Similarly, in Argentina, HLA-DRB1*1301 was the primary susceptibility allele, whereas HLA-DRB1*1302, which differs from HLA-DRB1*1301 by only 1 amino acid, appeared to be protective in children with Type 1 AIH.45 These findings therefore reveals that different HLA-DRB1 allotypes confer susceptibility to type 1 AIH in children and adults. This raises the possibility that pediatric and adult AIH, especially Type 1, may be triggered by different trigger factors even within the same ethnic community. It is clear that understanding which genes are implicated in the genesis and/or disease progression will obviously help to identify key pathways in AIH and provide better insights into its pathogenesis.

CONCLUSION

AIH is a “complex trait” disease. The exact mechanism of its pathogenesis is not fully known. Knowledge of the genetic predispositions for autoimmune hepatitis may, however elucidate the key pathogenic mechanisms, identify etiologic agents, characterize susceptible populations, predict prognosis, and target new therapies. Current hypotheses suggest that AIH is triggered by an environmental factor in a genetically susceptible host. Multiple genes may interact to produce a “permissive gene pool” that determines both disease risk and phenotype, across different ethnic groups. More research is needed because at present it is not known how many “susceptibility genes” may be present in AIH. The absence of large numbers of multiplex families is a problem for those trying to determine the exact role of genes in AIH. Traditionally genetic diseases are identified and mapped by ‘linkage analysis’ which is the gold standard for gene mapping. When such families are few, as in AIH, genetic ‘mapping’ may be performed by association analysis. Case control association analysis in such cases can produce very powerful results; it is also a practical option for the study of genes in diseases with a small heritable component. But the sample sizes required, will be very large as the quality of any case-control association study is directly proportional to the numbers studied. Future studies on AIH are needed to consider gene to gene interaction and also haplotype tagging. Because a better understanding of the disease would help discover specific and targeted immune-therapies with less side effects if and when the offending genes are identified in future.

Conflict of Interest: None to declare

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