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An audit of the presence of coeliac disease associated human leukocyte antigen haplotypes in renal and bone marrow transplant donors and recipients from the South African National Blood Services

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An audit of the presence of coeliac disease associated human leukocyte antigen haplotypes in renal and bone marrow transplant donors and recipients from the South African National Blood Services

Kitso-Lesedi Mrubata

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Masters of Medicine in Paediatrics

Johannesburg, 2020

Declaration

I, Kitso-Lesedi Mrubata, declare that this Research Report is my own, unaided work. It has been submitted for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

Kitso-Lesedi Mrubata

30th day of September 2020 in Johannesburg

Dedication

This is dedicated to my mom, Disemelo Maphate-Mrubata

Presentations arising from this research report

1. Mrubata, K, De Maayer T- An audit of the presence of coeliac disease associated human leukocyte antigen haplotypes in renal and bone marrow transplant donors and recipients from the South African National Blood Services, Faculty of Health Sciences Research Day and Postgraduate Expo Poster presentation (15 October 2020)
2. Mrubata, K, De Maayer T- An audit of the presence of coeliac disease associated human leukocyte antigen haplotypes in renal and bone marrow transplant donors and recipients from the South African National Blood Services e-poster presentation, Department of Paediatrics Research day (20 November 2020)

Abstract

Introduction

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 bear the strongest association with CD, and 90 - 95% of patients with CD bear these haplotypes. The absence of these haplotypes has high negative predictive value. The susceptibility of the South African population to CD has not been studied previously.

Methods

The South African National Blood Services database was used to analyse the prevalence of HLA DQ2.5 and DQ8 in potential donors and recipients of organ transplants.

Results

The overall prevalence of HLA DQ2.5 and HLA DQ8 was 19.8%. The prevalence was lower in Black subjects (15%) than Caucasians (28%). Mixed race (22%) and Indian (17%) subjects had intermediate prevalence. The was no significant difference between potential transplant donors and recipients.

Conclusion

The prevalence of HLA DQ2.5 and HLA DQ8 differed among South African study participants of different ethnicities and was lower than the reported world-wide prevalence of 30-40%.

Acknowledgments

I wish to express my sincerest gratitude to Dr Tim De Maayer for his valuable expertise, guidance and support. I am thankful to Dr Thandeka Ngcana, Ms Karin van den Berg, Mr Derrick Nelson and the South African Blood Bank Services who have all been instrumental in this research endeavour.

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List of Abbreviations

Anti-TG2	Anti-tissue Transglutaminase
CD	Coeliac Disease
CMJAH	Charlotte Maxeke Academic Hospital
EMA	Anti-endomysial Antibody
ESPGHAN	European Society of Gastroenterology, Hepatology and Nutrition
GVHD	Graft Versus Host Disease
HLA	Human Leukocyte Antigen
HREC	Human Research Ethics Committee
INF-G	Interferon Gamma
MHC	Major Histocompatibility Class
SAM	Severe Acute Malnutrition
SANBS	South African Blood Services
TNF-alpha	Tumour Necrosis Factor Alpha
WDGMC	The Wits Donald Gordon Medical Centre
WHO	World Health Organization

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Preface

This research report has been compiled in the “submissible format” in accordance with the University of the Witwatersrand Faculty of Health Sciences requirements for a research report. The abstract and manuscript has been prepared for submission to the Journal of Paediatric Gastroenterology, Hepatology and Nutrition.

Appendices include:

A. Author Guidelines for the Journal of Paediatric Gastroenterology, Hepatology and Nutrition
B. Original research protocol with extended literature review
C. Human Research Ethics Committee of the University of the Witwatersrand approval certificate and the SANBS HREC clearance certificates
D. Postgraduate committee approved protocol
E. Plagiarism report complied through Turnitin has a similarity index of 15%

Introduction

Coeliac Disease (CD) is an autoimmune condition, in which genetically predisposed individuals develop an immune mediated response to gluten resulting in enteropathy and other multi-systemic disorders (1).

The genetic system bearing the strongest disease association to CD are the Human Leukocyte Antigen (HLA) Major Histocompatibility Class II genes; HLA-DQ2.5 and HLA-DQ8 (2). Approximately 90-95% of CD patients carry HLA haplotypes 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 (3). The remaining 5% of CD patients without HLA DQ2.5 or DQ8 are thought to have a HLA-DQ2.2 haplotype (DQA1*02:01–DQB1*02:02) (4). Although several genomic areas outside the HLA region associated with CD have been identified, their contribution to predisposition to CD is relatively small (5). The strength in the association between HLA genetic factors and CD is supported by the effect of HLA gene dosing; individuals with HLA-DQ2.5 homozygosity have a comparatively five times higher risk of developing CD with a more severe phenotypical presentation (6).

The prevalence of CD associated HLA haplotypes varies according to population groups but CD is prevalent across various ethnic groups (7). Studies have reported the prevalence of HLA-DQ2.5 and HLA-DQ8 in Western and European populations to range between 30 and 40% (8).

In Southern Africa, the population's predisposition to and the prevalence of CD has not been reported. There is significant mortality and morbidity due to diarrhoeal illness and malnutrition, and symptoms associated with atypical CD are common (9,10). The ESPHGAN's guidelines for the screening of high risk groups are not always followed due to financial constraints, misinformation or misconceptions about CD particularly in relation to non-European ethnic groups (8).

The aim of this retrospective study was to report on the prevalence of HLA-DQ2.5 and-DQ8 within the South African population using HLA data collected by the South African

National Blood Services (SANBS) for the purposes of transplantation and to compare the findings to international literature.

Methods

The SANBS is currently at the forefront of HLA genotyping in South Africa and lends its services to other southern African countries such as Namibia and Botswana. The genotyping technology is used to assist the Sunflower Foundation (stem cell transplant registry), the South African Bone Marrow registry and in renal, heart and lung transplantation. HLA typing for all transplantation donors, potential donors and recipients is captured on the SANBS database. Data from 2014 until November 2019 from the electronic database developed in 2014 was analysed by the investigators, while ensuring the confidentiality of individuals within the study cohort.

Participants of the study included subjects of all ages in whom HLA typing had been performed for the purposes of transplantation, as either potential donors or recipients. Participants results and information was collected in an anonymised form and stratified according to gender, age, ethnicity and the presence or absence of HLA-DQ2.5 (encoded, DQB1*02 DQA1*05), HLA DQ8 (DQB1*03:02DQA1*03) haplotypes and HLA-DQ2.2 (DQA1*02:01–DQB1*02:02). Participant HLA results were divided in 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 and HLA-DQ8 and low risk when the patient had HLA-DQ2.2 haplotypes. To account for possible confounders participants were further stratified according to their diagnosis or reason for HLA typing.

Data was analysed using Stata® Intercooled version 11 (Statacorp, USA). Prevalence is reported with 95% confidence intervals (95% CI) and Chi square statistics were used to compare prevalence in different groups. A p-value less than 0.05 was considered significant.

Ethics

Ethical approval [M190670 2019/0481] was obtained from the Witwatersrand University's Human Research Ethics Committee and SANBS Human Research Ethics Committee.

Results

The records of 5746 persons who underwent HLA typing for the purposes of bone marrow and kidney transplantation were included. The median age of the 5383 subjects whose age was known was 36 years (Range 0 –91 years). Just over half (53%) of the subjects were male, 42% were female, and in the remaining 5%, gender was not specified. The most common reason for HLA testing was chronic renal failure requiring kidney transplant (38%), while 28% were tested as potential organ donors. A large proportion of patients (18%) did not have the reason for testing recorded. Many subjects did not have ethnicity recorded (38%), while Black participants made up 35%, Caucasians 14%, Indians 12%, and Mixed race just 1%. (see Table 1)

In total, 1135 (19.8%, 95% CI: 18.7 - 20.8%) were positive for HLA DQ2.5 or DQ8. Black subjects had a significantly lower prevalence (15.9%, 95% CI: 14.3 – 17.5%) than others ($p<0.001$), while White subjects (28.6%, 95% CI: 25.5 – 31.8%) were more likely to bear the haplotypes associated with a higher risk for developing CD ($p< 0.001$). Mixed race (22.0%, 95% CI: 11.1 – 32.9%), Indian (17.4%, 95% CI: 14.6 – 20.2%) and unspecified race (20.8%, 95% CI: 19 – 22.4%) groups 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 of the HLA DQ2.5 or DQ8 positive versus negative groups (median age 36 years in both groups, female gender in 44% vs 42% respectively).

There was no difference in the prevalence of HLA DQ2.5 or DQ8 positivity between potential organ donors versus recipients (20.2% vs 20.0%, $p=0.96$)

Two percent (124) of study subjects were homozygous for HLA-DQ2.5 or had both HLA-DQ2.5 and HLA-DQ 8 genotypes (high risk, Table 2). This group included 35 (1.7%) Black, one (1.7%) Mixed race, 12(1.7%) Indian and 26 (3.25%) White participants. The HLA-DQ2.2 haplotype which confers a low risk of coeliac disease was more prevalent among Indian subjects at 15% of the population group (low risk, Table 2).

Discussion

This retrospective audit sought to determine the prevalence of CD associated haplotypes using the HLA information of potential transplant donors and recipients within 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 Western countries which have a prevalence of 30-40%(8).

Within our study, prevalence of the CD associated HLA haplotypes was similar between male and female study participants. Typically, the prevalence of CD haplotypes DQ2.5 and DQ8 has no difference between male and female carriers given its inheritance pattern (2). A study by Mergiorni et al found that the percentage of HLA-DQ2.5 and HLA-DQ8 who were negative for CD was higher among males, and there were significance differences between males and females for paternal gene imprinting(11). Approximately 5% of our study participants did not specify their gender, which may have influenced results. There was no significant difference among the different age groups.

The study relied on existing data from potential transplant donors and recipients, the indication for recipients requiring transplant range from lymphoma to kidney disease. Considering the atypical extra-intestinal manifestations of CD, some conditions and indications for transplant may bear an association to CD resulting in bias. A meta-analysis performed by Wijarnpreecha et al showed a significantly increase risk of kidney disease in patients with CD (12). However, there was no significant difference in the prevalence of the CD associated haplotypes in potential organ donors versus recipients.

CD associated HLA haplotypes were identified across all ethnic groups within our study sample. The noted differences between ethnicities were; a higher prevalence within white and Mixed-race study participants 29% and 22% respectively and a prevalence of 16% among black study participants.

According to our research, this is the first description of the prevalence CD associated HLA haplotypes within a multiracial South African population sample. The results provided by our study regarding the population's genetic susceptibility to CD suggests that we may need to explore the potential for undiscovered CD in the South African

population. Alternatively, the prevalence of CD may be truly low in South Africa and an exploration of factors influencing this may be required.

Since a high prevalence of CD is noted in regions where wheat or barley form part of the staple diet, the Maghreb region in northern Africa being a frequently quoted example, the dietary patterns of a region must be considered (13). A study by Aronsson et al ~~in the Journal of American Medicine~~ concluded that an there is an association between increased gluten intake within the first 5 years of life and higher risk of CD and positive CD serology among genetically predisposed individuals (14). They did however suggest randomised controlled studies to confirm this dose-response relationship (14). ESPGHAN guidelines also recommended further study into the optimal amount gluten to be given during the weaning period but discouraged large amounts of gluten based on low level evidence (14,15). A survey done by the National Food Consumption Survey (NFCS) of South Africa showed that the most commonly consumed foods at the time were maize, tea, sugar and bread (16). Studies around nutritional transition suggest a westernisation in the diet of many Sub-Saharan Africans linked to multiple factors, improvements in household income being one of them. These studies suggest that as the local diet becomes comparable to our Western counterparts, we may expect an increase in case presentations of conditions such as CD (17).

The microbiome could play a primary role in the pathogenesis of CD among genetically predisposed individuals (18). Factors influencing the microbiome include age, diet, genetics and environment, and further study is required as to what the host microbiome risk factors are in relation to CD (19,20). Research on the microbiome in relation to chronic disease is a topic that requires further study in South Africa, its relationship to CD might present another reason for the low detection rate of CD.

Environmental enteropathy is a major cause of intestinal structural dysfunction among children in resource-limited countries (21). There is similarity between CD and environmental enteropathy in presentation, appearance on intestinal biopsy, and as causes of both malnutrition and stunting, and therefore the potential for misdiagnosis and overlap exists (22).

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 thus a low prevalence of diagnosed CD in Sub-Saharan Africa. 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.

Study Limitations

The data used to analyse the prevalence of CD had many cases in which the ethnicity of subjects was unknown, resulting in a small sample size in the less common population groups (e.g. Mixed-race group of 59). Self-reported ethnicity may also not be accurate in all cases. The study relied on a sample of existing data with various indications for transplant some of which may overlap or have association with the extra-intestinal manifestation of CD which may introduce selection bias.

Conclusion 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 in children and adolescents with symptoms associated with CD, and those who are with an increased risk for CD regardless of ethnicity, as suggested in the ESPGHAN guidelines (8). Heightened awareness and large population-based studies are required to further determine the prevalence of CD in Southern Africa that could potentially confirm or refute the hypothesis that many cases of CD may be undiagnosed in Southern Africa. Further study is required to investigate the prevalence of CD in South Africa and explore the reasons affecting its prevalence.

Declarations

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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References

1. Hill ID, Fasano A, Guandalini S, Hoffenberg E, Levy J, Reilly N, et al. NASPGHAN clinical report on the diagnosis and treatment of gluten-related disorders. J Pediatr Gastroenterol Nutr. 2016;63(1):156–65.

2. Brown NK, Guandalini S, Semrad C, Kupfer SS. A clinician's guide to celiac disease HLA genetics. Am J Gastroenterol. 2019;114(10):1587–92.

3. Megiorni F, Pizzuti A. HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: Practical implications of the HLA molecular typing. J Biomed Sci [Internet]. 2012;19(1):1. Available from: Journal of Biomedical Science

4. Mubarak A, Spierings E, Wolters V, Van Hoogstraten I, Kneepkens CMF, Houwen R. Human leukocyte antigen DQ2.2 and celiac disease. J Pediatr Gastroenterol Nutr. 2013;56(4):428–30.

5. Rachisan AL, Pirvan A, Miu N, Gheban D, Hrusca A, Samasca G, et al. Is HLA Typing a Diagnostic Tool in Celiac Disease? Int J Celiac Dis [Internet]. 2016;4(1):9–10. Available from: http://pubs.sciepub.com/ijcd/4/1/10/index.html

6. Tye-Din JA, Cameron DJS, Daveson AJ, Day AS, Dellsperger P, Hogan C, et al. Appropriate clinical use of human leukocyte antigen typing for coeliac disease: An Australasian perspective. Intern Med J. 2015;45(4):441–50.

7. Lionetti E, Catassi C. Co-localization of gluten consumption and HLA-DQ2 and -DQ8 genotypes, a clue to the history of celiac disease. Dig Liver Dis [Internet]. 2014;46(12):1057–63. Available from: http://dx.doi.org/10.1016/j.dld.2014.08.002

8. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 2020;70(1):141–56.

9. Statistics South Africa. Mortality and causes of death in South Africa: findings from death notification, 2016 | Statistical release P0309.3. 2016;(February):1–36.

Available from: http://www.governmentpublications.lib.uct.ac.za/news/mortality-and-causes-death-south-africa-findings-death-notification-2016

10. Reid AE, Hendricks MK, Groenewald P, Bradshaw D. Where do children die and what are the causes? Under-5 deaths in the Metro West geographical service area of the Western Cape, South Africa, 2011. South African Med J. 2016;106(4):359–64.

11. Megiorni F, Mora B, Bonamico M, Barbato M, Montuori M, Viola F, et al. HLA-DQ and susceptibility to celiac disease: Evidence for gender differences and parent-of-origin effects. Am J Gastroenterol. 2008;103(4):997–1003.

12. Wijarnpreecha K, Thongprayoon C, Panjawatanan P, Thamcharoen N, Pachariyanon P, Nakkala K, et al. Celiac disease and the risk of kidney diseases: A systematic review and meta-analysis. Dig Liver Dis [Internet]. 2016;48(12):1418–24. Available from: http://dx.doi.org/10.1016/j.dld.2016.08.115

13. Cataldo F, Montalto G. Celiac disease in the developing countries□: A new and challenging public health problem HISTORY AND ORIGIN OF CELIAC. 2007;13(15):2153–9.

14. Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, et al. Association of gluten intake during the first 5 years of life with incidence of celiac disease autoimmunity and celiac disease among children at increased risk. JAMA - J Am Med Assoc. 2019;322(6):514–23.

15. Szajewska H, Shamir R, Mearin L, Ribes-Koninckx C, Catassi C, Domellof M, et al. Gluten introduction and the risk of coeliac disease: A position paper by the european society for pediatric gastroenterology, hepatology, and nutrition. J Pediatr Gastroenterol Nutr. 2016;62(3):507–13.

16. Labadarios D, Steyn NP, Nel J. How diverse is the diet of adult South Africans□? Nutr J [Internet]. 2011;10(1):33. Available from: http://www.nutritionj.com/content/10/1/33

17. Mbogori T, Mucherah W. Westernization of Traditional African Diets and the

Development of Chronic Diseases in Africa. 2019;(October).

18. Nylund L, Kaukinen K, Lindfors K. The microbiota as a component of the celiac disease and non-celiac gluten sensitivity. Clin Nutr Exp [Internet]. 2016;6:17–24. Available from: http://dx.doi.org/10.1016/j.yclnex.2016.01.002

19. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature [Internet]. 2012;486(7402):222–7. Available from: http://dx.doi.org/10.1038/nature11053

20. Kalliomäki M, Satokari R, Lähteenoja H, Vähämiko S, Grönlund J, Routi T, et al. Expression of microbiota, toll-like receptors, and their regulators in the small intestinal mucosa in celiac disease. J Pediatr Gastroenterol Nutr. 2012;54(6):727–32.

21. Ali A, Iqbal NT, Sadiq K. Environmental enteropathy. Curr Opin Gastroenterol. 2016;32(1).

22. Kowsari K, Sali R, Khan MN, Adorno W, Ali SA, Moore SR, et al. Diagnosis of Celiac Disease and Environmental Enteropathy on Biopsy Images Using Color Balancing on Convolutional Neural Networks. Adv Intell Syst Comput. 2020;1069:750–65.

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

DEMOGRAPHIC CATEGORIES	Total (N= 5746)	HLA-DQ2.5 n (%)	HLA-DQ8 n (%)	HLA-DQ2.5 or DQ8: n (%)
Gender				
Female	2417	476 (20)	20 (1)	494 (20)
Male	3070	564 (18)	24 (1)	588 (19)
Unspecified	259	49 (19)	4 (2)	53 (20)
Age group				
0 – 17	836	135 (16)	6 (1)	141 (17)
18 – 35	1778	365 (21)	16 (1)	380 (21)
36 – 53	2003	378 (19)	18 (1)	395 (20)
54 – 71	753	147 (20)	4 (1)	151 (20)
72 +	13	3 (23)	0 (0)	3 (23)
Unspecified	363	61 (17)	4 (1)	65 (18)
Ethnicity				
Black	2034	321 (16)	3 (0)	324 (16)
White	800	216 (27)	14 (2)	229 (29)
Indian	690	113 (16)	7 (1)	120 (17)
Mixed Race	59	11 (19)	2 (3)	13 (22)
Unspecified	2163	428 (20)	22 (1)	449 (21)
Reason for HLA testing				
Chronic renal failure	2165	446 (21)	5 (0)	451 (21)
Donor	1623	302 (19)	25 (2)	327 (20)
Leukaemia	398	68 (17)	4 (1)	70 (18)
Aplastic Anaemia	118	19 (16)	0 (0)	19 (16)
Cardiac	100	25 (25)	0 (0)	25 (25)
Lung	45	9 (20)	2 (4)	11(24)
Other	269	39 (15)	6 (2)	45 (17)
Not specified	1028	181 (18)	6 (1)	187 (18)

* Percentages calculated as percentage of row total

Table 2: Coeliac disease risk categories stratified by ethnicity

Ethnicity	**High risk n (%)**	**Moderate risk n (%)**	**Low risk n (%)**
Black (n= 2034)	35 (2)	324 (16)	191 (9)
White (n= 800)	26 (3)	120 (15)	91 (11)
Indian (n= 690)	12 (2)	229 (33)	105 (15)
Mixed race (n= 59)	1 (2)	13 (22)	3 (5)
Unspecified (n= 2163)	50 (2)	449 (21)	255 (12)
Total (n= 5746)	124 (2)	1135 (20)	645 (11)

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

Appendix A: Publication author guidelines

Journal of Pediatric Gastroenterology and Nutrition

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ARTICLE TYPES

Rapid Communication: This article type allows for rapid review (within 10 days) and publication of original studies. Manuscripts considered for rapid review will be limited to reports judged to be of general scientific or public health importance. Authors submitting Rapid Communications must provide a detailed cover letter outlining the rationale for fast tracking their work. Authors must state whether the findings could alter current standards of patient care (e.g., finding efficacy or lack of efficacy of treatment), and/or if the findings suggest a novel mechanism or understanding of disease process (e.g., new susceptibility gene identification in *H pylori* organism).

Rapid Communications should contain no more than 3000 words, structured abstract with no more than 250 words and no more than four figures and tables combined (for example, a submission may include 4 figures, 1 figure and 3 tables, 4 tables, etc., but not 2 figures and 3 tables) and no more than 50 references. Submissions exceeding these parameters without justification or without a detailed cover letter explaining the rationale for a Rapid Communication will be returned to the author for correction prior to review. Extra material such as very detailed methods, tables or figures that are not needed by most readers may be submitted as Supplemental Digital Content without limitation on length (see below). Articles submitted for Rapid Communication but deemed to be more appropriate for standard submission will be returned for resubmission as an Original Article (below).

Original Articles: Original articles are full-length reports of original research. Original articles are accepted based on their scientific relevance, the originality of the work, and the priority of the work for *JPGN* and its readership. Authors should aim for accuracy, clarity, and brevity. Long introductions, repetition of data among tab figures, and the text, and unfocused discussions should be avoided.

Original research articles should be approximately 18 double-spaced, numbered pages, including the title page, references, figures, and tables. Failure to comply wit length restrictions may result in a delay in the processing of your paper. The following length targets (up to 3000 words for the text including Introduction, Methods, Results and Discussion) are recommended for Original Articles:

- Structured Abstract: maximum of 250 words
- Introduction: 1 page (about 250 words)
- Methods: 2-3 pages (up to 750-1000 words)
- Results: 2-3 pages (up to 750-1000 words)
- Discussion: 3-5 pages (up to 1000 words)
- References: limited to those critical and relevant to the manuscript (not more than 50)
- Tables and Figures: 4 total (legends limited no more than 100 words each)
- Additional/supplemental content may be submitted as "Supplemental Digital Content (SDC)", which has no space limitation (see section on SDC below).

Appendix B: Ethics clearance 1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr K-L Mrubata

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M190670

NAME: (Principal Investigator)	Dr K-L Mrubata
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Medical School University
PROJECT TITLE:	An audit of the presence of coeliac disease associated human leucocyte antigen haplotypes in renal and bone marrow transplant donors and recipients from the South African National Blood Services
DATE CONSIDERED:	28/06/2019
DECISION:	Approved unconditionally
CONDITIONS:	Study title change noted on 2019/08/16
SUPERVISOR:	Dr T de Maayer
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2019/07/11

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore reports and re-certification will be due early in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

Appendix B: Ethics clearance 2

Head Office or Zone
1 Constantia Boulevard
Constantia Kloof Ext 22, 1709

Postal Address: Private Bag X14, Weltevreden Park, 1715
Tel: 011 761 9000 Fax: 0866747666
Email: customerservice@sanbs.org.za

SOUTH AFRICAN NATIONAL BLOOD SERVICE NPC
Human Research Ethics Committee

OHRP Number : IORG0006278
FWA Registration Number : IRB00007553
SA NHREC Registration Number : REC-270606-013

Secretariat: Tel: 011 761 9096 | Valencia.Simmadari@sanbs.org.za

To : Dr Kitso-Lesedi Mrubata
Email : mrbkl1001@gmail.com

Dear, Dr Mrubata

DATE OF COMMITTEE MEETING : **10th September 2019**
PROJECT TITLE : AN AUDIT OF THE PRESENCE OF COELIAC DISEASE ASSOCIATED HUMAN LEUKOCYTE ANTIGEN HAPLOTYPES IN RENAL AND BONE MARROW TRANSPLANT DONORS AND RECIPIENTS FROM THE SOUTH AFRICAN NATIONAL BLOOD SERVICES
DECISION OF THE COMMITTEE : **APPROVED**
CLEARNACE CERTIFICATE NO. : **2019/0481**

1. Execution of the study must be compliant with applicable guidelines and policies.
2. Any amendment, extension or any other modifications to the protocol must be submitted to this Ethics Committee for approval prior to implementation.
3. The Committee must be informed of any serious adverse event, planned and unplanned termination of the study.
4. A progress report should be submitted yearly for studies longer than a year and a final report at completion of the study for both short term and long term studies.
5. Kindly refer to the SANBS HREC clearance certificate number on all future correspondence on this study to the HREC secretariat.
6. This approval is valid for 5 years from the date stated above.

COMMITTEE GUIDANCE DOCUMENTS:

- International Conference on Harmonization (ICH) Good Clinical Practices (GCP) Guideline (ICH, 1996), Ethics in Health Research: Principles, Structures and Procedures (SA Department of Health, 2015); Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa (SA Department of Health, 2006); Ethical Principles for Medical Research Involving Human: Declaration of Helsinki (World Medical Association, 2013); Reviewing Clinical trials: A Guide For Ethics Committees (Karlberg and Speers, 2010).

CHAIRPERSON: Prof J.N. Mahlangu

26 November 2019
DATE

Universal Blood Type: O-
Donates to: Everyone
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Appendix C: Turnit in report

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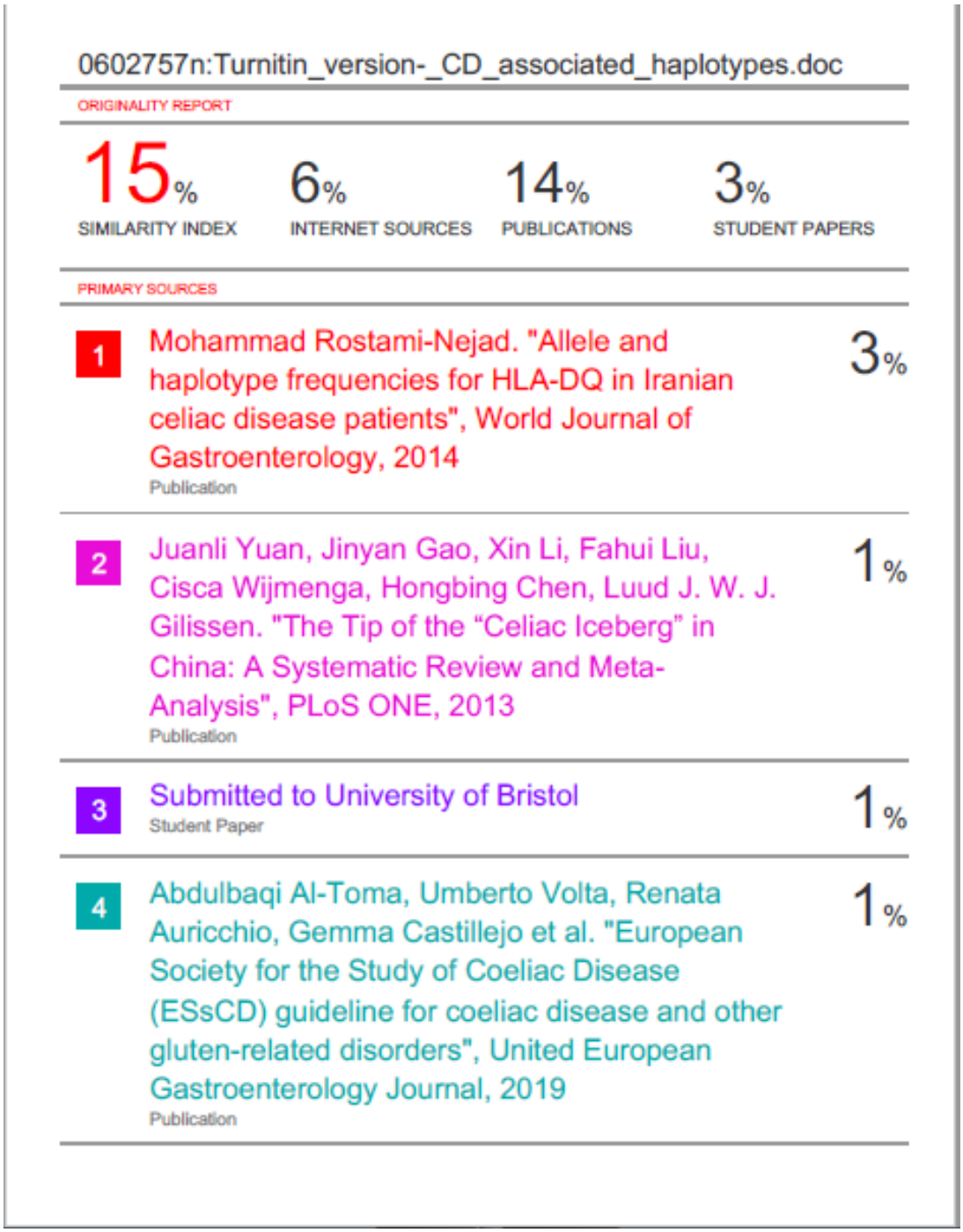

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ORIGINALITY REPORT

15%	6%	14%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Mohammad Rostami-Nejad. "Allele and haplotype frequencies for HLA-DQ in Iranian celiac disease patients", World Journal of Gastroenterology, 2014 Publication	3%
2	Juanli Yuan, Jinyan Gao, Xin Li, Fahui Liu, Cisca Wijmenga, Hongbing Chen, Luud J. W. J. Gilissen. "The Tip of the "Celiac Iceberg" in China: A Systematic Review and Meta-Analysis", PLoS ONE, 2013 Publication	1%
3	Submitted to University of Bristol Student Paper	1%
4	Abdulbaqi Al-Toma, Umberto Volta, Renata Auricchio, Gemma Castillejo et al. "European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders", United European Gastroenterology Journal, 2019 Publication	1%

Appendix D: Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Kitso-Lesedi Mrubata (Student number: 0802757N) am a student registered for the degree of Masters in Medicine (Paediatrics) in the academic year 2020.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 17/11/2020

1

Appendix D: Research Protocol

An audit of the presence of coeliac disease associated human leukocyte antigen haplotypes in renal and bone marrow transplant donors and recipients from the South African National Blood Services



Investigator

Dr. Kitso-Lesedi Mrubata

MBChB (UCT) DCH(SA)

Student no: 0602757N

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Paediatric Gastroenterologist and Senior Specialist

Rahima Moosa Mother and Child Hospital

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NOMENCLATURE

Anti-TG2	Anti-tissue Transglutaminase
CD	Coeliac Disease
CMJAH	Charlotte Maxeke Academic Hospital
EMA	Anti-endomysium
ESPGHAN	European Society of Gastroenterology, Hepatology and Nutrition
GVHD	Graft Versus Host Disease
HLA	Human Leukocyte Antigen
INF-G	Interferon Gamma
MHC	Major Histocompatibility Class
SAM	Severe Acute Malnutrition
SANBS	South African Blood Services
TNF-alpha	Tumour Necrosis Factor Alpha
WDGMC	The Wits Donald Gordon Medical Centre
WHO	World Health Organization

1. INTRODUCTION AND BACKGROUND

The development of an autoimmune condition requires an environmental trigger in the context of genetic predisposition [(1)]. Coeliac Disease (CD) is an autoimmune condition, in which individuals with the genetic predisposition have an immune mediated response to gluten exposure resulting in enteropathy and other adverse clinical outcomes [(2)].

Gluten is found in wheat, barley and rye products contains high levels of glutamine and proline residues which are known collectively as prolamines [(2)]. The environmental trigger for CD is these prolamines which cause a change in the intestinal mucosa resulting in eventual villous atrophy [(2)]. Clinical manifestations of CD are grouped in accordance with patient presentation which may be typical or atypical depending on the degree of villous atrophy and malabsorption that results [(3)]. In CD, often iceberg model is used to describe clinical presentation in which the tip corresponds to patients with classic malabsorption, while the more atypical presentations are included in the invisible and larger portion below the waterline [(4)]. Typical or classical gastrointestinal manifestations present at 6 to 18months of age, they may include chronic diarrhoea, steatorrhoea, bloating, malnutrition and growth faltering [(3)]. Atypical presentations are extra intestinal and include iron deficiency anaemia, osteoporosis, osteopenia, abnormal liver function tests (mainly a transaminitis), peripheral neuropathy and ataxia[(3,5)].The clinical manifestations of Coeliac Disease may cause severe morbidity and mortality if this condition remains untreated[(5)].

Diarrhoeal disease and severe acute malnutrition continue to be a source of mortality and morbidity in children under five in Southern Africa [(6)]. The clinical features of these

conditions overlap with those of the phenotypical presentation seen in patients with CD[2].A study performed at Prince Albert Luthuli Hospital in 2016 provided evidence for the screening of CD within our diabetic population furthermore the study(Prevalence of positive coeliac serology in a cohort of South African children with type 1 diabetes mellitus.) confirmed the low prevalence of symptoms within the chosen population of study [7]. A recent audit in a T1DM clinic in Johannesburg by the authors revealed 8% of the clinic's patients (all diagnosed with T1DM) had biopsy proven CD [8]. The prevalence of CD is underestimated worldwide [9]. This is largely due to lack of screening and under-investigation.

The development CD is multifactorial, it involves a combination of environmental factors and variations in multiple genes [4]. Some of the factors that have been identified include; viral infections which may alter gut permeability, the gut microbiome (natural flora in the gut), breastfeeding or timing of initiation of solids to the infant diet [4]. Factors supporting a strong genetic contribution include familial aggregation, an 83 -86 % pattern in monozygotic twins vs the 11% pattern in dizygotic twins as well as the association with other autoimmune conditions in the same individual or in different members of the same family (mainly type 1 diabetes, thyroiditis and multiple sclerosis)[4][10]. The HLA system is the main predisposing genetic factor in CD (11). Genetic predisposition for CD is associated specifically with class II HLA genes of MHC [1]. Various HLA haplotypes have been identified in CD, however the two that are predominant are the Human Leukocyte Antigens; HLADQ2 and HLADQ8[12].

Prolamines in the gut bind to HLA DQ2 and DQ8 on antigen-presenting cells leading to activation of cellular and humoral immunity [1]. This leads to the activation of T-Lymphocytes which produce cytokines (TNF-alpha and INF-G.) these activate the production of metalloproteinases by fibroblasts [1]. Metalloproteinases act on the intestinal cells brush border and digesting their intercellular matrix leading to villous atrophy and eventual crypt hypertrophy [1]. Simultaneously stimulated B cells that have also been activated produce autoantibodies that infiltrate the mucosa [1,13]. Individuals with HLA DQ2 and HLA DQ8 have an increased predisposition for developing this CD by 90% and 10% respectively [12].

The diagnosis of CD is confirmed by serological tests, searching for anti-TG2 and EMA auto-antibodies and more importantly identifying certain histological changes upon intestinal biopsy of the duodenum while the patient is on a gluten containing diet [2]. The ESPGHAN recently revised its guidelines for the diagnosis of CD in 2012[6]. Under these guidelines the omission of biopsy is permitted in the presence of CD related symptoms, convincing quantitative antibody levels and HLA typing-specifically the presence of HLA DQ2 or HLA DQ8[10]. This strengthens and ascertains the role of HLA in the pathophysiology and diagnosis of CD.

Knowledge and research around CD has been on a steady increase since it was first described in 2000 years ago, and its diagnosis and prevalence has increased proportionally [14]. The prevalence of CD is said to be 1% worldwide [2]. Although previously thought to be predominant among Caucasian populations, the geo-

epidemiology of CD has been shown to have variability between countries ranging from the USA, New Zealand and Australia to India, Egypt, Morocco, Libya, with the highest prevalence of CD being reported in the Saharawi population of Arab-Berber ethnicity living in Algeria at 5.6% CD[12]. Some postulations for this increased prevalence include; improved sensitivity and specificity of the serogical markers anti-TG2 and EMA used for the diagnosis [12]. The wider use of biopsy (the gold standard) has been cited as another reason with the development of the Marsh criteria used in to grade the enteropathy [3]. Environmental factors such as the introduction of gluten to many countries' diets have also been used to explain the rise of the CD diagnosis with countries such as Burkina Faso having almost no "Coeliacs" due their lack of wheat consumption [12]. Another explanation has been the mixing of populations and the transference of responsible HLA alleles [12]. CD requires the presence specific HLA class II alleles for its development, with 90% of patients being HLA DQ2 positive and 5% possessing the HLA- DQ8 haplotypes[10].Immunogenetic information is available in much of the developed world but has been poorly studied in the developing countries[12,15].Very little information is available with regards to the prevalence of HLA DQ2 and HLA DQ8 haplotypes in the South African setting and by inference the population's susceptibility to CD.

HLA typing and matching is useful and utilised most frequently for kidney and bone marrow transplantation [16]. Transplantation of foreign tissue or inadequately matched induces both humoral and cellular immune responses in the recipient, which leads to graft rejection or for bone marrow transplantation GVHD [16]. The SANBS is responsible for the majority of Gauteng, and the Free State province's HLA typing services. The SANBS database tests and stores information about the HLA profiles and the presence or HLA

haplotypes of individuals of various demographics for the purposes of successful transplantation, the HLA haplotypes tested include those associated with CD. The electronic database, Meditech, was developed 5 years ago and used to store donor and recipient information.

The purpose of this study to perform an audit of the prevalence of CD associated HLA DQ2 and DQ8 using the information of those having undergone investigation for renal or bone marrow transplant, potential donors and transplant recipients using the database provided by the SANBS. The collection and stratification of this Immunogenetic information will be done to gather more information with regards to the South African population's susceptibility to CD and support demand for more screening and diagnosis among high risk groups.

2. PROBLEM STATEMENT

In South Africa there is little evidence regarding the population's susceptibility to Coeliac Disease. There is a need to determine the proportion of our population that is susceptible to Coeliac Disease and assess the need to review our screening guidelines. In addition, there is a need to identify any differences in prevalence between different ethnic groups in South Africa.

3. AIM

To determine the prevalence of Coeliac Disease associated HLA DQ2 and DQ8 among potential donors and recipients for kidney transplant done at by SANBS during the past five years using the electronic database, and to stratify this information per ethnic group.

4. OBJECTIVES

1. To determine the percentage of kidney and bone marrow transplant potential donors and recipients who have HLA DQ2 and DQ8 haplotypes and compare with available literature.
2. To describe the demographics of patients with HLA DQ2 and DQ8 and compare them with available literature.

5. METHODS

a. STUDY DESIGN

A retrospective audit of the records of potential donors and recipients who were investigated for kidney and bone marrow transplant using the SANBS electronic database over a five-year period

b. SAMPLE POPULATION

i. INCLUSION CRITERIA

The sample population includes all potential donors and recipients investigated for renal and bone marrow transplant.

ii. EXCLUSION CRITERIA

Patients with incomplete records will be excluded from the study

c. SAMPLE SIZE

The estimated sample size for the proposed study is the number of potential kidney and bone marrow transplant donors and recipients from transplant investigations done previously that are available on the SANBS electronic database (me ditech).

d. **PROCEDURES**

Data collection will commence once ethical clearance is obtained from the Human Research Ethics Council of the University of the Witwatersrand and the SANBS ethics council. HLA and demographical data will be retrieved from patient records collected by the SANBS during the HLA typing service provided prior to bone marrow and renal transplantations. Only electronic data records from the past 5 years will be used to ensure anonymity of subjects, the primary investigator (Dr Kitso Mrubata) will be allowed access only to the relevant data necessary to fulfil the study aims and objectives. Data collected will include age, sex, ethnicity and presence of HLA-DQ8 and HLA-DQ2 haplotypes

a. **DATA HANDLING AND COLLECTION**

Study data will be collected and managed using REDCap electronic data capture tools hosted at the University of the Witwatersrand.

REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing:

1) an intuitive interface for validated data entry

2) audit trails for tracking data manipulation and export procedures

3) automated export procedures for seamless data downloads to common statistical packages

4) procedures for importing data from external sources. [17]

6. DATA ANALYSIS

Data will be analysed using Stata® Intercooled version 11. [18] Chi squared tests will be used to compare proportions of the population groups with HLA-DQ2 and HLA-DQ8.

Appropriate descriptive statistical analysis using percentages, means and standard deviations and medians and ranges will be used for demographic data. A statistician will be consulted for further assistance if needed.

7. LIMITATIONS

- The study is a retrospective audit of records and thus relies on data from existing records. These records may not be complete and may weaken the study.
- The sample is a convenience sample taken from Kidney and bone marrow and bone marrow transplant recipients and donors in whom HLA typing has already been investigated and is on the SANBS data base given the transplant criteria and indications of kidney transplant it may not be representative of the South African population.
- Demographic data including self-reported ethnicity will be used to classify different ethnic groups, introducing a possible source of bias.

8. ETHICAL CONSIDERATIONS

Risks: As this is a retrospective study, there will be no risk/s to participants.

Anticipated benefits: There are no direct benefits; except that the study will contribute further to medical knowledge.

Confidentiality: Anonymised data will be provided from the SANBS database and no identifying data will be extracted from the existing database.

Costs to participants: Participants will incur no extra costs.

Review board approval: The protocol will be submitted for approval by the Human Research Ethics Committee (HREC) (Medical), the SANBS ethics committee and University of the Witwatersrand prior to initiation of the study.

9. **TIMELINES**

	April 2019	May 2019	Jun 2019	Jul 2019	Nov 2019	Dec 2019	Jan2020	Feb 2020	Mar 2020	April 2020	May 2020	June 2020	July 2020	Aug 2020	Sep 2020
terature eview															
rotocol reparation															
rotocol ssessment															
thics approval															
ost graduate pproval															
ata collection															
ata analysis															
rite up report															
rite up paper															

10. COST AND FUNDING

The cost involved in the study is for stationery, printing and binding. This cost will be borne by the primary investigator as there is no funding for this study.

REFERENCES

1. Rachisan AL, Pirvan A, Miu N, Gheban D, Hrusca A, Samasca G, et al. Is HLA Typing a Diagnostic Tool in Celiac Disease? Int J Celiac Dis [Internet]. 2016;4(1):9–10. Available from: http://pubs.sciepub.com/ijcd/4/1/10/index.html

2. Hill ID, Fasano A, Guandalini S, Hoffenberg E, Levy J, Reilly N, et al. NASPGHAN clinical report on the diagnosis and treatment of gluten-related disorders. J Pediatr Gastroenterol Nutr. 2016;63(1):156–65.

3. Green PH, Jabri B. Coeliac disease. Lancet [Internet]. 2003;362(9381):383–91. Available from: https://www-sciencedirect-com.stanford.idm.oclc.org/science/article/pii/S0140673603140275

4. Megiorni F, Pizzuti A. HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: Practical implications of the HLA molecular typing. J Biomed Sci [Internet]. 2012;19(1):1. Available from: Journal of Biomedical Science

5. Leffler DA, Green PHR, Fasano A. Extraintestinal manifestations of coeliac disease. Nat Rev Gastroenterol Hepatol [Internet]. 2015;12(10):561–71. Available from: http://dx.doi.org/10.1038/nrgastro.2015.131

6. Reid AE, Hendricks MK, Groenewald P, Bradshaw D. Where do children die and what are the causes? Under-5 deaths in the Metro West geographical service area of the Western Cape, South Africa, 2011. South African Med J. 2016;106(4):359–64.

7. Tayob S, Pillay K, Tlou B, Ganie Y. Prevalence of positive coeliac serology in a

cohort of South African children with type 1 diabetes mellitus. South African J Child Heal. 2016;10(1):12–5.

8. Basson C ,Coeliac Disease in a South African Paediatric Type 1 Diabetes Mellitus clinic., 2017

9. Catassi C, Fasano A. Coeliac disease: The debate on coeliac disease screening-Are we there yet? Nat Rev Gastroenterol Hepatol [Internet]. 2014;11(8):457–8. Available from: http://dx.doi.org/10.1038/nrgastro.2014.119

10. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136–60.

11. Tye-Din JA, Cameron DJS, Daveson AJ, Day AS, Dellsperger P, Hogan C, et al. Appropriate clinical use of human leukocyte antigen typing for coeliac disease: An Australasian perspective. Intern Med J. 2015;45(4):441–50.

12. Lionetti E, Catassi C. Co-localization of gluten consumption and HLA-DQ2 and -DQ8 genotypes, a clue to the history of celiac disease. Dig Liver Dis [Internet]. 2014;46(12):1057–63. Available from: http://dx.doi.org/10.1016/j.dld.2014.08.002

13. Choo SY. The HLA system: Genetics, immunology, clinical testing, and clinical implications. Vol. 48, Yonsei Medical Journal. 2007. p. 11–23.

14. García Nieto V. A History of Celiac Disease. Celiac Dis Non-Celiac Gluten Sensit. 2014;45–59.

15. Piancatelli D, Ben El Barhdadi I, Oumhani K, Sebastiani P, Colanardi A, Essaid A. HLA Typing and Celiac Disease in Moroccans. Med Sci [Internet]. 2017;5(1):2. Available from: http://www.mdpi.com/2076-3271/5/1/2

16. Mahdil B mutar. A glow of HLA typing in organ transplantation. Clin Transl Sci. 2013;2(6):1.

17. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009 Apr;42(2):377–81.

18. StataCorp. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP; 2009.

Protocol Appendix A

Study no:

Sex Male Female

Age

Ethnicity:

)Q 2.5 (DQB1*02 DQA1*05)	No
)Q8(DQB1*03:02DQA1*03)	No
)Q2(DQB1*02 DQA1 not *05)	No

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NPC Registration No. 2000/026290/08

29 May 2019

To SRC / HREC

Re: Data for research

Subject to approval, this letter is to confirm that SANBS will be able to supply:
Dr Kitso-Lesedi Mrubata from WITS the following demographics and data for the study "The Prevalence of Coeliac disease associated Human Leucocyte Antigen haplotypes in Gauteng, South Africa":

Presence/absence of:

a. HLA DQ 2.5 (DQB1*02 DQA1*05), this is the most likely haplotype combination
b. HLA DQ8 (DQB1*03:02 DQA1*03)
c. HLA DQ2 (DQB1*02 DQA1 not *05)

1. We would only provide data that is already in electronic format, which is about 3 to 4 years.
2. We would be able to provide demographics; age, gender and were provided, race/ethnicity.

Purpose: To use for the above research purposes.

Signed:

Ute Jentsch
Lead Consultant Pathology (Specialized Lab Services and Quality Control), Medical

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