My task is to discuss idiopathic autoimmune hepatitis, which, by definition, is defined as a disease of unknown cause. But I
think as I proceed through this presentation, you will begin to identify themes that resonate quite nicely with what Dr. Utrecht has already mentioned.
My goals are actually to describe the advances that are transitioning autoimmune hepatitis from an idiopathic disease to an explainable state. Additionally, I will indicate that the transition is far from complete as new knowledge brings new questions.
explainable disease.

And I will also indicate that this transition is far from complete, as new knowledge actually brings new questions about the nature of this entity.
Idiopathic autoimmune hepatitis is an inflammatory liver disease, which, by definition, is of unknown cause. Now, it is

- Inflammatory liver disease
- Unknown cause
- Autoantibodies
- Hyper $\gamma$-globulinemia
- Interface hepatitis

characterized by the presence of autoantibodies, hyper gamma globulinemia, especially high levels of serum in globulinemia levels and, by the presence of interface hepatitis on microscopic examination.
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characterized by the presence of autoantibodies, hyper gamma blobulinemia, especially high levels of serum in globulinemia levels and, by the presence of interface hepatitis on microscopic examination.
Now, codified diagnostic criteria for definite autoimmune hepatitis requires the absence of viral markers. And there must be no
or low likelihood of alcohol-related or drug-induced disease. Additionally, the immune manifestations must be substantial, as reflected in serum autoantibody and gamma globulinemia levels and there must be no evidence of homeostasis, either biochemically, clinically, or histologically.

Now, liver disease is of similar immune manifestations but with known causes must be designated by their etiologic agent and, therefore, they must be classified separately from idiopathic autoimmune hepatitis, mainly because their treatments and their outcomes are different.
# Types of Autoimmune Hepatitis

<table>
<thead>
<tr>
<th>Type 1 AIH</th>
<th>Type 2 AIH</th>
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<tbody>
<tr>
<td>• ANA and/or SMA</td>
<td>• Anti-LKM1</td>
</tr>
<tr>
<td>• Adults and children</td>
<td>• Mainly children</td>
</tr>
<tr>
<td>• Most common</td>
<td>• Adults (USA), 4%</td>
</tr>
<tr>
<td>• Genetic predisposition (DRB1 alleles)</td>
<td>• Genetic predisposition (DQB1 alleles)</td>
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Now, two types of autoimmune hepatitis have been described, based, primarily on their serological markers. Type 1

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<td>(DRB1 alleles)</td>
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autoimmune hepatitis is characterized by the presence of antinuclear antibodies or smooth muscle antibodies. And Type 1 autoimmune hepatitis affects all age ranges and it is the most common form of this disease worldwide.
Type 2 autoimmune hepatitis is characterized by antibodies to liver, kidney, microsome type 1. It affects mainly European

<table>
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<tr>
<th>Susceptibility Alleles Implicated in Type 1 Autoimmune Hepatitis</th>
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<tbody>
<tr>
<td>Northern Europeans</td>
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<tr>
<td>North Americans</td>
</tr>
<tr>
<td>Mexicans (Mestizo)</td>
</tr>
<tr>
<td>Japanese &amp; Chinese</td>
</tr>
<tr>
<td>South Americans</td>
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Strettel MD et al: Gastroenterology 112:2028, 1997
DeBoer YS et al: Gastroenterology 147:443, 2014
children. And in fact, it is relatively uncommon in the United States both in children and in white North American adults with this disease. Interestingly, both types of genetic predispositions but they actually differ in regard to their susceptibility alleles.
Now the susceptibility alleles that have been implicated in Type 1 autoimmune hepatitis are DRB1*0301 and 0401 in white,
Northern European and North American patients.

DRB1*0404 and 0405 have been associated with an increased occurrence of Type 1 autoimmune hepatitis in Mexicans, Japanese and mainland Chinese. And HLA DRB1*1301 is the primary susceptibility allele in Argentina, Brazil, and Venezuela, especially in very young children. The susceptibility alleles that have been implicated in Type 2 autoimmune hepatitis are DRB1*07 in British, German, and South American patients and DRB1*03 and DB1*02 in Spanish patients. A report in the DQB1*0201 is in strong linkage to this equilibrium with DRB1*07 and DRB1*03. Therefore, it has been proposed as the principal genetic determinant of Type 2 autoimmune hepatitis. The diversity of these susceptibility alleles that have been associated with autoimmune hepatitis really suggest that individuals are selected to develop this disease by their genetic predisposition to respond to certain sensitizing antigens and that, in fact, because of these different susceptibility alleles, different sensitivity antigens are likely to generate the same clinical disease.
Susceptibility alleles do encode the antigen binding groove of Class II molecules of the major histocompatibility complex. And
the antigen binding groove, as depicted on this slide, actually can determine the nature of the antigen that is accommodated. Various amino-acid sequences coded by the susceptibility alleles indicate that the occurrence of type 1 autoimmune hepatitis in white North America and Northern European patients is strongly associated with a sixth immunoacid sequence, included as LLEQKR at positions 67 through 72 of the DR beta polypeptide chain of the Class II MHC molecule.
Now, the strongest association with Type 1 autoimmunity hepatitis in this population is actually the presence of a positively
charged lysine at the DR beta 71 position.
If we look at the susceptibility alleles that have already been described in North Americans, Northern Europeans, and Asians,
these susceptibility alleles all include a sixth amino acid sequence between positions DR beta and 72 that are the same or similar to the ones that I have just mentioned. The only exception is the substitution of a positively charged arginine encoded as an R for a positively charged lysine coded as a K at the DR beta 71 position. These findings suggest that patients with these susceptibility alleles may in fact respond to the same or similar sensitizing antigens.

In contrast, DRB1*1301, which I have just mentioned as the predominant susceptibility allele in South American patients, especially children, that susceptibility allele encodes a different six amino acid sequence in this DR beta 67 or 71 position, especially different in that it encodes a negatively charged glutamic acid encoded as an E in the DR beta 71 position.

Clearly, these different susceptibility alleles for the same disease in different ethnic populations and in different age groups suggests that the analyses of these susceptibility alleles and the engine binding groups that they encode might well provide some valuable clues about the nature of the sensitizing that actually causes this disease.
It is also important to note that multiple genetic polymorphisms have been described in idiopathic autoimmune hepatitis but...
their role is clearly unclear. Recently, a polymorphism for the SH2B3 gene has been described in a cohort of patients with Type 1 autoimmune hepatitis from Northern Europe. This analysis was done by genome-wide association studies.

The variant of SH2B3 may well affect immune reactivity by altering the activation of T cells affecting cytokine production and modifying the adaptive immune response.

Another variant, a variant of the CARD10 gene, has also been implicated in Type 1 autoimmune hepatitis in the same genome-wide association studies. And this variant might well affect pro-inflammatory signaling pathways. The important message here is that multiple polymorphisms have already been described in idiopathic autoimmune hepatitis and that many of these polymorphisms are not disease-specific. In fact, many do occur in multiple immune-mediated non-liver-related diseases and, in fact, they probably contribute to modulating the vigor of the inflammatory response but are not clearly essentially for the development of the disease.
Salient Features of CYP2D6

- CYP2D6 has 5 epitopes recognized by anti-LKM1
- Dominant sequence spans 193-212 in 93% British patients
- Homologies with HCV, CMV, HSV1


Now the cytochrome oxygenase CYP2D6 is now recognized as the principal target autoantigen of Type 2 autoimmune
hepatitis. Antibodies to liver kidney microsome in certain Type 1 inhibit the activity of this enzyme in vitro. Liver-infiltrating cytotoxic CD8 cells are sensitized specifically to CYP2D6 in patients with Type 2 autoimmune hepatitis. And human CYP2D5 administered by immunization or by infection with an adenovirus vector actually induces experimental autoimmune hepatitis in mice.
CYP2D6 has five epitopes, which are recognized by antibodies at LKM1 and the dominant sequence spans the positions 193

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<th>Key Contributing Factors in Autoimmune Hepatitis</th>
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<tr>
<td><strong>Molecular mimicry</strong></td>
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<tr>
<td>• Main cause for losing self-tolerance</td>
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<tr>
<td>• Mimicry between human and mouse CYP2D6 promotes loss of humoral and cellular tolerance</td>
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<tr>
<td><strong>Epitope spread</strong></td>
</tr>
<tr>
<td>• Reactivity to CYP2D6 early against closely homologous epitopes</td>
</tr>
<tr>
<td>• Reactivity later against neighboring and remotely homologous epitopes</td>
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</table>

and 212 on the recombinant CYP2D6 molecule. This sequence is recognized by antibodies to LKM1 in 93 percent of the British patients with Type 2 autoimmune hepatitis. Importantly, homologies exist between the epitopes associated with CYPD26 and amino acid sequences within hepatitis C virus, cytomegalovirus and herpes simplex virus type 1. Now, these homologies suggest that repeated or protracted infection or exposure with viral antigens that closely resemble self-antigens can overcome self-tolerance.

The prominent target autoantigen of Type 1 autoimmune hepatitis, which is the most common form worldwide is still unknown.
Animal studies have indicated that molecular mimicry is an important mechanism for losing self-tolerance in autoimmune

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<tr>
<th>Syndromes Characterized by Autoimmune Hepatitis</th>
<th>Implicated Autoantigen</th>
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<tbody>
<tr>
<td>Type 2 AIH</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>Autoimmune Polyglandular Syndrome Type 1</td>
<td>CYP1A2</td>
</tr>
<tr>
<td>Tienilic acid-induced AIH</td>
<td>CYP2A6</td>
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</tbody>
</table>

hepatitis. This mimicry between human and mouse CYP2D6 can actually lose of humoral and cellular tolerance to mouse CYP2D6 in experimental autoimmune hepatitis and actually induces the disease in these animals.

Epitope spread is also an important mechanism for sustaining or exacerbating this disease and animal studies have indicated that reactivity to CYP2D6 early in the course of the disease is directed against closely homologous epitopes to the mouse CYP2D6 but that reactivity later in the course of experimental autoimmune hepatitis begins to be directed at neighboring epitopes and remotely homologous epitopes.
Now, interesting to this group and to me is the fact that the principal autoantigens that have been implicated in the various
clinical syndromes associated with autoimmune hepatitis have all been drug metabolizing enzymes associated with
the P450 system.

Type 2 autoimmune hepatitis, the autoimmune hepatitis has been associated with autoimmune polyglandular
syndrome Type 1. The autoimmune-like hepatitis that has been induced by tienilic acid all have been associated
with drug metabolizing enzymes in the P450 system. So that clearly, the P450 system is pivotal to the emergence
this form of liver disease.
The cell mediators of idiopathic autoimmune hepatitis are components of the innate and adaptive immune systems. The cells

- Mainly immunosuppressive effects
- Natural thymic-derived but also inducible by antigen and TGF-β
- Deficient number and function in AIH
- Exact role in AIH controversial

Longhi MS et al: J Autoimmun 25:63-71, 2005
that are at the center of this very complex interactive network are the regulatory T cells and the natural killer T cells.
### Deficient Number of Tregs in Autoimmune Hepatitis

<table>
<thead>
<tr>
<th>Sampling Time</th>
<th>AIH (n=41)</th>
<th>Normal (n=18)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>2.5±0.2</td>
<td>6.8±0.7</td>
<td>0.000015</td>
</tr>
<tr>
<td>Remission</td>
<td>4.2±0.8</td>
<td>6.8±0.7</td>
<td>0.022</td>
</tr>
<tr>
<td>Total</td>
<td>3.3±0.4</td>
<td>6.8±0.7</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Longhi MS et al: J Hepatol 41:31, 2004

The regulatory T cells have broad immunosuppressive effects that have been really a hot focus of attention in idiopathic...
autoimmune hepatitis. These cells are natural thymic-derived cells but they can also be induced from naive conventional T lymphocytes by antigen exposure, by stimulation with transforming growth factor beta. The important thing is that the deficiencies in the number and function of these cells have been described in idiopathic autoimmune hepatitis but in fact these results have been recently challenged and that the exact role of the regulatory T cell in idiopathic autoimmune hepatitis is controversial.
The early studies described that a reduced number of the regulatory T cells in the peripheral circulation of patients with...
autoimmune hepatitis compared to normal healthy controls, regardless of the degree of inflammatory activity. These early studies also demonstrated that the addition of regulatory T cells to preparations of CD8 cells failed to significantly suppress the activity of the effector CD8 cells.
So, these studies really generated great interest in the regulatory T cells as a possible mechanism that could be a target.
population that could be manipulated and improved through various pharmacologic and cellular interventions. But the fact is that recent studies using more restrictive and rigorous definitions for regulatory T cells have actually contested these findings.
These studies demonstrated that the number of peripheral regulatory T cells in patients with autoimmune hepatitis actually
were similar to those of healthy normal individuals. And furthermore, the addition of regulatory T cells from patients with autoimmune hepatitis to preparations of effector T cells reduced the proliferative activity of the effector T cell population similar to normal controls.
The critical determinant of the activity of autoimmune hepatitis may relate to the relative balance between the activities of the natural killer (NK) and T cell markers.
regulatory T cells and the effector T cells, rather than to the absolute number or function of individual cell populations.
The natural killer T cells are really emerging as the key regulators of immune reactivity in this disease. The natural killer T
cells have dual personalities. They can respond very rapidly to cites of tissue injury within the liver and behave like an innate immune response and they can be sensitized to specific antigens and behave as an adaptive immune response. They have surface markers both of natural killer cells and conventional T cells and they have stimulatory and inhibitory actions that are, in fact, dependent on the nature of the sensitizing antigen, who like the lipids, actually sensitize these cells through CD1 molecules that are class 1 molecules of the major histocompatibility complex. And the nature of the lipid antigen, whether it be a ceramide or a sulfatide can actually determine the predominant action of the NK T cell population. So, the NK T cells are actually emerging as an exciting area that might lead to therapeutic manipulations by designing antigens that would elicit disease-specific functions.
The migration of inflammatory and immune cells to sites of tissue injury within the liver is actually orchestrated by a variety of
chemokines. But the chemokines CXCL9 and CXCL10 have been increased in autoimmune hepatitis and their levels have actually been closely associated with disease activity. The cytokine exotaxin-3 has also been increased in immune-mediated liver diseases compared to viral-related liver diseases. And in fact, this finding suggests that eosinophils are preferentially recruited to sites of tissue liver injury that are immune-mediated. The chemokines are currently being evaluated primarily as indices of disease activity and indices of treatment response.
Distinguishing Features of Autoimmune Hepatitis

- Self-perpetuating
- Strong genetic predisposition
- Spontaneous occurrence
- Deficient immune cell suppression
- Life-long activity and cirrhosis

Lastly, I would like to mention apoptosis, since apoptosis is the principal mechanism of how to cite loss in autoimmune
hepatitis. A receptor mediated extrinsic apoptotic pathway predominates in this disease and it mainly results in the activation of caspase-3 and 7, which result in the fragmentation of the nucleus. It is also important to note, however, that an intrinsic apoptotic pathway associated with mitochondrial dysfunction induced by reactive oxygen species also contributes to the apoptosis, mainly through activation of caspase, through the development of an apoptosome and then activation of caspase-9.

The apoptosis of hepatocytes has an important consequence, the release of apoptotic bodies, which can serve as allele antigens, activating the lymphocytes that can actually expand the inflammatory autoreactive and fibrotic responses in its self-amplification loop.
Key Unanswered Questions

• Does AIH have a cause or can it emerge spontaneously?
• Can triggering antigens be discovered and validated?
• What is latent AIH and does it exist?
• Can AIH be predicted and the risk mitigated or obviated?

I would like to close by emphasizing that idiopathic autoimmune hepatitis is an important model by which to begin to
understand immune-mediated liver injury. It is also a disease which can be distinguished from most forms of autoimmune diseases that have known causes, mainly by its self-perpetuating nature, its strong genetic predisposition, and its spontaneous occurrence.

It is also possible that deficiencies in the modulation of certain immune cell responses may distinguish the disease, as may propensities for life-long fluctuations in disease activity and progression to cirrhosis.
The key questions that I see as being unanswered as yet are: Does autoimmune hepatitis have a cause or does it emerge...
spontaneously? Can triggering exogenous antigens actually be discovered and validated? What is latent autoimmune hepatitis and does it exist? And can autoimmune hepatitis be predicted and the risk mitigated or obviated?

I think these are questions that offer great challenges that must be addressed by future investigation.
In conclusion, I hope I have indicated that autoimmune hepatitis actually reflects multiple imbalances in a complex
homeostatic network that involves cellular and molecular interventions; that genetic factor strongly influence antigen selection and immune reactivity; that the cytochrome monooxidase CYP2D6 is the target autoantigen of Type 2 autoimmune hepatitis but, in fact, the principal autoantigen of the dominant form of the disease, Type 1 autoimmune hepatitis, is still unknown; that deficiencies in the number and function of regulatory T cells have been described, they have been exciting, but they are now controversial; and in fact, natural killer T cells seem to be emerging as the key regulators of this disease.

Certainly autoimmune hepatitis has moved beyond the idiopathic stage but, clearly, its transition to a fully explained disease is far from complete.

Thank you very much.