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ORIGINAL ARTICLE

Autoimmunity predominates in a large South African cohort with addison's disease of mainly European descent despite long-standing disease and is associated with HLA DQB*0201

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Summary

Objective We sought to determine whether autoimmunity is the predominant cause of Addison's disease in South Africa and whether human leucocyte antigen (HLA) DQ association exists.

Design We compiled a national registry of patients from primary care, referral centres and private practices.

Patients A total of *144 patients, 94 of European descent, 34 Mixed Ancestry, 5 Asian and 11 Black Africans* (mean age 45·9 years, range 2·7–88 years; mean duration of disease 13·1 years, range 0–50 years) and controls were matched for gender and ethnicity. All potential causes were investigated.

Results Fifty one per cent of cases (74 patients) were autoimmune in aetiology. Either 21-hydroxylase autoantibodies (72 patients, 50% of entire patient group) or adrenocortical autoantibodies (35 patients, 24%) were present, while 23% of patients had both. None of the Asian ($n = 5$) or Black ($n = 11$) patients had evidence of autoimmune disease. Overall 8% of patients had tuberculosis, 4% adrenoleucodystrophy, 1% adrenocorticotrophic hormone resistance syndrome and 6% X-linked adrenal hypoplasia. In those with autoimmune disease primary hypothyroidism (47%), premature ovarian failure (8%) and type 1 diabetes (7%) were the most prevalent accompanying autoimmune conditions. HLA DQB1*0201 alleles predominated in the autoimmune group (DQB1*0201: 65% *vs* 43% of controls $P = 0{\cdot}017$) with the *0201/*0302 heterozygous genotype being the most prevalent (28% *vs* 8% $P = 0{\cdot}02$).

Conclusions While autoimmunity accounts for at least half of patients with Addison's disease in South Africa and is associated with HLA DQB1*0201, none of the Black Africans or Asians in this cohort had adrenal autoantibodies. Moreover, 21-hydroxylase autoantibodies were detectable in a higher proportion than adrenocortical autoantibodies, especially in those patients with a long history after disease onset.

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Introduction

Although an increasing prevalence of primary adrenal insufficiency has been reported in Western countries over the past four decades, it is unclear whether this constitutes a true rise in prevalence or heightened awareness.[1,2] Autoimmune Addison's disease is the most common form of primary adrenal insufficiency in Western countries, accounting for 68–94% of cases, sometimes assessed by adrenal autoantibody assays in excess of 10 years after the onset of clinical Addison's disease.[2–5] In an Italian study 70% of patients with previously designated idiopathic Addison's disease with a disease duration of less than 20 years were positive for adrenal autoantibodies, consistent with an autoimmune aetiology.[6] Positive adrenocortical and 21-hydroxylase autoantibodies were reported in up to 90% of patients with recent-onset autoimmune Addison's disease, compared with 0·3% among healthy Italian control subjects.[7–9]

In contrast, a single study from South Africa suggested a frequency of autoimmune Addison's disease of only 12%, with the majority of cases being idiopathic (42%) or tuberculosis related (34%); however, adrenal autoantibodies were not measured in that study.[10] Type 1 diabetes has been extensively studied and serves as a prototype for the study of other autoimmune conditions. As islet-cell autoantibodies (ICA) titres decline following diagnosis, only 5–10% of type 1 diabetes patients remain ICA positive

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10 years after diagnosis; it is likely that in Addison's disease studied long after diagnosis, a significant number of patients will be adrenal autoantibody negative.[11] African patients too may have a different autoantibody profile when compared with European patients, as many cases of ketosis-prone diabetes in African patients are negative for autoantibodies to glutamic acid decarboxylase (GAD) 65 and only 4 in 10 African-Americans are ICA positive with new-onset type 1 diabetes.[11–13]

We thus sought to determine the aetiology of Addison's disease in a large South African cohort and hypothesized that autoimmunity would be more common than previously described. In addition, as human leucocyte antigen (HLA) DQ alleles have previously been reported to associated with adrenal autoimmunity, especially in autoimmune polyglandular syndrome type 2 (APS 2) with Addison's disease and type 1 diabetes,[14,15] we examined whether there was a predisposition to autoimmunity based on HLA DQB1 genotypes.[16]

Patients and methods

Patients

This cross-sectional South African study was adapted from a previous protocol.[9] Over 9000 emails and 1500 letters requesting information on patients with Addison's disease were sent to general practitioners, specialist physicians and endocrinologists in both the private and public sectors. All academic and district hospitals throughout South Africa and almost half of the country's registered general practitioner population were contacted. The University of Cape Town Research and Ethics Committee approved the study, and each participating tertiary hospital received approval through their respective research and ethics committees. All consenting patients with confirmed Addison's disease (by clinical presentation, low basal cortisol and simultaneously elevated adrenocorticotrophic hormone (ACTH) concentration, or where indicated, a peak cortisol after 250 μg Synacthen® (Novartis Pharma AG, Basel, Switzerland) of <550 nmol/l associated with a raised plasma ACTH concentration exceeding 10·1 pmol/l) were enrolled.[10] A questionnaire was completed at enrolment to ascertain demographic and clinical data.

Autoimmune Addison's disease was diagnosed when subjects were positive for adrenocortical and/or 21-hydroxylase autoantibodies.[6] Tuberculosis-related adrenal insufficiency was diagnosed if there was a prior or current history of tuberculosis. [6]. Biopsy-proven or radiological evidence of metastatic disease indicated a metastatic aetiology of Addison's disease, while patients with sarcoidosis or iron overload and no other clear cause of hypoadrenalism had Addison's disease attributed to these conditions.[17] X-linked adrenal hypoplasia was diagnosed in boys with primary adrenal failure and salt loss in the first weeks of life associated with hypogonadotrophic hypogonadism. The diagnosis of ACTH resistance syndrome was made based on primary hypoadrenalism and isolated glucocorticoid deficiency, with normal mineralocorticoid function.[18,19] Patients with an AIDS-defining illness were classified as having AIDS-related Addison's disease. The diagnosis of idiopathic Addison's disease was reserved for patients in whom there was no obvious clinical cause, without an associated endocrinopathy or positive autoantibodies.[4] Sarcoidosis, iron overload and HIV were excluded clinically with appropriate laboratory studies. All patients had blood samples obtained for tissue transglutaminase, thyroid microsomal, antithyroglobulin, parietal cell, islet cell, ovarian, testicular, and placental antibody determination, as well as HLA DQB1 typing.

Serum autoantibodies

Adrenocortical autoantibodies were determined using indirect immunoflouresence on cryostatic sections of human adrenal glands.[20,21] Within 2 h of surgical removal, the substrate human adrenal gland is snap frozen in isopentane that has been cooled in a dry-ice acetone bath.

21-hydroxylase autoantibodies[22] were measured with a commercial immunoprecipitation assay based on ^{125}I-labelled recombinant human 21-OH (RSR Ltd., Cardiff, UK). 21-OHAbs levels above 1 U/ml were considered positive. The assay has high specificity for 21-hydroxylase antibodies and is more sensitive than the immunofluorescence assay for detection adrenal antibodies in cases with overt adrenal failure.[23]

Islet-cell autoantibodies were determined in an indirect immunofluorescence assay using cryostatic sections of human pancreas of blood group O. Values ≥10 JDF units were considered positive. In past Immunology of Diabetes Society (IDS) workshops, this ICA assay had a specificity of 100% and a sensitivity of 74·4% in patients with new-onset type 1 diabetes who were less than 30 year of age.[24,25]

The thyroid microsomal antibodies were measured using a haemagglutination assay, utilizing a kit, Thymune-M (Remel Europe Ltd, Dartford, Kent, UK). Results were considered positive if the titre exceeded 1:400.

Thyroglobulin autoantibodies (Anti-Tg): were measured using a haemagglutination assay, utilizing a kit, Thymune-T, (Remel Europe Ltd, Dartford, Kent, UK). Results were considered positive if the titre exceeded 1:100.

Tissue transglutaminase autoantibodies (AntiTTG) IgA antibodies against tissue transglutaminase were determined with a routine in-house ELISA at the Department of Clinical Immunology, University Hospital, Uppsala, using commercially available guinea pig transglutaminase and rabbit anti-IgA antibodies. The upper normal reference range, 95th percentile**,** was 6 kUnits/l.

Steroid cell cytoplasmic autoantibodies (StcAb) were measured by indirect immunofluorescence using as substrate human placenta, human testes and pregnant rabbit ovary. StcAb may react with syncytiotrophoblast in the placenta, Leydig cells in the testes, and/or the theca interna/granulosa cell layers of the Graafian follicles, and the corpora lutea of the ovaries. Some StcAb-positive sera react with all tissues whereas other sera may only react with one or two tissues.[26]

Parietal cell antibodies were measured by indirect immunofluorescence, using human stomach as substrate and read as positive or negative.[27]

Very Long-Chain Fatty Acids (VLCFA's)

The plasma VLCFA concentration was determined by capillary gas chromatography-mass spectrometry following procedures

described previously.[28,29] Normal values were determined in 50 healthy, similarly aged South African controls to the study population. (C26:0 0·5–2·1 μmol/l, C24:0 12·6–46 μmol/l, C22:0 33·9–157·5 μmol/l). Normal C24/C22 and C26/C22 ratios were calculated as 0·2–0·4 and 0·01–0·02, respectively. All abnormal results obtained were confirmed by an external laboratory (Lab. Genetic Metabolic Diseases, F0-225; Academic Medical Centre, University of Amsterdam, The Netherlands).[28,29]

HLA DQB1 genotyping

HLA DQB1 genotypes were determined using a previously described method.[30] Briefly, exon 2 of the DQB1 gene was amplified using PCR. The amplified PCR products were then separated by denaturing gradient gel electrophoresis. DQB1 alleles were determined by comparing the migration of bands from samples and standards with known identity. For each patient with Addison's disease, a healthy control matched for gender and ancestry was selected from adults attending the blood bank clinic.

Statistical methods

Patient characteristics were described as means with 95% confidence intervals for continuous variables and numbers of patients and percentages for binary and categorical variables. Data were compared between autoantibody groups using the univariate linear regression Wald test for continuous variables, and chi-squared tests for binary and categorical variables.

The relative risk compared to controls in HLA allele frequency was described for European ancestry patients without type 1 diabetes to avoid confounding by ethnicity or known associations with type 1 diabetes mellitus. Factors associated with an autoimmune aetiology in the study cohort were explored in univariate and multivariate logistic regression models.

The 21-hydroxylase autoantibody titres were transformed to a logarithmic scale, plotted and regressed against the duration of Addison's disease at enrolment in a two-way scatter plot. All analyses were conducted using STATA™ version 10.0 (Stata Corp., College Station, TX, USA).

Results

Seven patients with an original label of Addison's disease were excluded (two had a normal Synacthen® test, two had secondary hypoadrenalism, one had a bilateral adrenalectomy for Cushing's disease and two had suppression of the hypothalamic-pituitary adrenal axis, related to previous steroid use for another indication). A total of 147 patients were identified with 144 (61% women) patients with Addison's disease enrolled. Three patients declined citing personal reasons (see Fig. 1). The mean age at enrolment was 45·9 years (range 2·7–88; 95% confidence interval [CI] 42·5–49·4 years). The mean age of diagnosis was 32·8 years. Most patients had long-standing disease (mean of 13·1, range 0–50 years) at enrolment. Most patients (n = 109, 75%) were over 20 years of age at diagnosis and 49% were diagnosed more than 10 years earlier. Eighty-eight per cent were recruited from the major metropolitan centres of South Africa, often in close association with a major teaching hospital. The ethnicity of the cohort was heterogeneous: 65% European ancestry, 24% Mixed ancestry, 3% Asians and 8% Black South Africans.

Seventy per cent (n = 101) had identifiable causes of Addison's disease. Of the total cohort, 51% had autoimmunity based on the presence of 21-hydroxylase and/or adrenocortical autoantibodies, 8% had a history of tuberculosis, 4% had adrenoleucodystrophy, 1% had ACTH resistance syndrome and 6% had X-linked adrenal hypoplasia. A presumptive diagnosis of tuberculous adrenalitis was made in 8% of subjects; only two subjects had CT scans with both being suggestive of tuberculous adrenalitis. In 30% no specific

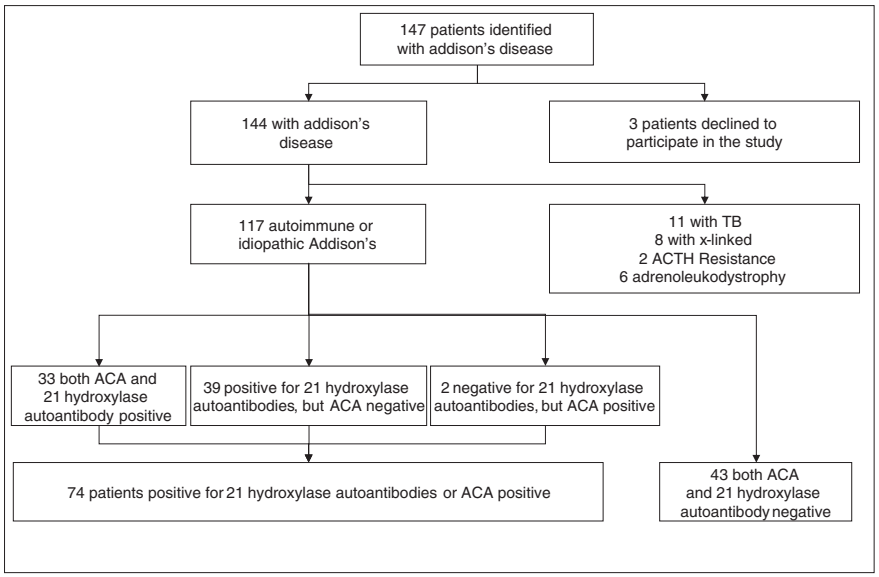

Fig. 1 Diagnostic categories of patients included in the study.

ACA – adrenocortical autoantibodies

aetiology was uncovered. There were no patients with sarcoidosis, iron overload, metastatic disease or AIDS-related Addison's disease.

Of the 74 patients with at least one adrenal autoantibody, 72 (96%) had autoantibodies to 21-hydroxylase, 35 (48%) had adrenocortical autoantibodies and 33 (45%) were positive for both antibodies. Of the 74 patients with adrenal autoimmunity, primary hypothyroidism was the most common associated clinical autoimmune condition in 47%, while premature ovarian failure (8%) and type 1 diabetes (7%) were the next most prevalent disorders. Hypoparathyroidism, pernicious anaemia, coeliac and Graves' disease, immune thrombocytopenic purpura, ulcerative colitis and vitiligo coexisted in 5% or fewer of those subjects positive for 21-hydroxylase and/or adrenocortical autoantibodies. In patients with antiadrenal antibodies, primary hypothyroidism was present in 20 of 32 patients (positive thyroid microsomal or antithyroglobulin antibodies), coeliac disease was present in two of six patients (positive tissue transglutaminase autoantibodies), pernicious anaemia was diagnosed in two of 11 patients with positive anti-parietal cell antibodies and although six patients had type 1 diabetes, only four of these patients had islet-cell autoantibodies. The proportions of the total 144 patient cohort with either APS 1 or APS 2 were 5 (3%) and 66 (46%), respectively. Overall, 11 (7·6%) patients had a first-degree relative with Addison's disease.

When comparing the Addison's disease patients with autoimmunity with those defined as idiopathic (Table 1), the former were younger at diagnosis and were more likely to have a positive family history of autoimmune disease. Among the subgroup designated as idiopathic Addison's disease, 20 patients (47%) had at least one other organ-specific autoantibody. It is likely that some of these subjects have lost serologic evidence of adrenal autoimmunity over time and adding this group to the autoimmune group would increase the proportion with autoimmune Addison's disease in the entire cohort to 65%. The disease duration obtained at enrolment was not different between the idiopathic group without antibodies and the autoimmune group.

The idiopathic group was older than the other two groups ($P = 0{\cdot}002$). Interestingly, none of the five Asian or 11 Black patients was positive for adrenal autoantibodies. More patients with adrenal autoantibodies had a history of foreign ancestry (first- or second-degree relative born in Europe or the USA).

An inverse correlation was present between titres of 21-hydroxylase autoantibodies and duration of disease since diagnosis (Fig. 2).

Table 1. Comparisons based on adrenal autoantibody status

	ACA(+) and/or anti-21(+)	ACA(−) and anti-21(−)	Comparison *P*-value
Patients in group (*N*)	74	43	
Gender – n (%)			
Female	54 (73)	30 (70)	0·710
Age at diagnosis			
Age in years (95% CI)	33·1 (29·3–36·9)	40·0 (34·6–45·5)	0·034
Duration of Addison's diagnosis at enrolment – *n* (%)			
New onset	6 (08)	7 (16)	0·573
1–4·9 years	18 (24)	9 (21)	
5–9·9 years	13 (18)	6 (14)	
≥10 years	37 (50)	21 (49)	
Ancestry – *n* (%)			
European	65 (88)	21 (49)	<0·001
Mixed	9 (12)	15 (35)	
Asian	0 (00)	2 (05)	
African	0 (00)	5 (12)	
Other antibodies – *n*/*N* (%)			
Any other autoantibody	46/74 (62)	20/43 (47)	0·100
Transglutaminase	4/73 (05)	2/43 (05)	0·846
GADA†	15/71 (21)		
Thyroid microsomal	24/74 (32)	9/43 (21)	0·183
Anti-thyroglobulin	8/74 (11)	2/43 (05)	0·251
Parietel cell	11/74 (15)	9/43 (21)	0·401
Islet cell	4/74 (05)	3/43 (07)	0·730
Ovarian	5/74 (07)	2/43 (05)	0·643
Testicular	1/74 (01)	0/43 (00)	0·444
Placental	1/74 (01)	3/43 (07)	0·106
Prevalence of other autoimmune diseases – *n* (%)			
Any auto-immune disease	44 (59)	27 (63)	0·722
Family history and ancestry – *n* (%)			
Family History‡	24 (32)	5 (12)	0·012
Foreign ancestry	36 (49)	13 (30)	0·052
Therapy – mean (mg) (95% CI)			
Hydrocortisone dose	24·8 (22·8–26·8)	21·0 (18·6–23·5)	0·026
Hydrocortisone dose/m^2	16·2 (11·2–21·2)	11·4 (9·5–13·4)	0·305
Fludrocortisone dose	0·1 (0·1–0·1)	0·1 (0·1–0·1)	0·904
Fludrocortisone dose/m^2	0·1 (0·0–0·1)	0·1 (0·0–0·1)	0·643

ACA, adrenocortical autoantibody; anti-21, 21 hydroxylase autoantibodies; GADA, glutamic acid decarboxylase 65 antibodies; CI, confidence interval; mg, milligrams; univariate linear regression wald test used for continuous variables, chi-square statistic for proportions.
†Only done in anti-21 hydroxylase autoantibody positive patients; ‡Presence of autoimmune disease in first or second-degree relatives.

HLA alleles and autoimmune Addison's disease

Compared to controls, the HLA DQB1*0201 allele was positively associated with autoimmunity in European ancestry patients without type 1 diabetes while the *0601 allele was negatively associated (Table 2). All patients with type 1 diabetes mellitus had alleles *0201,*0302 or *0301 and hence these patients were excluded to avoid bias. In sensitivity analyses, these associations persisted in the entire cohort, and because of small numbers, could not be adequately described in patients of Mixed, Black or Asian ancestry. When comparing all patients with autoimmune Addison's disease to all other patients with Addison's disease, the same crude associations are present (Table 3).

HLA DQB1 genotypes and autoimmune Addison's disease

The most common genotypes in all patients with Addison's disease were *0201/*0302 (19%) and *0201/*060x (10%). The *0201/*0302 genotype was more common in patients with autoimmune Addison's disease (28%), compared to controls without Addison's disease (8%, $P = 0{\cdot}002$). This was also the only genotype associated with autoimmune Addison's disease when compared to patients with Addison's disease of other aetiology (unadjusted odds ratio [OR] 3·2, 95% CI 1·3–8·3, $P = 0{\cdot}01$; Table 3).

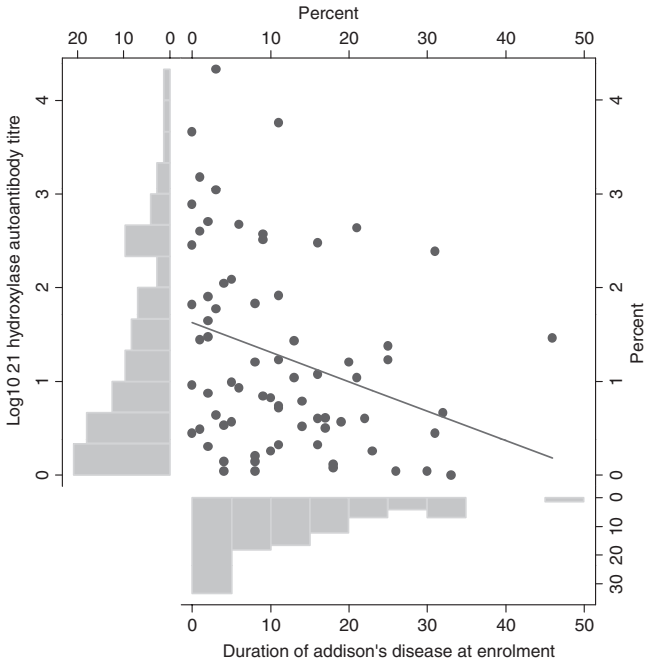

Fig. 2 Relationship between duration with Addison's disease and 21-hydroxylase autoantibody titre. The hstiograms indicated a skewed distribution of antibodies with the greatest number of patients having the lowest antibody titre (*y*-axis) and shortest duration of Addison's disease at enrolment (*x*-axis).

Table 2. Associations between autoimmune Addison's disease and human leucocyte antigen (HLA) subtypes in 55 patients of European ancestry without type 1 diabetes mellitus controls

Allele	Tested (*N*)	Autoimmune Addison's disease* Present (%)	Controls Present (%)	Relative risk	*P*-value**
0201	55	36 (65·4)	26 (43·3)	1·5	0·0174
0302	55	20 (36·3)	17 (28·3)	1·3	0·3571
0301	55	13 (23·6)	21 (35·0)	0·7	0·1822
0402	55	8 (14·5)	8 (13·3)	1·1	0·8512
0500	55	10 (18·1)	17 (28·3)	0·6	0·1995
060×	55	15 (27·2)	22 (36·6)	0·7	0·2814
0601	55	1 (1·8)	7 (11·6)	0·2	0·0381

*Six patients were homozygous for the same allele (4 × 0201, 1 × 0300, 1 × 0301), and one patient had 0401 which was not included in the table, resulting in 103 alleles reported for 55 patients.
**Two-sample test of proportions.

None of the HLA alleles or heterozygous genotypes were significantly associated with autoimmune aetiology in a multivariate analysis that was adjusted for sex and ancestry, although there was some evidence of a potential association with the *0201/*0302 heterozygous genotype (adjusted odds ratio [AOR] 2·4, $P = 0·105$) (Table 3).

Discussion

Since our original report 11 years ago in this *Journal,* we are able, with improved access to special investigations and application of these to an appreciably larger cohort, to contribute to the already known body of evidence. This study, the largest cross-sectional study of Addison's disease in sub-Saharan Africa in a heterogeneous population of predominantly European descent, refutes our earlier report suggesting that autoimmunity is an uncommon aetiology for Addison's disease in South Africa.[10] We have shown for the first time that adrenocortical antibodies are not nearly as detectable as 21-hydroxylase antibodies in monitoring Addison's disease long after the onset. Nevertheless, an inverse relationship exists between duration of Addison's disease and 21-hydroxylase antibody titres. Curiously, none of the Asians or Black subjects, albeit that both groups had small numbers, demonstrated adrenal autoantibodies. Addison's disease is likely being under diagnosed in South Africa, with potential grave consequences to some patients.

Over 50% of our cohort was classified as autoimmune on the basis of at least one positive adrenal autoantibody. If one considers long-standing Addison's patients who were adrenal autoantibody negative, but had other autoimmune clinical conditions as likely to have autoimmune Addison's, the prevalence of autoimmune Addison's rose to 65%. We found an inverse correlation between the titres of 21-hydroxylase autoantibodies and the duration of disease since diagnosis, confirming that the adrenal autoimmunity wanes with increasing time after diagnosis. Thus, consistent with other Western studies, our study confirms that autoimmunity is the most common cause of Addison's disease in South Africa, in a cohort that was predominantly of European descent.[3] On the other hand, very few Black patients were enrolled and none of them had autoimmunity, which may explain the similar prevalence to other Western countries.

The cross-sectional design and measurement of autoantibodies in many cases long after the initial diagnosis is the major limitation of our study. Previous studies have demonstrated a 20% decline in the prevalence of autoantibodies in subjects followed for two or more years after the diagnosis of Addison's disease.[3,7] Our study clearly demonstrates that 21-hydroxylase autoantibody titres decline with increasing duration of Addison's disease. The possibility therefore exists that patients who were adrenal autoantibody positive at diagnosis were antibody negative at recruitment into the study, thus falsely reducing the reported prevalence of autoimmune Addison's. A further limitation is that very few patients underwent a CT scan of the adrenal glands, an investigation of proven diagnostic utility, particularly in the setting of negative autoantibodies. The final limitation of the study relates to possible ascertainment bias. Although every effort was made to comprehensively identify patients with Addison's disease, we were unable to determine what proportion of cases were missed because of nonresponse, or whether or not there was any selection bias in those patients included in the cohort. The vast majority of the patients recruited for the study were of European ancestry, a minority were Black African and Asian, a profile which is unrepresentative of the South African population, where the majority of the population are Black Africans (79%) with smaller proportions of European ancestry (10%), Mixed (9%) races and Asians (2%). It is unclear whether the preponderance of European ancestry patients with Addison's disease represents a true higher prevalence of the disease

Table 3. Associations with autoimmunity in the Entire cohort with Addison's disease

	Univariate analyses*			Multivariate analysis		
Characteristic	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Female gender	2·9	1·4–5·7	0·003	3·0	1·2–7·1	0·015
Age of diagnosis (per 10 year increase)	1·0	0·9–1·2	0·836			
European ancestry	10·2	4·4–23·7	<0·001	10·1	3·9–25·8	<0·001
Any other associated autoimmune diseases	2·1	1·1–4·0	0·030			
A family history of autoimmune diseases	4·3	1·7–10·8	<0·001			
Foreign ancestry	2·5	1·3–5·1	0·008			
Pigmentation on diagnosis	2·3	1·1–5·0	0·033	2·5	0·9–6·9	0·068
Severe presentation	2·6	1·3–5·2	0·005			
Any other autoantibodies	3·6	1·7–6·7	<0·001	2·3	1·0–5·4	0·045
Presence of HLA types						
0201	2·6	1·2–4·7	0·012			
0302	2·0	0·8–3·6	0·127			
0301	1·0	0·4–1·6	0·486			
0402	2·5	0·5–5·5	0·365			
0500	0·8	0·2–1·3	0·154			
060×	0·8	0·3–1·3	0·218			
0601	0·7	1·3–8·8	0·007			
Presence of specific HLA combination						
0201/0302	3·2	1·3–8·3	0·010	2·4	0·8–7·1	0·105

*$n = 117$; excludes patients with known aetiology of Addison's disease other than autoimmunity.

in this population, or is an artefact reflecting more limited access of the Black population to health care with resultant missed diagnosis and/or underreporting.

The contributions of tuberculosis and other known causes of Addison's disease (8% and 18% of our cohort) are in agreement with other European studies.[3,9] While both South Africa and India are developing countries, our findings contrast starkly with data from an Indian cohort with adrenal insufficiency in which the prevalence of primary adrenal failure attributable to tuberculosis was considerably greater (50%) than that found in our study (8%).[31] Given the high background prevalence of tuberculosis and HIV (tuberculosis case finding was 1000/100 000 and antenatal HIV seroprevalence was 29% in South Africa in 2006), surprisingly few patients presented with either tuberculosis or HIV-related Addison's disease in our study.[32,33] However, an earlier study from a South African tertiary care hospital determined that primary hypoadrenalism is uncommon in a cohort of acutely ill, hospitalized patients with active pulmonary tuberculosis, supporting the contention that tuberculosis-related primary adrenal failure is relatively uncommon.[34]

The heterogeneity of the population group studied, likely contributes to the observed frequencies of adrenal autoimmunity. Consistent with most studies, the vast majority of our patients were of European ancestry, with Black and Asian participants constituting a small minority. None of our Black and Asian patients had adrenal autoantibodies. Black Africans with type 1 diabetes in sub-Saharan Africa similarly have decreased frequencies of islet autoantibodies compared with their European ancestry counterparts, suggesting that genetic differences exist for the lack of antibody response in both type 1 diabetes and Addison's disease or the aetiologies of these disorders are considerably different between these populations.[12,13]

Addison's disease is often associated with other autoimmune diseases including type 1 diabetes and thyroiditis, making it difficult to determine whether the observed HLA associations are attributed to the presence of other autoimmune disease or with Addison's disease specifically.[35,36] Our study excluded patients with autoimmune Addison's disease and associated autoimmune conditions to eliminate this source of bias. The sample sizes in almost all studies are small limiting study power and increasing the chance of a type 2 error. On the other hand, the observed associations could reflect random chance. In our regression analysis, we observed a positive association between Addison's disease and several HLA*DQB1 alleles: especially DQB1*0201 and *0302 and *0601 alleles and the *0201/*0302 genotype. The presence of type 1 diabetes in patients with APS 2 is reportedly strongly associated with DQB1*0201 and DQB1*0302 alleles. However, even after excluding Addison's patients with type 1 diabetes, we still observed an association with *0201 and *0302, confirming a previous report.[37] Even though our study has a relatively large sample size compared some previous studies, the study power is still limited and associations require confirmation in further larger studies.

In contrast to our study, 21-hydroxylase autoantibodies titres did not decline with increasing duration of disease among North Indian Caucasians.[31] However, these patients were studied for a much shorter time [mean duration of Addison's disease was 12 months (range 1–72 months), compared to 13·3 years (range 0·03–50 years)] in our study. The declining autoantibody titres over time has been attributed to the diminished antigenic stimulation because of the progressive destruction of the adrenal cortex.[31] A similar waning of autoimmunity occurs in coeliac disease where reduction of transglutaminase autoantibody levels is observed after introducing a gluten-free diet.[38] Similarly, thyroglobulin

autoantibody titres normalize once the thyroid is ablated for malignancy.[39] Finally, in type 1 diabetes ICA autoantibody prevalence declines with time after diagnosis, so that only 5–10% are ICA positive 10 years after diagnosis.[11]

This study is unique, as it represents a truly multicentre collaboration within South Africa. The goal of this study was to include and characterize all patients with a confirmed diagnosis of Addison's disease in South Africa. Given the known prevalence rates of Addison's disease in other countries, it is likely that this condition is still being under diagnosed. Based on a conservative prevalence of 0·6 in 100 000 individuals, over 250 patients should have been identified.[2,4] There is no known information on the prevalence of Addison's disease in Black African populations. The almost negligible contribution of patients with Addison's disease from rural areas suggests that the diagnosis is being missed or that patient survival is compromized in rural areas.

Conclusions

In summary, we have shown for the first time that despite the high prevalence of tuberculosis and HIV in South Africa, autoimmune Addison's disease is by far the most common cause of adrenal insufficiency in South Africa. HLA DQB*0201 and *0302 correlate with adrenal autoimmunity while a novel finding of this study is that *0601 may be protective. Specifically, both the Asian and the Black subgroups were negative for adrenal autoantibodies. Notwithstanding our best efforts to identify as many patients with Addison's disease as possible, it is highly likely that this condition is being under diagnosed. Despite measurement long after diagnosis, markers of autoimmunity persist in many patients with Addison's disease. 21-hydroxylase autoantibodies identified twice as many autoimmune Addison's disease patients as adrenocortical antibodies and therefore appear more sensitive markers of an autoimmune aetiology in patients with long-standing disease.

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Conflicting interests

None to declare.

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