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Identification of a subgroup of black South Africans with type 1 diabetes who are older at diagnosis but have lower levels of glutamic acid decarboxylase and islet antigen 2 autoantibodies

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Abstract

Aims To compare the age at diagnosis and prevalence of islet autoantibody [glutamic acid decarboxylase (65 kDa) 65 and islet antigen 2] positivity in black and white participants with type 1 diabetes in South Africa, and to analyse the relationship between age at diagnosis and the presence of autoantibodies.

Methods Participants were recruited from diabetes outpatient departments and autoantibodies to glutamic acid decarboxylase (65 kDa) and islet antigen 2 were measured by enzyme-linked immunosorbent assay.

Results We recruited 472 (353 black and 119 white) participants with type 1 diabetes. Age at diagnosis of diabetes was later in black (19.7 ± 10.5) than in white participants (12.7 ± 10.8 years; $P < 0.001$) with a median (interquartile range) disease duration of 5.0 (2.0–10.0) and 8.5 (4.0–20.0) years ($P < 0.001$), respectively. An older age at diagnosis (≥ 21 years) was more frequent in black (152 of 340, 45%) than in white participants (24 of 116, 21%; $P < 0.001$). The prevalence of islet antigen 2 autoantibodies was 19% (66/352) in black and 41% in white participants (48/118; $P < 0.001$). There was no significant difference in glutamic acid decarboxylase (65 kDa) autoantibody positivity between black (212/353, 60%) and white participants (77/117, 66%; $P = 0.269$). In black, but not white, participants the prevalence of both glutamic acid decarboxylase (65 kDa) and islet antigen 2 autoantibody positivity was significantly lower in participants diagnosed at age ≥ 21 years ($P < 0.001$ for both comparisons).

Conclusions The older age at diagnosis, lower prevalence of islet antigen 2 autoantibodies and a distinct subgroup of participants with type 1 diabetes with age at diagnosis of > 20 years in the black compared to white population suggest a difference in the immunological aetiology of type 1 diabetes in these two population groups.

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Introduction

Autoantibodies to the 65kDa isoform of glutamic acid decarboxylase (GAD65), tyrosine phosphatase-like insulinoma antigen 2 [islet antigen (IA)2], insulin and zinc transporter 8 (ZnT8) autoantibodies [1] are the most useful markers of the autoimmune process in type 1 diabetes. Over 90% of newly diagnosed individuals are positive for at least one of these autoantibodies. The 10-year risk of progression to type 1 diabetes increases with a higher number and titre of autoantibodies, a younger age at seroconversion and with the presence of high-risk HLA genotypes [2].

The prevalence of autoantibodies in participants with type 1 diabetes has been investigated in a variety of population groups. A study conducted in 194 white European people newly diagnosed with type 1 diabetes (aged < 40 years) found a frequency of 76% and 58% for GAD65 and IA2 autoantibodies, respectively. GAD65 and IA2 autoantibody positivity declined to 60% and 45%, respectively, after 4 years [3]. The prevalence of GAD65 and IA2 autoantibodies in 57 Japanese children (aged < 15 years) with new-onset type 1 diabetes was 75% and 72%, respectively, whilst in 97 adults (aged ≥ 18 years) the frequencies were 78% and

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What's new?

- Autoantibodies to islet cell-specific antigens are markers of type 1 diabetes development.
- There are limited data on the prevalence of type 1 diabetes-related autoantibodies in sub-Saharan African people with type 1 diabetes.
- A lower prevalence of islet antigen (IA)2 autoantibodies was found in black compared to white South Africans with type 1 diabetes.
- In black but not white participants the prevalence of glutamic acid decarboxylase (65 kDa) and IA2 autoantibody positivity was significantly lower in participants diagnosed at ≥ 21 years of age.
- These data suggest ethnic differences in type 1 diabetes immune-related aetiology which may give greater insight into the pathophysiology of the disease and allow the development of targeted interventions.

41%, respectively [4]. A study in Bangalore, India, found frequencies of 65% and 19% for GAD65 and IA2 autoantibodies, respectively, in 88 children diagnosed at < 18 years of age with a disease duration < 48 months [5]. Libman *et al.* [6] reported frequencies of 58% and 77% GAD65 autoantibody positivity, and 42% and 63% IA2 autoantibody positivity, in 437 newly diagnosed (before age 19 years) black and white participants in the USA, respectively [6]. The percentage of autoantibody positivity can thus vary depending on the individual's age at diabetes onset, duration of disease and ethnicity [7].

Only two studies have assessed the prevalence of GAD65 autoantibodies in South African participants with type 1 diabetes. A study from Johannesburg, South Africa reported that 44% of 100 black participants with type 1 diabetes of varying disease duration were GAD65 autoantibody-positive [8]. Rheeder *et al.* [9] showed that the prevalence of GAD65 autoantibodies was significantly lower in 43 black compared to 17 white South Africans presenting with diabetic ketoacidosis (33% vs 67%); however, no study has examined the prevalence of both GAD65 and IA2 autoantibodies in black individuals with type 1 diabetes. In addition, a South African study showed that the age at diagnosis in black participants with type 1 diabetes was older than that in white participants [10]. We therefore hypothesize that GAD65 and IA2 autoantibodies occur at lower frequencies in black compared to white individuals with type 1 diabetes and that this may be related to differences in age at diagnosis in the two ethnic groups. The aim of the present study was to determine the prevalence of GAD65 and IA2 autoantibodies and the relationship between autoantibody positivity and age at diagnosis, and to examine ethnic differences between black and white South Africans with type 1 diabetes.

Methods

Study population

Black and white South African participants with type 1 diabetes were recruited by convenience sampling from private and government diabetes clinics in Pretoria, Johannesburg and Durban over a 5-year period (November 2007 to December 2012). Anthropometric measurements were taken and demographic data collected using a questionnaire including date of birth, age at diagnosis and gender. Participants with known type 2 diabetes, secondary diabetes or gestational diabetes were excluded from the study.

Classification of diabetes

Participants with type 1 diabetes were classified using clinical criteria: age at diagnosis < 30 years and insulin therapy within the first year of diagnosis (therapy initiated immediately in children). Participants aged > 30 years at diagnosis but requiring insulin treatment within the first year of diagnosis and who were not obese were classified as having type 1 diabetes.

Autoantibody measurement

The concentrations of GAD65 and IA2 autoantibodies were measured using enzyme-linked immunosorbent assay (ELISA) kits (Kronus; Star, ID, USA) according to the manufacturer's instructions. All assays were run in the same laboratory and the interassay coefficient of variation was < 5% for both ELISAs. Each individual ELISA was run using samples from both ethnic groups and from participants with a range of ages at diagnosis. Participants were considered GAD65 and IA2 autoantibody-positive when the respective concentrations were ≥ 5 IU/ml and ≥ 15 IU/ml, based on the manufacturer's recommendations.

Statistical analysis

Data were analysed using R version 3.5.0 (2018-04-23) [11]. The Shapiro–Wilk *W*-test was used to test for normality of continuous variables, and those that were not normally distributed (age, age at diagnosis, duration of disease and BMI) were presented as median (interquartile range) and compared between groups using the Mann–Whitney *U*-test. Categorical data were compared between groups using the chi-squared test and were presented as proportions and percentages. Odds ratios were calculated with 95% CIs and *P* values using the mid-*P* method (using compareGroups in R). For all analyses, $P < 0.05$ was considered statistically significant. Multivariable logistic regression analyses were performed using autoantibody status as the dependent

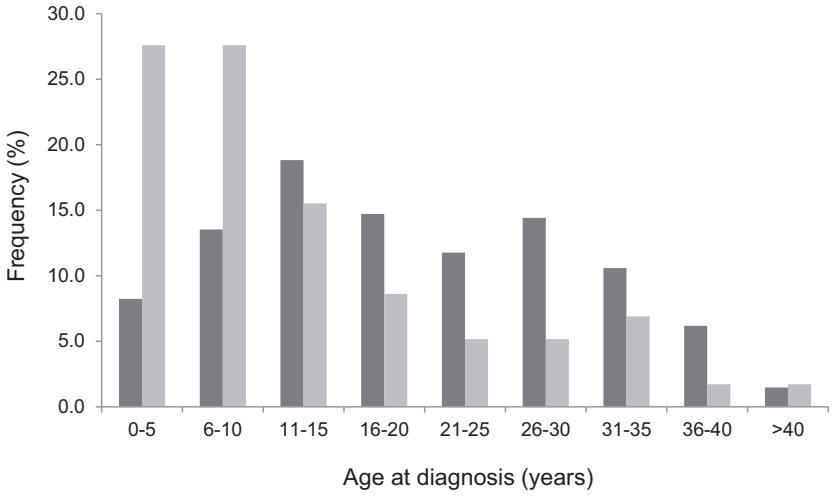

FIGURE 1 Frequency of affected participants by age at diagnosis in black and white South Africans with type 1 diabetes. Dark grey bars represent black participants and light grey bars represent white participants with type 1 diabetes.

variable. The independent variables were ethnicity, duration of disease, age at diagnosis and gender. The age thresholds of < 21 or ≥ 21 years were selected based on data from Kalk *et al.* [10], who showed a peak age of onset at 22–23 years of age in the black population and this was also the midpoint between the two peaks for age at diagnosis (Fig. 1) observed in the black participants in the present study.

A sample size calculation was performed based on the only data from South Africa comparing GAD65 autoantibody prevalence between black (33%) and white (67%) people with type 1 diabetes [9]. We assumed an underestimation of GAD65 autoantibody prevalence in the African population in this study and set the prevalence to 50%. Thus, the minimum number per group was calculated to be 65 participants. To account for any inaccuracies in our estimations we chose to recruit a minimum of 100 participants within the white type 1 diabetes group (fewer white than black people with type 1 diabetes attend the public hospitals from which the majority of participants were recruited).

Ethics

Ethics clearance was obtained from the University of the Witwatersrand Human Research Ethics Committee (M10978, M150885) as well as the University of Pretoria (85/2009) and the University of KwaZulu Natal (BE224/010). Informed consent was obtained from participants or parents/legal guardians where necessary.

Results

Clinical and laboratory characteristics

A total of 472 participants with type 1 diabetes [353 (75%) black and 119 (25%) white] were recruited for this study, with 74 participants (16%) recruited within 1 year of their diagnosis. The clinical and anthropometric characteristics of the study population are shown in Table 1. Black participants were significantly older at diagnosis ($P < 0.001$) and had a shorter duration of disease ($P < 0.001$). Amongst the

Table 1 Anthropometric and clinical characteristics among black and white participants with type 1 diabetes

Characteristic	Total (n = 472)	Black (n = 353)	White (n = 119)	$P^{\S}$
Age, years	26.2 (16.2–35.4)	26.9 (17.7–35.2)	23.3 (12.5–37.7)	0.129
Gender				0.059
Male, n (%)	224 (48)	158 (45)	66 (56)	
Female, n (%)	247 (52)	194 (55)	53 (44)	
Age at diagnosis*, years	16.0 (9.0–27.0)	19.0 (11.0–29.0)	8.5 (5.0–17.0)	<0.001
Disease duration†, years	6.0 (3.0–11.0)	5.0 (2.0–10.0)	8.5 (4.0–20.0)	<0.001
BMI‡, kg/m^2	23.4 (20.1–28.0)	24.0 (20.3–28.7)	22.4 (18.9–26.1)	0.023

Results are presented as median (interquartile range). Missing information for
*age at diagnosis (n = 17),
†disease duration (n = 16) and
‡BMI (n = 30).
§P value for comparison between black and white participants.

black participants, there were more women (n = 194, 55%), whereas amongst the white participants, men predominated (n = 66, 56%). White participants had a significantly lower BMI (P = 0.023).

Age at diagnosis

Figure 1 shows the distribution of age at diagnosis by ethnicity. In the white population, most participants (64/116, 55%) were diagnosed by 10 years of age. Only 22% of black participants (74/340) were diagnosed by 10 years of age (P < 0.001). Two distinct peaks for age at diagnosis were observed in the black population, 11–15 years and 26–30 years. A higher percentage of black compared to white participants (45%, 152/340 vs. 21%, 24/116; P < 0.001) were diagnosed at ≥ 21 years of age.

Autoantibody positivity, age at diagnosis and disease duration

The overall prevalence of autoantibody positivity was 65% (304/470), with 62% (289/470) GAD65 autoantibody-positive, 24% (114/470) IA2 autoantibody-positive and 21% of all participants (99/469) positive for both autoantibodies (Table 2). The prevalence of GAD65 autoantibodies was similar in black (60%, 212/353) and white participants (66%, 77/117) (P = 0.269). By contrast, significantly more white participants were IA2 autoantibody-positive than black participants (41%, 48/118 vs 19%, 66/352; P < 0.001). Furthermore, more white than black participants were GAD65 and/or IA2 autoantibody-positive (74%, 86/117 vs 62%, 218/353; P = 0.028) or GAD65 and IA2 autoantibody-positive (33%, 39/117 vs. 17%, 60/352; P < 0.001).

In both population groups the GAD65 and IA2 autoantibodies were more frequent in participants with an age at diagnosis of < 21 years (Table 3); however, these differences were only significantly different in the black population.

A significantly younger median age at diagnosis was found only in GAD65 and IA2 autoantibody-positive black participants when compared to autoantibody-negative participants (P < 0.001 for both comparisons; Table 4). No significant difference in disease duration was observed in either ethnic group when comparing those with or without GAD65 or IA2 autoantibodies (Table 4).

Autoantibody positivity and interaction of ethnicity with age at diagnosis and disease duration

The data in Tables 3 and 4 suggest that IA2 and GAD65 autoantibody positivity were associated with a lower age at diagnosis, but only in the black population; therefore, to determine whether the differential relationship between age at diagnosis and autoantibody levels was robust and not attributable to confounding, multivariable logistic regression models were developed using autoantibody status as the dependent variable and age at diagnosis, ethnicity, duration of disease and gender as independent variables (Table 5). Interactions between ethnicity and age at diagnosis and disease duration were also analysed. The models for GAD65 and IA2 autoantibody positivity for the combined ethnic groups (models 1 and 2, respectively) both demonstrate that the presence of these autoantibodies was associated with a lower age at diagnosis [odds ratio 0.95 (95% CI 0.93–0.97); P < 0.001, in both models]. In the IA2 autoantibody model only (model 2), autoantibody positivity was associated with shorter disease duration [odds ratio 0.97 (95% CI 0.94, 0.99); P = 0.015] and white ethnicity [odds ratio 2.86 (95% CI 1.72–4.77); P < 0.001]. When an interaction term for ethnicity × age at diagnosis was introduced into models 1 and 2, significant associations were observed with both GAD65 autoantibody positivity [odds ratio 1.08 (95% CI 1.03–1.12); P < 0.001] and IA2 autoantibody positivity [odds ratio 1.07 (95% CI 1.02, 1.12); P = 0.003]. An interaction term for ethnicity × disease duration was also added to both models and was found to be significant in model 1 [odds ratio 1.06 (95% CI 1.00, 1.11); P = 0.029], but not model 2 [odds ratio 0.99 (95% CI 0.94–1.05); P = 0.84]. To further investigate the interaction of ethnicity and age at diagnosis and disease duration, multivariable logistic regression models were set up for each antibody for each ethnic group (models 3–6; Table 5). There was a

Table 2 Frequency of glutamic acid decarboxylase (65 kDa) and islet antigen 2 autoantibody positivity in black and white participants with type 1 diabetes

	All participants n/N (%)	Black participants n/N (%)	White participants n/N (%)	Odds ratio (95% CI)*	$P^{\dagger}$
GAD65 autoantibody-positive	289/470 (62)	212/353 (60)	77/117 (66)	1.28 (0.83, 1.99)	0.269
IA2 autoantibody-positive	114/470 (24)	66/352 (19)	48/118 (41)	2.96 (1.88, 4.68)	<0.001
GAD65 and/or IA2 autoantibody-positive	304/470 (65)	218/353 (62)	86/117 (74)	1.71 (1.09, 2.76)	0.028
GAD65 and IA2 autoantibody positive	99/469 (21)	60/352 (17)	39/117 (33)	2.43 (1.50, 3.90)	<0.001

GAD65, 65kDa isoform of glutamic acid decarboxylase; IA2, islet antigen 2.
*Reference group was black participants;
$^{\dagger}P$ value for comparison of autoantibody positivity of white with black participants.

Table 3 Glutamic acid decarboxylase (65 kDa) and islet antigen 2 autoantibody positivity, based on age at diagnosis and duration of disease in black and white participants with type 1 diabetes

	Age at diagnosis (years)					
	Black participants (n = 340)			White participants (n = 116)		
	< 21 years (n = 188)	≥ 21 years (n = 152)	P	< 21 years (n = 92)	≥ 21 years (n = 24)	P
Median (IQR) disease duration, years	6.0 (2.0–11.0)	4.5 (2.0–9.0)	0.227	9.0 (4.8–21.0)	8.0 (4.0–16.2)	0.621
GAD65 autoantibody positivity, n/N (%)	137/188 (73)	64/152 (42)	<0.001	61/90 (68)	15/24 (63)	0.807
IA2 autoantibody positivity, n/N (%)	51/188 (27)	11/151 (7.3)	<0.001	40/91 (44)	8/24 (33)	0.480
GAD65 and/or IA2 autoantibody positivity, n/N (%)	143/188 (76)	64/152 (42)	<0.001	67/90 (74)	18/24 (75)	1.000

GAD65, 65kDa isoform of glutamic acid decarboxylase; IA2, islet antigen 2; IQR, interquartile range.

Table 4 Age at diagnosis and disease duration according to autoantibody status

	GAD65 autoantibodies					
	Black participants (n = 340)			White participants (n = 114)		
	GAD65 autoantibody-positive (n = 201)	GAD65 autoantibody-negative (n = 139)	P	GAD65 autoantibody-positive (n = 76)	GAD65 autoantibody-negative (n = 38)	P
Age at diagnosis, years	15.0 (10.0–23.0)	25.0 (16.0–32.5)	<0.001	10.0 (6.0–17.0)	7.0 (4.0–15.0)	0.108
Duration of disease, years	5.0 (2.0–9.0)	5.0 (3.0–11.0)	0.276	9.0 (4.0–20.0)	8.0 (5.0–14.0)	0.923
	IA2 autoantibodies					
	Black participants (n = 339)			White participants (n = 115)		
	IA2 autoantibody-positive (n = 62)	IA2 autoantibody-negative (n = 277)	P	IA2 autoantibody-positive (n = 48)	IA2 autoantibody-negative (n = 67)	P
Age at diagnosis, years	12.5 (8.2–17.8)	21.0 (12.0–30.0)	<0.001	10.0 (6.0–16.2)	8.0 (4.0–18.5)	0.523
Duration of disease, years	5.0 (2.0–9.0)	5.0 (2.0–11.0)	0.463	7.0 (4.0–14.0)	9.0 (5.0–21.0)	0.074

GAD65, 65kDa isoform of glutamic acid decarboxylase; IA2, islet antigen 2. Results are presented as median (interquartile range)

reduced risk of being autoantibody-positive with increasing age at diagnosis only in black participants (models 3 and 5), whilst increasing disease duration was associated with a lower risk of being positive for GAD65 autoantibodies only in black participants (model 3).

When participants diagnosed at age > 30 years [those with possible latent autoimmune diabetes in adults (LADA)] were excluded from models 1 and 2, minimal effects on the outcomes were observed.

Discussion

There are limited immunological and genetic data for type 1 diabetes in sub-Saharan African populations. The present study showed a difference in the distribution of age at diagnosis between black and white participants with type 1 diabetes, with two peaks (at 11–15 and 26–30 years) observed in the black participants whereas a single peak (0–10 years of age) was seen in the white participants. The prevalence of GAD65 autoantibody positivity was similar in black and white participants (60% vs 66%, respectively), but IA2 autoantibody positivity was significantly lower in the former group (19% vs 41%). In the black participants, autoantibody positivity was associated with a significantly younger age at diagnosis. In addition, the prevalence of autoantibody positivity was significantly lower in black but not in white participants with type 1 diabetes diagnosed at age ≥ 21 years.

A previous study from Johannesburg [10] found the peak age of onset in black people with type 1 diabetes to be 22–23 years, a decade later than that observed in white people with type 1 diabetes. Another South African study similarly

Table 5 Multivariable logistic regression models for risk factors associated with antibody positivity in the combined ethnic groups and in the individual ethnic groups

Model number	Ethnicity	Dependent variable	Independent variables	OR (95% CI); *P*
1	Black and white combined	GAD65 autoantibody positivity	Age at diagnosis	0.95 (0.93–0.97); <0.001
			Disease duration	0.99 (0.96–1.01); 0.295
			Ethnicity*	1.00 (0.60–1.67); 0.984
			Gender†	0.68 (0.46–1.00); 0.050
2	Black and white combined	IA2 autoantibody positivity	Age at diagnosis	0.95 (0.93–0.97); <0.001
			Disease duration	0.97 (0.94–0.99); 0.015
			Ethnicity*	2.86 (1.72–4.77); <0.001
			Gender†	0.87 (0.55–1.38); 0.558
3	Black	GAD65 autoantibody positivity	Age at diagnosis	0.93 (0.90–0.95); <0.001
			Disease duration	0.95 (0.92–0.99); 0.010
			Gender†	0.62 (0.39–1.00); 0.049
4	White	GAD65 autoantibody positivity	Age at diagnosis	1.00 (0.97–1.04); 0.816
			Disease duration	1.01 (0.98–1.05); 0.360
			Gender†	0.90 (0.42–1.94); 0.784
5	Black	IA2 autoantibody positivity	Age at diagnosis	0.92 (0.89–0.96); <0.001
			Disease duration	0.96 (0.92–1.01); 0.146
			Gender†	0.93 (0.52–1.67); 0.804
6	White	IA2 autoantibody positivity	Age at diagnosis	0.99 (0.96–1.02); 0.556
			Disease duration	0.97 (0.93–1.00); 0.053
			Gender†	0.80 (0.37–1.71); 0.560

GAD65, 65kDa isoform of glutamic acid decarboxylase; IA2, islet antigen 2; OR, odds ratio
*Ethnicity was coded as black 1, white 2.
†Gender was coded as male 1, female 2.

showed a peak age of onset of type 1 diabetes of 21–30 years in 86 black participants [12]. These findings were confirmed in the present study and were extended by the comparison of the distribution of age at diagnosis between white and black people with type 1 diabetes. This demonstrated very different distribution patterns, with the peak frequency for diagnosis occurring between birth and 10 years of age in the white participants and two peaks occurring in the black population at ages 11–15 and 26–30 years. The high frequency of diagnoses in participants aged < 10 years observed in the white participants was unexpected but may be attributable to the shift to a younger age at diagnosis that is being seen in European populations [13]. The reason for the low frequency of diagnoses in participants aged < 10 years in the black population is not known. It has been suggested that in African countries there may be high rates of mortality in individuals with type 1 diabetes diagnosed in infancy as a result of diabetic ketoacidosis and poor access to appropriate healthcare facilities, especially in rural areas [14]. This is less likely in South Africa where healthcare coverage and access to healthcare facilities is greater than in other regions of Africa [15], and, in the present study, all the clinics from which participants were recruited were based in urban areas. It is also possible that the autoimmune response is less aggressive in black compared to white participants, as illustrated by the lower frequency of IA2 autoantibodies in the former group. This may give rise to a longer prodromal period and hence a shift in the age of diagnosis of type 1 diabetes in the black population to an older age than that observed in white participants.

The reason for the lower prevalence of IA2 autoantibodies in black compared to white participants with type 1 diabetes is not known; however, IA2 autoantibody positivity has been shown to be associated with HLA-DR4 [16]. In addition, the 11th International Histocompatibility Workshop Study, which compared HLA class II allele frequencies between white and black participants with type 1 diabetes, showed that HLA-DR4 is more common in white than black individuals [17].

To our knowledge, this is the first report of the frequency of IA2 autoantibodies in South Africans with type 1 diabetes. The prevalence reported in the present study (19%) is within the range of other studies in sub-Saharan Africa, which reported frequencies of 6.4% (n = 47) [18], 10% (n = 302) [19] and 21% (n = 94) [20] in black people with diabetes from Cameroon [18,19] and Tanzania [20]. In addition, a study conducted in East African migrants resident in the USA showed a prevalence of 25% for those with type 1 diabetes [21]. The frequency of this autoantibody is higher in studies conducted in white individuals with type 1 diabetes (54–65%) [6,22–24], which mirrors the higher prevalence observed in white than black participants in the present study.

With regard to the prevalence of GAD65 autoantibodies in sub-Saharan African populations, a study conducted in Cameroon [18] showed a prevalence of 34% in 47 participants with type 1 diabetes. In a cohort of 94 individuals with type 1 diabetes from Tanzania, 30% of participants were GAD65 autoantibody-positive [20]. In the present study, the frequency of GAD65 autoantibodies in the black (60%) and

white (66%) South Africans with type 1 diabetes was not significantly different and was similar to that reported in white European and African American populations [6,25]. It is possible that the higher prevalence of GAD65 autoantibodies detected in our cohort compared to other studies from sub-Saharan Africa is due to differences in assays used (ELISA vs radioimmunoassay) with different specificity levels, the age at diagnosis of the participants, the diabetes classification of the participants and the disease duration.

The prevalence of GAD65 and IA2 autoantibody positivity was lower in both ethnic groups in individuals with an age at diagnosis ≥ 21 years, but these differences were only significant in the black population. Furthermore, both GAD65 and IA2 autoantibody positivity were associated with a significantly younger age at diagnosis of type 1 diabetes in the black but not the white participants. Multivariable logistic regression models confirmed these results, with a significant interaction observed between ethnicity and age at diagnosis in both the GAD65 and IA2 autoantibody regression models. Regression models for each ethnic group further showed that only in black participants was a significant negative relationship observed between increasing age at diagnosis and autoantibody positivity. These data suggest that the black participants with type 1 diabetes who are diagnosed at a later age are immunologically distinct from those diagnosed at an earlier age, but this immunological differentiation is not as pronounced in the white participants. These two subgroups of type 1 diabetes in the black population have not been observed previously and require further investigation. The regression models also showed that GAD65 autoantibody positivity fell with increasing disease duration only in the black participants with type 1 diabetes. This further emphasizes possible immunological differences in disease pathology in these two ethnic groups.

Participants with LADA can include individuals requiring insulin within the first year after diagnosis [26], and may therefore have been included within the group of participants diagnosed after 21 years of age. Although there are no definitive diagnostic criteria for LADA, many studies use an age threshold of > 30 years [27]; therefore, all participants who were aged > 30 years at diagnosis were excluded from the multivariable logistic regression models, and this had minimal effects on the outcomes. Thus, none of the associations demonstrated in the present study, particularly the negative association between age at diagnosis and antibody positivity observed in the black population, can be ascribed to the inclusion of participants with LADA.

Possible limitations of this study are the lower number of white participants with type 1 diabetes, the lack of data for other type 1 diabetes autoantibodies such as insulin and ZnT8 autoantibodies, the lack of data on socio-economic status and the low number of newly diagnosed individuals with type 1 diabetes. The insulin autoantibodies were not measured as most of the participants were already on insulin treatment and current insulin autoantibody assays are unable to distinguish these antibodies from those induced by exogenous insulin therapy [28]. The GAD65 and IA2 autoantibodies were measured in preference to ZnT8 autoantibodies because the former two antibodies are more commonly measured, particularly in those few studies performed in Africa, allowing us to make comparisons between this and previous studies. ZnT8 autoantibodies will, however, be measured in a future study. A further limitation of this study is that we did not perform HLA genotyping, but, again, this will be undertaken in a future study. It has been reported that HLA-DR3 is associated with GAD65 autoantibodies and HLA-DR4 with IA2 autoantibodies [16]. It is also known that HLA haplotypes differ based on ethnicity; the HLA DR3/DR4 heterozygous haplotype confers the greatest genetic risk in Europeans, whereas HLA DR7, DR9 and DR13 haplotypes are common in African Americans [29,30].

One of the strengths of this study is the large number of black participants with type 1 diabetes included; we believe this to be the biggest study conducted to date in sub-Saharan Africa. In addition, this is the first study to compare the prevalence of both GAD65 and IA2 autoantibodies in black and white individuals with type 1 diabetes in South Africa.

In conclusion, we observed an older age at diagnosis of type 1 diabetes in black than white participants. In addition, we showed there was a distinct subgroup of black individuals with type 1 diabetes with an age at diagnosis above 21 years who had lower levels of autoantibody positivity than participants diagnosed at an earlier age. This is a novel finding and requires further investigation. The lower level of autoantibody positivity in this group may reflect a less aggressive autoimmune response against the islet β cells, resulting in a longer prodromal period and a delay in clinical symptomology; however, this must be confirmed in future studies. Data on the immunological aetiology of type 1 diabetes in sub-Saharan African populations are sparse and the present study provides valuable new information on this topic.

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Competing interests

None declared.

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