Executive Summary:

The main objectives of Euradrenal were to study pathogenesis of autoimmune Addison's disease (AAD), develop an experimental animal model, describe the natural course, and improve diagnosis, treatment and follow-up of patients. This was achieved by bringing together twelve partners from seven countries and two associated industrial enterprises with broad and complementary expertise in experimental and clinical medicine, including genetics, and immunology.

Establishment of a European network of patient registries and biobanks enabled Euradrenal investigators to recruit 2868 patients during the project period providing a robust basis for sufficiently powered studies into the genetic, immunological and clinical aspects of AAD. Taking advantage of national public registers in many of the partners' countries we were able to study many aspects of the natural course of AAD. We could show that AAD patients overall had reduced bone mineral density (BMD) and that BMD was inversely correlated to daily replacement dose of glucocorticoid. Follow-up studies form Sweden and Italy found increased frequencies of fractures in the hip and spine, respectively.

Up to 15% of female patients experienced premature menopause. Antibodies against the steroidogenic enzyme side-chain cleavage enzyme could be used as a biomarker to predict the development of this malady. Fortunately many AAD women seem to retain viable ovarian follicular tissue for a period of time after premature menopause, creating a window of opportunity to conceive. Moreover birth rates were decreased and pregnancy complication such as premature delivery, low birth weights and Caesarean section were increased. Surprisingly pregnancy complications could be detected as early as 3 years before the diagnosis of AAD.

Search for susceptibility genes unveiled 7 new genes associated with AAD, and more may come to light as the final results of extensive human and canine sequencing projects are analysed. As of yet we have not found an Addison-specific gene.

In the first standardisation study of 21-hydroxylase antibody assays we compared the performance of both the commercial assay and various in-house assays. The results showed that the in-house assays performed just as well as the commercial, but the latter had overall higher specificity. Euradrenal studies showed that AAD patients have autoreative CD4 and CD8 T cells with reactivity against 21-hydroxylase in peripheral blood. Distinct HLA-restricted epitopes were found; preliminary data reveals that CD8 cells are able to kill adrenocortical cells. These cells could provide a future target for immunomodulatory therapy aimed at preventing development of AAD in patients still in the subclinical phase of the disease. In parallel a mouse model was developed by targeted deletion of 21-hydroxylase in medullary thymic epithelial cells thereby precluding the normal negative selection of autoreactive T cells. The mouse models will be invaluable instruments for further studies into the pathogenesis of AAD.

To aid physicians in the follow-up of patients AddiQoL, an Addison-specific quality of life instrument was developed, validated and translated into multiple European languages. AddiQoL was used in the multi-center trial comparing conventional oral treatment with subcutaneous infusion of hydrocortisone. Although not all analyses have been completed at the end of Euradrenal, we were able to show that the diurnal variations in cortisol and ACTH were largely normalized. Many patients felt much improved on this therapy, some to the extent of refusing to give up pump treatment. We believe subcutaneous infusion of hydrocortisone is a treatment alternative for a subgroup of patients not functioning well on standard replacement therapy.

To conclude, the Euradrenal consortium has made significant advances into understanding the pathogenesis and natural course of AAD. This knowledge has been translated into improved diagnostics, treatment and follow-up of patients now defined in European guidelines under publication. The network of patient registries and biobanks and the experimental models developed will provide investigators with important tools for future research on AAD. We are indebted to the European Commission for funding the project, the patients for participating, and to the patient organizations for their collaboration.

Project Context and Objectives:

Autoimmune Addison's disease (AAD) is a rare acquired endocrine and immunological disease (at a prevalence of about 10 per 100 000 inhabitants) characterised by the autoimmune destruction of the adrenal cortices. Untreated AAD is deadly, but if diagnosed in time and given replacement therapy with glucocorticoids and mineralocorticoids AAD patients may live near-normal lives.

AAD typically affects young individuals and more frequently women. A distinct feature of AAD is the high frequency (60-80 %) of co-existing organ-specific autoimmunity, most often endocrine diseases, but in many instances also affecting the genitourinary tract and the digestive system, e.g. autoimmune thyroid disease, type-1 diabetes, premature ovarian failure, and pernicious anaemia, respectively. This particular disease complex is designated autoimmune polyendocrine syndrome type-2 (APS-2), although a number of non-endocrine conditions including vitiligo, alopecia, and celiac disease also constitute typical features of this disorder. Hence, AAD serves as an instructive and highly relevant model disease for organ-specific autoimmunity.

Many of the symptoms and signs of Addison's disease are largely unspecific such as fatigue, nausea, abdominal pain and weight loss, and diagnosis is often delayed until a life-threatening acute adrenal crisis erupts. Research has been severely inhibited by lack of sufficiently powered studies and long-term follow-up of patients. Thus many aspects of the natural course of AAD have not been systematically studied in larger cohorts of patients. Likewise early detection and targeted prevention of AAD is hampered by a lack of detailed understanding of the pathogenic events leading to AAD.

Immunogenetic factors such as the presence of specific major histocompatibility complex (MHC) and CTLA-4 polymorphisms have been proposed to play a role in the pathogenic events that lead to adrenocortical destruction. Antibodies against 21-hydroxylase and other enzymes in the pathway of steroid synthesis are typically generated at early subclinical stages of the disease. These autoantibodies now serve as important clinical markers to diagnose AAD distinctively from other forms of primary adrenal failure.

An important role of T cell-specific responses in the development of AAD has been suggested by (i) the increased frequency of activated T cells in patients afflicted by AAD and (ii) findings of a defective regulatory T cell capacity in some patients with APS-2. While these single findings clearly imply a (central) role for the immune system in the events leading to AAD, a much more detailed understanding of the pathogenesis is warranted to identify and employ reliable biological markers for the identification of individuals at risk to develop AAD, and to devise new treatment modalities for disease prevention. The lack of development in this area is at least partly due to the absence of a small animal model of AAD which has greatly inhibited research.

The parameters currently available to guide steroid treatment are very crude and lack specificity. As a consequence and despite optimal replacement therapy with adrenal steroids following conventional best-practice, many patients experience a reduced quality of life and are unable to follow a regular work schedule. New ways to replace the lack of adrenal steroid and new biomarkers that reflect the action of steroids at the cellular level are greatly needed to improve therapy and hence the quality of life in the individual patient. Improvements in this area will also benefit other patient groups dependent on regular near physiological steroid treatment, e.g. patients with pituitary failure.

Although optimal steroid replacement is important, to prevent adrenal insufficiency or reverse a partial destruction of the adrenal cortex would be even better. There are no trials on the testing of immunomodulatory therapy in Addison's disease. Increased understanding of the pathogenesis may

provide the necessary understanding to devise totally new treatment modalities to prevent the development of AAD.

Finally, the line of action outlined in the proposed project dedicated to AAD can also be viewed as a model system both to study the lack of adrenal steroids and to study optimal replacement strategies in other diseases related to autoimmune and endocrine disorders.

To overcome the fundamental shortcoming in our present knowledge of AAD, the Euradrenal consortium brought together twelve beneficiaries from seven countries and two associated industrial partners with broad and complementary expertise in experimental and clinical medicine. In addition to have on board many of the most distinguished investigators in the field of ADD in Europe, Euradrenal included experts of canine genetics to exploit the natural occurring canine Addison's disease model to identify disease-relevant genes. The team also includes world-leading immunologists with expertise in studies of T lymphocytes in various disorders, and on small animal models of autoimmunity.

The Euradrenal project was organised into the following work packages (WP):

WP1. Patient registry and biobank

WP 2. Natural course of autoimmune Addison's disease

WP 3. Dog model of Addison's disease

WP 4. Mouse model of Addison's disease

WP 5. Genetics in humans

WP 6. Immune regulation

WP 7. Diagnosis, treatment and follow-up

WP 8. Dissemination of knowledge

WP 9. Project management

The proposed approach of integrating results from genetic, immunological, epidemiological and clinical studies on AAD should thus provide a future sound basis for novel strategies for early prevention, precise diagnosis, and optimal therapy of AAD.

Objectives of Euradrenal:

1. Establish a network of patient registries and biobanks on Addison's disease in Europe (WP1) in the involved research centres. The network will provide clinical information which describes the epidemiology and natural course of the disease (WP2) in order to overcome the current limitation of published studies which are without exception based on knowledge drawn from insufficiently sized patient cohorts. Hence the intended European-wide network of patient registries will be of a sufficient critical mass of patients for the research purposes outlined in this project. The detailed objectives are to:

- Organise current registries according to the common consortium form.
- Collect serum/plasma and DNA from all patients; RNA and blood cells from selected groups of AAD patients and controls.
- Merge the individual registries into a network of registries so that information can be shared among beneficiaries.

2. In recognition of the recent sequencing of the dog genome and the spontaneous high incidence of AAD in certain dog breeds such as the standard poodle and Portuguese water dog, identify genes associated with AAD in the dog, and use this information to find the relevant genes in humans and mice (WP3) (dog human mouse crosstalk). The detailed objectives are to:
    - Collect dogs with AAD and healthy controls from the same breed.
    - Perform genome wide single nucleotide polymorphism (SNP) analysis on multiple breed.
    - Identify the canine genes associated with AAD.
    - Identify and characterise orthologues of these candidate genes in humans and mice (WP3-5).
3. Identify human genes associated with AAD (WP5). Detection of allelic variants of genes conferring increased disease susceptibility in conjunction with immunological markers of autoimmune adrenalitis (e.g. autoantibodies against 21-hydroxylase) will be used to identify persons at risk of developing AAD. Individuals with such a risk profile will be candidates for future preventive treatment. The detailed objectives are to:
    - Seek polymorphisms and mutations in genes already known or suspected to be associated with AAD and other organ-specific autoimmune diseases exploiting the power provided by a large patient registry.
    - Perform a genome association analysis to identify alleles associated with disease in close interaction with similar analyses in dog (WP3).
    - Perform exome sequencing to identify rare variants of candidate genes suspected to confer disease susceptibility using novel high-throughput sequencing technologies.
4. Identify the mechanism(s) and kinetics operational in the immune-mediated destruction of the adrenal cortex (WP4 & 6). We will exploit recent advances in tetramer technology, use our understanding of cell-mediated autoimmunity in related/corresponding autoimmune diseases and employ our know-how to develop gene targeting directed to thymus epithelial cells to generate a novel mouse model of AAD. The detailed objectives are to:
    - Identify disease-relevant T cell epitopes in 21-hydroxylase using tetramer technology (WP6).
    - Identify the pathogenic events in T cell mediated destruction of the adrenal cortex in a novel mouse model (WP4).

- Study pathogenic T and regulatory T cells in patient samples and in the mouse model of AAD (WP4 & 6).

- Identify patients at risk of developing AAD by autoantibody screening and perform a standardization study of 21-hydroxylase antibody assays (WP6).

5. In order to reduce treatment-related mortality and morbidity, we will develop physiological and personalised steroid replacement (WP7) by implementing a treatment-sensitive quality of life instrument to monitor therapy, and provide European treatment guidelines and patient information. The detailed objectives are to:

    - Implement a novel treatment sensitive quality of life questionnaire (AddiQoL).

    - Develop sensitive stimulation test for early detection of AAD by adrenal cortex stimulation tests.

    - Study new ways to administer cortisol, i.e. slow-release preparation (in collaboration with a SME, see Appendix) and a continuous infusion system.

Project Results:

1 - Patient registries and biobanks

One of the important objectives and main achievements of Euradrenal is the establishment of network of European patient registries and biobanks on autoimmune Addison's disease (AAD) containing sera and DNA on all and RNA on selected patients. At the start of the project about 1200 patients were collected among the partners. During especially the first and second period this number was significantly expanded beyond the goal of 2000 patients to a total of 2868 patients at the closure of the 3rd reporting period.

Samples and clinical information is stored in the local registries and biobanks in Bergen, Uppsala/Stockholm, Newcastle, Frankfurt, Warsaw, Padua and Perugia. The registry information collected during Euradrenal is being compiled for completion in a joint publication presenting the whole European cohort. The publication will include data on clinical manifestations including endocrine co-morbidity, treatment, autoantibodies and genetics.

Status at the end of Euradrenal 31 March 2012:

Partner	Patients	Sera	DNA	RNA	Pedigrees
P1 Husebye	533	533	533	450	12
P2 Kämpe	290	290	290		
P3 Betterle	640	300	250		
P4 Pearce	355	249	249		12
P6 Badenhoop	240	240	240		20
P7 Kasperlik	228	222	216		
P8 Falorni	272	270	185		
P9 Hulting	310	310	310		
Sum	2868	2414	2207	450	30

The biobanks and registries were essential for the rest of the work in Euradrenal, reported on in the following sections.

Beyond Euradrenal the Euradrenal investigators plan to collect updated information and new samples on already included patients, include new patients, and add new partners from more countries in Europe to the network. To this end researcher from Spain, France, The Netherlands, and Slovakia have shown interest. Thus, this network could have a great impact on research on AAD also in the future.

2 - Natural course of autoimmune Addison's disease

A major effort of Euradrenal has been to describe the natural course of autoimmune Addison's disease to improve the often fragmented information based on small patient cohorts that was available at the start. Through Euradrenal a number of detailed descriptions of larger cohorts of AAD patients have been published, e.g. from Norway (Erichsen et al. PMID 19858318), Poland (Kasperlik-Zaluska et al. 2010), and Italy (Betterle et al., manuscript). In these publications gender aspects, age at diagnosis, autoantibody profile and associated endocrine and non-endocrine manifestations were presented. In addition to these general descriptions, we choose to concentrate our effort into 4 different areas taking advantage of nation-wide registries available in some of the investigator's countries, especially Sweden. These areas were:

1. Epidemiology and mortality
2. Quality of life and working ability
3. Women's health including fertility and sexuality
4. Bone health
5. Cardiovascular aspects

Epidemiology and mortality

We found a high prevalence (number of cases at a given point in time) of Addison's disease in Norway at 14.4 per 100,000 inhabitants (Erichsen et al. PMID 19011006) by scrutiny of diagnostic registries at all somatic hospitals in Norway, confirming earlier regional statistics from Western Norway (Løvås & Husebye, PMID 12072049). This is one of the highest numbers ever reported. These figures were later confirmed by the Swedish survey on Addison's disease reporting a prevalence of 13.1 per 100,000 and an incidence (number of new cases per year) of 0.6 per 100,000 inhabitants per year (Björnsdottir et al, manuscript).

Furthermore, by retrieving data from the Technikerkrankenkasse (TK), one of Germany's largest health care insurance providers covering nearly 10% of the population, the prevalence of Addison's disease was found to range between 10 and 12.2 per 100,000 (Meyer et al, manuscript). Incidence figures showed a steady increase of 18% over the four year period 2007-2011. The prevalence was lower in men (7.3-8.5 per 100,000 with an increase of 14%) than in women (12.9-16 per100,000 with an increase of 19%). Autoimmune co-morbidities (up to eleven diagnoses) were found in 59% which is similar to most cross-sectional studies reported so far. This is the first report of epidemiological data on Addison's disease from Germany.

Previous studies using prescription of medication databases to estimate prevalence of revealed similar numbers from Italy (11.7 per 100,000; Laureti et al, PMID 10323417). Even if many areas are missing we find a higher prevalence and evidence of increased incidence of Addison's disease in Europe. It will be important to use the patient registries for epidemiological surveillance to see how the epidemiology develops in the future.

Mortality

Euradrenal studies have made significant contributions to understanding mortality in Addison's disease. Using Swedish hospital admission and death registries, Bensing and co-workers (Bensing et al. PMID18727712) found that mortality was more than doubled and especially high in the subgroup with autoimmune polyendocrine syndrome type 1 (APS-1) with an odds ratio of 4.6. A study from Norway did not report overall increase in mortality, but identified a subgroup of males diagnosed before the age of 30 years, with doubled mortality rate. Addison's disease interpreted as an acute adrenal crisis, infections, and sudden death were the main death causes, while cardiovascular deaths were not increased (Erichsen et al. PMID 19011006). The latter finding has now been confirmed in an Italian study of Addison patients revealing that overall mortality in Addison's disease and APS-2 was not increased (Betterle et al, manuscript).

Quality of life (QoL) and working ability

In a national survey in Norway using the quality of life questionnaire SF-36, we found that vitality, general health and physical role was low for all patient categories; the reductions were most pronounced for patients with AAD and type-1 diabetes (Erichsen et al. PMID 19858318). The presence of thyroid disease in addition to AAD did not affect the SF-36 scores. Mental health and bodily pain scores were comparable to that of the background population. This is the largest cohort of AAD patients investigated with SF-36 so far (Erichsen et al. PMID 19858318).

Since SF-36 is a general QoL questionnaire not specific for any particular disease, we choose as part of the Euradrenal effort to develop an Addison-specific QoL instrument to be used in the treatment and follow-up of Addison patients. We first completed an English version with the acronym AddiQoL (Løvås et al. PMID 20016050). We then went on to translate it into Norwegian, Swedish, Italian, German and Polish versions by state-of-the-art techniques. These were administered to large cohorts of patients with Addison's disease in each country for evaluation of validity and reliability and to improve the questionnaire further. After removal of six problematic items, Rasch analysis confirmed a uni-dimensional construct with high reliability, very well targeted to the patient group.

Test-retest reliability for patient subgroups in Norway, Italy and Sweden was excellent. Correlation between the AddiQoL scores and scores from SF-36 and another commonly used questionnaire Psychological General Well Being (PGWB) were high using Norwegian and Swedish patients. In addition to the standard 30 item questionnaire AddiQoL-30 a short version AddiQoL-8 reflecting general health and fatigue was developed (Øksnes et al. PMID 22090270). AddiQoL has received much interest and is now being translated into more European languages including Spanish, Dutch and Slovak.

The first study with AddiQoL beyond the validation process employed 200 German patients with AAD and confirmed that female patients with AAD have significantly lower QoL scores than males. The findings also suggested that latency between first symptoms and diagnosis of AAD may negatively influence QoL for long periods of time (Meyer G et al 2012, abstract, manuscript in preparation).

Overall working ability was reduced in AAD patients. Thirty percent of the working age patients received full (7%) or part time (23%) disability benefits, as compared to 11% (8.5% full, 2.5% part time) in the general population. The percentage of working disability was 19% in patients with

isolated Addison's disease and 36% in APS-2 (Erichsen et al. PMID 19858318). We interpret these data that most Addison patients retain a fairly good working capacity, but they seam to be sensitive to stressful work environments and shift work, although the individual differences between patients are large.

Women's health including fertility and sexuality

In the Norwegian survey published in 2009 we found that 6.7% of the females had premature ovarian insufficiency (POI) (Erichsen et al. PMID 19858318). Recent numbers from Italy confirms and extends these observations revealing a frequency of up to 15% and that steroidogenic antibodies (e.g. anti-side chain cleavage enzyme (SCC-Ab) are predictive marks for premature ovarian failure (Reato et al. PMID 21677034). However, in contrast to non-autoimmune POI, AAD patients with this condition seem to retain a pool of follicles for a period of time. Falorni and co-workers (La Marca et al. PMID 19622621) found that AAD patients had much higher levels of anti-mullerian hormone (AMH) at diagnosis reflecting a preserved follicle pool. The years following menopause AMH levels fell sharply. With the aim of addressing the question whether this inverse correlation was the consequence of a progressive decline of the follicular pool during the natural history of POI or represented the existence of distinct subpopulations of women with POI associated to AAD, the Perugia group (Falorni et al. PMID 22417127) performed a follow-up study on 11 women that demonstrated that AMH levels declined at an average rate of 50% per year. Even so, the presence of follicles at diagnosis is very important from a clinical point of view, since it indicates that there is a window of opportunity to rescue follicles that can be used to induce future pregnancies, as opposed to the situation in other causes of POI.

Irrespective of the increased frequency of POI, birth rates are reduced in women with Addison's disease. In a first of its kind study from Norway using birth registry data the standardized incidence rate for birth was significantly reduced (0.69) compared to the background population (Erichsen et al PMID 20610594). In a similar Swedish study (Björnsdottir et al. PMID 20718774) the seminal finding was that among AAD women the frequency of preterm delivery, low birth weight and caesarean sections were increased. Surprisingly, this increase could be detected as early as 3 years before the diagnosis of AAD.

Finally sexuality in women with ADD was addressed for the first time in a systematic way employing the sexual activity questionnaire (SAQ). To our surprise we did not find reduced sexuality in Addison patients. On the contrary these women reported that they were just as sexually active as controls and had increased pleasure and less discomfort despite low androgen levels (Erichsen et al. PMID 20610594).

Bone health

Since Addison patients are treated with glucocorticoids it has been a concern if this treatment adversely affects bone mineral density (BMD) and increases fracture risk. Earlier studies have been hampered by generally low number of participants. As part of the Euradrenal project we reported reduced BMD in patients with Addison's disease and indications that polymorphisms in genes regulating steroid action influences BMD (Løvås et al. PMID 19282465). BMD revealed an inverse correlation to steroid dose, and was especially low in those taking synthetic potent glucocorticoids

such as prednisolone. Despite reduced BMD, vertebral fractures were not more prevalent then in the background population. In contrast, a follow-up registry study from Sweden reported significantly increased frequencies of hip fractures in Addison patients with an excess risk of about 50% (Björnsdottir et al. PMID 20861125). Furthermore, preliminary results from a cohort study in Padova reported an increased frequency of vertebral fractures, but numbers of participants are being expanded in order to reach firm conclusions (Betterle et al., manuscript).

Cardiovascuclar aspects

A study on metabolic alterations showed higher prevalence of central adiposity, impaired glucose tolerance and dyslipidemia in Addison patients (Giordani et al. PMID 19620820). Although not proven, this could at least partly be a consequence of over replacement of glucocorticoids. Prospective studies employing the network of European patient registries and biobanks should be able to answer this question.

3 - Dog models of Addison's disease

The study of the spontaneous canine model of Addison's disease involved both immunological and genetic aspects. The genetic studies aimed at exploiting the fact that several dog breed have increased incidence of Addison's disease, and the way dogs are breed to retain certain phenotypic properties, give long stretches of linkage disequilibrium. Thus, the number of individuals needed to obtain sufficiently powered studies is much lower than in human studies.

Identification of the canine adrenal autoantigen

In human the enzyme 21-hydroxylase (21OH) is the main autoantigen in AAD. Preliminary evidence revealed that 21OH was not a target in the dog. Thus, we sought to identify the canine autoantigen by immunoscreening of a canine adrenal cDNA expression library. The library was synthesized with high titer and with large inserts (i.e. the majority > 2000 bp) making it ideal for our screening purposes. Dog sera for screening were selected using an immunofluoresence assay against dog adrenal cortex. Positive sera (containg antibodies against adrenal cortex) were taken forward to the screening procedure. A novel canine autoantigen was identified, namely the protein vigilin which shows homology with hRNP family of proteins, which are known autoantigens in systemic lupus erythematosus, a systemic autoimmune disease. It has been suggested that vigilin is an intracellular cholesterol-transporter and involved in steroid synthesis. Finding a totally different autoantigen in dog is not overly surprising. It is known from the autoimmune regulator (Aire) knockout mouse, a model for the human disease APS-1 that autoantigens differ between mouse and man.

As a supplement we screened dog sera against human 21-hydroxylase antibodies synthesised by in vitro transcription translation without any positive reactivity confirming earlier preliminary observations. Larger cohorts of dogs will be screened to establish the frequency of vigilin antibodies in different breeds (Landegren, Kämpe et al., manuscript).

Genome wide association studies (GWAS)

One of the dog breeds with susceptibility for Addison's disease is the standard poodle. A GWAS was performed on 112 Addisonian and 116 control standard poodles. In the first analyses, no susceptibility genes were identified. However, after reviewing the details of each case and including only those with confirmed Addison's disease with supposedly autoimmune etiology, a region of association was identified on dog chromosome 17 which is outside the dog histocompatibility complex region. Subsequently various other breed with susceptibility to Addison's disease, including Portuguese Water Dogs, Border Collie, Bearded Collie, West Highland White Terrier, Weathen Terrier, Cocker Spaniel and Springer Spaniel were collected for GWAS. Using high density SNP genotyping on six of these pedigrees and breed-matched, Addison's-free controls revealed multiple susceptibility regions. Altogether, we identified approximately 200 novel genes across 11 canine chromosomes (Short et al., manuscript). Further candidate gene analyses were carried out on 249 SNPs across 43 candidate genes. SNPs were genotyped in 216 cases and 495 controls in fourteen pedigree dog breeds. Analyses show SNP associations in 27 of the genes. Some genes show multiple SNP associations for a gene in single a breed while others show associations in multiple breeds (e.g. TAF5L, CP21A, GC, PTPN22). Fourteen SNPs have significant associations in two or three different breeds.

In addition to GWAS, hypothesis-driven association studies were undertaken to compare susceptibility genes in dogs and men. As the human MHC region contains the genes that have the highest risk for human AAD, we investigated the canine MHC, known as DLA (for Dog Leukocyte Antigen). In total, 420 dogs with Addison's disease and 4158 healthy controls were characterised for their DLA class II haplotypes, including DLA-DRB1, DQA1 and DQB1. The results reveal a complicated pattern of DLA associations, with some haplotypes being raised in cases from several breeds, while other haplotypes were decreased, and at least one haplotype being raised in some breeds but lowered in others. It is clear that the canine MHC (i.e. DLA) has an influence on AD susceptibility in dogs, but further analysis is required to confirm the details. Taken together our results strengthens the notion that the pathogenesis of Addison's disease in dog and man has many similarities, and that it is a relevant model for the human disease

4 - Mouse models of Addison's disease

Research into the pathogenesis of AAD has been severely inhibited by the lack of a small laboratory animal model. One of the main objectives of the Euradrenal team was therefore to establish such a model that could provide the scientific community with a tool to study not only pathogensis, but also to test immunomodulary treatments. In our endeavour to create such a model we used two different strategies, (i) immunisation of mouse with 21OH, the main autoantigen of AAD, and (ii) a transgenic model based on targeted deletion of 21OH in thymic medullary epithelial cells to evade central deletion of autoreactive clones.

Mouse models by immunisation

An improved purification method of 21OH in the baculovirus expression system was developed (Bratland et al. PMID 19329278) for immunisation experiments. The following immunisation protocols were employed:

1. Injection of recombinant 21OH emulsified in the Sigma Adjuvant System, along with stimulating anti-CD40 antibodies, poly IC and interferon. Mice were injected subcutaneously on day 0 and boosted on day 10. Interferon producing T cells responsive to 21OH peptides could be detected, but no detectable lymphocytic infiltration of the adrenals, nor was any detectable 21OH autoantibodies observed.

2. Injection of in vitro activated and antigen-loaded dendritic cells (DCs). In this approach we established DC in vitro from bone marrow precursors of C57Bl/6 mice. The DCs were pulsed with antigen and then activated by lipopolysaccaride (LPS) and stimulated with anti-CD40 antibodies. T cell responses as measured by interferon were generally at the same levels, or slightly weaker than those observed using the former strategy and some mild lymphocytic infiltration was observed in the adrenals.

3. Injection of necrotic insect cells infected by recombinant baculovirus encoding 21OH. In addition to the general adjuvant effect of necrotic cells, baculovirus has been shown to stimulate the innate immune system through the TLR9 receptor. We also added a second TLR ligand, poly IC which acts as synthetic RNA (ligand of TLR3) to the immunization mixture. With this approach lymphocytic infiltration was observed in one third of the mice. Corticosterone levels (the mouse cortisol) were also reduced.

We now believe we have identified a reasonable immunization strategy capable of invoking strong in vivo as well as in vitro immune responses in the C57Bl/6 mice to use in the animal models.

Models based on targeted deletion of the 21-hydroxylase gene

To delete the 21-hydroxylase gene specifically in medullary thymic epithelial cells we generated a gene targeting vector to alter the genomic sequence and allow for the loss of exon 3 to 10 following a loxP-Cre effected recombination. This vector was then successfully synthesized and electroporated into mouse embryonic stem cells in two versions. In the first the expression of the 21OH protein was lost; in the second the modified locus (prior to recombination) also expressed the membrane-bound form of ovalbumin (mOVA) which serves, for experimental purposes, as an antigen surrogate for 21OH. Offspring that is either heterozygous or homozygous for the targeted locus (designated 21OH +/f and 21OH f/f, respectively) were viable and born at the expected Mendelian distribution.

An supplementary ADD disease model foresees the generation of mice that not only lack mOVA expression in TEC whilst continuing to express this surrogate self-antigen in the adrenal cortex, but that also transgenically express a T cell antigen receptor (TcR, known as OT-I) specifically recognizing an OVA derived peptide (SIINFEKL) when presented by MHC class I molecules.

Thus, as outlined in the original application, two genetically distinct disease models have been generated to investigate at the cellular leve,l the immunopathology of ADD. Their usefulness remains to be proven in projects beyond Euradrenal.

5 Genetics in humans

At the onset of Euradrenal a few disease associated genes were known, but none were specific for AAD as similar associations also has been reported in other autoimmune diseases. Our objective was twofold, to use the network of biobanks to perform sufficiently powered studies to provide robust replication of genetic associations at genes known or suspected to be associated with AAD, and take advantage of new sequencing technologies in a hypothesis-free attempt to find rare gene variants associated with ADD.

Studies of candidate loci

Initial candidate gene studies focussed on well-established genomic loci with preliminary evidence for an etiological contribution in previous studies of AAD. These loci included the HLA, PTPN22 and CTLA4. Two collaborative studies on PTPN22 was published (Skinningsrud et al. PMID18301444; Roycroft et al. PMID18710467) and a third on HLA (Skinningsrud et al. PMID21816777). Importantly, the HLA associations of AAD have been significantly refined by this work.

A detailed analysis of 16 markers spanning the CTLA4 locus (CD28-CTLA4-ICOS interval) was performed. Association at both CD28 and CTLA4 alleles was found in a cohort of 700 Norwegian and UK AAD patients. Regression analysis of the full haplotypes indicated, however, that variation within CTLA4 itself is driving the association. A manuscript has been prepared (Bøe-Wolff A et al. manuscript), but final genotypes are being performed to confirm the findings in additional Euradrenal AAD cohorts in order to strengthen the certainty of the association.

As well as these well-characterized loci, several more novel loci were studied, on the basis of their involvement with other autoimmune conditions such as type-1 diabetes, vitiligo or rheumatoid arthritis. These included additional candidate genes such as NALP1 (Magitta et al. PMID18946481), CIITA/ CLEC16A (Skinningsrud et al. PMID18593762) PD-LI (Mitchell et al. PMID19850680) and CD226 (Gan et al. PMID: 21521299). Three suspected AAD susceptibility loci were robustly confirmed in datasets of more than double previous experimental approaches and a further 4 new AAD loci were identified for the first time. A high-profile review of this information was published (Mitchell AL, Pearce SH. Nature Rev Endocrinology PMID22290360).

A larger candidate gene study was subsequently performed, taking a poll of biologically plausible candidates based on information from fundamental immunology, as well as from existing associations in different autoimmune conditions. Out of more than 50 possible candidates, consortium partners voted for a top 20 genes (including: CYP27B1, CYP2R1, CYP24A1, GATA3, IFIH1, IL17A, IL17RA, IL21, IL23A, NFKB1, NFATC2, Rel, RORA, ROR, STAT2, STAT4, TBX21, VDR). Assays for a total number of 150 SNPs were entered into design software with a

total of 120 assays being selected by haplotype tagging principles in 4 separate pools, each of which was genotyped by Sequenom. In a 2-phase design, the initial experiment took place with 700 samples from the UK and Norway, with a replication phase consisting only of the associated 21 SNPs from the first round with Swedish, Polish, German and Italian patient samples and their respective healthy control groups. In total, 2001 AAD DNA samples were genotyped with 1898 healthy controls, the largest AAD genetics experiment ever undertaken. The genotype data amongst the diverse cohorts were heterogeneous, so a meta-analysis using a random effects model was employed.

New loci confirmed to be associated with AAD, included CYP27B1, STAT4, Rel and GATA3. Analysis by subphenotypes, including isolated AAD versus APS-2 and excluding individuals who were not 21OH-Ab positive confirms these findings with minor alterations in p values. This extends the number of AAD loci up to 14, from an initial 7 before the start of the consortium, GATA3 being a novel susceptibility locus for autoimmunity. We are currently working on functional assays to understand the exact role that GATA3 variation in the immune system biology.

Exome sequencing of ADD patients

In order to find rare genetic variants with association to AAD, we sequenced 200 exomes, all from AAD patients(including APS-2. As a first round of analysis we did not plan to include ethnically matched controls, but relied on comparisons with available exome data sets, in particular those of the 1000 genomes project for cost-effectiveness reasons. However, it is clearly important to have access to ethnically matched controls which can be subjected to more targeted genotyping/sequencing to rule out the possibility that identified rare variants are in fact population specific markers not represented in the 1000 genomes.

Bioinformatic analysis is still ongoing, but preliminary analysis has shown that the capture is working fine with >90% of targeted bases being sequenced and >80% of targeted sequences giving >20x sequence coverage. Preliminary analysis of variants identified in a few AAD patients demonstrate that we find the expected number of variants compared to the human genome reference sequence with approximately 22,000 single nucleotide variants (18,000 high quality (HQ)) of which some are presumably deleterious.

Further bioinformatic and biostatistical analyses will focus on identifying rare and presumably damaging variants using tools such as PolyPhen as well as on aggregate analysis of all 200 patients. Prior to this we will eliminate genes known to produce technical artefacts. Based on these analyses we will compile a list of candidate genes for harbouring rare disease susceptibility variants which can be the subject of targeted sequencing in the Euradrenal case control collection. We believe this project has the potential to identify novel disease susceptibility genes.

Data integration between dog and man and pathway analysis

Identification of 7 novel AAD susceptibility loci during the course of the Euradrenal project, on top of the 7 that were known or suspected prior to the work, has given us the opportunity to construct a dendrogram of biochemical and immunological relationships between the identified protein products. Using ingenuity pathway analysis, including biofunction and canonical pathway analyses, the direct protein-protein interactions can be highlighted, along with indirect interactions. These analyses have suggested the IL12- IL18-receptor axis, CARDs 6 and 8, and TLR10 as other potential key players for future investigation in AAD.

Genetic prediction in subclinical ADD

Using the candidate gene experiments to give us a lead that might be used in disease prediction is clinically relevant, especially when we hopefully in the near future can offer immunomodulatory

therapy to reverse autoimmune adrenalitis and overt AAD. The strongest link proves to be MHC, with the haplotypes HLA DRB1*03:01 and DRB1*04:04, particularly in heterozygous combination conferring an odds ratio for AAD of 32, which could be a useful predictive marker for disease progression (Erichsen PMID 19011006). There is also an independent contribution from HLA-B however, suggesting that the aetiological allele might lie between HLA-DRB and HLA-B (the MHC class III region) (Skinningsrud PMID 21816777).

One line of investigation that has been opened up by Euradrenal's results relates to copy number variants. These are defined as genomic insertions/ deletions or rearrangements that are subcytogenetic but >1 kilobases in size. As well as a systematic survey of copy number variants across the genome (publ. 29), we have made detailed analysis of the RCCX duplication in the MHC class III region of chromosome 6p21. This area is close to the HLA DR/ DQ region and so alleles and haplotypes in the RCCX would be expected to have some association with AAD, through bystander effects. The RCCX duplication contains the gene for 21OH, the key enzyme autoantigen in AAD (CYP21B) and the duplication means that most alleles also carry a second 21OH transcript, which is a processed pseudogene (CYP21P). Although the CYP21P is not believed to have any function', it is conserved down to the mouse, suggesting that it does have an important role in physiology. Of 299 UK AAD patients studied, 49 (16.4%) had both RCCX alleles that contained only CYP21B, that is with no CYP21P gene. This compared to 10 of 298 healthy controls (3.0%); $p<0.0001$, OR 5.6. Similar results have been confirmed in the Norwegian cohort and individuals who are hemizygous for CYP21P carriage are concordantly increased in AAD subjects. Thus, copy number of CYP21P genes may be the aetiological allele in MHC, and also should prove useful in predicting individuals who are at most risk of AAD. However, functional work is ongoing to examine why CYP21P deficiency might predispose to AAD, or whether these effects are solely related to the association at nearby MHC class I and II alleles.

6 Immune regulation

The main autoantigen in AAD, 21OH, was reported for the first time in 1992 by one of the partners (Winqvist et al. PMID 1351548). Subsequent studies revealed that the majority of patients with Addison's disease were 21OH-Ab positive taken as evidence that the etiology in deed was autoimmune. However the antibodies themselves were not directly involved in inhibiting adrenocortical function or tissue destruction. Sporadic reports indicated that the autoimmune pathogenesis involved T cells as central players. One of the main objectives of Euradrenal was therefore to unravel in more detail how autoimmune destruction of the adrenal takes place.

The identification of T cell epitopes in AAD patients

Based on findings in related diseases we started out with the hypothesis that T cells and B cells target the same protein, namely 21OH. To test for T cell reactivity against 21OH we used the baculovirus-expressed and highly purified 21OH described in chapter 6. We found robust T cell proliferation responses and interferon gamma production in patients as opposed to controls (Bratland et al. PMID 19890026). Altogether about 50% of the patients' T cells respond to 21OH with predominantly CD4-positive cells. The response was restricted to patients with 21OH-Ab and the response was associated with the disease susceptible MHC genotype DR3-DQ2/DR4-DQ8.

Using a panel of peptides from 21OH, we were able to localize an immunodominant epitope (21OH342-361) partly overlapping the steroid bind site in 21OH, a conserved part of the protein. In silico simulations of binding revealed that the epitope binds to the high-risk MHC class II haplotype HLADR4*0404 which shows a particular strong association to AAD. In type-1 diabetes HLADR4*0401 is associated with disease, and 21OH342-361 does not bind to this HLA molecule (Bratland et al. PMID 19890026).

In parallel we mapped epitopes on 21OH with propensity to bind to of HLA-A*0201 restricted CD8+ T cell using a computerized motif prediction algorithm for HLA-A*0201. Subsequently, the predicted 9-mer epitopes were synthesized and tested in in vitro stimulation assays. By this approach, we have managed to identify four epitopes in the C-terminal portion of 21OH which upon stimulation of peripheral blood mononuclear cells (PBMC) result in activation of CD8+ T cells measured by gamma interferon production.

To assess the relevance of these epitopes in a larger cohort of patients with Addison's disease and controls, a series of overlapping 18-amino acid peptides spanning the whole 21OH protein were utilized. By means of a 14-day recall assay, we expanded 21OH specific T cells from peripheral blood mononuclear cells (PBMC)with the pool of 21OH peptides. We then used the individual peptides to perform an intracelullar cytokine staining assay using Live/Dead, CD4, CD8, CD3, IFN, TNF, IL-2 and IL-17 to find evidence of activation. The majority of patients showed a dominant CD8 response to peptides 27 and 34 and a lesser CD4 response to peptide 17. This was shown to be a response specific to AAD patients, with no such responses detectable in PBMC from healthy donors or type-1 diabetes patients.

As the adrenal gland is almost completely destroyed by the time patients are diagnosed with AAD, it was interesting that patients continued to show significant T-cell responses to 21-hydroxylase epitopes upon restimulation. Therefore, we assessed whether the peptides responses identified above could be detected ex vivo. By using ELISPOT assays we were able to show that IFN producing cells in PBMC from AAD patients responded to the same peptides as in the recall assay. Once again, PBMC from both healthy volunteers and type-1 diabetes patients were negative.

As the responses to peptides 27 and 34 were predominantly CD8 T-cell mediated and CD8 T cells generally recognise shorter peptide fragments, we synthesised overlapping nonamers to focus on the epitopes that may be involved in this response. Peptide 27f (LLNATIAEV) is a classical A2 epitope and elicited a strong ex vivo response as expected since this patient was HLA-A2 positive. Peptide 27f was then refolded with A2 and 2M proteins and conjugated to streptavidin-APC to make a tetramer that could be used to stain T cells. This enabled us to sort cells by flow cytometry and then expand to generate a T-cell line 99% specific for the peptide.

Our results to date have identified hot spot peptide sequences on 21OH that appear to be the focus of T-cell responses. These responses were shown to be both CD8 and CD4 dominated by the former. Euradrenal have with these series of experiments, provided novel information on the role of autoreactive T cells and opens up for future experiments focusing on how these T cells cause destruction of the adrenal gland and ultimately the onset of AAD. Moreover, information on these cells could help us to find new immunomodulatory therapy aimed at stopping or possibly reversing adrenocortical destruction.

Define autoantibody spectrum in AAD patients

An autoantibody screen was performed in the Euradrenal selection of Addison's patients to see if there were variations throughout Europe. The frequencies of 21OH-Ab positive patients are surprisingly similar in the different populations, centring around 85% in Norwegian, Swedish, Italian, German and UK patients. SCC-Ab correlated to premature ovarian failure and testing for these antibodies should be performed in Addison females in childbearing ages, since SCC-Ab positivity predicts early menopause and loss of fertility.

Antibodies have also been analysed in other autoimmune disease. More than 6000 sera from the type-1 diabetes genetics consortium (T1DGC), an international network to perform GWAS analysis, have been analysed for 21OH and other APS-2-related antibodies (Baker et al, in press). Finally, we have tested Norwegian and UK cohorts of patients with myasthenia gravis (MG), both late-onset (LOMG), early onset (EOMG) and MG with thymoma for AAD and APS-1-related autoantibodies. We have found that a substantial number of patients with MG had antibodies typical for AAD (21OH-Ab) and APS-1(e.g. tryptophan hydroxylase antibodies) (Bøe Wolff et al. submitted). These results reveal that there are similarities between these diseases and that the thymus where central tolerance is developed, plays a key role.

Standardised autoantibody test

Based on a study by Falorni and co-workers (Falorni et al. PMID 21570358) looking at inter-laboratory concordance in assay of 21OH-Ab, we aimed to evaluate qualitative and quantitative concordance of results among different laboratories. Altogether 13 different laboratories across Europe and 1 laboratory in the United States participated as listed below. A mixture of research laboratories and hospital routine laboratories were recruited in collaboration with RSR, a SME of Euradrenal (and also a participating center):

Participating Centres:

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A mixture of commercial immunoradiometric kit (RSR) (labs ED, GH, IN, NI, ON) and in house immunoradiometric assays (labs AA, EH, EV, ID, IL, GR, SS, TA, WI) were used to test Addison and control sera.

Overall, the RSR commercial kit showed a lower coefficient of variation as compared to in-house assays, though two research assays had coefficient of variations lower than 5%. The best diagnostic accuracies were observed in two in-house assays. However, the best RSR assay had similar diagnostic accuracy. Overall, the labs using the RSR assays showed good diagnostic sensitivity, but in most of cases showed low diagnostic specificity. The opposite trend was observed for many in-house assays, which showed superior diagnostic specificity and somewhat lower diagnostic sensitivity than the RSR kit. The recalculation of an optimised cut-off value improved the diagnostic accuracy of all assays.

	Diagnostic sensitivity	Diagnostic specificity	Diagnostic accuracy (AUC of ROC curve)
1. WI (in-house)	66/69 (96%)	51/51 (100%)	0.978(95%CI:.933-.996)
2. IL (in-house)	64/69 (93%)	51/51 (100%)	0.964(95%CI:.913-.989)
3. AA (in-house)	65/69 (94%)	49/51 (96%)	0.951(95%CI:.896-.982)
4. ED (RSR kit)	63/69 (91%)	50/51 (98%)	0.947(95%CI:.890-.979)
5. EV (in-house)	58/69 (84%)	51/51 (100%)	0.920(95%CI:.857-.962)
6. TA (in-house)	57/69 (83%)	50/51 (98%)	0.903(95%CI:.836-.950)
7. SS (in-house)	55/69 (80%)	51/51 (100%)	0.900(95%CI:.836-.950)
8. IN (RSR kit)	60/69 (87%)	46/51 (90%)	0.886(95%CI:.815-.937)
9. EH (in-house)	55/69 (80%)	49/51 (96%)	0.879(95%CI:.807-.931)
9. GR (in-house)	59/69 (85%)	46/51 (90%)	0.879(95%CI:.806-.931)
11. NI (RSR kit)	65/69 (94%)	33/51 (65%)	0.795(95%CI:.711-.863)
12. ON (RSR kit)	63/69 (91%)	22/51 (57%)	0.741(95%CI:.653-.816
13. GH (RSR kit)	66/69 (96%)	15/51 (29%)	0.625(95%CI:.532-.712)

14. ID (in-house)	41/69 (59%)	27/51 (53%)	0.562(95%CI:.468-.652)

Exploring the role of vitamin D on adrenal autoimmunity

Vitamin D deficiency contributes to autoimmune disease and significant associations with the genes controlling its activation and metabolism have been reported amongst others in type-1 diabetes, multiple sclerosis and AAD. Besides its role in bone mineralisation vitamin D is an important regulator of T-lymphocyte and macrophage interaction thus involved in the early steps of immune mediated endocrine organ destruction. In order to define the vitamin D status and its genetics we screened patients with AAD from five European countries and correlated the findings with genotypes for genes involved in the metabolism and action of vitamin D, namely the vitamin D receptor (VDR), the cholesterol synthesis enzyme 7-dihydrocholesterol reductase (DHCR7), the degrading enzyme 24-hydroxylase CYP24A1 and the activating enzyme 1-a-hydroxylase CYP27B1.

Using a tag-SNP approach 1048 AAD cases (Germany n = 237; Italy n = 349; Norway n = 378, UK n = 45, and n=39 Poland) were studied for vitamin D system genotypes and a subgroup of these for the vitamin D status (n=712) using a RIA (DIASORIN) for 25(OH)D3. Furthermore the vitamin D activation was determined through measuring the active form 1,25(OH)D3 in the serum from 1115 patients and controls from Germany and Norway. Taqman genotyping was performed in addition for CYP27B1 (rs10877012), VDR ApaI (rs7975232) and DHCR7 (rs12785878) polymorphisms for samples from Germany (330 cases, 292 controls), and Norway (358 cases, 362 controls).

Overall 54-61% patients were vitamin D insufficient or severely (9-17%) deficient (<20 or <10ng/ml). The median in all European cohorts was <20 ng/ml and only 8-13% of patient reached sufficient 25(OH)D3 levels > 30ng/ml. The 25(OH)D3 was significantly lower in CYP27B1 (rs10877012) CC homozygotes compared to heterozygotes in German patients and carriers of the allele C had significantly higher thyroid peroxidase antibody (TPO-Ab) titers. DHCR7 (rs12785878) GG homozygotes were significantly more frequent in Norwegian patients in comparison to matched controls (p=0.02). Vitamin D deficient (<20ng/ml) patients showed significantly less frequent alleles of CYP24A1 (p=0.01) and VDR (p<0.05) in comparison to controls, whereas the group with vitamin D levels > 20ng/ml showed no significant differences for these genotypes. Vitamin D activation for its active 1,25D correlated strongly with its baseline level.

These data suggest a contribution of the vitamin D system genotypes to autoimmune Addison's disease depending on the vitamin D status. Since vitamin D deficiency is highly prevalent a targeted supplementation strategy may be necessary to achieve sufficiency in Addison's disease (Badenhoop et al, manuscript and poster at the Endocrine Society´s meeting in Housten, June 2012).

Significantly higher levels of 1,25D are observed in healthy controls in comparison to patients with Addison's disease. Patients with Addison's disease are therefore more affected by vitamin D deficiency by having an impaired activation to the most active metabolite 1,25D. This may not only be relevant for the immune dysfunction, but also for bone physiology and susceptibility to osteoporosis. Stratifying for gender we observe a trend for a lower formation of 1,25D in female patients (34 pg/ml) versus female controls (45 pg/ml) as compared to male patients (43 pg/ml) in the group with a severe vitamin D deficiency (25D <10ng/ml, p=0.09).

A clinical pilot study to investigate the T-lymphocyte and cytokine regulation effects of high dose vitamin D therapy (4000U/d or placebo switched after 3 months in a double-blind cross-over randomised design) is ongoing in patients with chronic autoimmune Addison's disease.

7 - Diagnosis, treatment and follow-up

Many patients with Addison's disease report reduced quality of life and working ability despite being given best practice treatment. A likely explanation is that today's standard replacement therapy is unable to restore the normal diurnal variation in cortisol levels. We have used multiple approaches to improve diagnosis and treatment of Addison's patients. First we developed and implemented a treatment-sensitive and Addison-specific quality of life questionnaire AddiQoL described in chapter 2. We them moved to improve therapy by more physiological dosing of glucocorticoids using subcutaneous infusion of cortisol and to optimize testing of pre-clinical adrenal dysfunction.

Prior the Euradrenal one of the partner performed a proof of concept study showing that subcutaneous cortisol infusion was technically feasible and able to reconstitute the diurnal variation in serum cortisol levels in patients with Addison's disease (Løvås and Husebye, PMID 17609409). In Euradrenal we performed an unblinded, cross-over designed multi-center trail comparing conventional hydrocortisone treatment with subcutaneous infusion as illustrated in below.

The principal aims of this study was to evaluate the effects of CSHI (physiological) replacement as compared to conventional (unphysiological) replacement on parameters of energy, bone and glucocorticoid metabolism, QoL (SF-36, W-BQ12 and AddiQoL-36), sleep (Pittsburgh Sleep Quality Index (PSQI), sleep diary and actigraphy).

Investigators in Bergen, Stockholm and Uppsala participated in the study. Twenty patients were recruited in Bergen and 10 each in Stockholm and Uppsala, altogether 40 patients. Analyses of data are ongoing and all conclusions are not yet made at the end of Euradrenal. However, 10 patients were admitted for 24 hour sampling to show that (i) a diurnal pattern of cortisol could be obtained, (ii) ACTH levels could be near normalised.

Most of the patients tolerated pump treatment and many felt much improved, even to the degree of refusing to stop. We believe that pump treatment is an treatment option for patients not responding to the regular replacement regimen on tablets. With the future improvement of pump technology, this treatment will become much easier too.

In conclusion, a near normal diurnal variation in cortisol and ACTH could be obtained. The analysis of quality of life including the use of the newly developed AddiQoL questionnaire, sleep and metabolic parameters are under analysis and will be presented in 3 planned manuscripts.

Potential Impact:

Dissemination of results has been an important and major task in the Euradrenal consortium and will continue to be even after the project period is completed. Our commitment to this task is illustrated by the dedication of a work package to this work. The plan for use and dissemination of foreground has taken place on multiple levels and through a range of channels. Dissemination has included:

Scientific publications

Altogether 49 papers have been published as a direct result of the Eurandreal consortiums' work. The majority have been published in the leading endocrinological, immunological and genetic journals. At least 20 additional papers are likely to be published in the near future as there is a lag in time between completion of studies and actual publication of results. Many of the most exciting results have not yet been published including our novel findings regarding the mouse model of Addison's disease, the latest findings on autoreative T cells and replacement therapy with subcutaneous infusion of hydrocortisone. As an outcome of our final symposium, a series of reviews will be published including the clinical guidelines in Journal of Internal Medicine (impact factor 7) in August/September 2012.

Dissemination through internet

Almost 3000 patients were included in Euradrenal studies. Without their collaborative spirit and willingness, none of the Euradrenal studies would have been possible to perform. A summary of the main results of Euradrenal is posted on our website in English, we are in the process of translating it into the various partner's own language. The information will be updated as new studies are published

Euradrenal website

The http://www.euradrenal.org website has been operational throughout the project including news, sections for health professionals, patients, families and the public. The domain will continue to operate and be updated.

Euradrenal workshops

Two workshops have been organized by Euradrenal, one dedicated to European patient organisations and one organized together with the Annual Meeting of The Norwegian Endocrine Society open to endocrinologists from all over Europe. At both workshops representatives from the European organisation contributed with their own presentations creating a collaborative atmosphere between patients and investigators and between participants from different countries this has transformed Euradrenal into a truly European endeavour that will continue to develop in the coming years. The programmes of both conferences is posted below

Partnership with patient organisations

A close collaboration with European patient organisations has been built and will continue to be an effective way to disseminate foreground. Since many of the beneficiaries are consultants for their national patient organisations, information is easily conveyed and many of the findings of Euradrenal are spread on these organisations own web pages. A particular spin-off of this partnership is the development of a new and very simple steroid card, now implemented for both adults and children in Sweden and Norway. We aim to spread this card to other countries in Europe with the ultimate goal to make a new European standard. We will continue to foster the close relationship with the patient organisations

Exploitation of results

Euradrenal has produced results in many area concerning AAD spanning from epidemiology to molecular mechanisms in pathogenesis and associated genes. It has generated a network of patient registries and biobanks and small animal models of AAD.

Exploitation of these results will take place in many areas

Resources for future research

1. Biobank and patient registry
2. Small animal models

Information of pathogenesis could be exploited in following areas:

1. Early diagnosis of progression in subclinical Addison's disease by T cell tests
2. Immunomodulatory treatment to stop progression of subclinical AAD by targeting autoreative T cells and B cells

List of Websites:

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