From the Department of Molecular Medicine and Surgery
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EPIDEMIOLOGICAL STUDIES ON COMORBIDITY, HERITABILITY AND CO-AGGREGATION IN ORGAN-SPECIFIC AUTOIMMUNE DISEASES

Jakob Skov

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Epidemiological studies on comorbidity, heritability and co-aggregation in organ-specific autoimmune diseases

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By

Jakob Skov

Principal Supervisor:
Associate Professor **Sophie Bensing**
Karolinska Institutet
Department of Molecular Medicine and Surgery, and Department of Endocrinology, Karolinska University Hospital, Solna

Co-supervisor(s):
Professor **Olle Kämpe**
Karolinska Institutet
Department of Medicine, Solna, and Department of Endocrinology, Karolinska University Hospital, Solna, and K.G. Jebsen Center for Autoimmune Diseases, University of Bergen, Norway

Professor **Jonas F Ludvigsson**
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics, and Department of Pediatrics, Örebro University Hospital

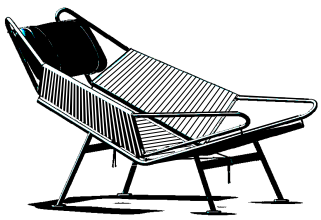
Doctor **Anders Sundström**
Swedish Medical Products Agency
Uppsala, Sweden

Opponent:
Emeritus Professor **Anthony P Weetman**
University of Sheffield
The Faculty of Medicine, Dentistry and Health

Examination Board:
Associate Professor **Mikael Lantz**
Lund University
Department of Endocrinology
Skåne University Hospital

Associate Professor **Jonas Spaak**
Karolinska Institutet
Department of Clinical Sciences, Danderyd Hospital

Associate Professor **Sara Öberg**
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics, and Harvard T.H. Chan School of Public Health, Department of Epidemiology



“The good chair is a task one is never completely done with”

Hans J. Wegner

ABSTRACT

Autoimmunity is caused by loss of tolerance, whereby the immune system fails to distinguish self from non-self. The autoimmune panorama entails more than 100 diseases that collectively affect close to 5% of the total population, with a higher prevalence in women than in men. Depending on which tissues are targeted, autoimmunity is classified as organ-specific or systemic. For unknown reasons, organ-specific autoimmunity often targets the endocrine system, leading to organ failure and lifelong need of hormone replacement.

Most autoimmune diseases show familial clustering, but co-aggregation of different disorders in family members, and co-occurrence of multiple diseases in a single individual, is also common. This points to shared etiologies in autoimmunity. Twin studies can help explain to what extent genetic and environmental influences contribute to differences in traits, and can thus provide a framework for genetic studies. In autoimmunity, twin studies have helped explain genetic contributions (heritability) for some diseases, but for many they are still lacking. In other scientific fields, twin studies have also been used to shed light on disease overlap, but no attempts of explaining clustering of autoimmunity using twins have been published.

In this thesis we explore different aspects of organ-specific autoimmunity. In Study I, we use the Swedish Twin Registry to demonstrate that the heritability for Addison's disease is very high, making it suitable for further genetic studies. In Study II we use national health registries to explore cardiovascular morbidity for Addison's disease, and show that women are at increased risk of cardiac events. We also demonstrate a dose-dependent correlation between adrenal hormone replacement-therapy and risk of cardiovascular disease. Study III addresses genetic versus non-genetic components to clustering in seven autoimmune diseases, and we find evidence of genetic factors explaining co-aggregation. In Study III we also validate previous estimates of heritability in autoimmunity by using a larger cohort of twins than ever before used. In Study IV, we take a closer look at Hashimoto's thyroiditis and Graves' disease, again using twins. Results imply that they are distantly related diseases, in contrast with current dogma. We also find that heritability is higher in men than in women, most notably for Hashimoto's thyroiditis. This could mean that the mechanisms leading to disease differ between the sexes.

LIST OF SCIENTIFIC PAPERS

I. Skov J, Höijer J, Magnusson PKE, Ludvigsson JF, Kämpe O, Bensing S. Heritability of Addison's disease and prevalence of associated autoimmunity in a cohort of 112,100 Swedish twins. Endocrine. 2017 Dec;58(3):521-527

II. Skov J, Sundström A, Ludvigsson J, Kämpe O, Bensing S. Sex-Specific Risk of Cardiovascular Disease in Autoimmune Addison Disease - A Population-Based Cohort Study. J Clin Endocrinol Metab. 2019 Jun 1;104(6):2031-2040.

III. Skov J, Eriksson D, Kuja-Halkola R, Höijer J, Guðbjörnsdóttir S, Svensson A-M, Magnusson P, Ludvigsson J, Kämpe O, Bensing S. Heritability and overlap in seven organ-specific autoimmune diseases: a population-based twin study.
Eur J Endocrinol. 2020 May;182(5):473-480.

IV. Skov J, Calissendorff J, Eriksson D, Ludvigsson J, Magnusson P, Kämpe O, Bensing S, Kuja-Halkola R. Limited genetic overlap between Hashimoto´s thyroiditis and Graves´ disease in Swedish twins: a population-based cohort study. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

AD	Addison's Disease
AID	Autoimmune Disease
AIRE	Autoimmune Regulator
AITD	Autoimmune Thyroid Disease
APS-1	Autoimmune Polyendocrine Syndrome Type 1
APS-2	Autoimmune Polyendocrine Syndrome Type 2
ATC	Anatomical Therapeutical Chemical classification system
BACH2	BTB Domain and CNC Homolog 2
BMI	Body Mass Index
CDR	Cause-of-Death Registry
CeVD	Cerebrovascular Disease
CLEC16A	C-Type Lectin Domain Containing 16A
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
CVD	Cardiovascular Disease
DHEA	Dehydroepiandrosterone
DM-1	Type-1 Diabetes
DM-2	Type-2 Diabetes
DZ	Dizygotic
FCRL3	Fc Receptor-Like Protein 3
FOXE1	Forkhead Box Protein E1
GWAS	Genome-Wide Association Study
HDL-C	High-Density Lipoproteins
HLA	Human Leukocyte Antigen
ICD	International Statistical Classification of Diseases
IHD	Ischemic Heart Disease
MZ	Monozygotic
NDR	The National Diabetes Register
NPR	The National Patient Register
PPV	Positive Predictive Value
PTPN22	Protein Tyrosine Phosphatase, Non-Receptor Type 22
SPDR	The Swedish Prescribed Drug Register
STR	The Swedish Twin Register
TGab	Thyroglobulin Autoantibodies
TPOab	Thyroid Peroxidase Autoantibodies
TPR	The Total Population Register
21OH	21-hydroxylase

1 INTRODUCTION

1.1 AUTOIMMUNITY

1.1.1 The discovery of autoimmunity

Prevention of infectious disease, through discrimination of self from non-self is the critical task of the immune system. Failure to do so, and the ensuing consequences were hypothesized by Paul Ehrlich at the turn of the nineteenth century. He coined the condition we today know as autoimmunity "horror autotoxicus", and predicted that is was a biological impossibility. Over the following decades mounting evidence in support of a misdirected immune response sometimes targeting own tissues had little scientific impact, and it was not until the early 1960s that the concept of autoimmunity was fully accepted.

Today we know that approximately 5% of the global population will suffer from autoimmune disease (AID) at some time in life [1, 2]. While some AIDs are transient, most are chronic, often manifesting at a young age, leading to considerable morbidity over time.

The original definition of what constitutes autoimmunity, based on the Koch's postulate of infections, was outlined by Witebsky [3]. He stated that autoimmunity was the result of an autoantibody or cell-mediated immune reaction; that a corresponding antigen-target could be identified, and that transfer of autoantibodies would induce a similar response in experimental animals. Over the years increased understanding of immune mechanisms such as naturally occurring autoantibodies, T-cell mediated disease, and delineation of autoimmunity from autoinflammation has led to revisions of this definition [4, 5].

Direct evidence of autoimmunity through transfer of autoantibodies is rare. We are limited to a select few experiments of nature for such proof: neonatal forms of Graves' disease (GD), Myasthenia gravis and Pemphigus vulgaris are all caused by transplacental passage of autoantibodies from the mother to the fetus, demonstrating transfer of autoimmunity [6]. However, most autoimmune phenotypes are caused by autoantigen-specific T-cells rather than antibodies, and human-to-human transfer of disease is rarely feasible [7, 8], making direct evidence difficult to obtain. Sometimes, animal-models can provide conceptual evidence, but for many diseases the link to an autoimmune etiology hinges on circumstantial evidence such as infiltration of immune cells in tissue samples taken from affected individuals, response to immunosuppressant therapy, strong association to HLA genotypes, or co-aggregation with other AIDs [4].

1.1.2 Systemic and organ-specific autoimmunity

Depending on the tissues targeted, AIDs are classified as either systemic or organ-specific, with the former affecting tissues in multiple organs and the latter restricted to single organs. The distinction is far from clear-cut, and not mirrored in distinct etiologies, as both organ-specific and systemic diseases sometimes overlap in families and individuals. It is also important to note that T-cell mediated and autoantibody-driven diseases are found in both systemic and organ-specific autoimmunity [6]. For unknown reasons, endocrine organs are often the target in organ-specific autoimmunity. Based on data from the Danish population, endocrinopathies account for approximately 30% of the total autoimmune burden [1].

In systemic autoimmunity, therapy is focused on disease modulation. Similar strategies, and attempts of altogether reversing the destructive process through immunomodulation, have been tried in organ-specific diseases such as type-1 diabetes (DM-1) and Addison's disease (AD), but with limited success [9]. Consequently, some 130 years after the introduction of thyroid extracts for treating myxedema [10], clinical strategies in endocrine autoimmunity are still centered around adequate hormone replacement.

1.1.3 Gender-differences in autoimmunity

For most AIDs there is a clear sex-difference in prevalence, with females more affected than males (Figure 1). The severity of autoimmune manifestations from the same disease may also differ between the sexes [11].The underlying mechanisms are only partly understood, but both genetic and non-genetic effects have been suggested. Minor differences in how males and females react to common infections, with a stronger cellular and humoral response in women, have been shown [12]. Mechanisms related to sex hormones, X-chromosome inactivation, and microchimerism (transfer of cells from the fetus to the mother and vice-versa during pregnancy), among others, have also been suggested [11], but the net effect in terms of explained difference in prevalence from these factors is currently unknown.

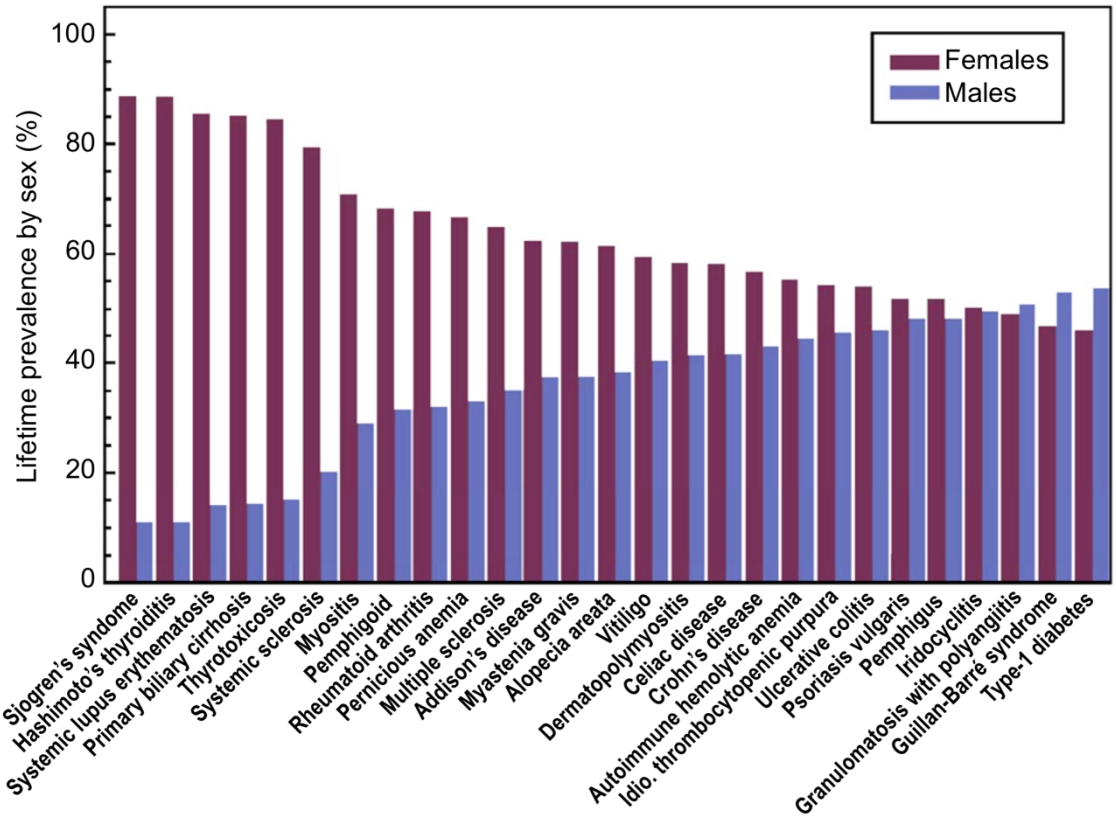


Figure 1. Prevalence of autoimmune diseases in Denmark by sex. Adapted from J. Autoimmun. 2007;29(1):1. Eaton et al. *Epidemiology of autoimmune diseases in Denmark* with permission from Elsevier.

1.1.4 Co-occurrence and co-aggregation of autoimmunity

Co-occurrence, defined as multiple traits occurring in one individual, is not uncommon in autoimmunity. This is most striking in rare monogenic disorders [13]. Autoimmune polyendocrine syndrome type-1 (APS-1), first summarized in a case series in 1956 [14], is a case in point. Here, multiple AIDs, including primary hypoparathyroidism and AD, typically manifest at an early age along with other phenotypic criteria [15]. However, most AIDs are complex disorders, with multiple genetic and environmental factors contributing to disease, and sometimes to co-occurrence of diseases. On an individual level, we usually cannot predict the onset of autoimmunity, or which AID will develop, but patterns of co-occurrence vary, where some AIDs rarely overlap whereas isolated disease is the exception in others [2].

Co-aggregation, defined as either identical or related traits recurring in family members of index cases, is also common in autoimmunity, with multigenerational pedigrees often displaying a variety of manifestations (Figure 2) [16, 17]. Co-aggregation implies a shared etiology, but families typically share both genes and environment, and discriminating genetic from non-genetic influences requires studies specifically designed to do so.

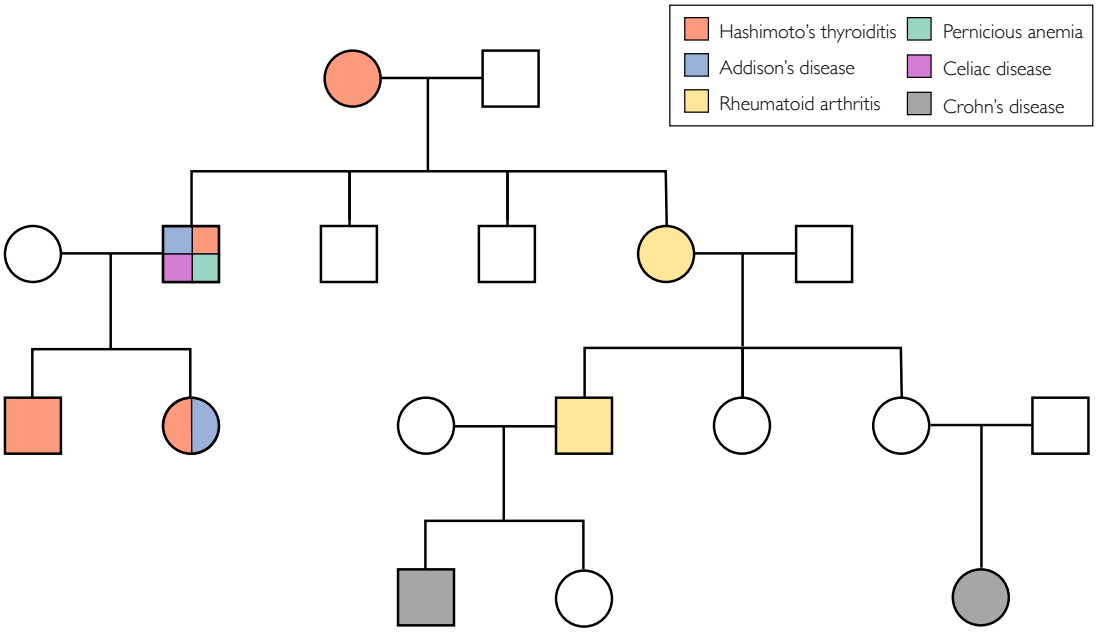


Figure 2. Co-aggregation of autoimmune diseases in a family pedigree. Two members of the family are patients of the author of this thesis.

1.2 MODEL DISEASES FOR ORGAN-SPECIFIC AUTOIMMUNITY

1.2.1 Addison's disease

AD is caused by an autoimmune destruction of the adrenal cortex. This results in loss of glucocorticoid and mineralocorticoid production which is universally fatal without adequate hormone substitution [18]. AD is a rare disorder, with prevalence estimates ranging from 5/100 000 in Japan to approximately 20/100 000 in the Scandinavian countries [19-27]. Monogenic AD due to APS-1 accounts for a small minority of cases, whereas sporadic (complex) disease is more common. Similar to APS-1, individuals with complex AD often develop autoimmune comorbidities, [28-30] (Figure 3) in what constitutes autoimmune polyendocrine syndrome type 2 (APS-2)[15]. Despite different etiologic origins, autoantibodies against 21-hydroxylase (21OH), a key-enzyme in the steroid synthesis, is a hallmark of both monogenic and complex AD. The female to male ratio of complex AD is approximately 3:2, with incidence rates peaking between 30-50 years of age [30].

Glucocorticoid replacement therapy in AD typically consist of hydrocortisone tablets twice or thrice daily with doses amounting to 20-30 mg/day [31]. Intermittent oral dosing does not restore diurnal patterns of cortisol release, with peak levels early in the morning and then gradually decreasing serum concentrations with a nadir around midnight [32]. Mineralocorticoid replacement typically consist of fludrocortisone 0.1 mg once daily. For women, with no gonadal source of testosterone, adrenal destruction also leads to androgen deficit. Several small randomized trials have examined the effects of treatment with the weak androgen dehydroepiandrosterone (DHEA), but they have not been able to consistently

demonstrate improvements in somatic parameters or well-being [33-36]. DHEA is therefore used sparingly in Sweden today. In addition to androgen deficit, a minority of women with AD also develop primary ovarian insufficiency, preceded by autoantibodies against side-chain cleavage enzyme, leaving them deplete of sexual hormones without adequate substitution [30].

1.2.2 Autoimmune thyroid diseases

Hashimoto´s thyroiditis (HT), initially defined as lymphadenoid goiter without any reference to thyroid function [37], is nowadays generally used to describe chronic autoimmune hypothyroidism, regardless of thyroid size. This nomenclature is used in this text. HT, together with autoimmune hyperthyroidism or GD, collectively referred to as autoimmune thyroid diseases (AITDs), represent two of the most common forms of autoimmunity. The prevalence of AITD has increased over recent decades, and now most likely exceeds 5% among women in northern-European countries [38, 39]. Precise estimates are lacking, as a clear distinction of HT from subclinical hypothyroidism is hard to attain using health-registers. Moreover, prescription patterns indicate increased use of levothyroxine in recent years beyond what can be expected from an increased incidence alone [40]. Whereas AD typically manifests as part of APS-2, AITDs are often unique manifestations of autoimmunity (Figure 3). This is counter-intuitive, given that AITDs often co-occur with other AIDs [1, 41-45], but reflects their high prevalence compared to most other autoimmune disorders. Both HT and GD are more common in women than in men, with female to male ratios of 1:4-10 [46, 47]. This indicates that the underlying mechanisms leading to disease may well be different between the sexes.

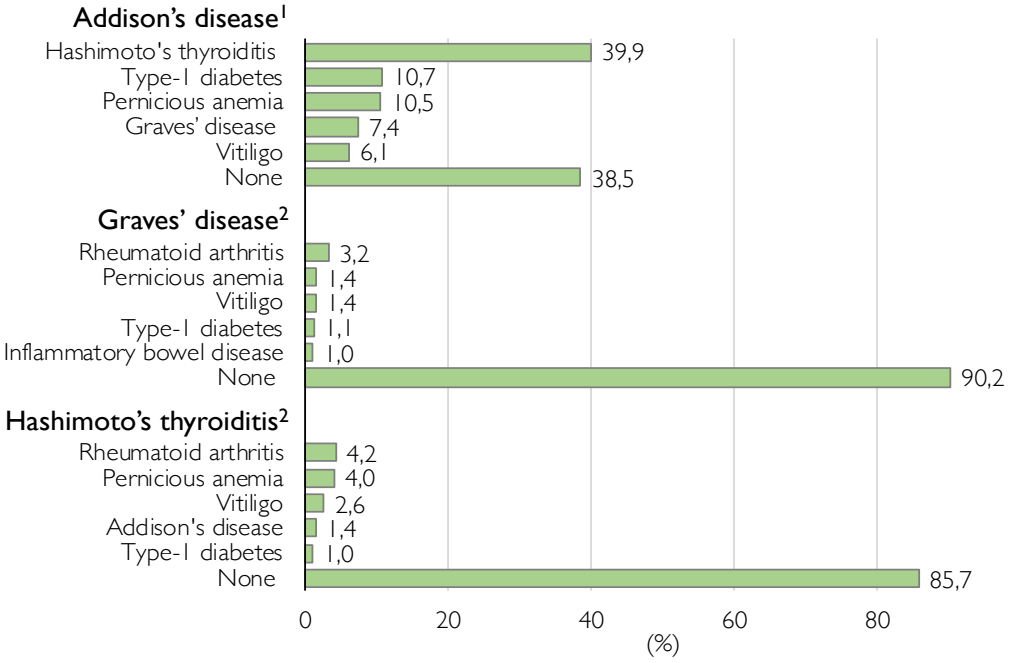


Figure 3. Prevalence of autoimmune co-morbidities among patients with Addison's disease, Graves' disease and Hashimoto's thyroiditis. [1]Ref [30] [2]Ref [48].

1.3 TWIN STUDIES

1.3.1 The evolution of twin studies

Twin studies, whereby similarities in traits or diseases in monozygotic (MZ) and dizygotic (DZ) twin pairs are compared to elucidate the influence of "nature versus nurture", were first envisioned in the late nineteenth century [49]. The fundamental twin methods used today build on models established in the 1920s and 1930s [50, 51]. The classic twin model hinges on the assumption that MZ twins have virtually identical genomes, that DZ twins share on average half of their segregating alleles, that MZ and DZ twins share environments to an equal extent, and that twins do not differ from the general population with regards to the examined trait. Some of these assumptions have come into question [52], but on closer examination, they have appeared valid [53]. The value of using twin-studies to explore traits and diseases soon became apparent, leading to the founding of national twin registers, starting with the Danish Twin Register in 1954 [54]. Twin methodology has evolved over time, with studies in behavioral sciences, psychology and psychiatry leading the way [55, 56]. In these fields, similar to genetic studies, epidemiological data has been used not only to detect genetic influences on individual traits, but also to study the nature and strength of disease-overlap [57, 58].

1.3.2 Heritability

A higher concordance (i.e. proportion of twins sharing a trait) in MZ than in DZ twins indicate that genetic effects contribute to this trait. The magnitude of genetic influence is often presented as heritability, a useful but sometimes misinterpreted statistic. The heritability of a trait is the proportion of the total variance for the trait in the examined population that is attributable to genetic effects. Heritability therefore varies from 0 to 1, sometimes presented on a percentile scale (0-100%). A heritability of 1 suggests that genetic effects explain all the observed variance in the population, whereas a heritability of 0 indicates that environmental factors are responsible for all observable variance.

It is important to note that heritability does not reflect what *causes* a trait, only what causes the *variation* in a trait in a given population at a given point in time. Heritability is a group statistic, and provides no information as to the importance of genes in causing disease in an individual. Nor does it account for fundamental genetic or environmental factors shared by all individuals in the observed population, as they do no contribute to variance [59]. Still, estimates of heritability provide important information on the influence of genes in explaining how we differ with regards to traits and diseases.

1.3.3 Twin studies in autoimmunity

The methods used for calculating heritability are best suited for traits that are common and rich in information, such as biometric data (height and body weight), or behavioral data, that can be measured on a continuous or ordinal scale. Under such circumstances estimating heritability is feasible even in cohorts of modest size. Unfortunately, AIDs fulfill none of these criteria, as they are all infrequent or rare, and typically represent dichotomies (disease or no disease) carrying little information. Consequently, estimating heritability for AIDs require large twin registries, available in recent decades only.

The first formal assessment of heritability for an organ-specific AID was a Swedish twin-study on Crohn's disease and ulcerative colitis published in 1988 [60]. Since then, many AIDs have been the subject of examination, but due to their low prevalence, estimates of heritability have generally been imprecise [61]. For other AIDs, estimates are still lacking. A common attribute of most AIDs so far studied is that heritability explains a majority of the observed variance (Table 1).

Despite ample epidemiological data on co-occurrence and co-aggregation of AIDs, twin studies have not been used to quantify genetic and environmental influences on disease-overlap. Besides increasing our understanding of how diseases interrelate, studying etiologic overlap could help us predict how likely patients and their relatives are of developing not only single diseases, but any one in a group of closely related disorders.

Table 1. Heritability in organ-specific autoimmune diseases

Disease	Heritability, % (95% CI)[a]	Reference
Type-1 diabetes	72	[62]
	88 (78-94)	[63]
Hashimoto's thyroiditis[b]	73 (46-89)	[64]
Graves' disease	79 (64-90)	[65]
Celiac disease	57-87	[66]
	75 (55-96)	[67]
Vitiligo[c]	72	[68]
	46 (41-51)	[69]
	49 (47-51)	[70]
Crohn's disease	100 (34-100)	[60]
	89	[71]
Multiple sclerosis	64 (28-77)	[72]

[a]When reported. [b]Based on titers of thyroid autoantibodies. [c]Derived from family-based studies.

1.4 GENETIC STUDIES IN AUTOIMMUNITY

Among the earliest insights into the genetics of autoimmunity was the strong association with variants of the human leukocyte antigen-complex (HLA). These discoveries came about through linkage analysis in pedigrees with a high prevalence for the AIDs in question [73, 74]. Haplotypes in the HLA-region typically confer the largest risk in complex AIDs, making them easier to identify, whereas other allelic variants are harder to detect. Consequently, few other genes were identified using these methods [75]. Instead, candidate gene approaches helped progress the understanding of autoimmunity [76], and in the last two decades genome wide association studies (GWAS) and whole genome sequencing has added to the list of polymorphisms associated with AIDs [77]. Most of the alleles identified in organ-specific autoimmunity locate to genetic regions involved in regulation of the adaptive immune response [78], whereas only a small minority locate to genes specific to the target tissue itself [79, 80]. Interestingly, most loci are pleiotropic, influencing the risk of more than one AID [81, 82], hinting at a partly shared genetic origin contributing to overlap in autoimmunity. This is also consistent with the autoimmune clustering observed in individuals and in families [1, 2, 16, 83, 84].

1.5 ETIOLOGY OF ORGAN-SPECIFIC AUTOIMMUNE DISEASES

1.5.1 Addison's disease

Primary adrenal insufficiency due to APS-1 is caused by mutations in the autoimmune regulator gene (AIRE) [85, 86], whereas sporadic AD is a complex disease, with a non-Mendelian pattern of inheritance. HLA DR3-DQ2 and DR4 are the predominant risk factors in sporadic AD [87-89], but several other loci involved in immune regulation have been implicated. These include allelic variants of the CTLA4 [90-92], PTPN22 [93], BACH2 [94, 95] and most recently the AIRE-gene [96]. Most, but not all [95, 96] of these alleles have been identified through candidate-gene studies based on findings in other autoimmune diseases, including thyroid disorders (Table 2). So far, no GWAS in AD has been published.

Familial aggregation of complex AD has been shown in both Norwegian and Swedish cohorts [29, 30], and several case-reports describe concordance for AD in monozygotic twin pairs [97-100]. These data, in line with studies demonstrating high heritability for other AIDs, indicate genetic influence on the occurrence of AD. So far, no environmental factors have been credibly linked to AD, and no formal evaluation of the relative genetic and environmental influence on AD have been undertaken.

Table 2. Non-HLA genes associated with organ-specific autoimmunity				
Gene	Autoimmune disease			
	Addison's disease	Hashimoto's thyroiditis	Graves' disease	Other organ-specific autoimmune diseases
CTLA4	[101]	[102, 103]	[104, 105]	Type-1 diabetes, myasthenia gravis, vitiligo [106]
PTPN22	[101]	[107, 108]	[109, 110]	Type-1 diabetes, celiac disease, multiple sclerosis [108, 110, 111]
BACH2	[94, 95]	-	[112]	Type-1 diabetes, Crohn's disease, celiac disease, vitiligo [113, 114]
CLEC16A	[115]	[116]	-	Type-1 diabetes, multiple sclerosis [117, 118]

Examples of genes implicated in Addison's disease, autoimmune thyroid diseases and other organ-specific autoimmune diseases.

1.5.2 Autoimmune thyroid diseases

Epidemiologic data on the contribution of genetic and non-genetic factors to HT and GD mostly stem from studies of the Danish Twin Registry [64, 65, 119, 120]. These report a heritability of 79% (95% CI 64-90%) for GD. Estimates of heritability for overt HT are lacking, but heritability for autoantibodies against thyroid peroxidase (TPOab) and thyroglobulin (TGab) in euthyroid subjects explain 73% (95% CI 46-89%) of observed variance. Iodine-intake and smoking are known to have substantial influence on the risk of AITDs [121, 122], but still, environmental factors explain only modest proportions of variance (21% for GD and 27% for HT). The Danish study on euthyroid subjects reported a lower heritability for TGab in men compared to women [64], but so far, no study on overt thyroid disease has been powered to detect etiologic differences between the sexes.

Extrapolating the heritability of overt HT from data on thyroid autoantibodies is not without risks, something the authors of the Danish twin study point out [64]. Not all individuals with titers against thyroid autoantigens progress to overt HT [39], and neither TPOab nor TGab are unique to HT, as they are common in patients with GD as well [123]. Moreover, autoantibodies can arise from different etiologies, exemplified by 21OH autoantibodies found in both APS-1 and APS-2, and rheumatoid factor found in both systemic lupus erythematosus and juvenile idiopathic arthritis despite little genetic overlap [82].

Linkage- and candidate-gene studies have been relatively successful in identifying susceptibility-loci in AITD. Both gene-variants central to immune regulation and to thyroid function have been associated with increased risk of disease [124]. GWAS have helped validate previous findings and added to the list, with loci related to the HLA-complex, PTPN22, CTLA4 and FCRL3 and FOXE1 among others implicated in more than one GWAS [125].

1.5.3 Etiologic overlap in autoimmune thyroid diseases

HT and GD are generally considered to be closely related diseases [124, 126-128], and many features do indeed support a common etiology: transition from one disease to the other does occur [129, 130], family-studies have demonstrated accumulation of both diseases in relatives of index cases [48, 131, 132], and MZ twin pairs with HT in one twin and GD in the other twin have been reported [133-135]. Furthermore, genetic overlap is evident from GWAS-data [125], and TPOab and TGab, a hallmark of HT, are found in patients with GD as well.

However, there are features suggestive of separate etiologies: the phenotype in GD is autoantibody-mediated, whereas HT results from cytotoxic destruction of the thyroid gland. As previously stated, shared autoantibody-profiles are not synonymous with a shared etiology either, and while GWAS-data is consistent with genetic overlap, the proportion of heritability so far explained by pleiotropic loci is small. Moreover, many of the genes implicated are also relevant to other AIDs, supporting a broad autoimmune tautology rather than a common genetic origin to AITDs (Table 2). Still, without objective estimates of disease-overlap, this remains speculative.

1.6 CARDIOVASCULAR COMORBIDITY IN ADDISON´S DISEASE.

For AD, cohorts large enough to explore long-term outcomes have only emerged over the last two decades, and many fundamental questions related to health are still unanswered. For AITDs, many of these questions have been addressed [136-140], and will therefore not be discussed further in this thesis.

After the introduction of glucocorticoid and mineralocorticoid replacement in the 1950s, patients diagnosed with AD were generally considered at modestly increased risk of death, primarily due to acute adrenal crises[141]. This notion was challenged in 2006, with a population-based Swedish study demonstrating that mortality was more than twice that in the general population[142]. Diagnostic records did not provide clear answers as to why, but cardiovascular and infectious diseases appear to explain most of the excess mortality [142, 143]. DM-1, present in approximately 10% of the Swedish AD-population [30], is associated with cardiovascular disease, but adjusting for diabetes had marginal impact on results [142]. Data from Norway have partially corroborated these observations, demonstrating no overall increase in mortality, but with an increased risk of cardiovascular death among individuals diagnosed with AD before the age of 40 years [144].

Concerns of a possible connection between glucocorticoid replacement-doses and cardiovascular disease (CVD) has emerged over time [141, 145, 146]. Prolonged exposure to high doses of glucocorticoids is known to increase cardiovascular risks [147], and glucocorticoid replacement doses in AD are generally much higher than physiological release of cortisol [148, 149]. Data on secondary adrenal insufficiency caused by pituitary diseases also points to a dose-associated increase in risk of death [150, 151]. The effects of fludrocortisone on cardiovascular outcomes in AD have not been discussed or explored in a wider context, despite the obvious risks associated with primary aldosteronism [152]. Case-reports on heart failure due to overdosing in patients newly diagnosed with AD highlight the dangers of mineralocorticoid excess [153-155], but long-term outcomes of different regimens are unknown.

Studies on cardiometabolic risk factors such as adiposity, glucose metabolism, lipid metabolism and blood-pressure have yielded conflicting data. The largest studies to date, reporting on Swedish patients with AD, have found increased prescriptions of lipid-lowering agents and antihypertensives, but lower body mass index (BMI) and lower blood-pressure compared to controls [26, 30]. In other cohorts, AD has been be associated with dyslipidemia, higher BMI, abdominal obesity, and poor glucose metabolism in an inconsistent manner [156-158].

2 AIMS

2.1 OVERALL AIMS

The aim of this thesis was to explore the etiology of organ-specific autoimmune diseases, to quantify etiologic overlap in organ-specific autoimmunity, and to examine cardiovascular comorbidity in AD.

2.2 SPECIFIC AIMS

Study I. To estimate the heritability of AD and to explore patterns of autoimmune comorbidity using data from the Swedish Twin Registry.

Study II. To examine incident cardiovascular disease (including cerebrovascular disease) in AD, and to investigate the effects of glucocorticoid and mineralocorticoid dosing.

Study III. To estimate heritability and genetic influences on co-aggregation in seven organ-specific autoimmune diseases using data from the Swedish Twin Registry.

Study IV. To estimate the heritability for HT and GD by sex, and to estimate co-heritability between HT and GD using bivariate twin methodology.

3 MATERIALS AND METHODS

3.1 REGISTERS

The Swedish personal identity number [159] allows for linkage to the National registries of health kept by the National Board of Health and Welfare, and to registers containing demographic information kept by Statistics Sweden.

The Total Population Register (Study II)

The Total Population Register (TPR) was started in 1968. It contains data on birth, death, name-change, marital status, family relationships, place of residence, and national and international migration. Coverage is virtually complete [160].

The Swedish National Patient Register (Study I-IV)

The Swedish National Patient Register (NPR) contains inpatient information dating back to 1964, with nationwide coverage since 1987. It collects data on hospital admission dates, discharge dates, discharge codes and procedural codings for surgery classified according to the International Statistical Classification of Diseases (ICD). As of 2001, data on hospital-based outpatient care (but not primary healthcare) is also included. The diagnostic accuracy is generally very good [161].

The Swedish Prescribed Drug Register (Study I-IV)

The Swedish Prescribed Drug Register (SPDR) contains data on all prescription drugs dispensed in Sweden from July 2005 onwards [162]. It includes information on dispensed quantity, dosage, and type of drug classified according to the Anatomical Therapeutical Chemical (ATC) classification system. It does not include information on drugs used for inpatient care or drugs sold over the counter. Unlike the NPR which does not include information on primary health care, the PDR includes information on all drugs dispensed.

The Cause-of-death Registrer (Study II)

The cause-of-death registry (CDR) contains data since 1961 on all deceased persons who at the time of death were registered in Sweden. Recorded variables include underlying cause of death, multiple causes of death, nature of injuries associated with death, and basis for statement of cause of death. Diagnoses are coded according to the international version of the ICD. Coverage is >99.5% [163]. Diagnostic accuracy is acceptable for deaths related to cancer and ischemic heart diseases (80-90%), but lower for all other conditions (<80%) [164].

The Swedish Twin Registry (Study I, III, IV)

The Swedish Twin Registry (STR) is the world's largest twin resource. It was started in the late 1950s and to date contains information on more than 216,000 individual twins born in Sweden between 1886 and 2015 [165]. The registry is voluntary with legal guardians usually contacted by the time twins turn 9 years of age. Historically, coverage has been very high, but it has dropped in recent years [165]. Zygosity has been determined for more than 86,000 twin pairs using a validated intra-pair similarity algorithm, DNA, or being of opposite sex. The STR conducts regular updates of national health data on twins that have at some point agreed to participate in studies. Biobank material (saliva, capillary blood or venous blood) is available for more than 50,000 twins.

The National Diabetes Register (Study III, IV)

The National Diabetes Register (NDR) is a health quality register established in 1996 for the purpose of collecting information on and monitoring diabetes care [166]. It documents health data including type of diabetes, therapy, and complications on an individual level. The NDR is maintained by the Swedish Society for Diabetology with financial support from all Swedish regions. Coverage is nationwide and nearly complete.

3.2 CASE ASCERTAINMENT

Adrenal failure is a heterogenic condition with many potential mechanisms of disease. Secondary failure due to pituitary or hypothalamic diseases, and long-term glucocorticoid treatment are common causes of adrenal malfunction. Trauma, infectious diseases, and genetic conditions can also lead to adrenal. Not even autoimmune adrenal failure is not synonymous with complex AD. The different forms are easily mixed up, resulting in erroneous ICD-coding. Moreover, if AD cannot be confirmed or rejected without confirmatory testing, is sometimes ICD-coded as AD pending further investigation. This leaves traces in the NPR that cannot be erased. A Norwegian study using hospital records as gold standard for diagnosing AD reported a positive predictive value (PPV) of 87% for ICD-codes indicating AD [144], whereas a Swedish study found that 28% of patients with a diagnosis of AD in the NPR also had diagnostic records suggestive of pituitary disease or other conditions associated with AD (unpublished data from [26]). To further improve diagnostic accuracy, investigators in the Swedish study excluded patients without filled prescriptions of glucocorticoids and mineralocorticoids in the SPDR. By default, this excluded approximately 12% of true AD cases that do not use mineralocorticoids [30], but the cohort dropped by 40% in size, indicating that misclassification is very common. We adopted a similar strategy, but required multiple (≥2) prescriptions of mineralocorticoids for inclusion (**Study I-III**). However, among patients with samples in the STR-biobank (**Study I, III**), presence

or absence of 21OH autoantibodies in serum were used to validate AD regardless of substitution therapy.

For GD, a corresponding ICD-code was used for inclusion unless diagnostic records indicated other forms of hyperthyroidism (**Study I, III-IV**). In **Study I, III-IV**, a diagnosis of HT required a corresponding ICD-code provided diagnostic records did not indicate other causes of hypothyroidism. For patients alive in 2006, with the SPDR in operation, multiple (≥2) dispensations of levothyroxine (ATC H03AA) were required to validate a diagnosis of HT. In the absence of ICD-codes for HT or other causes of hypothyroidism, multiple prescriptions of levothyroxine were sufficient for a diagnosis of HT in patients with concurrent DM-1 or AD, as HT is often not coded in this setting. In **Study II,** individuals with a diagnosis of HT in the NPR or with multiple (≥2) dispensations of levothyroxine in the SPDR fulfilled criteria for HT if they did not have diagnostic records indicating other causes of hypothyroidism.

Celiac disease (CD), vitiligo (VI), atrophic gastritis and pernicious anemia (both referred to as AG) were ascertained through recordings of relevant ICD-codes in the NPR. ICD-codes indicating non-autoimmune etiology were used as exclusions to improve diagnostic accuracy. Multiple (≥2) prescriptions of vitamin B12 (ATC B03BA) were required for a diagnosis of AG in patients alive in 2006 (**Study III**).

If patients had multiple (≥2) correct ICD-codes, multiple (≥2) exclusion codes were required for exclusion (**Study III-IV**).

In **Study I**, a diagnosis of DM-1 was based on a corresponding ICD code in combination with multiple (≥2) filled prescriptions of insulin for patients alive in 2006. In **Study II**, DM-1 was defined by a corresponding ICD-code. Among subjects with ICD-codes for both DM-1 and Type-2 diabetes (DM-2), the last recorded diagnosis before start of follow-up was considered correct. All other subjects with multiple fillings of antidiabetic agents were considered to have DM-2. In **Study III** and **Study IV**, DM-1 was ascertained by cross-matching the STR with the NDR.

3.3 STATISTICAL METHODS

Tetrachoric correlations

A commonly used measure of co-variance for dichotomous traits is the tetrachoric correlation. When analyzing tetrachoric correlations the liability threshold model may be used, whereby dichotomous outcomes are assumed to reflect an underlying normally distributed liability that is due to multiple genetic and environmental factors. This distribution is assumed to have a mean 0 and a variance 1 in the general population. Individuals above a certain threshold will develop the disease, whereas individuals below the threshold will not. The correlation is then calculated as the similarity between twins within pairs, by zygosity (Figure 4). A higher tetrachoric correlation in MZ than in DZ pairs is indicative of genetic influences [59]. This metric is the basis of subsequent calculations of heritability.

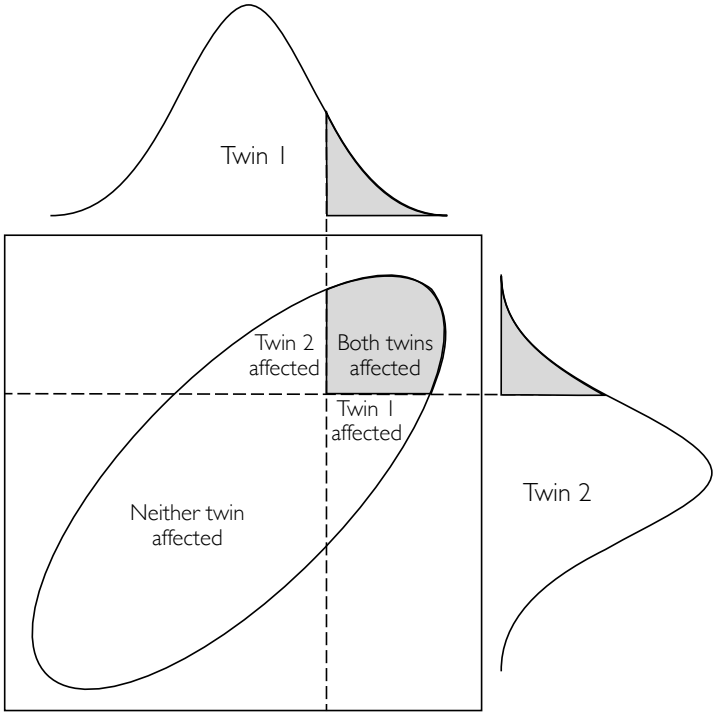


Figure 4. Tetrachoric correlation of twin pair data with dashed lines indicating thresholds of normally distributed liabilities.

Structural equation modeling

Under the assumption of the classic twin model (outlined in section 1.3.1), the observed variance of a trait can be decomposed into four variance components: additive genetic factors (A), non-additive genetic factors due to dominance or epistasis (D), environmental factors that are shared by twins in a pair (C), and environmental factors that are unique to individuals (E). Using structural equation modeling, explanatory models based on these sources of variance are tested to find the model with the best fit [52]. Combinations of (C) and (D) are confounded in twins and cannot be estimated simultaneously. Hence, the ACE-model accounting for common environmental influences (C) or the ADE-model that considers genetic dominance (D) instead, must be tested separately. In the ACE-model (Figure 5A),

the estimate of (A) is equal to the narrow sense heritability h^2, calculated from additive genetic (a^2), common environmental (c^2), and unique environmental (e^2) components of variance as follows:

$$h^2 = a^2 / (a^2 + c^2 + e^2)$$

When using the liability-threshold model for dichotomous traits, variance = 1, hence:

$$a^2 + c^2 + e^2 = 1;\ h^2 = a^2$$

By excluding components of variance from the full models, submodels (typically AE and E) are tested to find the best-fitting model. In twin studies on AIDs, more often than not, the AE-model has provided the best fit [63, 65, 167, 168].

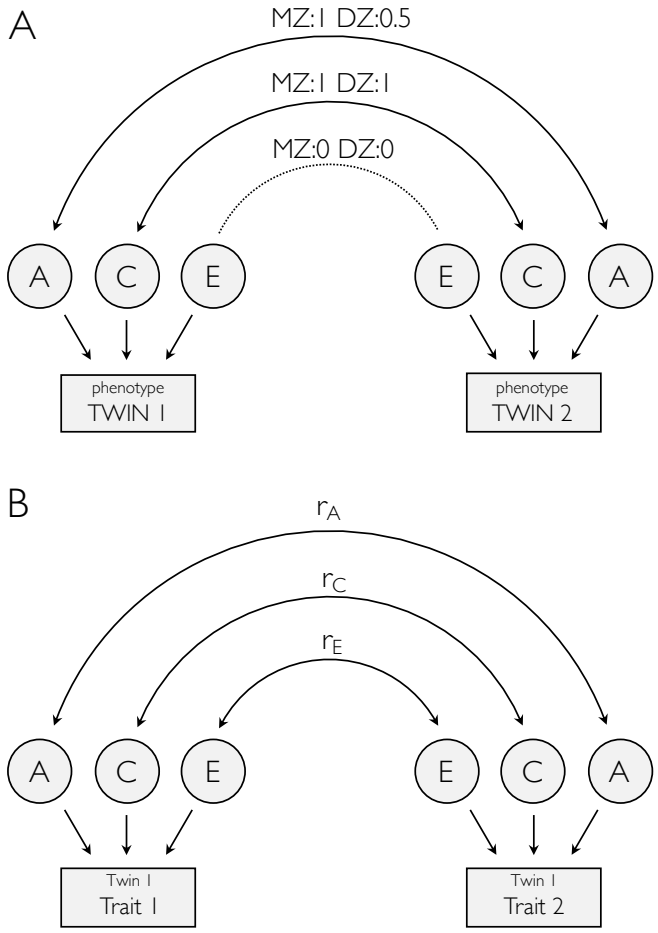


Figure 5A. A path diagram of a univariate ACE-model in which the three latent variables influence variation (A = additive genetic influences, C = common environmental influences, E = unique environmental influences) Double-headed arrows indicate the correlations among variables in monozygotic (MZ) and dizygotic (DZ) twin pairs.
Figure 5B. A path diagram of a bivariate ACE-model in an individual twin. Correlations between the additive genetic, shared environmental, and nonshared environmental influences are denoted by r_A, r_C and r_E. The genetic correlation represents the extent to which genetic influences on trait 1 are correlated with genetic influences on trait 2. Though not illustrated here, there are genetic and environmental correlations between the two members of a twin pair for both trait 1 and trait 2.

When multiple traits are assessed in twin pairs, structural equation models can be extended to analyze the covariance between these traits (Figure 5B). In **Study IV**, we used bivariate models to explore the genetic and environmental architecture of disease-overlap in HT and GD

Biometric twin models were fitted using R (R Foundation for Statistical Computing) in **Study I, III and IV**, and with OpenMx software [169] run within the R environment [170] in **Study III and IV**.

Poisson regression

The Poisson regression is a generalized linear model form of regression analysis suited for modeling count based data of rare events, as these tend to follow a Poisson distribution (as opposed to more common events which tend to be normally distributed). In **Study I**, Poisson regression was used to calculate the likelihood of MZ twin pairs developing the same disease (positive concordance), compared to DZ twin pairs, expressed as incidence rate ratios.

Cox proportional hazard models

Cox proportional hazard is a regression model used in survival analysis for investigating the effect of several variables upon the time a specified event takes to happen. In **Study II**, Cox regression models were used to calculate hazard ratios for cardiovascular events in individuals with AD compared to matched controls. To explore correlations between replacement therapy and cardiovascular outcomes, dummy variables of hormone replacement regimens stratified into low, medium or high doses were added to the models. In **Study III and IV**, Cox regression models were used to estimate the risk of twins developing AIDs after an AID was diagnosed in the co-twin. In these models, date of birth, year of start of observation, date of diagnosis or date of right-censoring were used as covariates. Twins were considered unexposed until the time of diagnosis in the co-twin and exposed thereafter.

3.4 METHODS SUMMARY

3.4.1 Study I

By cross-matching information in the STR with the NPR we identified twins with a diagnosis of AD from 1964 to 2012. Among suspected cases with biobank samples, presence of 21OH autoantibodies was used for inclusion. Among suspected cases without biobank samples prescription patterns in the SPDR were used to validate a diagnosis of AD. We also collected information on autoimmune comorbidities. Based on disease patterns in MZ and DZ twins, concordance rates and heritability estimates were calculated, and similarities in co-occurrence of AIDs in MZ versus DZ pairs were explored.

3.4.2 Study II

All adult individuals in Sweden with a diagnosis of AD from 1964-2013 were identified in the NPR. The diagnosis was then validated by exclusion of individuals with ambiguous diagnoses in the NDR or with insufficient prescriptions of hormone replacement in the SPDR. Each subject with AD was matched with 10 population controls. Incident CVD was analyzed prospectively from 2006 to 2013, and correlations between glucocorticoid and mineralocorticoid intake and CVD were evaluated. Case-fatality rates from CVD in subjects with AD and in matched controls were calculated.

3.4.3 Study III

By cross-matching information in the STR with the NPR we identified twins with a diagnosis of HT, GD, VI, AG, DM-1, CD or AD from 1964 to 2015, and included all individuals alive (or not yet born) in 1976. Diagnoses were validated using prescription data in the SPDR, and by cross-matching with the NDR. Univariate heritability was calculated for each disease. Attempts of estimating bivariate heritability for disease-pairs were mainly unsuccessful due to insufficient data. Instead, HRs were calculated for twins developing the same or a different disease as compared to their co-twin, with differences between MZ and DZ pairs used to estimate the genetic influence on co-aggregation.

3.4.4 Study IV

Using the methods outlined in Study III, all twins with a validated diagnosis of HT or GD were identified. Univariate heritability was calculated by sex, and bivariate heritability was calculated to quantify genetic and non-genetic influences on the etiologic overlap between HT and GD.

4 RESULTS

4.1 STUDY I

A total of 29 individual with AD were identified in a cohort of 112,000 twins. The mean age at diagnosis of AD was 38 years, 15/29 (52%) of patients were female, and autoimmune comorbidity was present in 18/29 (62%) patients. 14 patients were part of MZ twin pairs, and 15 were part of DZ pairs. Five out of nine (5/9) MZ pairs and zero out of fifteen (0/15) DZ pairs were concordant for AD. The probandwise concordance for MZ twins was 0.71 (95% CI 0.40– 0.90) and the heritability was estimated at 0.97 (95% CI 0.88–0.99), with the AE-model providing the best fit.

Patterns of autoimmune co-aggregation in twin pairs affected by AD differed by zygosity, with MZ pairs displaying a higher degree of disease-concordance (Figure 6). This was reflected by an incidence rate ratio of 15 (95% CI 1.8–116) for MZ pairs becoming disease-concordant compared to DZ pairs.

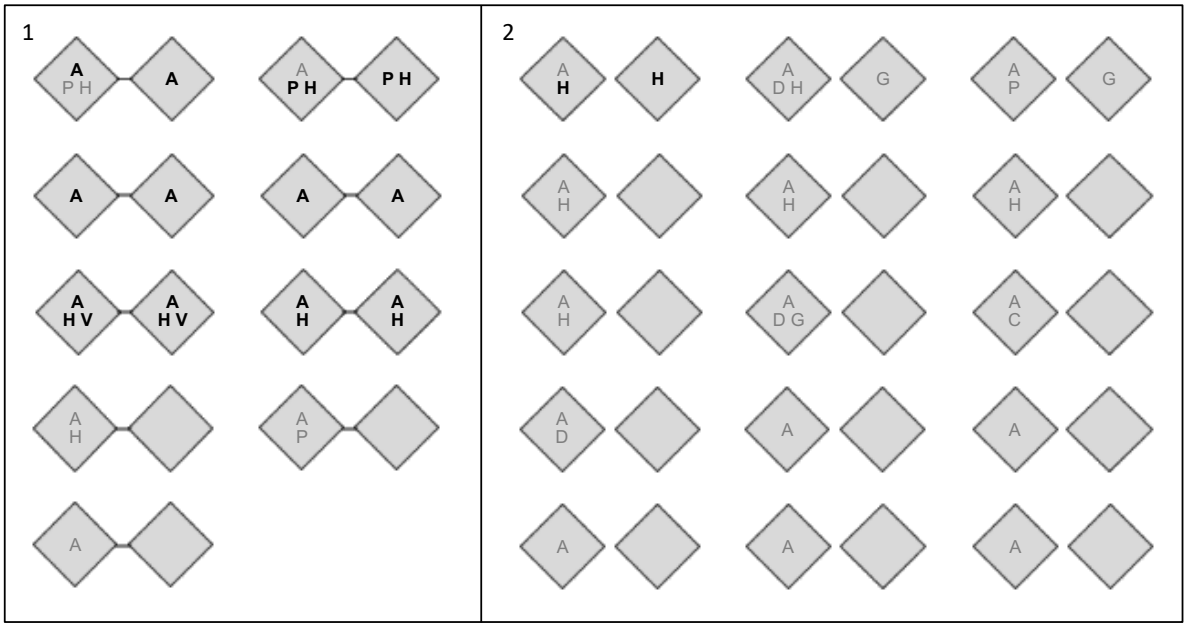


Figure 6. Organ-specific autoimmune diseases in monozygotic (1) and dizygotic (2) twin pairs with Addison's disease. (A) Addison´s disease, (H) Hashimoto's thyroiditis, (G) Grave´s disease, (D) Type-1 diabetes, (C) celiac disease, (P) pernicious anemia, (V) vitiligo. Concordant diagnoses in bold letters. Disease patterns are unique for most twin sets. To conceal identity, sex is not displayed. Reprinted from reference [171] under Creative Commons Attribution License CC-BY 4.0

4.2 STUDY II

In total, 1500 subjects with AD and 13,758 matched controls were identified using national health registers. Diagnostic records and prescription-patterns indicated that 23% of patients with AD had hypertension, 12.6% had dyslipidemia and 17.7% had diabetes (DM-1 or DM-2) (Table 3).

Adjusted HR for CVD among patients with AD compared to matched controls was 1.20 (95% CI 0.95-1.51). Stratifying CVD into ischemic heart disease (IHD) and cerebrovascular disease (CeVD), by sex, indicated that CVD was primarily driven by IHD in women (Table 4). The 30-day case-fatality rate in IHD was 41% in patients with AD, and 28% in matched controls (p=0.04). No difference in case-fatality rate was observed for CevD.

The mean dispensed dose of hydrocortisone was 29.6 mg/day, and of fludrocortisone 0.09 mg/day, with slightly higher doses in men. Stratifying glucocorticoid intake into tertiles and fludrocortisone into halves revealed an incremental increase in aHR for CVD with higher doses. This effect was more pronounced in women than in men (Figure 7).

Table 3. Age and comorbidities

	Addison's disease			Controls		
	Total	Women	Men	Total	Women	Men
Individuals, n (%)	1 500	818 (54.5)	682 (45.5)	13 758	7487 (54.4)	6271 (45.6)
Age at start of follow-up median (IQR)	50 (37-63)	54 (40-67)	47 (33-59)	49 (36-62)	52 (39-65)	45 (33-57)
Hypertension, n (%)	353 (23.5)	134 (16.4)	219 (32.1)	2 815 (20.5)	1,770 (23.6)	1,045 (16.7)
Dyslipidemia, n (%)	189 (12.6)	113 (13.8)	76 (11.1)	1,219 (8.9)	683 (9.1)	536 (8.5)
Diabetes, n (%)	266 (17.7)	140 (17.1)	126 (18.5)	649 (4.7)	362 (4.8)	287 (4.6)
Hashimoto's thyroiditis, n (%)	542 (36.1)	364 (44.5)	178 (26.1)	501 (3.6)	438 (5.9)	63 (1.0)
COPD, n (%)	18 (1.2)	12 (1.5)	6 (0.9)	113 (0.8)	72 (1.0)	41 (0.7)

COPD – Chronic obstructive pulmonary disease

Table 4. Unadjusted and adjusted HRs for cardiovascular events in subjects with AD vs matched controls, stratified by sex.

	Addison's disease (n=1,500)		Controls (n=13,758)				
Outcome	Events, n	Events/ 1000 PY	Events, n	Events/ 1000 PY	a HR[a]	95% CI	p-value
CVD[b,c]							
All	94	10.7	563	7.0	1.20	0.95-1.51	0.13
Male	40	10.3	270	7.6	1.05	0.74-1.50	0.79
Female	54	12.7	293	7.6	1.35	0.98-1.85	0.06
IHD							
All	71	8.1	338	4.2	1.61	1.22-2.12	0.001
Male	28	7.0	181	5.0	1.16	0.75-1.78	0.50
Female	43	9.8	157	3.9	2.15	1.49-3.10	<0.0001
CeVD							
All	44	5.0	343	4.3	0.88	0.63-1.23	0.46
Male	19	4.7	144	3.9	0.88	0.53-1.50	0.63
Female	25	5.6	199	4.9	0.88	0.56-1.37	0.57

[a]Adjusted for diabetes and chronic obstructive pulmonary disease. [b]CVD included IHD and CeVD. [c]Number of events do not equal the sum of IHD and CeVD because both can occur in the same individual.

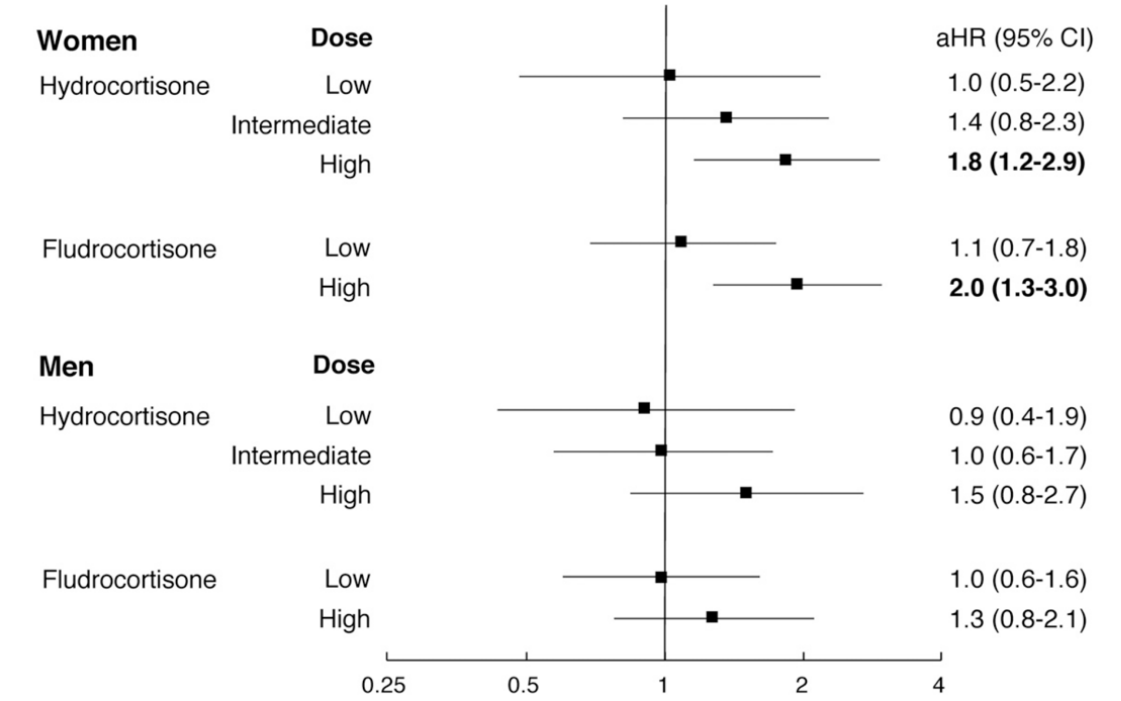


Figure 7. aHRs for CVD in subjects with AD vs matched controls by sex and by hydrocortisone/fludrocortisone dosing. Adjusted for diabetes and COPD in all models, and tertiles of hydrocortisone dosing or halves of fludrocortisone dosing as appropriate. Reprinted from reference [172] under Creative Commons Attribution License CC-BY 4.0

4.3 STUDY III

In a cohort of 110,814 twins we identified 3,882 individuals (3.5%) with at least one of the prespecified AIDs. HT was the most common disease with a prevalence of 2,4% in women and 0.5% in men, and AD was the rarest with a total of 28 cases. Cross-trait concordance (different AIDs in co-twins) was found in 153 twin pairs. Univariate heritability was calculated for each AID, and for an aggregate considering all AIDs as one phenotype (Table 5). The AE-model provided the best fit for all diseases except for GD where the ADE was marginally better. However, the broad sense heritability (A+D) was similar for the AE and the ADE model, and we therefore only presented results from the AE models (Table 5).

Attempts of calculating bivariate heritability for disease-pairs were mostly unsuccessful. Instead, familial aggregation and co-aggregation analyses were performed using Cox regression models. These demonstrated increased risk of not only diseases present in co-twins, but also of other AIDs. This effect was more pronounced in MZ than in DZ twins, demonstrating genetic influences on co-aggregation (Table 6).

Table 5. Estimated heritability and environmental factors from best-fitting models.

Disease	Proportions of variance			
	A	95% CI	E	95% CI
Hashimoto's thyroiditis	0.64	0.58-0.70	0.36	0.30-0.43
Atrophic gastritis	0.38	0.23-0.53	0.62	0.47-0.77
Celiac disease	0.91	0.87-0.94	0.09	0.06-0.13
Graves' disease	0.60	0.49-0.71	0.40	0.29-0.51
Type-1 diabetes	0.81	0.73-0.89	0.20	0.12-0.27
Vitiligo	0.65	0.50-0.81	0.35	0.19-0.50
Addison's disease	0.97	0.91-1.00	0.03	0.00-0.09
Any of the above diseases	0.69	0.65-0.73	0.31	0.27-0.35

A - additive genetic effects. E - effects form unique environment, not shared by twins.

Table 6. Familial aggregation and co-aggregation of autoimmunity

Index disease	Index disease			Non-index disease		
	aHR	95% CI	p-value	aHR	95% CI	p-value
Hashimoto's thyroiditis						
MZ	11.5	8.6-15.4	< 0.001	2.9	1.7-5.1	< 0.001
DZ	4.1	3.1-5.4		1.4	0.8-2.5	
Atrophic gastritis						
MZ	9.5	4.8-18.7	0.019	3.2	1.8-5.7	0.005
DZ	2.8	1.3-6.0		1.1	0.6-2.1	
Celiac disease						
MZ	124.0	80.7-192	< 0.001	2.2	1.0-4.9	< 0.001
DZ	15.8	10.3-24.4		2.6	1.5-4.4	
Graves' disease						
MZ	31.7	17.9-56.0	< 0.001	3.8	2.3-6.3	< 0.001
DZ	2.0	0.6-6.1		1.5	0.8-2.7	
Type-1 diabetes						
MZ	145	85-248	< 0.001	4.4	2.3-8.6	< 0.001
DZ	10.4	4.5-23.7		2.4	1.4-4.1	
Vitiligo						
MZ	77.5	28.4-212	< 0.001	3.0	0.7-12.0	0.060
DZ	0.0	0.0-0.0		2.4	0.7-7.6	
Addison's disease						
MZ	971.0	380-2477	< 0.001	9.2	2.4-35.4	0.299
DZ	0.0	0.0-0.0		10.0	2.4-40.8	
Any disease						
MZ	10.1	8.4-12.2	< 0.001	n.a.		-
DZ	2.8	2.4-3.4		n.a.		

Adjusted hazard ratios for developing index and non-index diseases if co-twin has autoimmune disease. Index disease – same disease as present in co-twin. Non-index disease – any of the other six diseases studied. Adjusted for date of birth, year of start of observation, date of diagnosis or date of right-censoring

4.4 STUDY IV

In a cohort of 110,814 twins we identified 1,683 (1.5%) twins with HT and 558 (0.5%) with GD. Both diseases were more common in women than in men with a prevalence of 24.0/1000 in women and 5.2/1000 in men for HT, and 8.9/1000 in women and 1.8/1000 in men for GD. HT and GD was present in 1,545 and 536 twin pairs respectively, with 31 pairs affected by both diseases (HT in one twin and GD in the other). In quantitative genetic modeling, the AE-models preferred over the ACE-models in both univariate and bivariate estimates of heritability. Hence, adjusted results from univariate AE-models are displayed in Table 7 and the bivariate AE-model are displayed in Figure 8. In the bivariate model, the additive genetic correlation (r^A) amounted to 0.35 (95% CI 0.20-0.50) and the unique environmental correlation (r^E) to -0.56 (95% CI -0.89- -0.22). The negative r^E partly reflects the case ascertainment process, where a previous diagnosis of GD was considered an exclusion criterion for HT. Etiologic overlap was small, with 8% of observed variance for both HT and GD explained by genetic factors shared with the other disease, and 11% of variance explained by shared environmental factors.

Table 7. Univariate estimates of explained variance for Hashimoto's thyroiditis and Graves' disease, by sex.

	Additive genetic effects			Non-shared environmental effects		
	A	95% CI	p-value	E	95% CI	p-value
Hashimoto's thyroiditis						
All	0.65	(0.60-0.70)		0.35	(0.30-0.40)	
Women	0.60	(0.54-0.66)	<0.001	0.40	(0.34-0.46)	<0.001
Men	0.90	(0.82-0.97)		0.10	(0.03-0.18)	
Graves' disease						
All	0.63	(0.54-0.72)		0.37	(0.28-0.46)	
Women	0.63	(0.52-0.73)	0.085	0.38	(0.27-0.48)	0.085
Men	0.79	(0.63-0.96)		0.21	(0.04-0.37)	

Models adjusted for age categories and sex when appropriate. p, p-value for difference between men and women tested using a Wald test. Age categories were < 1920, 1920-1939, 1940-1959, 1960-1979, > 1979; except Graves' in men, where categories were collapsed to <1940, 1940-1959, >1959 due to low prevalence.

	Explained variance	Hashimoto's thyroiditis	Graves' disease
A	Total	0.65 (0.61-0.70)	0.63 (0.55-0.72)
	Shared	0.08 (0.01-0.15)	0.08 (0.01-0.14)
	Not shared	0.57 (0.49-0.66)	0.56 (0.44-0.67)
E	Total	0.35 (0.30-0.40)	0.37 (0.28-0.46)
	Shared	0.11 (-0.02-0.24)	0.11 (-0.02-0.25)
	Not shared	0.24 (0.10-0.37)	0.25 (0.09-0.41)

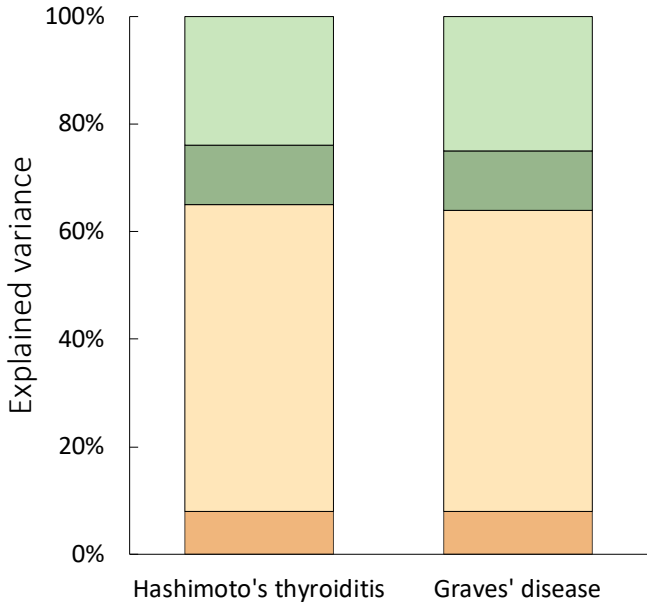


Figure 8. A - additive genetic effects. E – environmental effects not shared by co-twins. 95% confidence intervals in parenthesis. A is equivalent to heritability.

5 DISCUSSION

5.1 HERITABILITY IN ORGAN-SPECIFIC AUTOIMMUNITY

Combining phenotypical data with information on family-relations has helped us understand a great deal of how genes influence traits at a population-level. Most's AIDs so far explored are relatively common. AD on the other hand is rare, and even with a large twin registry such as the STR, we barely had enough data for calculating heritability. Our findings in **Study I** of a heritability near 1 for AD indicates that genetics effects are responsible for nearly all the observed variance. However, it is important to reiterate that a high heritability does not equate to near Mendelian patterns of inheritance, or exclude that environmental triggers are necessary for developing AD. It does however indicate that genetic influences are important, and that GWAS or similar studies would most likely be successful in identifying genes important in AD.

Current estimates of heritability for many of the AIDs so far explored stem from the late 1990s and the first decade of this century. The twin cohorts used back then were smaller than those we have at our disposal today. This forced investigators to extrapolate information from small cohorts od diseased twins, much like we did in **Study I**. For AD, a more precise estimate based on twin-data would require the combined efforts of many national registries, but for other AIDs, the STR now harbors a twin-cohort large enough for more precise estimates.

In **Study III**, we assembled information on seven common organ-specific AIDs. For all AIDs, the twin-cohort provided a larger sample of affected twins than available in any previously published study, allowing for robust estimates of heritability. For VI, our results complement previous estimates from family-based studies on subjects of mixed ethnic origin. For AG, our estimates are the first published, but results should be interpreted with caution, as case ascertainment for this diagnosis is difficult using registry data. For most other AIDs, our results were in line with previous results, but with a lower level statistical uncertainty due to larger sample sizes.

In **Study IV**, we looked in depth at the heritability of GD and HT. Our results suggest that genetic effects explain a larger proportion of variance in men than in women in both HT and GD, but the differences between the sexes were statistically significant for HT only. Whether different sets of genes or different magnitudes of effect of the same genes explain these findings is still unknown, as our data was insufficient for a qualitative analysis.

5.2 CO-AGGREGATION IN ORGAN-SPECIFIC AUTOIMMUNITY

Clustering of autoimmunity is common, but we are only just beginning to understand the underlying mechanisms. GWAS have provided evidence of pleiotropic genes influencing autoimmunity, but their combined effects explain only minor proportions of disease-overlap. At present, genomics has provided an explanatory model for how genes contribute co-aggregation in autoimmunity more than an actual explanation to observed variance.

Twin studies can be used to quantify genetic and environmental components in disease overlap. In autoimmunity, no such attempts have been published, most likely due to the large cohorts needed for such undertakings. In **Study I**, patterns of autoimmune clustering in MZ twins were strikingly similar, with concordance for 20 out of 28 autoimmune manifestations, whereas DZ twins did not display coherence in terms of phenotypes (Figure 6). An intriguing but speculative interpretation of this observation, is that pleiotropic loci interact to produce discrete risk profiles that are identical in MZ co-twins, but differ between DZ co-twins, consistent with the 'common variant/multiple disease' hypothesis [173]. This implies that with greater understanding of the genetic underpinnings of autoimmunity, we would in theory be able to predict what AIDs may develop in individuals prone to autoimmunity.

To further explore genetic and environmental influences on AIDs, we examined disease-overlap in a wider context in **Study III**. Overall, cross-trait concordance was surprisingly rare, present in only 153 out of 55,407 twin pairs (0.3%). Still, patterns of familial co-aggregation indicated an increased risk of both the same and of other AIDs than present in co-twins. Of note, co-aggregation appeared to be more common in MZ than in DZ pairs for most diseases, highlighting that genetic influences are important in co-aggregation.

In **Study IV**, with sufficient data for an in-depth examination of thyroid autoimmunity, we performed a bivariate analysis of heritability. Our findings of limited etiologic overlap between HT and GD are at odds with several studies reporting on co-occurrence (in individuals) of AITD.

We know that environmental triggers are essential to complex AIDs. This is illustrated by concordance rates for most AIDs well below 50% in MZ twins. This must be kept in mind when dissecting etiologic overlap using data on co-occurrence of diseases that affect the same organ. Both HT and GD have a profound effect on the thyroid gland, most likely altering expression and exposure of potential antigens. This could act as an environmental trigger, increasing the risk of additional thyroid autoimmunity. A similar mechanism is on display after radioiodine ablation of the thyroid gland. This leads to increased levels of circulating TRab [174], and sometimes induces or exacerbates thyroid associated orbitopathy [175].

Family-based studies on co-aggregation are perhaps better suited for evaluating disease-overlap, and most so far published imply that co-aggregation is common [48, 176-178]. However, most of these studies rely on hospital-based sampling in one form or another, making them vulnerable to selection bias. The only population-based study we have found reports a modest increase in risk of GD in probands if either a sibling or parent has HT [179]. Interestingly, the risk increases dramatically when HT is present in both a parent and a sibling.

5.3 CARDIOVASCULAR MORBIDITY IN ADDISON'S DISEASE

In the general population, the risk of CVD is closely linked to cardiometabolic risk factors such as smoking, hypertension, dyslipidemia, diabetes and obesity. Such factors likely contribute to the increased risk of IHD observed in **Study II**, but they do not offer a comprehensive explanation. In **Study II**, the use of antihypertensives was less common in subjects with AD compared to controls, whereas use of lipid-lowering agents was slightly more common. We did not have information on smoking or obesity among study participants, but previous reports on Swedish patients with AD suggest that smoking is rare (4%), that obesity (BMI $\geq$25 kg/m^2) is actually more common in matched controls, and that visceral fat content is similar in both groups [30, 180]. Diabetes, affecting nearly 18% of patients with AD in **Study II**, and only 5% of controls, is certainly responsible for part of the unadjusted risk, considering the high mortality observed in patients with diabetes and AD [181], but the increase in hazard for CVD remained after adjustment for this condition.

Atherogenic lipid-profiles, with higher levels of triglycerides and lower levels of high-density lipoprotein (HDL-C) are more common in patients with AD than in matched controls despite more frequent use of lipid-lowering agents [157, 158, 180]. This could be a mediator of CVD, but does not explain the difference in outcome for men and women, as dyslipidemia shows no convincing sex-differences as a cardiovascular risk factor [182, 183].

Concurrent autoimmunity could potentially explain the female preponderance in CVD. HT, more common in women than in men, and premature ovarian failure, are both linked to CVD. Androgen deficiency is perhaps a less appealing explanation, with no convincing evidence of lasting improvement in metabolic markers from DHEA-substitution [35, 36, 184], and with testosterone linked to shorter life spans in men [185].

Arguably, adverse effects from adrenal hormone replacement is the best explanation to the excess risk of IHD observed. We found an incremental increase in risk of CVD with higher glucocorticoid and mineralocorticoid replacement doses, and this effect appeared to be more prominent in women (Figure 7).

If other mechanisms contribute to CVD in AD is unknown, as most data on cardiovascular complications of glucocorticoids stem from studies on their use for anti-inflammatory purposes [186, 187]. Importantly, individuals included in such studies most likely retain an underlying capacity to secrete cortisol and maintain rudimentary diurnal and ultradian patterns, which is not the case in patients with AD. In line with our findings, over-substitution of glucocorticoids and mineralocorticoids may also be of greater concern in women than in men, since physiologic levels cortisol [188] and aldosterone [189, 190] appear to be lower in women.

The lack of a significant increase in risk of CVD in the overall cohort of patients with AD, but large effects in subgroups calls for caution, as the risk of random findings increases with subgroup-analyses. What speaks in favor of a causal relationship between replacement therapy and CVD is the dose-dependent effect, and indirect evidence in support of such mechanisms from findings in similar diseases and treatments. This is also true for our results of increased case-fatality rate in patients with AD, based on quite few cases. However, we know from other settings that patients with AD are at increased risk of dying in case of severe illness [143, 144, 191], and it seems reasonable that this would be true for IHD as well.

5.4 METHODOLOGICAL CONSIDERATIONS

5.4.1 Internal validity

Validation studies have indicated PPVs of 85-95% for diagnoses in the NPR, including AD [161]. In our experience, these numbers are overly optimistic. Disruption of endocrine function can occur in many different ways and at different levels of the hormonal axis (hypothalamic, pituitary, end-organ), but still result in similar phenotypes. We used strict diagnostic criteria outlined in section 3.2 to circumvent this problem. A challenge in validating HT is that most patients do not attend hospital-based clinics for thyroid check-ups, and therefore leave no diagnostic records in the NPR unless they seek hospital-care for other reasons. In contrast, liberal prescribing policies have left traces of levothyroxine-use in the SPDR for close to 10% of adult Swedish women (https://sdb.socialstyrelsen.se/if_lak/). Therefore, using prescriptions of levothyroxine to validate HT in the absence of an exclusion diagnosis in the NPR is still questionable.

In all works presented in this thesis, we have used strict inclusion criteria for diagnoses later used as variables in analytical statistics, but taken a more relaxed approach with diagnostic variables used in descriptive statistics only (HT in **Study II** for example). The purpose was to maximize specificity at the cost of reduced sensitivity for analytical variables, as a reduced specificity would invariably have led to unreliable estimates of heritability and relative risks [192, 193].

Using the SPDR to validate AD is essential, but it is not without consequence, as it can introduce immortal time bias [194]. In **Study II**, all patients diagnosed with AD before the introduction of the SPDR had to be alive in 2005 for validation. This in fact "immortalized" them, as all patients dying before 2005 were automatically excluded. The obvious strategy to eliminate immortal time is to include incident cases only (diagnosed after 2005), but that would have left us with a cohort of only 400 patients, leaving the study severely underpowered. Instead, we chose to match controls and subjects with AD and at the date of a first diagnostic record of AD in the NPR, and to exclude controls dying before the start of the SPDR, in essence immortalizing them as well. This left us with a comparatively healthy cohort, which may have led us to underestimate absolute risks, but enabled us to calculate more accurate relative risks.

5.4.2 External validity

Estimates of heritability belong to the population on which they are based, and should in theory be extrapolated to other settings with caution. In reality, they have proven quite stable across populations with reasonably similar socioeconomic environments [195]. For AITDs, a cause of concern is iodine status which can have a significant impact on the prevalence of thyroid autoimmunity, and which does differ considerably between countries and regions. AITDs are also heterogenic across ethnicities, with slightly different genetic mechanisms contributing to disease, and with different disease prevalences [127, 196]. The same is true for AD, with considerable genetic heterogeneity between populations [197].

Heritability has its limitations. The most obvious is that genetic studies in medicine have not been able to account for more than a minority of it. This has raised questions about the unexplained or 'missing heritability'. The narrow sense heritability (h^2) rests on the assumption that alleles influence phenotypic traits independently of each other in an additive manner. This is not always true, gene-gene interactions through dominance and epistasis have been demonstrated [198, 199], but the magnitude of this effect is unknown. Gene-environment interactions and epigenetic effects have also been suggested as explanations to missing heritability [200, 201]. However, with sample-sizes in GWAS growing, larger proportions of heritability are explained, either by a multitude of loci with small individual effects, [202, 203] or by rare variants with a large impact [204]. In the end, additive effects may still provide the best answer for most traits [205].

6 CONCLUSIONS AND IMPLICATIONS

In **Study I**, the probandwise concordance for AD in MZ twins was over 70%, meaning that co-twins of diseased twins are more likely than not to develop AD. In the rare instance of a MZ twin developing AD, it is therefore important to be wary of future symptoms consistent with AD in the co-twin. A direct consequence of a high MZ and low DZ concordance is that heritability will be high, for AD close to 1. This suggests that it is a disease well suited for future GWAS or whole genome sequencing.

In **Study II**, we found that women with AD are at increased risk of IHD, and that this risk correlates with hormone replacement doses. Similar findings have been reported for secondary adrenal insufficiency, underscoring the importance of using the lowest doses compatible with well-being, and treating cardiovascular risk factors at an early stage, in women in particular.

Many family studies have demonstrated overlap in AIDs, without differentiating genetic from environmental causes. In **Study III**, we provide the first objective evidence of genetic influences on disease clustering using epidemiological data. This corroborates findings from GWAS on overlap in AIDs, but more detailed studies are needed to better our understanding of how AIDs interrelate, and to help guide future genetic studies.

In **Study IV**, we demonstrate that etiologic overlap in GD and HT is modest at most, and that they should be considered as separate diseases. Moreover, we find that heritability plays a larger role in men than in women with AITD. The modest overlap between HT and GD reported in **Study IV** means that cross-trait concordance was rare, occurring in only 31 twin pairs. While our results most likely suffice to rule out major genetic overlap in AITDs, they need to be validated in other settings. Still, future genetic studies will most likely benefit from treating GD and HT, in men and in women, as separate disorders. Ideally, they should be powered to detect genetic effects in each group separately, as they may be quite different.

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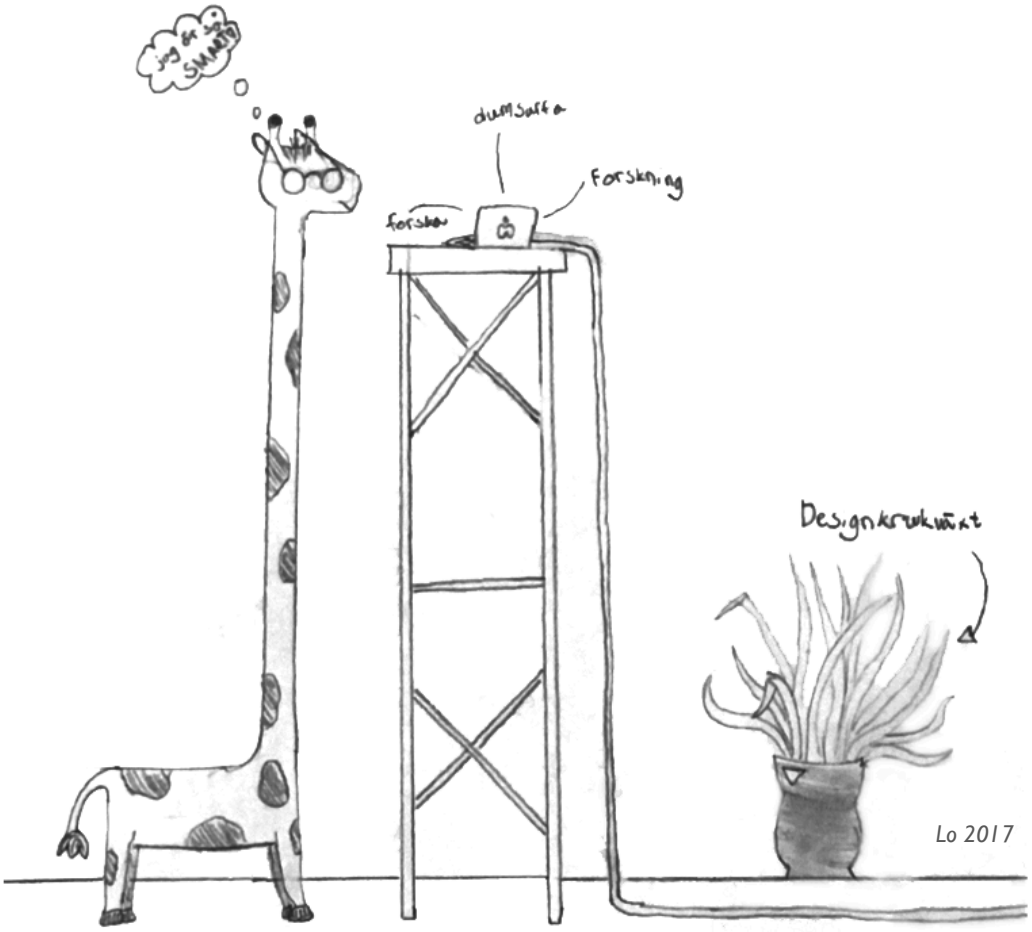

8 REFERENCES

1. Eaton, W.W., et al., *Epidemiology of autoimmune diseases in Denmark.* J Autoimmun, 2007. **29**(1): p. 1-9.
2. Cooper, G.S., M.L. Bynum, and E.C. Somers, *Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases.* J Autoimmun, 2009. **33**(3-4): p. 197-207.
3. Witebsky, E., et al., *Chronic thyroiditis and autoimmunization.* J Am Med Assoc, 1957. **164**(13): p. 1439-47.
4. Rose, N.R. and C. Bona, *Defining criteria for autoimmune diseases (Witebsky's postulates revisited).* Immunol Today, 1993. **14**(9): p. 426-30.
5. McGonagle, D. and M.F. McDermott, *A proposed classification of the immunological diseases.* PLoS Med, 2006. **3**(8): p. e297.
6. Rowley, M.J. and S.F. Whittingham, *The Role of Pathogenic Autoantibodies in Autoimmunity.* Antibodies, 2015. **4**(4): p. 314-353.
7. Lampeter, E.F., S.R. McCann, and H. Kolb, *Transfer of diabetes type 1 by bone-marrow transplantation.* Lancet, 1998. **351**(9102): p. 568-9.
8. Minchinton, R.M., et al., *Autoimmune thrombocytopenia acquired from an allogeneic bone-marrow graft.* Lancet, 1982. **2**(8299): p. 627-9.
9. Mastrandrea, L.D., *An Overview of Organ-Specific Autoimmune Diseases Including Immunotherapy.* Immunol Invest, 2015. **44**(8): p. 803-16.
10. Murray, G.R., *Note on the Treatment of Myxoedema by Hypodermic Injections of an Extract of the Thyroid Gland of a Sheep.* Br Med J, 1891. **2**(1606): p. 796-7.
11. Ngo, S.T., F.J. Steyn, and P.A. McCombe, *Gender differences in autoimmune disease.* Front Neuroendocrinol, 2014. **35**(3): p. 347-69.
12. Klein, S.L. and K.L. Flanagan, *Sex differences in immune responses.* Nat Rev Immunol, 2016. **16**(10): p. 626-38.
13. Johnson, M.B., A.T. Hattersley, and S.E. Flanagan, *Monogenic autoimmune diseases of the endocrine system.* Lancet Diabetes Endocrinol, 2016. **4**(10): p. 862-72.
14. Esselborn, V.M., et al., *The syndrome of familial juvenile hypoadrenocorticism, hypoparathyroidism and superficial moniliasis.* J Clin Endocrinol Metab, 1956. **16**(10): p. 1374-87.
15. Husebye, E.S., M.S. Anderson, and O. Kampe, *Autoimmune Polyendocrine Syndromes.* N Engl J Med, 2018. **378**(12): p. 1132-1141.
16. Cardenas-Roldan, J., A. Rojas-Villarraga, and J.M. Anaya, *How do autoimmune diseases cluster in families? A systematic review and meta-analysis.* BMC Med, 2013. **11**: p. 73.
17. Somers, E.C., et al., *Autoimmune diseases co-occurring within individuals and within families: a systematic review.* Epidemiology, 2006. **17**(2): p. 202-17.
18. Dunlop, D., *Eighty-Six Cases of Addison's Disease.* Br Med J, 1963. **2**(5362): p. 887-91.

19. Olafsson, A.S. and H.A. Sigurjonsdottir, *Increasing Prevalence of Addison Disease: Results from a Nationwide Study.* Endocr Pract, 2016. **22**(1): p. 30-5.
20. Meyer, G., et al., *Increasing prevalence of Addison's disease in German females: health insurance data 2008-2012.* Eur J Endocrinol, 2014. **170**(3): p. 367-73.
21. Laureti, S., et al., *Is the prevalence of Addison's disease underestimated?* J Clin Endocrinol Metab, 1999. **84**(5): p. 1762.
22. Kong, M.F. and W. Jeffcoate, *Eighty-six cases of Addison's disease.* Clin Endocrinol (Oxf), 1994. **41**(6): p. 757-61.
23. Mason, A.S., et al., *Epidemiological and clinical picture of Addison's disease.* Lancet, 1968. **2**(7571): p. 744-7.
24. Nerup, J., *Addison's disease--clinical studies. A report fo 108 cases.* Acta Endocrinol (Copenh), 1974. **76**(1): p. 127-41.
25. Willis, A.C. and F.P. Vince, *The prevalence of Addison's disease in Coventry, UK.* Postgrad Med J, 1997. **73**(859): p. 286-8.
26. Bjornsdottir, S., et al., *Drug prescription patterns in patients with Addison's disease: a Swedish population-based cohort study.* J Clin Endocrinol Metab, 2013. **98**(5): p. 2009-18.
27. Lovas, K. and E.S. Husebye, *High prevalence and increasing incidence of Addison's disease in western Norway.* Clin Endocrinol (Oxf), 2002. **56**(6): p. 787-91.
28. Betterle, C., et al., *Addison's disease: a survey on 633 patients in Padova.* Eur J Endocrinol, 2013. **169**(6): p. 773-84.
29. Erichsen, M.M., et al., *Clinical, immunological, and genetic features of autoimmune primary adrenal insufficiency: observations from a Norwegian registry.* J Clin Endocrinol Metab, 2009. **94**(12): p. 4882-90.
30. Dalin, F., et al., *Clinical and immunological characteristics of Autoimmune Addison's disease: a nationwide Swedish multicenter study.* J Clin Endocrinol Metab, 2016: p. jc20162522.
31. Lovas, K. and E.S. Husebye, *Replacement therapy for Addison's disease: recent developments.* Expert Opin Investig Drugs, 2008. **17**(4): p. 497-509.
32. Buckley, T.M. and A.F. Schatzberg, *Review: On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: Normal HPA axis activity and circadian rhythm, exemplary sleep disorders.* Journal of Clinical Endocrinology & Metabolism, 2005. **90**(5): p. 3106-3114.
33. Gurnell, E.M., et al., *Long-term DHEA replacement in primary adrenal insufficiency: a randomized, controlled trial.* J Clin Endocrinol Metab, 2008. **93**(2): p. 400-9.
34. Christiansen, J.J., et al., *Dehydroepiandrosterone substitution in female adrenal failure: no impact on endothelial function and cardiovascular parameters despite normalization of androgen status.* Clin Endocrinol (Oxf), 2007. **66**(3): p. 426-33.
35. Rice, S.P., et al., *Effects of dehydroepiandrosterone replacement on vascular function in primary and secondary adrenal insufficiency: a randomized crossover trial.* J Clin Endocrinol Metab, 2009. **94**(6): p. 1966-72.

36. Srinivasan, M., et al., *Effect of dehydroepiandrosterone replacement on lipoprotein profile in hypoadrenal women.* J Clin Endocrinol Metab, 2009. **94**(3): p. 761-4.
37. Hashimoto, H., *Zur Kenntniss der lymphomatösen Veränderung der Schilddrüse (Struma lymphomatosa).* Arch Klin Chir, 1912. **97**: p. 219–248.
38. Leese, G.P., et al., *Increasing prevalence and incidence of thyroid disease in Tayside, Scotland: the Thyroid Epidemiology Audit and Research Study (TEARS).* Clin Endocrinol (Oxf), 2008. **68**(2): p. 311-6.
39. Vanderpump, M.P., et al., *The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey.* Clin Endocrinol (Oxf), 1995. **43**(1): p. 55-68.
40. Rodriguez-Gutierrez, R., et al., *Levothyroxine overuse: time for an about face?* Lancet Diabetes Endocrinol, 2017. **5**(4): p. 246-248.
41. Triolo, T.M., et al., *Additional autoimmune disease found in 33% of patients at type 1 diabetes onset.* Diabetes Care, 2011. **34**(5): p. 1211-3.
42. Sheth, V.M., Y. Guo, and A.A. Qureshi, *Comorbidities associated with vitiligo: a ten-year retrospective study.* Dermatology, 2013. **227**(4): p. 311-5.
43. Hemminki, K., et al., *Familial associations of rheumatoid arthritis with autoimmune diseases and related conditions.* Arthritis Rheum, 2009. **60**(3): p. 661-8.
44. Barcellos, L.F., et al., *Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study.* Lancet Neurol, 2006. **5**(11): p. 924-31.
45. Somers, E.C., et al., *Are individuals with an autoimmune disease at higher risk of a second autoimmune disorder?* Am J Epidemiol, 2009. **169**(6): p. 749-55.
46. McLeod, D.S. and D.S. Cooper, *The incidence and prevalence of thyroid autoimmunity.* Endocrine, 2012. **42**(2): p. 252-65.
47. Abraham-Nordling, M., et al., *Incidence of hyperthyroidism in Sweden.* Eur J Endocrinol, 2011. **165**(6): p. 899-905.
48. Boelaert, K., et al., *Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease.* Am J Med, 2010. **123**(2): p. 183 e1-9.
49. Galton, F., *The History of Twins, As A Criterion Of The Relative Powers of Nature And Nurture(1,2).* International Journal of Epidemiology, 2012. **41**(4): p. 905-911.
50. Liew, S.H., et al., *The first "classical" twin study? Analysis of refractive error using monozygotic and dizygotic twins published in 1922.* Twin Res Hum Genet, 2005. **8**(3): p. 198-200.
51. Bell, A.E., *Heritability in retrospect.* J Hered, 1977. **68**(5): p. 297-300.
52. Tenesa, A. and C.S. Haley, *The heritability of human disease: estimation, uses and abuses.* Nat Rev Genet, 2013. **14**(2): p. 139-49.
53. Kendler, K.S., et al., *A test of the equal-environment assumption in twin studies of psychiatric illness.* Behav Genet, 1993. **23**(1): p. 21-7.
54. Skytthe, A., et al., *The Danish Twin Registry in the new millennium.* Twin Res Hum Genet, 2006. **9**(6): p. 763-71.
55. Boomsma, D., A. Busjahn, and L. Peltonen, *Classical twin studies and beyond.* Nat Rev Genet, 2002. **3**(11): p. 872-82.

56. Ronald, A. and R.A. Hoekstra, *Autism spectrum disorders and autistic traits: a decade of new twin studies.* Am J Med Genet B Neuropsychiatr Genet, 2011. **156B**(3): p. 255-74.
57. Lichtenstein, P., et al., *Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study.* Lancet, 2009. **373**(9659): p. 234-9.
58. Hettema, J.M., et al., *A population-based twin study of the relationship between neuroticism and internalizing disorders.* Am J Psychiatry, 2006. **163**(5): p. 857-64.
59. Rijsdijk, F.V. and P.C. Sham, *Analytic approaches to twin data using structural equation models.* Brief Bioinform, 2002. **3**(2): p. 119-33.
60. Tysk, C., et al., *Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking.* Gut, 1988. **29**(7): p. 990-6.
61. Bogdanos, D.P., et al., *Twin studies in autoimmune disease: genetics, gender and environment.* J Autoimmun, 2012. **38**(2-3): p. J156-69.
62. Kyvik, K.O., A. Green, and H. Beck-Nielsen, *Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins.* BMJ, 1995. **311**(7010): p. 913-7.
63. Hyttinen, V., et al., *Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study.* Diabetes, 2003. **52**(4): p. 1052-5.
64. Hansen, P.S., et al., *The relative importance of genetic and environmental effects for the early stages of thyroid autoimmunity: a study of healthy Danish twins.* Eur J Endocrinol, 2006. **154**(1): p. 29-38.
65. Brix, T.H., et al., *Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts.* J Clin Endocrinol Metab, 2001. **86**(2): p. 930-4.
66. Nistico, L., et al., *Concordance, disease progression, and heritability of coeliac disease in Italian twins.* Gut, 2006. **55**(6): p. 803-8.
67. Kuja-Halkola, R., et al., *Heritability of non-HLA genetics in coeliac disease: a population-based study in 107 000 twins.* Gut, 2016.
68. Hafez, M., L. Sharaf, and S.M. Abd el-Nabi, *The genetics of vitiligo.* Acta Derm Venereol, 1983. **63**(3): p. 249-51.
69. Das, S.K., et al., *Studies on vitiligo. II. Familial aggregation and genetics.* Genet Epidemiol, 1985. **2**(3): p. 255-62.
70. Zhang, X.J., et al., *Characteristics of genetic epidemiology and genetic models for vitiligo.* J Am Acad Dermatol, 2004. **51**(3): p. 383-90.
71. Halfvarson, J., *Genetics in twins with Crohn's disease: less pronounced than previously believed?* Inflamm Bowel Dis, 2011. **17**(1): p. 6-12.
72. Westerlind, H., et al., *Modest familial risks for multiple sclerosis: a registry-based study of the population of Sweden.* Brain, 2014. **137**(Pt 3): p. 770-8.
73. Grumet, F.C., et al., *Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus. A possible genetic predisposition to disease.* N Engl J Med, 1971. **285**(4): p. 193-6.
74. Singal, D.P. and M.A. Blajchman, *Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus.* Diabetes, 1973. **22**(6): p. 429-32.

75. Hugot, J.P., et al., *Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease.* Nature, 2001. **411**(6837): p. 599-603.
76. Lander, E.S. and N.J. Schork, *Genetic dissection of complex traits.* Science, 1994. **265**(5181): p. 2037-48.
77. Deitiker, P. and M.Z. Atassi, *Non-MHC genes linked to autoimmune disease.* Crit Rev Immunol, 2012. **32**(3): p. 193-285.
78. Zhernakova, A., S. Withoff, and C. Wijmenga, *Clinical implications of shared genetics and pathogenesis in autoimmune diseases.* Nat Rev Endocrinol, 2013. **9**(11): p. 646-59.
79. Dechairo, B.M., et al., *Association of the TSHR gene with Graves' disease: the first disease specific locus.* Eur J Hum Genet, 2005. **13**(11): p. 1223-30.
80. Bennett, S.T. and J.A. Todd, *Human type 1 diabetes and the insulin gene: principles of mapping polygenes.* Annu Rev Genet, 1996. **30**: p. 343-70.
81. Cotsapas, C., et al., *Pervasive sharing of genetic effects in autoimmune disease.* PLoS Genet, 2011. **7**(8): p. e1002254.
82. Li, Y.R., et al., *Genetic sharing and heritability of paediatric age of onset autoimmune diseases.* Nat Commun, 2015. **6**: p. 8442.
83. Johar, A., et al., *Definition of mutations in polyautoimmunity.* J Autoimmun, 2016.
84. Hemminki, K., et al., *Familial association between type 1 diabetes and other autoimmune and related diseases.* Diabetologia, 2009. **52**(9): p. 1820-8.
85. Finnish-German, A.C., *An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains.* Nat Genet, 1997. **17**(4): p. 399-403.
86. Nagamine, K., et al., *Positional cloning of the APECED gene.* Nat Genet, 1997. **17**(4): p. 393-8.
87. Myhre, A.G., et al., *Autoimmune adrenocortical failure in Norway autoantibodies and human leukocyte antigen class II associations related to clinical features.* J Clin Endocrinol Metab, 2002. **87**(2): p. 618-23.
88. Skinningsrud, B., et al., *Multiple loci in the HLA complex are associated with Addison's disease.* J Clin Endocrinol Metab, 2011. **96**(10): p. E1703-8.
89. Ross, I.L., et al., *HLA similarities indicate shared genetic risk in 21-hydroxylase autoantibody positive South African and United States Addison's disease.* Tissue Antigens, 2014. **84**(4): p. 361-9.
90. Donner, H., et al., *Codon 17 polymorphism of the cytotoxic T lymphocyte antigen 4 gene in Hashimoto's thyroiditis and Addison's disease.* J Clin Endocrinol Metab, 1997. **82**(12): p. 4130-2.
91. Vaidya, B., et al., *Association analysis of the cytotoxic T lymphocyte antigen-4 (CTLA-4) and autoimmune regulator-1 (AIRE-1) genes in sporadic autoimmune Addison's disease.* J Clin Endocrinol Metab, 2000. **85**(2): p. 688-91.
92. Blomhoff, A., et al., *Polymorphisms in the cytotoxic T lymphocyte antigen-4 gene region confer susceptibility to Addison's disease.* J Clin Endocrinol Metab, 2004. **89**(7): p. 3474-6.
93. Roycroft, M., et al., *The tryptophan 620 allele of the lymphoid tyrosine phosphatase (PTPN22) gene predisposes to autoimmune Addison's disease.* Clin Endocrinol (Oxf), 2009. **70**(3): p. 358-62.

94. Pazderska, A., et al., *A variant in the BACH2 gene is associated with susceptibility to autoimmune Addison's disease in humans.* J Clin Endocrinol Metab, 2016: p. jc20162368.
95. Eriksson, D., et al., *Extended exome sequencing identifies BACH2 as a novel major risk locus for Addison's disease.* J Intern Med, 2016. **280**(6): p. 595-608.
96. Eriksson, D., et al., *Common genetic variation in the autoimmune regulator (AIRE) locus is associated with autoimmune Addison's disease in Sweden.* Sci Rep, 2018. **8**(1): p. 8395.
97. Heggarty, H., *Addison's disease in identical twins.* Br Med J, 1968. **1**(5591): p. 559.
98. Russell, G.A., et al., *Autoimmune Addison's disease and thyrotoxic thyroiditis presenting as encephalopathy in twins.* Arch Dis Child, 1991. **66**(3): p. 350-2.
99. Simmonds, J.P. and J. Lister, *Auto-immune Addison's disease in identical twins.* Postgrad Med J, 1978. **54**(634): p. 552-4.
100. Fairchild, R.S., R.N. Schimke, and N.I. Abdou, *Immunoregulation abnormalities in familial Addison's disease.* J Clin Endocrinol Metab, 1980. **51**(5): p. 1074-7.
101. Mitchell, A.L. and S.H. Pearce, *Autoimmune Addison disease: pathophysiology and genetic complexity.* Nat Rev Endocrinol, 2012. **8**(5): p. 306-16.
102. Ikegami, H., et al., *The association of CTLA4 polymorphism with type 1 diabetes is concentrated in patients complicated with autoimmune thyroid disease: A multicenter collaborative study in Japan.* Journal of Clinical Endocrinology & Metabolism, 2006. **91**(3): p. 1087-1092.
103. Donner, H., et al., *Codon 17 polymorphism of the cytotoxic T lymphocyte antigen 4 gene in Hashimoto's thyroiditis and Addison's disease.* Journal of Clinical Endocrinology & Metabolism, 1997. **82**(12): p. 4130-4132.
104. Vaidya, B., et al., *The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus.* Human Molecular Genetics, 1999. **8**(7): p. 1195-1199.
105. Yanagawa, T., et al., *CTLA-4 GENE POLYMORPHISM ASSOCIATED WITH GRAVES-DISEASE IN A CAUCASIAN POPULATION.* Journal of Clinical Endocrinology & Metabolism, 1995. **80**(1): p. 41-45.
106. Burn, G.L., et al., *Why is PTPN22 a good candidate susceptibility gene for autoimmune disease?* FEBS Lett, 2011. **585**(23): p. 3689-98.
107. Eriksson, N., et al., *Novel associations for hypothyroidism include known autoimmune risk loci.* PLoS One, 2012. **7**(4): p. e34442.
108. Criswell, L.A., et al., *Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes.* Am J Hum Genet, 2005. **76**(4): p. 561-71.
109. Velaga, M.R., et al., *The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease.* J Clin Endocrinol Metab, 2004. **89**(11): p. 5862-5.
110. Smyth, D., et al., *Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus.* Diabetes, 2004. **53**(11): p. 3020-3.

111. Scalapino, K.J. and D.I. Daikh, *CTLA-4: a key regulatory point in the control of autoimmune disease.* Immunol Rev, 2008. **223**: p. 143-55.
112. Medici, M., et al., *Identification of Novel Genetic Loci Associated with Thyroid Peroxidase Antibodies and Clinical Thyroid Disease.* Plos Genetics, 2014. **10**(2): p. 13.
113. Marroqui, L., et al., *BACH2, a candidate risk gene for type 1 diabetes, regulates apoptosis in pancreatic beta-cells via JNK1 modulation and crosstalk with the candidate gene PTPN2.* Diabetes, 2014. **63**(7): p. 2516-27.
114. McAllister, K., et al., *Identification of BACH2 and RAD51B as rheumatoid arthritis susceptibility loci in a meta-analysis of genome-wide data.* Arthritis Rheum, 2013. **65**(12): p. 3058-62.
115. Skinningsrud, B., et al., *Polymorphisms in CLEC16A and CIITA at 16p13 are associated with primary adrenal insufficiency.* J Clin Endocrinol Metab, 2008. **93**(9): p. 3310-7.
116. Muhali, F.S., et al., *Polymorphisms of CLEC16A Region and Autoimmune Thyroid Diseases.* G3-Genes Genomes Genetics, 2014. **4**(6): p. 973-977.
117. Awata, T., et al., *Association of Type 1 Diabetes with Two Loci on 12q13 and 16p13 and the Influence Coexisting Thyroid Autoimmunity in Japanese.* Journal of Clinical Endocrinology & Metabolism, 2009. **94**(1): p. 231-235.
118. Booth, D.R., et al., *The expanding genetic overlap between multiple sclerosis and type I diabetes.* Genes and Immunity, 2009. **10**(1): p. 11-14.
119. Brix, T.H., et al., *A population-based study of Graves' disease in Danish twins.* Clin Endocrinol (Oxf), 1998. **48**(4): p. 397-400.
120. Brix, T.H., K.O. Kyvik, and L. Hegedus, *A population-based study of chronic autoimmune hypothyroidism in Danish twins.* J Clin Endocrinol Metab, 2000. **85**(2): p. 536-9.
121. Laurberg, P., et al., *Iodine intake as a determinant of thyroid disorders in populations.* Best Pract Res Clin Endocrinol Metab, 2010. **24**(1): p. 13-27.
122. Wiersinga, W.M., *Smoking and thyroid.* Clin Endocrinol (Oxf), 2013. **79**(2): p. 145-51.
123. Nakamura, H., et al., *Prevalence of interrelated autoantibodies in thyroid diseases and autoimmune disorders.* Journal of Endocrinological Investigation, 2008. **31**(10): p. 861-865.
124. Simmonds, M.J., *GWAS in autoimmune thyroid disease: redefining our understanding of pathogenesis.* Nature Reviews Endocrinology, 2013. **9**(5): p. 277-287.
125. Hwangbo, Y. and Y.J. Park, *Genome-Wide Association Studies of Autoimmune Thyroid Diseases, Thyroid Function, and Thyroid Cancer.* Endocrinol Metab (Seoul), 2018. **33**(2): p. 175-184.
126. Antonelli, A., et al., *Autoimmune thyroid disorders.* Autoimmun Rev, 2015. **14**(2): p. 174-80.
127. Lee, H.J., et al., *Immunogenetics of autoimmune thyroid diseases: A comprehensive review.* J Autoimmun, 2015. **64**: p. 82-90.
128. Bliddal, S., C.H. Nielsen, and U. Feldt-Rasmussen, *Recent advances in understanding autoimmune thyroid disease: the tallest tree in the forest of polyautoimmunity.* F1000Res, 2017. **6**: p. 1776.

129. Gonzalez-Aguilera, B., et al., *Conversion to Graves disease from Hashimoto thyroiditis: a study of 24 patients.* Arch Endocrinol Metab, 2018. **62**(6): p. 609-614.
130. Sjolin, G., et al., *The Long-Term Outcome of Treatment for Graves' Hyperthyroidism.* Thyroid, 2019. **29**(11): p. 1545-1557.
131. Tomer, Y., et al., *Common and unique susceptibility loci in Graves and Hashimoto diseases: results of whole-genome screening in a data set of 102 multiplex families.* Am J Hum Genet, 2003. **73**(4): p. 736-47.
132. Strieder, T.G., et al., *Prediction of progression to overt hypothyroidism or hyperthyroidism in female relatives of patients with autoimmune thyroid disease using the Thyroid Events Amsterdam (THEA) score.* Arch Intern Med, 2008. **168**(15): p. 1657-63.
133. Ilicki, A., C. Marcus, and F.A. Karlsson, *Hyperthyroidism and hypothyroidism in monozygotic twins: detection of stimulating and blocking THS receptor antibodies using the FRTL5-cell line.* J Endocrinol Invest, 1990. **13**(4): p. 327-31.
134. Jayson, M.I., et al., *Thyrotoxicosis and Hashimoto goitre in a pair of monozygotic twins with serum long-acting thyroid stimulator.* Lancet, 1967. **2**(7505): p. 15-8.
135. Chertow, B.S., W.J. Fidler, and B.L. Fariss, *Graves' disease and Hashimoto's thyroiditis in monozygous twins.* Acta Endocrinol (Copenh), 1973. **72**(1): p. 18-24.
136. Biondi, B. and G.J. Kahaly, *Cardiovascular involvement in patients with different causes of hyperthyroidism.* Nat Rev Endocrinol, 2010. **6**(8): p. 431-43.
137. Brandt, F., et al., *Morbidity before and after the diagnosis of hyperthyroidism: a nationwide register-based study.* PLoS One, 2013. **8**(6): p. e66711.
138. Jabbar, A., et al., *Thyroid hormones and cardiovascular disease.* Nat Rev Cardiol, 2017. **14**(1): p. 39-55.
139. Lillevang-Johansen, M., et al., *Duration of over- and under-treatment of hypothyroidism is associated with increased cardiovascular risk.* Eur J Endocrinol, 2019. **180**(6): p. 407-416.
140. Thvilum, M., et al., *Type and extent of somatic morbidity before and after the diagnosis of hypothyroidism. a nationwide register study.* PLoS One, 2013. **8**(9): p. e75789.
141. Arlt, W. and B. Allolio, *Adrenal insufficiency.* The Lancet, 2003. **361**(9372): p. 1881-1893.
142. Bergthorsdottir, R., et al., *Premature mortality in patients with Addison's disease: a population-based study.* J Clin Endocrinol Metab, 2006. **91**(12): p. 4849-53.
143. Bensing, S., et al., *Increased death risk and altered cancer incidence pattern in patients with isolated or combined autoimmune primary adrenocortical insufficiency.* Clin Endocrinol (Oxf), 2008. **69**(5): p. 697-704.
144. Erichsen, M.M., et al., *Normal overall mortality rate in Addison's disease, but young patients are at risk of premature death.* Eur J Endocrinol, 2009. **160**(2): p. 233-7.

145. Johannsson, G., et al., *Adrenal insufficiency: review of clinical outcomes with current glucocorticoid replacement therapy.* Clin Endocrinol (Oxf), 2015. **82**(1): p. 2-11.
146. Crown, A. and S. Lightman, *Why is the management of glucocorticoid deficiency still controversial: a review of the literature.* Clin Endocrinol (Oxf), 2005. **63**(5): p. 483-92.
147. Walker, B.R., *Glucocorticoids and cardiovascular disease.* Eur J Endocrinol, 2007. **157**(5): p. 545-59.
148. Esteban, N.V., et al., *Daily cortisol production rate in man determined by stable isotope dilution/mass spectrometry.* J Clin Endocrinol Metab, 1991. **72**(1): p. 39-45.
149. Kerrigan, J.R., et al., *Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis.* J Clin Endocrinol Metab, 1993. **76**(6): p. 1505-10.
150. Zueger, T., et al., *Glucocorticoid replacement and mortality in patients with nonfunctioning pituitary adenoma.* J Clin Endocrinol Metab, 2012. **97**(10): p. E1938-42.
151. Sherlock, M., et al., *ACTH deficiency, higher doses of hydrocortisone replacement, and radiotherapy are independent predictors of mortality in patients with acromegaly.* J Clin Endocrinol Metab, 2009. **94**(11): p. 4216-23.
152. Savard, S., et al., *Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study.* Hypertension, 2013. **62**(2): p. 331-6.
153. Bhattacharyya, A. and D.J. Tymms, *Heart failure with fludrocortisone in Addison's disease.* J R Soc Med, 1998. **91**(8): p. 433-4.
154. Campean, R., et al., *Takotsubo-like syndrome triggered by fludrocortisone overdose for Addison's disease: a case report.* J Med Case Rep, 2016. **10**(1): p. 281.
155. Willis, F.R., G.C. Byrne, and T.W. Jones, *Fludrocortisone induced heart failure in Addison's disease.* J Paediatr Child Health, 1994. **30**(3): p. 280-1.
156. Stewart, P.M., et al., *Exploring Inpatient Hospitalizations and Morbidity in Patients with Adrenal Insufficiency.* J Clin Endocrinol Metab, 2016: p. jc20162221.
157. Giordano, R., et al., *Metabolic and cardiovascular profile in patients with Addison's disease under conventional glucocorticoid replacement.* J Endocrinol Invest, 2009. **32**(11): p. 917-23.
158. Ross, I.L., et al., *Increased cardiovascular risk in South African patients with Addison's disease.* Horm Metab Res, 2013. **45**(12): p. 905-10.
159. Ludvigsson, J.F., et al., *The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research.* Eur J Epidemiol, 2009. **24**(11): p. 659-67.
160. Ludvigsson, J.F., et al., *Registers of the Swedish total population and their use in medical research.* Eur J Epidemiol, 2016. **31**(2): p. 125-36.
161. Ludvigsson, J.F., et al., *External review and validation of the Swedish national inpatient register.* BMC Public Health, 2011. **11**: p. 450.
162. Wettermark, B., et al., *The new Swedish Prescribed Drug Register--opportunities for pharmacoepidemiological research and experience from the first six months.* Pharmacoepidemiol Drug Saf, 2007. **16**(7): p. 726-35.

163. Johansson, L.A. and R. Westerling, *Comparing Swedish hospital discharge records with death certificates: implications for mortality statistics.* Int J Epidemiol, 2000. **29**(3): p. 495-502.
164. Johansson, L.A., C. Bjorkenstam, and R. Westerling, *Unexplained differences between hospital and mortality data indicated mistakes in death certification: an investigation of 1,094 deaths in Sweden during 1995.* J Clin Epidemiol, 2009. **62**(11): p. 1202-9.
165. Zagai, U., et al., *The Swedish Twin Registry: Content and Management as a Research Infrastructure.* Twin Res Hum Genet, 2019. **22**(6): p. 672-680.
166. Gudbjornsdottir, S., et al., *The National Diabetes Register in Sweden: an implementation of the St. Vincent Declaration for Quality Improvement in Diabetes Care.* Diabetes Care, 2003. **26**(4): p. 1270-6.
167. Kaprio, J., et al., *Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland.* Diabetologia, 1992. **35**(11): p. 1060-7.
168. MacGregor, A.J., et al., *Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins.* Arthritis Rheum, 2000. **43**(1): p. 30-7.
169. Neale, M.C., et al., *OpenMx 2.0: Extended Structural Equation and Statistical Modeling.* Psychometrika, 2016. **81**(2): p. 535-49.
170. Team, R.C., *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2012.* URL http://www. R-project. org, 2018.
171. Skov, J., et al., *Heritability of Addison's disease and prevalence of associated autoimmunity in a cohort of 112,100 Swedish twins.* Endocrine, 2017.
172. Skov, J., et al., *Sex-Specific Risk of Cardiovascular Disease in Autoimmune Addison Disease-A Population-Based Cohort Study.* J Clin Endocrinol Metab, 2019. **104**(6): p. 2031-2040.
173. Becker, K.G., *The common variants/multiple disease hypothesis of common complex genetic disorders.* Medical Hypotheses, 2004. **62**(2): p. 309-317.
174. Laurberg, P., et al., *TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study.* Eur J Endocrinol, 2008. **158**(1): p. 69-75.
175. Traisk, F., et al., *Thyroid-associated ophthalmopathy after treatment for Graves' hyperthyroidism with antithyroid drugs or iodine-131.* J Clin Endocrinol Metab, 2009. **94**(10): p. 3700-7.
176. Dittmar, M., et al., *Increased Familial Clustering of Autoimmune Thyroid Diseases.* Hormone and Metabolic Research, 2011. **43**(3): p. 200-204.
177. Villanueva, R., et al., *Sibling recurrence risk in autoimmune thyroid disease.* Thyroid, 2003. **13**(8): p. 761-764.
178. Manji, N., et al., *Influences of age, gender, smoking, and family history on autoimmune thyroid disease phenotype.* J Clin Endocrinol Metab, 2006. **91**(12): p. 4873-80.
179. Hemminki, K., et al., *The epidemiology of Graves' disease: evidence of a genetic and an environmental contribution.* J Autoimmun, 2010. **34**(3): p. J307-13.

180. Bergthorsdottir, R., et al., *Visceral Fat and Novel Biomarkers of Cardiovascular Disease in Patients with Addison's Disease; a case-control study.* J Clin Endocrinol Metab, 2017.
181. Chantzichristos, D., et al., *Mortality in patients with diabetes mellitus and Addison's disease: a nationwide, matched, observational cohort study.* Eur J Endocrinol, 2017. **176**(1): p. 31-39.
182. Appelman, Y., et al., *Sex differences in cardiovascular risk factors and disease prevention.* Atherosclerosis, 2015. **241**(1): p. 211-8.
183. Di Angelantonio, E., et al., *Major Lipids, Apolipoproteins, and Risk of Vascular Disease.* Jama-Journal of the American Medical Association, 2009. **302**(18): p. 1993-2000.
184. Arlt, W., et al., *Dehydroepiandrosterone replacement in women with adrenal insufficiency.* N Engl J Med, 1999. **341**(14): p. 1013-20.
185. Min, K.J., C.K. Lee, and H.N. Park, *The lifespan of Korean eunuchs.* Curr Biol, 2012. **22**(18): p. R792-3.
186. Wei, L., T.M. MacDonald, and B.R. Walker, *Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease.* Ann Intern Med, 2004. **141**(10): p. 764-70.
187. Souverein, P.C., et al., *Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study.* Heart, 2004. **90**(8): p. 859-65.
188. Roelfsema, F., et al., *Impact of age, sex and body mass index on cortisol secretion in 143 healthy adults.* Endocr Connect, 2017. **6**(7): p. 500-509.
189. Toering, T.J., et al., *Sex differences in renin-angiotensin-aldosterone system affect extracellular volume in healthy subjects.* Am J Physiol Renal Physiol, 2018. **314**(5): p. F873-F878.
190. Miller, J.A., L.A. Anacta, and D.C. Cattran, *Impact of gender on the renal response to angiotensin II.* Kidney Int, 1999. **55**(1): p. 278-85.
191. Smans, L.C., et al., *Incidence of adrenal crisis in patients with adrenal insufficiency.* Clin Endocrinol (Oxf), 2016. **84**(1): p. 17-22.
192. Bishop, S.C. and J.A. Woolliams, *On the genetic interpretation of disease data.* PLoS One, 2010. **5**(1): p. e8940.
193. Copeland, K.T., et al., *BIAS DUE TO MISCLASSIFICATION IN ESTIMATION OF RELATIVE RISK.* American Journal of Epidemiology, 1977. **105**(5): p. 488-495.
194. Shariff, S.Z., et al., *The secret of immortal time bias in epidemiologic studies.* J Am Soc Nephrol, 2008. **19**(5): p. 841-3.
195. Visscher, P.M., W.G. Hill, and N.R. Wray, *Heritability in the genomics era--concepts and misconceptions.* Nat Rev Genet, 2008. **9**(4): p. 255-66.
196. McLeod, D.S., et al., *Variation in rates of autoimmune thyroid disease by race/ethnicity in US military personnel.* JAMA, 2014. **311**(15): p. 1563-5.
197. Mitchell, A.L., et al., *Association of autoimmune Addison's disease with alleles of STAT4 and GATA3 in European cohorts.* PLoS One, 2014. **9**(3): p. e88991.
198. Carlborg, O. and C.S. Haley, *Epistasis: too often neglected in complex trait studies?* Nat Rev Genet, 2004. **5**(8): p. 618-25.

199. Phillips, P.C., *Epistasis--the essential role of gene interactions in the structure and evolution of genetic systems.* Nat Rev Genet, 2008. **9**(11): p. 855-67.
200. Fraga, M.F., et al., *Epigenetic differences arise during the lifetime of monozygotic twins.* Proc Natl Acad Sci U S A, 2005. **102**(30): p. 10604-9.
201. Lango Allen, H., et al., *Hundreds of variants clustered in genomic loci and biological pathways affect human height.* Nature, 2010. **467**(7317): p. 832-8.
202. Boyle, E.A., Y.I. Li, and J.K. Pritchard, *An Expanded View of Complex Traits: From Polygenic to Omnigenic.* Cell, 2017. **169**(7): p. 1177-1186.
203. Yengo, L., et al., *Meta-analysis of genome-wide association studies for height and body mass index in similar to 700 000 individuals of European ancestry.* Human Molecular Genetics, 2018. **27**(20): p. 3641-3649.
204. Marouli, E., et al., *Rare and low-frequency coding variants alter human adult height.* Nature, 2017. **542**(7640): p. 186-190.
205. Polderman, T.J., et al., *Meta-analysis of the heritability of human traits based on fifty years of twin studies.* Nat Genet, 2015. **47**(7): p. 702-9.