Genetic Characterization of Chicken Models for Autoimmune Disease

ANNA-STINA SAHLQVIST
Abstract


Autoimmune diseases are endemic, but the disease mechanisms are poorly understood. A way to better understand these are to find disease-regulating genes. However, this is difficult as the diseases are complex, with several genes as well as environmental factors influencing the development of disease. A way to facilitate the search for genes responsible for the diseases is to use comparative genomic studies. Animal models are relatively easy to analyze since control of environment and breeding are obtained.

The University of California at Davies – line 200 (UCD-200) chickens have a hereditary disease that is similar to systemic sclerosis. Using a backcross between UCD-200 chickens and red junglefowl (RJF) chickens we identified three loci linked to the disease. The loci contained immune-regulatory genes suggested to be involved in systemic sclerosis in humans, as well as a previously unidentified linkage between systemic sclerosis in UCD-200 chickens and IGFBP3.

The Dark brown (Db) gene enhances red pheomelanin and restricts expression of eumelanin in chickens. The Db phenotype is regulated by an 8 kb deletion upstream of SOX10. Pigmentation studies are potentially useful when trying to identify pathogenic mechanisms and candidate genes in vitiligo.

The Obese strain (OS) of chickens spontaneously develops an autoimmune thyroiditis which closely resembles human Hashimoto’s thyroiditis. By using an intercross between OS chickens and RJF chickens, we found several disease phenotypes that can be used in an ongoing linkage analysis with the goal to find candidate genes for autoimmune disease. An important phenotype to record and add to the linkage analysis is autoantibodies against thyroid peroxidase, since this phenotype is a key feature in Hashimoto’s thyroiditis. Previous attempts to measure these titres in OS chickens have failed, hence an assay was developed for this purpose.

Keywords: Autoimmune disease, Hashimoto’s thyroiditis, systemic sclerosis, comparative genomics, linkage analysis, OS chickens, spontaneous autoimmune thyroiditis, UCD-200 chickens.

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"'The Guide says there is an art to flying’, said Ford, ‘or rather a knack. The knack lies in learning how to throw yourself at the ground and miss.’"

-Douglas Adams, Life the Universe, and Everything
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


*The authors contributed equally to the work.

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<tbody>
<tr>
<td>ACA</td>
<td>Anti-topoisomerase I</td>
</tr>
<tr>
<td>AECA</td>
<td>Anti-endothelial cell antibody</td>
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<td>AIRE</td>
<td>Autoimmune regulator</td>
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<td>AITD</td>
<td>Autoimmune thyroid disease</td>
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<tr>
<td>APS-1</td>
<td>Autoimmune polyendocrine symptom type 1</td>
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<tr>
<td>ARA</td>
<td>Anti-RNA polymerase III</td>
</tr>
<tr>
<td>ATA</td>
<td>Anti-topoisomerase I</td>
</tr>
<tr>
<td>BC</td>
<td>Backcross</td>
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<tr>
<td>BLK</td>
<td>B lymphoid tyrosine kinase</td>
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<td>bp</td>
<td>Base pair</td>
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<tr>
<td>CA2</td>
<td>Second colloid antigen</td>
</tr>
<tr>
<td>CCP</td>
<td>Complement control protein</td>
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<tr>
<td>CCR8</td>
<td>Chemokine C-C motif receptor 8</td>
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<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>cM</td>
<td>centi Morgan</td>
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<tr>
<td>COL1A2</td>
<td>Collagen, type 1, alpha 2</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Cytotoxic T lymphocyte-associated factor 4</td>
</tr>
<tr>
<td>Db</td>
<td>Dark brown</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cell</td>
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<tr>
<td>EXOC2</td>
<td>Exocyst complex component 2</td>
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<tr>
<td>FOXP3</td>
<td>Forkhead box P3</td>
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<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
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<tr>
<td>GWAS</td>
<td>Genome wide association studies</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<tr>
<td>HT</td>
<td>Hashimoto’s thyroiditis</td>
</tr>
<tr>
<td>IBD</td>
<td>Identical by descent</td>
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<tr>
<td>IGFBP3</td>
<td>Insulin-like growth factor-binding protein 3</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IRF</td>
<td>Interferon regulatory factor</td>
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<tr>
<td>ITT</td>
<td><em>In vitro</em> transcription and translation</td>
</tr>
<tr>
<td>KCNA5</td>
<td>Potassium voltage-gated channel, shaker-related subfamily</td>
</tr>
<tr>
<td>LD</td>
<td>Linkage disequilibrium</td>
</tr>
<tr>
<td>LOD</td>
<td>Logarithm of odds</td>
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<tr>
<td>Lyp</td>
<td>Lymphoid tyrosine phosphatise</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MNC</td>
<td>Mononuclear cell</td>
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<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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</tr>
<tr>
<td>NK</td>
<td>Natural killer (cells)</td>
</tr>
<tr>
<td>NLRP1</td>
<td>NACHT, LRR and PYD domains-containing protein 1</td>
</tr>
<tr>
<td>OS</td>
<td>Obese strain</td>
</tr>
<tr>
<td>PTPN22</td>
<td>Protein tyrosine phosphatase 22</td>
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<tr>
<td>QTL</td>
<td>Quantitative trait locus</td>
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<tr>
<td>RJF</td>
<td>Red jungle fowl</td>
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<tr>
<td>SAT</td>
<td>Spontaneous autoimmune thyroiditis</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SOCS1</td>
<td>Suppressor of cytokine signalling 1</td>
</tr>
<tr>
<td>SOX</td>
<td>SRY (sex determining region-Y)-box 10</td>
</tr>
<tr>
<td>SSc</td>
<td>Systemic sclerosis</td>
</tr>
<tr>
<td>Tab</td>
<td>Thyroid autoantibody</td>
</tr>
<tr>
<td>TCR</td>
<td>T cell receptor</td>
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<tr>
<td>Tg</td>
<td>Thyroglobulin</td>
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<tr>
<td>TGF-β</td>
<td>Tumour growth factor β</td>
</tr>
<tr>
<td>TGFBR1</td>
<td>Transforming growth factor, beta receptor 1</td>
</tr>
<tr>
<td>T&lt;sub&gt;H&lt;/sub&gt;</td>
<td>T helper (cell)</td>
</tr>
<tr>
<td>TNFAIP3</td>
<td>Tumor necrosis factor, alpha-induced protein 3</td>
</tr>
<tr>
<td>TNFSF4</td>
<td>Tumor necrosis factor (ligand) superfamily, member 4</td>
</tr>
<tr>
<td>TPO</td>
<td>Thyroid peroxidase</td>
</tr>
<tr>
<td>T&lt;sub&gt;reg&lt;/sub&gt;</td>
<td>Regulatory T cells</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid stimulating hormone</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>UCD-200</td>
<td>University of California at Davies – line 200</td>
</tr>
<tr>
<td>WL</td>
<td>White Leghorn</td>
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</tbody>
</table>
Introduction

Autoimmune diseases are endemic, complex diseases dependent on both genetic and environmental factors. There are three criteria for autoimmune disease [1]:

- Direct evidence from transfer of pathogenic antibody or pathogenic T cells
- Indirect evidence based on reproduction of autoimmune disease in experimental animal models
- Circumstantial evidence from clinical clues

As most autoimmune diseases are complex with several underlying genes and a substantial environmental impact it has been hard to elucidate the disease mechanisms. Large family materials are required and the involvement of environmental factors has further complicated the attempts to map the causative genes.

Comparative genomics have been proved to enable the mapping of genetic regions of interest in complex diseases [2]. The use of animal models gives an excellent opportunity to explore the disease conditions in a more controlled environment and provide easy monitored family conditions.

Immunology

Tolerance

The development and maintenance of immunological tolerance is based on a combination of central and peripheral mechanisms that are affecting both the T and B cell compartment. In the thymus, medullary epithelial cells, under the influence of the autoimmune regulator protein (AIRE), express tissue specific antigens that are presented to the developing thymocytes in the negative selection process [3]. This leads to the elimination of self-reactive thymocytes. Self reactive thymocytes that have escaped the negative selection can be deleted or neutralized in the periphery by regulatory T cells (Tregs) that have been formed in the thymus in response to weak stimulation against self antigens [4], [5]. The clinical importance of the central and peripheral tolerance is exemplified by the two rare monogenous diseases auto-
immune polyendocrine syndrome type 1 (APS 1) and immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), that result from the distinct failure of the central and peripheral tolerance checkpoints, respectively [6], [7]. APS 1 is a consequence of mutations in the AIRE gene distributing the negative selection and IPEX is caused by FOXP3 mutations leading to a lack of Tregs [8].

In the bone marrow, immature B cells that recognize self antigens with high avidity reactivate their recombination genes, leading to receptor editing and avoidance of self reactivity [9]. Failure of receptor editing leads to apoptosis, and a weak signal through the B cell receptor leads to anergy. In the periphery, B cells with autoreactive B cell receptors can arise through somatic hypermutation in germinal centres. However, the self-reacting B cells are excluded from entering the follicles and die due to the lack of survival signals [9].

Autoimmunity

Autoimmune diseases can be mediated by autoantibodies and/or by autoreactive T cells. Tissue damage results from a direct attack on the cells bearing the antigen, from immune-complex formation or from local inflammation. T cells can be directly involved in inflammation or cellular destruction and are also required for sustained autoantibody responses. B cells are important antigen-presenting cells needed for a sustained autoantigen-specific T cell response. The constant presence of an autoantigen can lead to chronic inflammation, which in turn leads to release of more autoantigens due to the tissue damage. Nonspecific effector cells that respond to cytokines and chemokines are also attracted which results in a progressing self-destructive process. Self-antigens that were not the initial autoantigens can become secondary targets in a later stage of the disease due to the increased numbers of B cells and dendritic cells (DCs) at the site of inflammation. These can present new autoantigens to autoreactive T and B cells, i.e. epitope spreading [10]. Activated effector T cells specific for self-peptide:self-MHC complexes cause local inflammation through the activation of macrophages or damages the tissue directly. Affected tissues are heavily infiltrated by T lymphocytes and macrophages [9].

Autoimmune diseases can be divided into two subgroups: organ-specific and systemic. In organ-specific autoimmune diseases, the autoantigens are themselves organ-specific, e.g. thyroid peroxidase (TPO) in Hashimoto’s thyroiditis [11], whereas in systemic autoimmune diseases the autoantigens are ubiquitous and abundant in many cells in the body.
The avian immune system

The thymus in birds is similar to that of eutherian mammals. The germinal centres of bird lymphoid organs are large and well defined [12].

Birds don’t have lymph nodes, but they have similar structures consisting of a central sinus, which is surrounded by a shell of lymphoid tissue that contains germinal centres. The lymph node-like structure in birds have no external capsule [12].

**Bursa of Fabricius**

The bursa of Fabricius is a primary lymphoid organ that can only be found in birds. It is a gut-associated lymphoid tissue connected to the cloacal lumen by a dorsal duct and it is largest in size 1-2 weeks after hatch. The bursa functions as a maturation and differentiation site for B cells and immature cells migrate from the bone marrow to the bursa [13]. The location of the bursa leads to an exposure to environmental antigens such as bacteria and intestinal contents, and due to this antigen trapping, the bursa cannot be seen as a pure primary lymphoid organ. Furthermore, it performs some level of antibody synthesis and contains a small amount of T cells [14].

**Immunoglobulins**

The IgY immunoglobulins are the major antibodies produced by chickens. They are rearranged in B cells and then constantly synthesized and transferred to the yolk where they accumulate [15]. Three avian immunoglobulin classes have been identified so far: IgM, IgA and IgY. The Fc region of IgY was recently crystallized, and has structural features of both IgG and IgE [16]. IgY is systemic rather than secretory. It is also found in duodenal contents, tracheal washings and seminal plasma [17]. Like the mammalian immunoglobulins, IgY consists of two heavy and two light chains [18].

**Systemic sclerosis**

Systemic sclerosis (SSc) is a systemic autoimmune connective tissue disease clinically characterized by progressive fibrosis in the skin and internal organs. The disease is rare with a prevalence of 26 per 100,000 adults in the United States [19]. It is more common in women than in men and in Afro-Americans than in Caucasians [20]. In 2001, Arnett et al. found that SSc recurred in 1.6% of families compared to an estimated population risk of 0.026%, a significant increase suggesting that SSc is a heritable disease [19]. The disease is characterized by the triad of inflammation and autoimmunity, endothelial dysfunction and fibrosis [21]. This leads to microvascular alterations, perivascular inflammatory infiltrates, the presence of multiple autoantibodies and ultimately fibrosis of the skin and several internal organs, *e.g.*
kidneys and lungs [22]. The antibodies include anti-topoisomerase I (ATA), anti-centeromere (ACA), and anti-RNA polymerase III (ARA), which subcategorize patients. There are two types of scleroderma: Diffuse in which skin, lungs, GI tract, heart, and kidneys are affected; and limited which affects the skin of elbows and knees, face, and neck. In limited scleroderma, the main autoantibodies are ACA, whereas in diffuse scleroderma high titres of ATA are more common [23].

T cells in the skin produce cytokines that stimulate the production of collagen. Stimulation of fibroblasts is essential for the disease process [22]. The skin of SSc patients is tight, reddish, and scaly. Patients with substantial skin symptoms are often prone to have affected internal organs as well. More than 80% of the patients have vascular alterations and Raynaud’s phenomenon, which gives a de-colourization of fingers and toes in response to cold [24]. Renal fibrosis as a result of systemic sclerosis is a poor prognostic, often leading to death [25].

Hashimoto’s thyroiditis

Hashimoto’s thyroiditis is a common organ-specific autoimmune disease, which mainly affects women in the age of 30-50 years. The disease is complex and several genes as well as environmental factors are required for disease development [26]. It was the first disease to be recognized as an autoimmune disease [27] [28] and was first described by Dr. Hakaru Hashimoto in 1912 [29].

Hashimoto’s thyroiditis is characterized by serum autoantibodies against TPO, a key enzyme in thyroglobulin production [11]. The disease mechanisms involve infiltration of the thyroid gland by T and B cells reactive to thyroid antigens, which results in the production of thyroid autoantibodies. The exact etiology of thyroid autoimmunity is not known but many suggestions have been made. CD4+ cells affect the activation of CD8+ cells and the production of inflammatory cytokines leads to further recruitment and migration of macrophages to the thyroid gland [30]. The destruction of the thyroid gland can be seen histologically as diffuse, parenchymal lymphocyte infiltration and secondary germinal centres. At a later stage the thyroid can become fibrotic [31].

The destruction of the thyroid gland by immune cells and autoantibodies leads to an impaired ability to produce thyroid hormones. This results in weight gain, depression, cold sensitivity, fatigue, hair loss, infertility, etc [31].

Early disease signs are usually hard to detect since they are unspecific, leading to an incorrect diagnosis. To obtain a correct diagnosis the levels of thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine
(T4), and autoantibodies against thyroglobulin (Tg) and TPO can be measured.

Immunogenetics

There is a strong familial clustering of autoimmune diseases and they share a number of causative candidate genes [32]. The shared genes are mainly immune-regulating, but in some cases two or more diseases may also have the same target organ susceptibility genes. Hence autoimmune diseases also share etiologic pathways and disease mechanisms. These include genetic associations with human leukocyte antigen (HLA) and reactivity to self-antigens.

Candidate genes in systemic sclerosis

The candidate genes suggested to affect SSc can be divided into the subgroups immune regulating genes and tissue-specific genes.

Transforming growth factor-beta (TGF-β) stimulates collagen production and is strongly suggested to have a role in the development of SSc. It inhibits proliferation and effector functions of T cells, proliferation of B cells, and activation of macrophages. It activates fibroblasts which increase their collagen synthesis [33].

The following genes have also been suggested to be involved in the development of systemic sclerosis:

- **Tumor necrosis factor (ligand) superfamily, member 4 (TNFSF4)** is expressed in DCs and enables amplification of Th2 cell differentiation [34].
- **B lymphoid tyrosine kinase (BLK)**, which is involved in B cell lymphocyte development, differentiation, and signaling [35].
- **Tumor necrosis factor, alpha-induced protein 3 (TNFAIP3)** encodes an ubiquitin-editing enzyme which inhibits NF-kappa B activation and tumor necrosis factor (TNF)-mediated apoptosis [36].
- **Potassium voltage-gated channel, shaker-related subfamily, member 5 (KCNA5)** mediates voltage-dependent potassium ion permeability [37].
- **Protein tyrosine phosphatase, non-receptor type 22 (PTPN22)** encodes Lyp (lymphoid tyrosine phosphatase), which acts as an inhibitor of T cell receptor (TCR) signal transduction. Expressed in hematopoietic cells [38].
- **NRL family, pyrin domain containing 1 (NLRP1)** is an apoptosis protein [39].
- **Cluster of differentiation 247 (CD247)** is involved in the assembly and expression of TCR and signal transduction upon antigen triggering. A
low expression of this molecule gives an impaired immune response [40].

- **Interferon regulatory factor 8 (IRF8)** regulates expression of the FAS receptor which is involved in apoptosis of myeloid cells [41].
- **Collagen, type 1, alpha 2 (COL1A2)** encodes one of the chains of type 1 collagen [42].
- **SRY (sex-determining region Y)-box containing gene 5 (SOX5)** is a transcription factor in embryonic development and involved in determination of cell fate. Activates transcription of COL2A1 [41].
- **Signal transducer and activator of transcription factor 4 (STAT4)** is a transcription factor required for the development of T_{H1} cells [43], [44].

**Candidate genes in Hashimoto’s thyroiditis**

Similar to the candidate genes identified in SSc, the identified susceptibility genes in Hashimoto’s thyroiditis can be divided into two groups: immune-modulating genes and thyroid-specific genes [26], [45].

Using both the candidate gene approach and whole genome linkage studies, autoimmune thyroid disease (AITD) susceptibility genes have been identified and confirmed. Among these are: **HLA-DR, CD40, cytotoxic T lymphocyte-associated factor 4 (CTLA-4), protein tyrosine phosphatase-22 (PTPN-22), Tg and thyroid-stimulating hormone receptor (TSHR)**. These genes are all members of the immunological synapse, as receptors or signalling pathway molecules, or as presented peptides [26]. Sequence variations in the binding cleft of MHC II molecules represent a general paradigm as a susceptibility factor to several autoimmune conditions [46]. However, data on HLA haplotypes in Hashimoto’s thyroiditis have been less definite than in Grave’s disease [47], [48].

**CD40** polymorphisms have been tested in Hashimoto’s thyroiditis and no association was found [49]. This is not surprising since the disease has a large T_{H1} component.

**CTLA-4** is a major negative regulator of T cell-mediated humoral response [50], and has several times been shown to be associated with all AITD phenotypes. Blocking **CTLA-4** leads to enhanced proliferation of T cells and production of interleukin (IL)-2 [51]. In Hashimoto’s thyroiditis, it confers susceptibility to production of thyroid autoantibodies (Tabs) [52]. Tabs represent the preclinical stage of AITDs, it is thus possible that **CTLA-4** predisposes to the development of thyroid autoimmunity. It still unclear by what mechanisms **CTLA-4** polymorphisms confer to susceptibility to autoimmunity.

A R620W mutation in **PTPN22** has been shown to be associated with Hashimoto’s thyroiditis. **PTPN22** encodes the Lyp protein which is a powerful inhibitor of T cell activation [53].
It is however clear that additional genes contribute to the susceptibility to the disease, the different phenotypes, disease severity, and response to therapy [26].

Genetic markers
Genotyping refers to the process by which DNA is analyzed to determine which genetic variant (i.e. allele) is present for a certain marker. A DNA marker can be any type of variation in the genome and can be located within a gene or in any another part of the genome. An ideal marker should display high levels of polymorphism and preferably be codominant, i.e. all three genotypes can be scored: AA, Aa, aa.

Microsatellites and single nucleotide polymorphisms (SNPs) are the two main types of markers used today. Microsatellites are polymorphic di, tri and tetra repeats, for instance (CA)n. SNPs are polymorphic nucleotides in the DNA sequence (e.g. an A to G change). There are a wide variety of methods for SNP scoring, such as pyrosequencing and genotyping using the array format (for instance the Illumina Golden Gate Assay, [54]). The method of choice is depending on the number of markers. Microsatellites are more polymorphic (and give more information per marker) than SNPs, hence a large number of SNPs are required compared to microsatellites when performing linkage studies. A marker is fully informative if one of the founder lines has a different allele in comparison with the other founder line.

Linkage maps
A linkage map displays the linear order and the relative distances between loci, and is based on recombination fractions at meiosis and the likelihood for two loci to be inherited together in linkage groups (linkage). Hence, the resolution depends on the number of informative meioses. The recombination fraction (Θ) is the proportion of meioses in which two loci are separated by recombination. Two loci are in complete linkage if Θ = 0, and Θ = 0.5 for loci showing independent segregation. If Θ = 0.5 the loci are either far apart on the same chromosome or situated on different chromosomes. The map distance between two loci is given in centi Morgan (cM). One cM is defined as 1% recombination between two loci [55].

Linkage analysis
Genetic linkage is the tendency of alleles or loci to be inherited together from one generation to the next. It occurs as non-random segregation of loci
during meiosis and the degree of linkage between two loci is dependent on the number of crossovers that occur between them during meiosis. This process is imposed in linkage analysis, which reveals chromosomal regions that co-segregate with an investigated trait. In order to link a specific locus to a trait, a genetic linkage map is needed. If two markers in the map co-segregate they are in linkage with each other. This information reveals loci that are linked to the phenotype of interest, as the genomic position of the marker is known.

If the distance between two genetic markers is big, the probability that there will be recombination between them during meiosis increases.

**Pedigrees**

A family material or pedigree can be used to study the inheritance of a phenotype. One of the advantages with model organisms is that a large number of offspring (several thousand if required) can be generated. A pedigree consists of parental animals with different phenotypes that are crossed to generate a heterozygous F1 generation. The F1 individuals are then intercrossed resulting in an F2 generation segregating for all loci explaining phenotypic differences between the parental populations. Chromosomes are inherited from one generation to the next and recombination between the parental chromosomes occurs (Figure 1). Another way to construct a pedigree is to backcross the F1 animals to one of the parental populations. A backcross (BC) is appropriate for mapping of dominant loci, whereas an intercross is more preferable for mapping of co-dominant loci as it generates twice as many informative meioses [56].

High parental heterozygosity gives an extensive meiotic segregation of alleles to the offspring. Different alleles at certain loci may be nearly fixed in two outbred populations due to different selection pressures. Hence, the offspring will have high allelic heterozygosity at trait loci. In incomplete dominance, the heterozygous phenotype is an intermediate between the two homozygous forms.
Figure 1. F$_2$ intercross between Obese strain (OS) and red jungle fowl (RJF) chickens. An F$_1$ generation is produced by crossing the two divergent lines (F$_0$). The F$_1$ generation is subsequently intercrossed, generating recombinant offspring (F$_2$).

Quantitative trait locus

A quantitative trait locus (QTL) is a chromosomal region that harbours one or more genes affecting a quantitative trait, *i.e.* length, weight, *etc.* In a QTL analysis, genetic information and phenotypic data are combined to identify QTL regions. The analysis is performed unbiased, without any prior knowledge of involved genes.

QTL analysis

To perform a QTL analysis a linkage map, including genetic markers ordered after genotyping individuals in a three-generation pedigree, and recorded phenotypes from the individuals are necessary. The QTL analysis searches for association between marker alleles and trait phenotypes using interval mapping. The two breeds in the pedigree are assumed fixed for alternative alleles at a QTL position. Using flanking marker information, a coefficient is calculated for all F$_2$ individuals and at every cM in the genome based on the probability of inheriting one allele from each of the F$_0$ individuals. The logarithm of odds (LOD) score tells the odds of linkage for a
marker to the investigated phenotype. It is calculated as the logarithm of the odds that the marker and phenotype are linked rather than unlinked; \( \log_{10} = \text{odds in favour of linkage/odds against linkage} \). The significance threshold of the LOD score is usually 3 (odds in favour of linkage 1000:1). LOD \(< -2 \) is usually considered as significant evidence against linkage to that region, whereas LOD=0 when meioses are uninformative. Either maximum likelihood or least squares is used. The least square method requires less computer power than maximum likelihood. The phenotypes are regressed onto the coefficients [57] and the phenotype effects caused by sex, batch, husbandry etc. are corrected for by including them as covariates. For each QTL the additive, dominance and parent of origin effect is estimated. A threshold is computed by permutation tests by randomizing the data. Significant thresholds are usually set to 1\% and 5\% genome wide significance, where 1\% respectively 5\% of detected QTLs are expected to be false positives.

**Finemapping**

When a phenotype has been mapped to a chromosomal region, that region needs to be narrowed to minimize the number of candidate genes. To finemap the region, more markers are added to the region of interest. The resolution of the region depends on the mode of inheritance of the phenotype, the recombination frequency in the region and the size of the pedigree used for mapping. When no more recombination events can be found between the closest markers and the phenotype of interest, intercrossing of the F2 generation can be performed, creating more generations with recombination occurring in every generation. This generates material that gives the opportunity to identify minimum shared haplotype that is identical by descent (IBD) between individuals/breeds sharing the phenotype. In the resulting region, candidate genes are sequenced to identify the causative mutation(s).

**Linkage analysis versus association studies**

Linkage is a relation between loci, whilst association is a relation between alleles and phenotypes. This means that linkage is a specifically genetic relationship, but association is a statistical observation and may therefore have different causes.

To perform a linkage analysis, a family material is required, while in association studies, unrelated cases are compared to unrelated controls (it can be family or population based). The goal of association studies is to identify markers with alleles that are more common among cases than controls, or vice versa. Population-based association uses chi square (\( \chi^2 \)) test statistics.
Linkage is more powerful when searching for loci with alleles that have a high impact on the phenotype, and these tend to be rare in populations. Variants with a smaller impact are subsequently not detected [58]. Association studies are more powerful than linkage analysis for common diseases with low-penetrance risk alleles. However, the rare alleles usually don’t explain a larger disease risk [58].

Genome coverage for association analysis requires more than 300,000 markers, whereas complete coverage for linkage analysis requires approximately 400 markers.

Genome wide association studies (GWAS) do not identify the causative alleles themselves, only alleles that are in linkage disequilibrium (LD) with the causative ones [59]. Linkage analysis on the other hand, is used to map a gene to a chromosomal region. The limiting factor in linkage analysis is usually the number of meioses, not the number of markers [58].

**Comparative genomics**

**Model organisms**

Domestic animals are useful when investigating phenotypic variation due to thousands of years of manmade selection. They have a wide variety of mutations affecting their phenotypic traits [60] [2]. Some of these traits are monogenic and are inherited according to Mendelian expectations, e.g. some familial heritable disorders. Polygenic phenotypes, or complex traits, are harder to dissect than monogenic ones and are influenced by both environmental factors and alleles at several different loci.

**Chicken (Gallus gallus)**

The red jungle fowl (RJF) (Figure 2) was domesticated several thousands of years ago and is thought to be the ancestor to the domestic chicken [61]. Genetic studies of chickens have been carried out for more than 100 years [62].
Chickens have 38 pairs of autosomes and one pair of sex chromosomes. The chromosomes are referred to as micro- and macro-chromosomes due to their great variety in length. Females are the heterogametic sex (Z/W) and males are homogametic (Z/Z) [63].

There are several advantages in using chickens as disease models, e.g. a small and dense genome (~1GB) and a high recombination rate: 2.5-21 cM per Mbp (compared to 1cM per Mbp in humans). In 2004 the chicken genome sequence as well as a genetic variation map with 2.8 million SNPs were published [64], [65]. A previous cross between White Leghorn (WL) and red jungle fowl chickens revealed the power of using chickens as a model for detecting a variety of genes affecting complex traits [66], [67].

Animal models

The University of California at Davies – line 200 chicken

The University of California at Davies – line 200 (UCD-200) chickens (Figure 3) is the only animal model displaying all clinical, histopathological, and serological phenotypes seen in human SSc. They spontaneously develop a hereditary SSc-like condition at an early age. The first signs of disease are comb lesions and –necrosis, which is usually visible the first days after hatch. Other disease phenotypes include microvascular alterations, perivascular mononuclear cell infiltration, fibrosis of skin and viscera, and production of autoantibodies, such as ATA, ACA, and ARA [22].

The UCD chicken was first described in 1942, and the line was further established in the 1980ies [68].
UCD-200 chickens develop skin inflammation early in life, followed by fibrosis of the dermis, subcutaneous fat, and muscle. Fibrosis of internal organs such as esophagus, small intestine, lungs, heart, kidneys, and reproductive organs are also observed. Furthermore, thymic development has been shown to be abnormal, probably leading to defects in the negative selection of T cells. In addition to this, the UCD-200 has abnormalities in the subcapsular regions of the thymus as well as in the MHC class II expression in the cortex [69].

The skin of UCD-200 birds has lymphocytic cell infiltrates. Both CD4+ and CD8+ cells are present, but CD4+ cells are more abundant. Anti-endothelial cell antibodies (AECA) can be detected before disease onset (in human SSc these autoantibodies are present early in the disease development), and represents a key feature in the induction of primary endothelial cell injury [70].

The microvascular alterations of UCD-200 chickens lead to chronic ischemia, eventually affecting the internal organs. Endothelial cells are the main target of the autoimmune attack, and the subsequent apoptosis of these cells initiates the development of SSc together with accumulated infiltrating mononuclear cells (MNCs) and fibrosis [71], [72].

Mononuclear cells (MNCs) from lesional skin produce fibroblast-activating cytokines, which leads to increased production of collagen and glycosaminoglycans (GAG) [73].

The Obese strain of chickens

The Obese strain (OS) of chickens (Figure 4) develops spontaneous autoimmune thyroiditis (SAT) during the first weeks of life. The disease mimics human Hashimoto’s thyroiditis in several serological, immunological and histological aspects [74]. The typical phenotypes are small but obese bodies; long, silky feathers; small combs and wattles (due to delayed sexual matur-
ity); low fertility; and a phlegmatic behaviour, corresponding well to the disease phenotypes seen in patients with Hashimoto’s thyroiditis. The OS birds were originally chosen for breeding based on the visible disease symptoms. They originate from normal WL layers and the line was first described in 1955 [75]. OS chickens have thus been used as a model for Hashimoto’s thyroiditis during more than 50 years.

**Figure 4.** OS female. Note the long, silky feathers and moderate sexual attributes (i.e. small and pale comb and wattles).

The destruction of the thyroid gland and appearance of MNC infiltrates start at a few weeks of age and the thyroid is completely destructed at 1-2 months of age [76] [77]. OS birds display autoantibodies against Tg, the second colloid antigen (CA2), microsomal thyroid antigens, and thyroid hormones. Complement binding autoantibodies against Tg are transferred from the hen to its chicken via the egg yolk [78]. The immune system of OS birds shows a general hyperreactivity against exogenous and autologous antigens, and against T cell mitogens. This is probably due to hyperproduction of IL-2 and hyperexpression of the IL-2 receptor (IL-2R) as well as increased levels of IL-15 [79], [80], [81].

Positive T cell selection occurs in thymic nurse cells and these are deficit in OS chickens [82], leading to an abnormal immune reaction.

In addition to the defect T regulatory mechanisms, OS chickens also have a distorted immunoenocrine communication, intensifying the immunologic hyperreactivity [83].

Despite extensive previous experiments, the mechanisms and genes underlying SAT remain to be discovered. However, the OS chickens represent the best available animal model for Hashimoto’s thyroiditis, as they develop the disease spontaneously. Experiments have been conducted to study thyroiditis in a number of mouse strains, but the disease has to be induced in these models, and may thus alter the disease outcome [84], [85], [86].
Aim of the thesis

The aim of this thesis was to identify regions in the genome that are responsible for the development of autoimmune diseases using two chicken disease models.

Present investigations

The OS cross

Pedigree

A three-generation intercross between two RJF males and eight OS females, and one OS male and three RJF females was generated. The 36 F₁ individuals were intercrossed to make 856 F₂ individuals. The RJF and OS chickens are fixed for many different visual phenotypes, for instance size, body weight, and colour. The OS birds used in this study were homozygous for the MHC haplotype B13.

Genotyping

356 SNP markers of which 344 had information contents >0.5 were genotyped in all F₀, F₁ and F₂ individuals using the Illumina Golden Gate Assay (Illumina Inc., San Diego, CA, USA) [87]. The SNPs covered 29 autosomes and the sex chromosome Z, leaving the microchromosomes and the larger part of the W chromosome uncovered.

Construction of genetic maps

The CRIMAP software version 2.4 [88] and the functions build, flips and fixed were used to test the order of the markers and their relative distances along the chromosomes. Chrompic was used to reveal unlikely recombination events. The sex averaged autosomal map spanned 2358 cM, with an average marker spacing of 6 cM.

The UCD-200 cross

Pedigree

Eight UCD-200 individuals (four males and four females) and eight RJF birds (four females and four males) were used to generate a F₁ population
consisting of 22 individuals (two males and 20 females). The F1 individuals were backcrossed to 12 UCD-200 chickens (four males and eight females) generating 471 BC individuals in 26 full-sib families.

Genotyping
Genotyping was performed by the SNP&SEQ Technology Platform in Uppsala, Sweden, using the Illumina Golden Gate assay [87].

Construction of genetic maps
A genetic linkage map was built using the CRIMAP software [15]. The map included 299 SNP markers evenly distributed over 26 autosomes, and the Z chromosome. The markers were checked for non-Mendelian inheritance errors using the option prepare in CRIMAP and ordered according to the May 2006 (v2.1) chicken genome assembly [89]. A sex-averaged linkage map was built using the fixed option in CRIMAP. Markers that were not included in the genome assembly were incorporated at the most likely position in the linkage map according to the results from a two-point linkage analysis using the twopoint option in CRIMAP. The functions flips and chrompic were used to evaluate the order of the markers. The total sex-averaged linkage map covered 2623 cM.

Paper I
Mapping QTL affecting a systemic sclerosis-like disorder in a cross between UCD-200 and red jungle fowl chickens

Aim
To search for genetic loci regulating a systemic sclerosis-like disease in UCD-200 chickens.

Results and discussion
In our backcross between UCD-200 chickens and RJF chickens, none of the F1 individuals showed any signs of disease, but in the BC population 36% of the individuals showed signs of early avian SSc, and 61% were affected by late SSc. The main disease phenotypes were comb lesions, followed by swollen neck skin and lost feathers, but no macroscopic signs of visceral damage in the heart, kidneys or esophagus were detected. A significant difference in disease frequency between the sexes was revealed as the homogametic males were more affected than the heterogametic females. Disease incidence was significantly lower in females with UCD-200 maternal grandmothers but there was no correlative effect in males. Since there was no difference in disease severity between sexes in pure UCD-200 chickens, there might be a protective effect against the disease on the W chromosome.
In humans, females are more prone to get SSc. This correlates with our findings, as females are the homogametic sex in humans.

Three different loci were found to be linked to the SSc phenotypes in UCD-200 chickens. Two suggestive QTLs for early SSc were found; one on chromosome 2 (Figure 5) and one on chromosome 12. For late SSc, one suggestive QTL was found on chromosome 2 at the same position as the one found for early SSc (Figure 5), and one on chromosome 14.

No genome-wide significant QTL was found. This may be due to the fact that several of the chromosomes lacked markers.

There was no evidence of epistasis. Considering the relatively small population size in the study, one should however not rule out the possibility of such effects. As 12 of the chromosomes lacked markers (mostly microchromosomes which are recombination hotspots), which also decreases the power to find epistatic interactions.

The suggestive loci on chromosome 2 contained orthologues of five immune-regulating genes suggested to be involved in human SSc; TGFBR1, IGFBP3, EXOC2-IRF4, COL1A2, and CCR8. TGFBR1 binds TGFβ1, which is involved in cell proliferation and differentiation, and is an effective profibrotic cytokine. In a recent genome wide association study, EXOC2-IRF4 was suggested to be associated with human SSc [40]. COL1A2 regulates the synthesis of type I collagen, which has a fundamental role in human SSc. CCR8, found to be involved in early SSc in our study, is involved in autoimmune diseases in general. CCR8 is important for migration of immune cells to inflammatory sites. A previously unknown association between SSc and the IGFBP3 gene was also found on chromosome 2. IGFBP1 affects apoptosis, proliferation, and cell mobility and adhesion.

The suggestive QTL on chromosome 14 contained the orthologue of SOCS1, which is involved in the negative feedback loop that attenuate cytokine signaling.

These results prove the usefulness of the UCD-200 chicken as an animal model for human SSc. Further studies, adding more markers to the uncovered chromosomes, might discover epistatic interactions as well as significant QTLs regulating autoimmune disease in general and SSc in particular.
Figure 5. QTL-scan for chromosome 2. Solid line shows results for early SSc, and dashed line for late SSc.

Paper II

The dark brown plumage color in chickens is caused by an 8.3-kb deletion upstream of SOX10

Aim

To look for genes and mutations responsible for the Dark brown phenotype in a cross between OS and RJF chickens.

Results and discussion

The Dark brown (Db) mutation controls plumage colour patterns in chickens. The Db mutation reduces the expression of black eumelanin and enhances the expression of red pheomelanin in specific parts of the plumage. In our OS/RJF cross, the F2 individuals showed a vast variation in plumage colour and patterns. Of the 756 individuals scored, 161 had the Db phenotype. In males, the Db phenotype was divided into two groups: Type 1 males had a bright brown/reddish breast but no pheomelanin in the tail feathers, whereas type 2 males had a similar but less pronounced phenotype. Db females were bright orange over the entire body, except for the tail feathers. The initial linkage analysis was restricted to the 41 Db females as there was a complete linkage to chromosome 1, while a substantial recombination was discovered in males, probably due to phenotyping errors.
Since no causative mutation was found in the exons of SOX10, we looked at the conserved regions flanking the gene. The same region that is missing in Hry mice [90] was also deleted in the F2 individuals with the Db phenotype. When resequencing two RJF db+ individuals and six individuals from other lines with the Db phenotype a 12.8 kb haplotype shared among all resequenced Db birds was found. Hence, all polymorphisms outside of this haplotype could be excluded as causative mutations. One of the RJF birds had a haplotype identical to the Db haplotype, but without the deletion and the associated 10 bp insertion. Since the 8 kb deletion is the only sequence difference between the Db and the db+ chromosomes, this proves that the deleted region is causing the Db phenotype.

Forty-nine out of sixty-two type 1 males were homozygous for the deletion and 13/62 were heterozygous Db. On the contrary, 49/58 males in the type 2 group were heterozygous for the deletion. This implies that the Db locus is co-dominant as it gives a more distinct phenotype in homozygous individuals. Furthermore, the Db phenotype seems to be influenced by the sex of the chicken, as all females were homozygous for the deletion.

A previous study describes a deletion of a conserved element upstream of SOX10 in Hry mice, suggested to have a function in the SOX10 expression during the migration and development of melanocytes [91]. Interestingly, this deletion corresponds to one found in this study.

Paper III

Development of an assay for measurement of thyroid peroxidase autoantibodies in the Obese strain of chickens – an animal model for human Hashimoto’s thyroiditis

Aim
The aim of paper III was to develop an assay for measurement of autoantibodies against TPO in OS chickens. No commercial assays are available for this procedure, but it would be a valuable phenotype to add to our ongoing QTL analysis. Autoantibodies against TPO are the hallmark of human Hashimoto’s thyroiditis (HT).

Results and discussion
RNA from a healthy WL chicken was extracted. The TPO gene was cloned using reverse-transcriptase PCR (RT-PCR) and subsequently subcloned into the commercial vector pTNT. The transmembrane part of chicken TPO was removed to enable expression in a radioligand-binding assay described elsewhere [92]. The truncated protein was expressed in the in vitro transcription and translation (ITT) system. Sera from OS chickens, patients with HT positive for TPO autoantibodies, and negative controls (sera from healthy humans, RJF, WL, Brown line (BL), and Light brown line (LBL) chickens)
were used for immunoprecipitation (IP). To verify the results, a thyroid gland from a healthy BL chicken was stained for anti-microsomal antibodies. In addition to this, thyroid glands from OS and RJF chickens were hematoxylin-eosin stained to look for mononuclear infiltration.

The amount of OS birds positive for autoantibodies against TPO was surprisingly low: five out of 108 individuals. A WL chicken also tested positive in the assay. One out of 21 human patients with HT was positive, but none of the human negative controls showed any presence of autoantibodies. Thyroid glands from OS chickens revealed MNC infiltration, whereas healthy individuals were negative for this phenotype. In the immunohistochemical staining, OS chickens and HT patients were positive for anti-microsomal antibodies, while the negative controls were not.

The low amount of individuals with anti-TPO was somewhat unexpected, as this phenotype is the hallmark of HT in humans. A reason for this may be that the transmembrane part of TPO had to be removed to allow expression in the ITT system. Only the complement control protein (CPP) and myeloperoxidase (MPO) parts were expressed, as these have proved to contain the main epitopes for TPO autoantibodies in humans [93]. This might have removed epitopes or changed the folding of the protein, thereby deleting conformational epitopes. On the other hand, autoantibodies usually recognize evolutionary conserved protein sequences [94], [95], [96]. To circumvent this problem, other expression systems can be used, but the ITT is a fairly stable, cheap, and easy method, and the result from the assay was confirmed by the anti-microsomal antibody staining and the degree of MNC infiltration of the thyroid gland. The sera used for IP was taken when the birds were 9 and 28 weeks of age. The result might have been different if other ages also had been checked. It must also be taken into consideration that other autoantigens than the ones in human HT can be involved in SAT in chickens. Still, the TPO autoantibody phenotype will be a useful addition in our ongoing QTL analysis.
Paper IV

Disease phenotypes in the Obese strain of chickens

Aim
To evaluate disease phenotypes in SAT using the OS/RJF intercross.

Results and discussion
In this study, 567 F₂ individuals hatched in four batches were analyzed for the phenotypes mononuclear cell infiltration of the thyroid gland, body weight at three different ages, and visual phenotypes at three different ages and correlations between the different phenotypes were calculated. The variation in lymphocytic cell infiltration between eight adjacent histological sections from the same thyroid gland was analyzed in a subset of the F₂ birds. The infiltration rate varied considerably between thyroid sections from the same individual, illustrating the importance of using several sections of the thyroid gland from each individual. In the further analysis, eight thyroid sections each for 567 F₂ individuals were scored. 88% of the birds had an overall infiltration rate below 10%. No significant difference in infiltration rate was seen between the four batches or between males and females. The
The mean infiltration rate of the entire population was 4.8%, which correlates with results from a previous intercross between OS and WL birds [97]. Batch 2 of the F2 generation was not treated with thyroxine and interestingly, a significantly lower amount of the batch 2 birds died prematurely than birds in the other batches. This could be explained by environmental differences, such as varying housing conditions.

The birds that died before 200 days of age had a significantly higher infiltration rate than the birds that were sacrificed at 200 days of age, indicating that the hypothyroidism was the major cause of death among those individuals.

No correlation between infiltration and body weight was found at any of the three ages measured, even though OS birds are smaller than WL birds from which they originate. However, the healthy RJF birds are in turn smaller than OS birds and that complicates the analysis of the body weight phenotype in this cross.

The weight gain between 46 and 200 days of age was compared between batch 2 and batches 1 and 4. This showed a significantly higher weight increase between the ages measured in batch 1 and 4, which were treated with thyroxine, thus confirming the ability of thyroxin to decrease the disease phenotype low body weight secondary to hypothyroidism.

The visual phenotype (i.e. small but obese body constitution; phlegmatic behavior; small and pale comb and wattles; and long, silky feathers) was scored at 9, 21, and 28 weeks of age. This phenotype showed strong correlation with a higher infiltration rate at all three ages. There was also a correlation between the visual phenotype and a lower body weight at 28 weeks of age. This was expected, as one of the components of the visual scoring is a smaller body size.

The results from this study will be used in an ongoing QTL analysis, where the infiltration rate will be used as the main disease phenotype. The body weight and visual phenotypes will be used as covariates as well as independent phenotypes. Epistasis will be included and the results from the QTL analysis will be used in search of candidate genes for autoimmune thyroiditis. Ultimately, these results can be compared with human Hashimoto’s thyroiditis, aiming to find causative genes and disease pathways in humans.
Future perspectives

This thesis describes an attempt to unravel the genetics of autoimmune diseases, mainly Hashimoto’s thyroiditis and SSc. For this purpose chicken disease models, developing the respective disease spontaneously, were used.

In the UCD-200/RJF backcross, we found three suggestive loci controlling the disease development (paper I). The first goal is to search for significant loci and look for epistasis. Genotyping more markers, especially on the poorly covered microchromosomes, and repeating the QTL analysis would increase the possibility to find epistasis. In order to do so, the assembly of the chicken genome needs to be updated. Another approach is to increase the number of individuals in the analysis. The next step will be to finemap the regions in order to facilitate the search for candidate genes. The ultimate goal is to search for disease-causing mutations and test their functions in other animal models as well as in humans. This may in turn assist attempts to the define disease pathways and improve the treatment of the disease (or hopefully to prevent it from developing at all).

In the intercross between RJF and OS chickens, an 8 kb deletion upstream of \textit{SOX10} controlling the Dark brown (Db) phenotype was discovered (paper II). A way to increase the knowledge of the function of this mutation is to perform expression studies. Hopefully this will help to unravel how the deletion affects the distributions of eumelanin and pheomelanin. Pigmentation studies can be useful for studies of the depigmentation process in vitiligo. \textit{SOX10} is a suggestive candidate gene for this disease and it would be interesting to see whether the mutation found here or mutations in the vicinity of the found deletion affect the development of vitiligo. As many autoimmune diseases appear in clusters, this would facilitate the search for general immune-regulatory genes.

Adding more phenotypes to QTL analyses increases the opportunity to find disease-regulatory loci. As one of the key features of Hashimoto’s thyroiditis in humans is the development of autoantibodies against TPO, this is an interesting phenotype to add to the ongoing QTL analysis of the OS/RJF intercross. Commercially available assays for measurements of these titers are not functional with chicken serum. Our radioligand-binding assay (paper III) showed that a subgroup of OS chickens has these autoantibodies. However, the use of truncated TPO needed for protein expression in this assay may lack conformational epitopes, thereby generating false negatives. A way to circumvent this problem is to use other protein expression systems. On the
other hand, we must consider the possibility that autoantibodies against TPO may not be a key disease phenotype in OS chickens. Either way, this is an interesting phenotype to add as a covariate to the QTL analysis.

The phenotypes and the correlations between them, studied in the OS/RJF intercross (paper IV) add more knowledge about the constitution of spontaneous autoimmune thyroiditis in OS chickens. An obvious future task is to add these phenotypes to the ongoing QTL analysis. The main purpose of this QTL analysis is the same as in the UCD-200/RJF backcross (paper I); to find candidate genes, both general immune-regulating and target organ-specific ones. Following this is the search for causative mutations and to perform comparative studies in mouse models and human with the goal to find ways to prevent development of Hashimoto’s thyroiditis.

För att underlätta sökandet av sjukdomsreglerande gener kan man använda sig av djurmodeller. Hos sådana kan man lätt få stora familjer och kan dessutom kontrollera miljön. De sjukdomsgener man hittar hos djuren kan därefter jämföras med humant material och på så sätt underlätta sökandet efter sjukdomsorsaker. I den här avhandlingen har jag använt mig av två hönslinjer för att lättare hitta gener bakom Hashimotos sjukdom och systemisk skleros; Obese strain (OS), vilka spontant utvecklar en autoimmun tyreoidit som i allt väsentligt liknar Hashimotos sjukdom, och University of California at Davies - line 200 (UCD-200), som får en systemisk sklerosliknande sjukdom. Dessa linjer korsades med friska röda djungelhöns (RJF), vilka är vilda släktingar till tamhöns. Genom att använda genetiska markörer och statistiskt koppla specifika sjukdomstecken till dessa kan man finna regioner (loci) i genomet som innehåller sjukdomsreglerande gener. För varje generation sker rekombination mellan kromosomer och med hjälp av denna kan regionernas storlek minska, vilket gör det enklare att finna enskilda kandidatgener.
Om fler gener som påverkar autoimmuna sjukdomar kan hittas blir det lättare att förstå sjukdomsmekanismerna och därmed blir det även lättare att hitta bra botemedel.


I delarbete II använde vi korsningen mellan OS-höns och RJF-höns och identifierade en mutation uppströms SOX10-genen som ger ett specifikt mönster i kallat Dark brown (Db) i fjäderdräkten. Mutationen bestod av ett 8 kilobaser stort fragment av ett konserverat och tidigare identifierat regulatoriskt element som reglerar utvecklingen och distributionen av pigmentceller. Vidare visade sig Db nedärvas dominant i tuppar och recessivt i hönor.

Pigmenteringsstudier är intressanta ur ett autoimmunt perspektiv eftersom gener som styr pigmentering är potentiella sjukdomsgener vid autoimmuna sjukdomar riktade mot pigmenteade celler. Människor med den autoimmuna sjukdomen vitiligo, vilken leder till depigmenteader delar av huden, har ofta även andra autoimmuna sjukdomar, exempelvis Hashimotos sjukdom. Genom att jämföra kandidatgener mellan de olika sjukdomarna kan man finna dels gener som reglerar den generella autoimmuna reaktionen, och dels gener som styr den autoimmuna reaktionen till respektive målogran.

I delarbete III utvecklade vi en metod för att mäta förekomsten av autoantikroppar mot tyreoperoxidas (TPO) i serum från OS-höns, vilket är ett av de första tecknen på autoimmun tyreoidit hos människor. De metoder som finns tillgängliga för att mäta detta sjukdomstecken i andra arter har visat sig vara ineffektiva hos höns. Vi utgick från en tidigare etablerad metod, kallad In vitro transkription och translation (ITT) följt av immunprecipitering med sera från sjuka höns, friska höns, friska människor och människor med Hashimotos sjukdom, och modifierade metoden för att passa mätningar av autoantikroppar mot TPO i höns. För att konfirmera att ITT:n fungerade jämfördes resultaten med mätningar av infiltrationsgrad av sköldkörteln, vilket är ett säkert tecken på autoimmun tyreoidit hos människor, samt med jämföringar mot mikrosomala antikroppar i sköldkörteln. Resultaten visade att ITT:n är en robust mätmetod för detta sjukdomstecken hos höns, dock hade ett förvånande litet antal av OS-hönsen autoantikroppar mot TPO. Detta kan bero på att vi tog bort delar av TPO för att kunna uttrycka proteinet i vår assay. En annan orsak kan vara att det finns ett annat nyckelprotein för utvecklandet av autoimmun tyreoidit i OS-hönsen jämfört med människor.
Denna fenotyp kan ändå visa sig vara användbar i en pågående studie där vi söker efter regioner i genomet som reglerar utbrott av sjukdomen.

**Delarbete IV** beskriver förekomsten av olika sjukdomstecken i korsningen mellan OS- och RJF-höns. Vi undersökte 567 fåglar ur den tredje generationen i korsningen med avseende på infiltrationsgrad av sköldkörteln vid avlivning samt kroppsvikt och yttre sjukdomstecken vid tre olika åldrar. Rena OS-höns uppvisar en rad yttre sjukdomstecken, såsom små men feta kroppar, små och bleka kammar, ett flegramiskt beteende och långa, silriga fjädrar.

Sköldkörtlarna delades i flera snitt per individ och infiltrationsgraden i varje snitt mättes. Detta visade att det behövdes fler snitt från samma sköldkörtel för att få ett tillförlitligt resultat när den totala infiltrationsgraden mättes. Vi använde oss av åtta snitt från varje sköldkörtel för vidare analyser. Vi fann ingen korrelation mellan kroppsvikt och infiltrationsgrad, men mellan yttre sjukdomstecken och infiltrationsgrad fanns en stark koppling. Tillskott av sköldkörtelhormon visade sig ge en kraftigare viktökning, vilket indikerar att detta kan saktas ned den autoimmuna reaktionen.

Resultaten från denna studie kommer att användas i ett pågående försök att finna regioner i genomet som innehåller gener vilka påverkar utvecklingen av autoimmun tyreoidit.
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If you have now finished reading the acknowledgements you are welcome to take a peek at the rest of the thesis too.
Flopjod by Rolf Sahlqvist
References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.