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The prevalence of coeliac disease-associated human leukocyte antigens in South African transplant donors and recipients

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Background. Coeliac disease (CD) is an autoimmune condition occurring in genetically predisposed individuals exposed to an environmental trigger. The human leukocyte antigen (HLA) haplotypes *HLA-DQ2.5* and *HLA-DQ8* have the strongest association with CD, and 90 - 95% of CD patients bear these haplotypes. The susceptibility of the South African (SA) population to CD has not been studied previously.
Objectives. To describe the genetic propensity of the SA population to CD.
Methods. The South African National Blood Service database was used to analyse the prevalence of *HLA-DQ2.5* and *HLA-DQ8* in potential donors and recipients of organ transplants. Self-reported ethnic group was used to estimate the prevalence among different population groups.
Results. The overall prevalence of *HLA-DQ2.5* and *HLA-DQ8* was 19.8%. The prevalence was lower in black participants (15.9%) than in whites (28.6%). Coloured (22.0%) and Indian (17.4%) participants had an intermediate prevalence. There was no significant difference between potential transplant donors and recipients.
Conclusions. The prevalence of *HLA-DQ2.5* and *HLA-DQ8* differed among SA study participants of different ethnicities. However, the notion that CD does not occur in black South Africans owing to lack of a genetic predisposition is incorrect.

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Coeliac disease (CD) was previously thought to be a disease of people of European descent, but this perception has changed in the past few decades.[1] More recently, CD has been described to be prevalent in the Middle East, South America and North Africa.[1] There is a notable lack of studies on CD in sub-Saharan Africa, and the perception that CD does not exist in black southern African patients persists. The disease is therefore often not often considered in patients with compatible clinical presentations such as chronic diarrhoea and malnutrition, two common conditions in South Africa (SA).[2] Furthermore, atypical presentations of CD (e.g. iron deficiency, altered bone metabolism, elevated serum liver enzymes, short stature) have been shown to be common, leaving much of the CD 'iceberg' unrecognised.[3]

The genetic system bearing the strongest disease association with CD is the human leukocyte antigen (HLA) major histocompatibility class II genes *HLA-DQ2.5* and *HLA-DQ8*.[4] Approximately 90 - 95% of CD patients carry HLA haplotype *HLA-DQ2.5* heterodimers (encoded by *DQA1*05* and *DQB1*02* alleles) or *HLA-DQ8* (*DQB1*03:02*) in combination with a *DQA1*03* variant.[5] The remaining 5% of CD patients without *HLA-DQ2.5* or *HLA-DQ8* are thought to have an *HLA-DQ2.2* haplotype (*DQA1*02:01-DQB1*02:02*).[6] Although several genomic areas outside the HLA region associated with CD have been identified, their contribution to predisposition to CD is relatively small.[5]

Objectives

In southern Africa, the general population's predisposition to CD has not been reported. The objective of this retrospective study was to report on the prevalence of *HLA-DQ2.5* and *HLA-DQ8* in the SA population using HLA data collected by the Tissue Immunology Laboratory at the South African National Blood Service (SANBS) for the purposes of transplantation.

Methods

Ethical approval was obtained from the University of the Witwatersrand Human Research Ethics Committee (ref. no. M190670) and the SANBS Human Research Ethics Committee (ref. no. 2019/0481). All patients typed as part of the tissue immunology work-up or for inclusion on the bone marrow registry (through the Sunflower Fund) were included. HLA typing was performed by low- to medium-resolution Luminex typing utilising a Labtype rSSO kit (One Lambda, Thermo Fisher Scientific, USA) and acquired on LabScan 200 and Flex Map 3D (Thermo Fisher Scientific, USA). Briefly, this process utilises bead-complexed sequence-specific oligonucleotides to define genetic HLA typing. HLA class II data were expressed in a two-digit format. Data were recorded on the SANBS electronic database and extracted in a de-identified format. Where known, patient ethnicity, gender and age and reason for typing were also included. Ethnicity was self-reported and classified

as per Statistics South Africa into one of four categories: black, white, coloured and Indian.[7]

The presence or absence of *HLA-DQ2.5* (encoded *DQB1*02 DQA1*05*), *HLA-DQ8* (*DQB1*03:02DQA1*03*) and *HLA-DQ2.2* (*DQA1*02:01–DQB1*02:02*) haplotypes was determined. Participant HLA results were divided into three risk groups according to their HLA class II status: high risk when the participant was homozygous for *HLA-DQ2.5* or bore both *HLA-DQ8* and *HLA-DQ2.5* haplotypes, moderate risk when the participant haplotypes were heterozygous for *HLA-DQ2.5* or *HLA-DQ8*, and low risk with *HLA-DQ2.2* haplotypes.

Data were analysed using Stata Intercooled version 11 (StataCorp, USA). Where appropriate, descriptive statistics were computed, including mean and standard deviation for parametric and median and interquartile range (IQR) for non-parametric continuous data. Prevalence is reported with 95% confidence intervals (CIs), and χ^2 statistics were used to compare prevalences in different groups. A p-value <0.05 was considered significant.

Results

The records of 5 746 persons who underwent HLA typing were included. The median (IQR) age of the 5 383 participants whose age was known was 36 (24 - 48) years (range 0 - 91 years). The most common reason for HLA testing was chronic renal failure requiring kidney transplantation (37.7%), while 28.3% were tested as potential organ donors; 17.9% did not have the reason for testing recorded. Further general population characteristics are set out in Table 1.

In total, 1 135 participants (19.8%; 95% CI 18.7 - 20.8) were positive for *HLA-DQ2.5* or *HLA-DQ8*. Blacks had a significantly lower prevalence (15.9%; 95% CI 14.3 - 17.5) than whites (28.6%; 95% CI 25.5 - 31.8 (OR 2.11; 95% CI 1.74 - 2.56); p <0.001). Coloureds (22.0%; 95% CI 11.1 - 32.9), Indians (17.4%; 95% CI 14.6 - 20.2) and those of unspecified race (20.8%; 95% CI 19 - 22.4) had an intermediate prevalence of the *HLA-DQ2.5* or *HLA-DQ8* haplotypes (Table 1). There were no differences observed between the age and gender in the *HLA-DQ2.5*- or *HLA-DQ8*-positive v. negative groups (median age 36 years in both groups, female gender in 43.5% v. 41.7%, respectively).

There was no difference in the prevalence of *HLA-DQ2.5* or *HLA-DQ8* positivity between potential organ donors v. recipients (20.2% v. 20.0%; p=0.96).

Of the study subjects, 2.2% (n=124) were homozygous for *HLA-DQ2.5* or had both *HLA-DQ2.5* and *HLA-DQ8* genotypes (high risk, Table 2). This group included 35 (1.7%) black, 1 (1.7%) coloured, 12 (1.7%) Indian and 26 (3.3%) white participants. The *HLA-DQ2.2* haplotype, which confers a low risk of CD, was more prevalent among Indians at 15.2% (low risk, Table 2).

Discussion

This retrospective audit sought to determine the prevalence of CD-associated haplotypes using the HLA information from potential transplant donors and recipients in the SANBS database. We observed that the prevalence of *HLA-DQ2.5* and *DQ8* haplotypes in this study sample was almost 20%, which is lower than reported in studies performed in many Western countries, which have a prevalence of ~30%.[5]

CD-associated HLA haplotypes were identified across all ethnic groups. Significant differences between ethnicities were observed,

Table 1. Summary of study participant characteristics according to demographic category

Demographic categories	Total (N=5 746), n	*HLA-DQ2.5*, n (%)	*HLA-DQ2.5* or *HLA-DQ8*, n (%)
Gender			
Female	2 417	476 (19.7)	494 (20.4)
Male	3 070	564 (18.4)	588 (19.2)
Unspecified	259	49 (18.9)	53 (20.5)
Age group (years)			
0 - 17	836	135 (16.1)	141 (16.9)
18 - 35	1 778	365 (20.5)	380 (21.4)
36 - 53	2 003	378 (18.9)	395 (19.7)
54 - 71	753	147 (19.5)	151 (20.1)
≥72	13	3 (23.1)	3 (23.1)
Unspecified	363	61 (16.8)	65 (17.9)
Ethnicity			
Black	2 034	321 (15.8)	324 (15.9)*
White	800	216 (27.0)	229 (28.6)*
Indian	690	113 (16.4)	120 (17.4)
Coloured	59	11 (18.6)	13 (22.0)
Unspecified	2 163	428 (19.8)	449 (20.8)
Reason for HLA testing			
Chronic renal failure	2 165	446 (20.6)	451 (20.8)
Donor	1 623	302 (18.6)	327 (20.1)
Leukaemia	398	68 (17.1)	70 (17.6)
Aplastic anaemia	118	19 (16.1)	19 (16.1)
Cardiac transplant	100	25 (25.0)	25 (25.0)
Lung transplant	45	9 (20.0)	11(24.4)
Other	269	39 (14.5)	45 (16.7)
Not specified	1 028	181 (17.6)	187 (18.2)

Percentages calculated as percentage of row total.
*p<0.001.

Table 2. Coeliac disease risk categories stratified by ethnicity

Ethnicity	High risk, *n* (%)	Moderate risk, *n* (%)	Low risk, *n* (%)
Black (*N*=2 034)	35 (1.7)	324 (15.9)	191 (9.4)
White (*N*=800)	26 (3.3)	229 (28.6))	91 (11.4)
Indian (*N*=690)	12 (1.7)	120 (17.4)	105 (15.2)
Coloured (*N*=59)	1 (1.7)	13 (22.0)	3 (5.1)
Unspecified (*N*=2 163)	50 (2.3)	449 (20.8)	255 (11.8)
Total (*N*=5 746)	124 (2.2)	1 135 (19.8)	645 (11.2)

Percentages calculated as percentage of row total.
High risk = homozygous for *HLA-DQ2.5* or *HLA-DQ2.5* and *HLA-DQ8*-positive; Moderate risk = *HLA-DQ2.5*- or *HLA-DQ8*-positive; Low risk = *HLA-DQ2.2*.

with higher prevalences in white and coloured participants, at 28.6% and 22.0%, respectively, and a prevalence of 15.9% among black participants. This finding would suggest that all groups are susceptible to CD.

An HLA-associated predisposition is not sufficient to cause CD, as demonstrated in prospective studies by Liu *et al*.,[8] where 3% and 11% of children with heterozygous and homozygous *HLA-DQ2.5* haplotypes, respectively, developed CD by age 60 months. Dietary gluten intake is an obvious prerequisite. In SA, maize remains the most consumed staple food, but wheat consumption is not far off the world consumption rate (60 v. 66 kg/capita/year).[9] The role of gluten intake during the first 5 years of life in the development of CD remains controversial, but a recent prospective multinational cohort study followed 6 605 children who were *HLA-DQ2.5*- or *HLA-DQ8*-positive. The authors conclude that gluten intake >1 g/d above the reference amount was associated with an increased absolute risk of CD of 7.2%.[10] Although the gluten intake in young SA children remains undocumented, the 1999 National Food Consumption Survey[11] revealed that bread was among the foods most commonly eaten by children aged between 1 and 9 years. However, the timing of gluten introduction to infants at risk for CD and the role of breastfeeding do not appear to have a protective effect.[12]

Further factors that may influence the development of CD include gastrointestinal infections, other genetic factors, the gut microbiome, the innate immune system, and host-microbiome interactions.[12,13] These factors remain poorly understood, and it is unclear how they may influence the development of clinical CD in SA. However, it may be hypothesised that higher exposure to gastrointestinal infections, less antibiotic use and a different microbiome may be protecting South Africans.

Alternatively, a lack of awareness and the false belief that CD does not occur in black ethnic groups may lead to a lowered detection rate and therefore a low prevalence of diagnosed CD in sub-Saharan Africa. This is supported by Paruk *et al*.,[14] who reported a similar prevalence of CD in SA black patients with type 1 diabetes as studies from Western countries. Poor access to serological screening tests and confirmatory endoscopy may have an additional effect, as not all healthcare centres are well resourced or adequately equipped in our setting. Overall, further study is required to investigate the prevalence of CD in SA and explore the reasons affecting its prevalence or lack thereof.

Study limitations

The study relied on a sample of existing data with various indications for transplantation, some of which may overlap or have association with the extraintestinal manifestations of CD. However, there was no difference in the CD-associated HLA haplotypes of donors and recipients. Self-reported ethnicity may not be accurate in all cases, and ethnicity was unknown in a large percentage of participants, resulting in poor representation of minority groups. It is not known whether under-reporting of ethnicity was more prevalent in any group.

Conclusions and recommendations

This study has shown that the prevalence of the CD-associated haplotypes in potential donors and recipients of organ transplants in the SANBS database is 20%. CD-associated haplotypes were more common in white participants, but were present in all population groups. We recommend that clinicians screen for CD using anti-tissue transglutaminase antibodies in children and adults with symptoms associated with CD, and in those who are at an increased risk for CD (e.g. type 1 diabetes mellitus, autoimmune thyroid disease), regardless of ethnicity, as suggested in international guidelines.[15,16] Positive antibody results should be confirmed with endoscopy and duodenal biopsies, at least until the true prevalence of CD in SA is better understood.

Furthermore, heightened awareness and large population-based studies are required to determine the prevalence of CD in southern Africa.

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Author contributions. KLM: study design, data analysis, writing of initial manuscript; DN: data retrieval and manuscript review; PB: study design and manuscript review; EM: manuscript review; TDM: study design, data analysis, manuscript review.

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