

GENOMICS STUDY OF ANTI-TUBERCULOSIS DRUG-INDUCED HYPERSENSITIVITY REACTIONS

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Dedication

I would like to express my sincere gratitude to Allah for making it possible for me to accomplish my goals. Exceptional thanks to my wonderful, understanding, loving husband Dr Hussein Mwanga and our beautiful twins Adeel and Adeelah, I love you very much.

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Abstract

Introduction: All first-line anti-tuberculosis drugs can be associated with all phenotypes of cutaneous adverse drug reactions (CADR). Second-line drugs are associated with much poorer outcomes. Thus, identifying the offending drug in poly-pharmacy is difficult. Re-challenge with the drug is the gold standard in identifying the offender, however poses unacceptably high risk of CADR recurrence. Population and drug-specific genomics help identify those susceptible to adverse reactions to a drug facilitating avoidance of the drug.

Objective: To investigate the genomic susceptibility in patients with confirmed rifampicin and or isoniazid-associated hypersensitivity reactions using both genome-wide association studies and candidate gene approaches.

Methods: A case control study using 14 patients with previous tuberculosis-associated CADR who were re-challenged with first-line anti-tuberculosis drugs and subsequently developed re-challenge reactions to either isoniazid or rifampicin as cases. These were compared with 30 controls who had tolerated rifampicin and isoniazid during the re-challenge process (12 patients, Group 1a) and consecutive patients who had been on TB treatment for at least 12 weeks without developing any adverse drug reaction (18 patients, Group 1b) and 200 black South Africans from the general population. HLA genotypes of the samples were determined by SeCore® HLA Sequence based typing (Invitrogen, Life technologies, USA), and potential ambiguities were resolved by sequencing-based typing.

Results: We found HLA-B*58:02 (OR=3.6; 95% CI: 1.4-8.99) and HLA-DRB1*09:01 (OR=15.3; 95% CI: 2.1-113.1) to be significantly more prevalent in patients who developed rifampicin and isoniazid-associated CADR as compared to black South African general population. However, we found no significant associations between HLA genotype and rifampicin/isoniazid-associated CADR when we compared the cases to our study controls that had tolerated rifampicin and isoniazid. HLA-B *58.02 was not

found to be statistically associated with HIV positive status ($p=0.42$) and DRESS phenotype ($p=0.6279$). The majority of our cases were black Africans. Approximately 80% of our cases and controls were HIV-infected. DRESS/DIHS was the prevalent phenotype of CADR, accounting for approximately 80% of cases and controls.

Discussion: To our knowledge, this is the first study to show an association between HLA-B*58:02 and HLA-DRB1*09:01 alleles and severe cutaneous adverse drug reactions secondary to rifampicin and isoniazid in an African population. We identify 2 candidate HLA alleles that need confirmation of their association in African patients who develop rifampicin or isoniazid-associated CADR in larger studies. The value of identifying candidate alleles could lead to CADR preventative screening prior to initiating anti-tuberculosis therapy in black South Africans. The HLA-B*58:02 noted in our cases and controls tolerant of the drugs might not be associated with CADR but could be a reflection of the HIV status and control in HIV-TB co-infected persons.

Conclusion: HLA-B*58:02 and HLA-DRB1*09:01 may be associated with rifampicin and isoniazid-associated CADR. Alternately HLA-B*58:02 may be associated with HIV status rather than CADR. A sufficiently powered study is needed to confirm this association.

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Abbreviations

TB: Tuberculosis

HIV: Human immunodeficiency virus

WHO: World health organization

ADR: Adverse drug reactions

MDR: Multi drug resistance

MTB: Mycobacterium tuberculosis

HHV6: Human herpes virus type 6

CADR: Cutaneous adverse drug reactions

DRESS: Drug reaction with eosinophilia and systemic symptoms

DIHS: Drug induced hypersensitivity syndrome

SJS: Stevens Johnson syndrome

TEN: Toxic epidermal necrolysis

BSA: Body surface area

HLA: Human leukocyte antigen

GSTM1: glutathione-S-transferase M1

NAT2: N-acetyltransferase 2

CYP2E1: Cytochrome P450 2E1

RIF: Rifampicin

INH: Isoniazid

ETH: Ethambutol

PZA: Pyrazinamide

LTT/LTA: Lymphocyte transformation test/assay

Chapter 1: Introduction and Literature review

1: Literature review and background to the study

1.1. Tuberculosis

Tuberculosis (TB) is a worldwide infectious disease, caused by *Mycobacterium tuberculosis* (MTB). Commonly MTB affects the lungs however other organs can also be affected dependent on the person's ability to control the primary infection (Ghon focus). In 2013, there were 9 million people globally who developed TB. Of these, approximately 5.15 million were men, 3.3 million were women and 0.55 million were children. The majority of the reported TB cases were in Asia (56%) and Africa (29%) (World Health Organization 2014). South Africa was among the six countries with the highest absolute number of incident cases of TB with estimated absolute incident cases of 410000–520000 in 2013. The other countries were India (2.0 million–2.3 million), China (0.9 million–1.1 million), Nigeria (340 000–880000), Pakistan (370000–650000) and Indonesia (410000–520 000) (World Health Organization 2014). Considering incident rates, South Africa with 560 (776-980) new cases of TB per 100 000 population remained in the top ten countries with the highest estimated population based incidence rates for 2013. Eight of the top ten countries with high population based incidence rates were on the African continent whose people appear to carry the global population burden of TB (World Health Organization 2014).

Tuberculosis is one of the leading causes of death in the developing world. Due to human immunodeficiency virus (HIV) infection and poor socio-economic conditions in these regions, global attempts to control TB have been minimally effective and the incidence continues to increase (Department of Health Republic of South Africa 2014). In 2013 there were 1.5 million estimated TB deaths. Approximately 60% (900 000) of those dying from TB were men, 510 000 were women and 90 000 were children. African, South East Asian and Western Pacific WHO regions accounted for more than 75% of the TB deaths (World Health Organization 2014). This data excludes cases that

were missed by the notification health system, mainly in the developing world. It is evident from these statistics that TB remains one of the major threats to global health.

The HIV pandemic has resulted in a steep increased trajectory of cases co-infected with TB worldwide. Of the 9 million TB cases reported in 2013, 1.1million (13%), were co-infected with HIV. Africa was the WHO region with the highest number of TB cases living with HIV (World Health Organization 2014). It was estimated that approximately 34% of TB cases were co-infected with HIV in Africa, accounting for 78% (800 000) of TB-HIV co-infection worldwide (World Health Organization 2014). WHO data show that 1.5 million people died from TB in 2013, and 360 000 (24%) of these deaths were in people who were co-infected with HIV (World Health Organization 2014). Despite constituting only 0.7% of the world's population, South Africa accounts for about 25% (300 000) of the estimated global caseload of HIV associated TB (Wood et al. 2011). The annual risk of acquiring TB in an HIV infected individual is 10% compared to a lifetime risk of 10% in an HIV-unaffected counterpart (Department of Health Republic of South Africa 2014). Infection with HIV increases the risk of dissemination of recent MTB infection and of reactivation of latent MTB infection by 5 to 15% annually (Department of Health Republic of South Africa 2014).

The burden of HIV and TB co-infection has resulted in multiple new TB management problems, including an increase in reported cases of multi drug resistance (MDR) TB. Wells and colleagues reported the association of HIV and (MDR) TB which resulted in complexity in the management of TB patients and hence poor infection control (Wells et al. 2007). The risk factors in HIV and TB co-infected patients for developing MDR TB were advanced immunosuppression, poor adherence to first line TB medication including the intermittent use of TB treatment, drug interactions, adverse drug reactions and poor gastrointestinal absorption of TB drugs (Wells et al. 2007).

1.2. Adverse drug reactions

There are various definitions for what constitutes an adverse drug reaction (ADR). In 1998, Pirmohamed and colleagues defined ADR as 'unpleasant effects of the drug beyond its expected therapeutic effects which occur during clinical use (Pirmohamed et al. 1998). The term adverse drug effect (ADE) and ADR are often used interchangeably to mean the same thing but the ADE is the expected effect of the drug and an ADR is an unexpected reaction that occurs in a patient (Aronson & Ferner 2005).

Adverse drug reactions were initially divided into type A (augmented pharmacology) or type B (bizarre, idiosyncratic). Type A (Augmented) reactions represent an augmentation of the pharmacological actions of a drug. They are predictable, dose dependent and are therefore readily reversible on reducing the dose or withdrawing the drug. Type A reactions account for the majority of ADRs and includes side effects and toxic effects. Type B (Bizarre) reactions in contrast are rare, bizarre, unpredictable and are not dose dependent nor related to known drug pharmacology. They include immune mediated hypersensitivity reactions (IgE-mediated urticaria and immune complex vasculitis) and idiosyncratic reactions which are severe and life threatening such as SJS/TEN and DRESS where the pathogenesis is not clear-cut.

The importance of time-dependent reactions that occur after prolonged or chronic use, on drug withdrawal, or with a delayed action relative to when the drug was used need additional classification groups, as does drug failure. Type C (Chronic) reactions are related to time on medication and cumulative dose (glucocorticoid suppression of hypothalamic-pituitary-adrenal axis by glucocorticoid). Type D (Delayed) reactions becoming apparent sometime after the use of the drug and include teratogenesis and carcinogenesis. They are often dose-related. Type E (Withdrawal) reactions occur when the drug is withdrawn for whatever reason. Type F (Failure) reactions occur because of drug interactions leading to failed therapy. They are dose related (Todd 2006; Edwards & Aronson 2000). ADR are usually diagnosed on clinical grounds from the temporal relation between the start and finish of drug treatment and the onset and resolution of the reaction. A culprit drug is identified if the signs and symptoms of ADR occurred after initiating the drug and resolves after withdrawing the drug and recur again on re-challenge with the same drug.

Adverse drug reactions contribute substantially to patient morbidity and hospitalization in South Africa. This is added to by the burden of HIV and TB co-infection. A South Africa study showed that HIV-uninfected persons are less likely to be admitted to hospital with adverse drug reactions compared to those who are HIV-infected (Mehta et al. 2008). Polypharmacy (more than 7 drugs) commonly ARVs, anti-tuberculosis drugs and co-trimoxazole were the main implicated drugs in most of the ADR admissions and deaths in four South African hospitals (Mouton et al. 2014). Mouton and colleagues reported drug induced renal failure and drug-induced liver injury were the most common adverse drug reactions related to death in HIV patients admitted to hospital for ADRs in the same survey of the 4 hospitals in South Africa (Mouton et al. 2014). Of the 24 patients who developed drug-induced renal failure, tenofovir was the most commonly implicated culprit drug in 14 (61%) cases followed by co-trimoxazole in 3 cases, rifampicin in 2 cases, furosemide in 2 cases, co-amoxiclavulanic acid in 2 cases and ibuprofen, enalapril, spironolactone, ciprofloxacin, aciclovir and indomethacin 1 case each. Ten patients developed drug-induced liver injury. Seven cases were due to anti TB drugs (rifampicin, isoniazid and/or pyrazinamide) and 3 cases were due to co-trimoxazole either alone or together with other drugs. Fluconazole, erythromycin and sodium valproate accounted for 1 case each (Mouton et al. 2014). This data reflects the significant role played by anti-tuberculosis drugs in HIV associated ADR.

1.3. Cutaneous adverse drug reactions

A cutaneous adverse drug reaction (CADR) is the skin manifestations of an adverse drug reaction. There are different phenotypes of CADR ranging from mild self-resolving reactions to severe life threatening ones. Essentially any skin disease can be a manifestation of an adverse cutaneous reaction. Among the milder phenotypes are, exanthematous drug eruptions; IgE mediated reactions like urticaria; photo accentuated drug reactions; classic fixed drug reaction; vasculitis; neutrophilic drug reactions like acute generalized exanthematous pustulosis (AGEP) and acute febrile neutrophilic dermatoses (Sweet's syndrome). The severe or life threatening drug reactions include drug reaction with eosinophilia and systemic symptoms (DRESS) also referred to as

drug induced hypersensitivity syndrome (DIHS); generalized bullous fixed drug eruptions (GBFDE); Stevens-Johnson syndrome (SJS); toxic epidermal necrolysis (TEN) and lichenoid drug reactions (LDR).

Exanthematous, maculopapular or morbilliform drug reactions mostly occur 7 to 14 days after starting medications or earlier in cases of re-introduction of the same offending medication. Clinically they presents with an erythematous, maculopapular, measles-like eruption (Figure 1), urticarial plaques or annular, targetoid lesions. The mucosal surfaces are spared. The rash resolves within one or two weeks with no systemic symptoms, often despite ongoing treatment.



Figure 1: Morbilliform rash

Urticaria (Figure 2) is characterized by transient, erythematous, infiltrated papules and plaques with no epidermal change. The lesions last for less than 24 hours and resolve with no epidermal or post inflammatory changes. Angioedema (Figure 3) is acute oedema of dermal, subcutaneous and mucosal tissues and can be life-threatening if associated with asphyxia. Anaphylaxis is an acute life threatening type I hypersensitivity or IgE dependent drug reaction characterized by systemic manifestations including

hypotension and tachycardia which may occur together with or without urticaria and angioedema (Kemp et al. 2008).



Figure 2: Urticaria



Figure 3: Angioedema

Photosensitivity drug reactions (Figure 4) could either be photo-toxic (non-immune) or photo-allergic (immune mediated). The regions of the body affected are the sun-exposed surfaces of the face (forehead, zygoma, nose, lower lip and chin), the ears and cervical triangle. Sparing of the submental, posterior auricular and periorbital regions is

strongly supportive. Photo-toxic drug reactions usually occur within hours after exposure to the culprit drug together with light. This clinically resembles exaggerated or acute sunburn and can occur in any person taking a drug known to cause phototoxic reactions. Photo-allergic drug reactions usually develop within days of exposure to the culprit drug together with ultraviolet radiation. They are type IV hypersensitivity reactions and clinically may resemble eczema or lichenoid reactions (Glatz & Hofbauer 2012).



Figure 4: Photosensitivity drug reaction

Lichenoid drug reactions (LDR) occur within months to years of exposure to the culprit drug but may require exposure to the culprit drug together with ultraviolet radiation. It is clinically characterised by burning, itchy, lilac macules that progresses to become violaceous to purple, flat-topped, scaly papules often involving the flexural surfaces. Both the skin and mucosae may be involved. It resolves with or without post inflammatory hyperpigmentation on withdrawal of the drug (Figure 5).



Figure 5: Lichenoid drug reaction

A fixed drug eruption may occur on the skin or mucosae within minutes of culprit drug ingestion. They clinically presents as an itchy, well circumscribed, round, erythematous to purple patch often with an outer rim of erythema in the active state. They can blister and they resolve leaving slatey grey-black post-inflammatory hyperpigmentation (Figure 6). They occur as solitary or multiple patches. Re-exposure to the offending drug leads to recurrence at the same site or the development of new lesions elsewhere. Occasionally it can be life-threatening and confused with SJS/TEN if it is bullous and generalized (Figure 7) (Lipowicz et al. 2013).



Figure 6:
Fixed drug eruption



Figure 7:
Generalised bullous fixed drug eruption

SJS and TEN are severe, potentially life threatening drug reactions that occur between one to two weeks after exposure to the culprit drug. They are clinically characterized by mouth, eyes and genitalia involvement together with epidermal necrosis and varying degrees of detachment on a background of erythema. In SJS, the skin detachment is <10% body surface area (BSA) (Figure 8) compared to >30% BSA in TEN (Figure 9). SJS/TEN overlap is characterized by epidermal necrosis of 10%-30% (Roujeau 1994). SJS and TEN may be associated with systemic disease or with complications that could either be acute or chronic. Acute complications that account for the high mortality include bacterial systemic infection, metabolic dysfunction and fluid loss. Mortality risk can be assessed on the day of admission and again 3 days later by applying the TEN-specific severity-of-illness score (SCORTEN) (Guégan et al. 2006). Nail dystrophies, dyspigmentation, sicca symptoms, mucosal adhesions, oesophageal strictures, hypertrophic scars and blindness are chronic anticipated sequelae in some cases especially in those with severe disease (Roujeau 1994) .

The mortality rate varies. Studies have shown that SJS mortality ranges between 0% and 12%, while that of TEN ranges from 30% to 46% (Sekula et al. 2013; Saka et al. 2013; Kannenberg et al. 2012; Roujeau & Stern 1994). The mortality rate may increase depending on the extent of the BSA involved, the patient's age and other SCORTEN parameters (Harr & French 2010). A large prospective cohort study of severe SJS/TEN requiring hospitalization from several centres in Europe, reported a 6 week mortality rate of 23% overall; 12% for SJS, 29% for SJS/TEN overlap and 46% for TEN (Sekula et al. 2013). In this study a history of malignancy and the older age of the affected patients contributed to the higher mortality reported. In a prospective cohort study undertaken in South Africa, a comparable mortality rate of 24% for all forms of SJS and TEN was reported. When sub analysis were done, the TEN mortality rate was 40% which was statistically significant ($p=0.001$) when compared to the 0% of SJS (Kannenberg et al. 2012). Comparable findings were reported for sub-Saharan Africa in a retrospective study of a cohort collected from several African countries in which 74% were less than 50 years of age. An overall mortality of 12% was found; 5% for SJS, 9%

for SJS/TEN overlap and 41% for TEN. HIV-infection was comorbidity in 73% of the 22 deaths (Saka et al. 2013).



Figure 8: Stevens-Johnson syndrome



Figure 9: Toxic epidermal necrolysis

DRESS/DIHS is a severe CADR which occurs two to six weeks after exposure to the offending drug. It is clinically characterized by fever, oedema (especially of the face), a morbilliform rash (Figure 10), lymphadenopathy, leukocytes abnormalities and internal organ involvement. The most commonly affected internal organ is the liver which may result in severe hepatitis, liver failure, encephalopathy and sometimes death. A mortality rate for DRESS/DIHS was reported to be 10% from RegiSCAR data from around the world (Kardaun et al. 2013).



Figure 10: Drug reaction with eosinophilia and systemic symptoms

2. Pathogenesis

2.1. Adverse drug reaction pathogenesis

Multiple factors influence the occurrence of disease, its clinical picture and outcome. This concept best explains the pathogenesis of unexpected reactions to drugs. Only a proportion of those exposed to an infectious agent will develop clinical disease, and similarly not all those exposed to a drug develop a drug reaction. Other cofactors of causation need to be variably present, which, on their own, do not necessarily produce the effect (Todd 2006; Sullivan & Shear 2001)

The pathogenesis of CADR is slowly unraveling. The mechanism by which a drug leads to immune mediated adverse reaction (pharmaco-immune concept, modified Gel and Coombs hypersensitivity reactions) can be explained by either hapten hypothesis or P I concept. The drug or its metabolites must be recognized by the immune system in the context of self. Several mechanisms have been shown to be involved in drug/major histocompatibility complex (MHC)-peptide binding on antigen-presenting cells for initiation of an immune response, both innate and adaptive and cause localized or systemic reactions. The drug acts as a hapten (the chemically reactive drug itself binds

covalently to carrier proteins which may include the MHC-peptide complex), prohapten (the drug must be metabolised to a reactive hapten) and non-hapten (non-covalent, labile binding to the MHC-peptide complex) or the p-I concept (pharmacological interactions of drug with immune receptors concept, drugs can bind directly to T-cell receptors TCR). On presentation or binding to T-cells via shared or restricted TCRs (HLA-drug-TCR interaction), multiple cellular triggers are required for the propagation of an immune response resulting in an expansion of lymphocyte clones (T and B cells), the releases of cytotoxic proteins (granulysin, perforin, granzyme etc.), and apoptosis(Pichler et al. 2010). All reactions are T-cell-regulated, but the effect is dependent on antibody-mediated effector functions or more T-cell/cytokine dependent functions.(T and B cells) and cause localized or systemic reactions The “danger” hypothesis proposes that following initiation of the acquired immune system, tolerance (possibly involving T-reg cells) is the normal default response and that “danger” signals are necessary for propagation and the subsequent development of an immune response. “Danger” signals can be produced by the innate immune system or any inflammatory episode as a result of cellular stress (e.g., drug/toxic metabolites, cell damage, bacterial products) (Todd 2006; Uetrecht 1999).

The cutaneous reactions that can occur as a result of immune stimulation by a single drug are influenced by the efferent output following T-cell activation. Preferential activation of T-cell populations with distinct functions can lead to cytotoxic T-cells (CD4+ cells in morbilliform reactions, CD8+ cell in vesico-bullous reactions) can orchestrate the skin reaction by release of cytokines/chemokines which in turn activate or recruit specific cells into the skin (interleukin 4 and 5 for eosinophil infiltrates, interleukin 8 for neutrophil infiltrates, interferon- γ for monocyte infiltrates) (Pichler et al 2010).

The dynamic synergistic interaction of constitutional and acquired factors, determines whether one develops a drug reaction. The unique combination of cofactors present at the time of drug exposure in a particular individual may explain why an adverse event may not recur on re-challenge.

The relationship between drug reactions and immunogenetics was recently proposed by Chung and colleagues who showed that certain polymorphisms of the HLA genes predispose to drug hypersensitivity reactions. These included HLA-B*15:02 and carbamazepine (CBZ)-induced SJS/TEN and HLA-B* 58:01 and allopurinol-induced SJS/TEN and DRESS (Chung et al. 2007). More recent studies have shown that drugs presented by specific HLA alleles are recognized by specific T cell receptors leading to activation of cytotoxic T lymphocytes and cytotoxic signal expression (Chung et al. 2008). These HLA genetic associations with immune-mediated cutaneous drug reactions are complex, drug specific and ethnicity specific.

HLA-B*15:02 has been shown to be present in 100% of Han Chinese who develop CBZ-induced SJS/TEN but in only 3% of CBZ-tolerant patients and in 8.6% of the general population of Han Chinese from Taiwan (Hung et al. 2006; Chung et al. 2004). HLA-B*15:02 is present in 75% of Malay patients with CBZ-induced SJS/TEN but in only 15.7% of normal controls (Chang et al. 2011). In a study done in Europe only four cases (25%) were HLA-B*15:02 allele positive among 12 cases of CBZ-induced SJS/TEN and all four were of Asian origin (Lonjou et al. 2006). Screening for HLA-B*15:02 before prescribing CBZ in Taiwan has significantly reduced the number of cases of SJS/TEN in the country (Ferrell & McLeod 2008). This is probably applicable for Malaysia and for Asians living abroad.

HLA-B*58:01 was reported to be present in all 51 patients (100%) with allopurinol-induced SJS/TEN and DRESS in Han Chinese but in only 15% (20 of 135) of the allopurinol tolerant group and 20% (19 of 93) of the general healthy population from Taiwan (Hung et al. 2005). In Thai population, twenty seven (100%) allopurinol-induced SJS/TEN patients who were examined carried HLA-B*58:01 whereas only seven (13%) of the tolerant control patients had this allele (Tassaneeyakul et al. 2009).

HLA-B*57:01 was reported as present in 14 (78%) of the 18 patients with abacavir hypersensitivity, and in four (2%) of the 167 abacavir tolerant patients from a Western Australian cohort study (Mallal et al. 2002).

The presence of HLA-B*13:01 was proposed as a predictor of dapsone hypersensitivity in patients with leprosy. It was present in about 2% to 20% of Chinese persons with leprosy and dapsone hypersensitivity reactions, 1.5% of a similar cohort in Japan, 1% to 12% of Indians with leprosy and dapsone hypersensitivity, and 2% to 4% of a similar Southeast Asian cohort but 0% in European and African cohorts (Zhang et al. 2013).

Clearly there are population, drug and CADR specific HLA haplotypes that are not generalizable globally.

2.2. Pathogenesis of adverse reactions to TB drugs

Sharma and colleagues have demonstrated the association of HLA class II haplotypes associated with anti-tuberculosis drug induced liver injury. These include HLA-DRB1*03 for isoniazid, HLA-DQA1*01:02 for rifampicin, and HLA-DQB1*02:01 for ethambutol (Sharma et al. 2005).

Other studies have shown the association between isoniazid-induced hepatitis and genetic polymorphisms of drug metabolism enzymes, including cytochrome P450 2E1 (CYP2E1), N-acetyltransferase 2 (NAT2), and glutathione-S-transferase (GST) M1 (GSTM1). Individuals with CYP2E1 gene activity are able to convert isoniazid to toxic metabolites, including hydrazine, while GSTM1 and NAT2 convert the toxic metabolites to non-toxic metabolites (Huang et al. 2002). Huang and colleagues reported polymorphisms of the NAT2 gene as a susceptibility risk factor for anti-tuberculosis drug-induced hepatitis. N-acetyltransferase 2 is responsible for acetylation of INH toxic metabolites. Polymorphisms promoting slow acetylators have a more than two-fold risk of developing INH-induced hepatitis when compared with fast acetylators in a Taiwanese population (Huang et al. 2002). The following risk factors for the accumulation of hydrazine, the toxic metabolite of isoniazid, have been reported by Fukino and colleagues and include NAT2 slow acetylator phenotype, high concentration of serum rifampicin, and GST M1 null genotype (Fukino et al. 2008).

The clinical presentation of TB-associated CADR varies with different studies elaborating different types of CADR due to TB drugs. Tan and colleagues reported on 47 patients who developed TB associated CADR. A morbilliform eruption was seen in 72.3%, 8.5% had urticaria and the rest developed exfoliative dermatitis, erythema multiforme and a lichenoid eruption (Tan et al. 2007). Lehloenya and colleagues reported on the spectrum of TB-medication associated severe CADR requiring hospital admission. The spectrum of CADR included DIHS/DRESS (38%), SJS (26%), TEN (20%), SJS/TEN overlap (8%), and lichenoid drug reactions (5%) (Lehloenya et al. 2011). In a study from Togo, among 8 cases of severe CADR due to rifampicin and isoniazid, 3 developed SJS and 5 developed TEN (Pitche et al. 2005).

The clinical presentation of anti-tuberculosis associated drug reactions is likely to be immune-mediated, since re-challenging with the same drug typically shortens the incubation period and results in more severe manifestations (Hung et al. 2006; Chung et al. 2004).

The CADR are more frequent to occur in HIV infected patients compared to un-infected person. A South Africa study showed that HIV-uninfected persons are less likely to be admitted to hospital with adverse drug reactions compared to those who are HIV-infected (Mehta et al. 2008). Polypharmacy (more than 7 drugs) commonly ARVs, anti-tuberculosis drugs and co-trimoxazole were the main implicated drugs. There is a high incidence of TB-associated severe CADR in HIV-infected patients in the South African population (Marks et al. 2009). A study from a primary care clinic in South Africa showed that severe CADR to anti-tuberculosis drugs occurred in 13% of non-HIV-infected patients and 27% of those who were HIV-infected (Marks et al. 2009). Lehloenya and colleagues reported that 92% of the severe TB-associated CADR admitted to a tertiary hospital in South Africa from January 2001 to April 2009 were HIV co-infected patients (Lehloenya et al. 2011).

3. Tuberculosis management

First line anti-tuberculosis therapy with rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB) is effective with excellent cure rates and results in less recurrence of TB after treatment (Rezakovic et al. 2014). However it is associated with CADR. Several studies demonstrate a significant increase of TB CADR due to the increased burden of TB co-infected HIV individuals requiring treatment. From a retrospective study done in Penang hospital an incidence rate of 5.7% for CADR was reported. Pyrazinamide was the commonest offending drug (2.38%), followed by streptomycin (1.45%), ethambutol (1.44%), rifampicin (1.23%) and isoniazid (0.98%) (Tan et al. 2007). CADR were more likely to occur in HIV-infected people with polypharmacy (>7 drugs) and those with autoimmune disorders (Tan et al. 2007). In a similar retrospective study done in South Africa, among patients admitted to hospital with severe CADR, rifampicin was the most commonly implicated drug, followed by isoniazid, pyrazinamide and lastly ethambutol. Respectively these drugs were responsible for 57%, 22%, 13% and 4 % of severe CADR (Lehloenya et al. 2011).

Adverse drug reactions caused by anti-tuberculosis drugs can lead to interruption of therapy, treatment failure and/or the risk of drug-resistance (Rezakovic et al. 2014). Drug resistance complicates the management of tuberculosis and represents one of the most important emerging challenges in the control of TB worldwide (Glaziou et al. 2013).

The consequences of TB-associated CADR are thus significant and include increased mortality and morbidity and the need for second line anti-tuberculosis therapy.

Second line anti-tuberculosis therapy is associated with a higher incidence of toxicities and is less effective (Rezakovic et al. 2014). Despite this, it is still considered as a treatment option for multidrug resistance TB and repeat infections. These second line anti-tuberculosis drugs are associated with their own range of severe CADR. They are also associated with multiple drug hypersensitivity reactions which pose a major long term therapeutic challenge when needed for patients with proven allergies to first line

agents. In a study from South Africa among patients admitted with CADR, streptomycin and ofloxacin were related to multiple drug hypersensitivity CADR in 4% (1/23) of the cases each (Lehloenya et al. 2011).

Switching to second line anti-tuberculosis treatment results into poorer compliance due to the anxiety of taking treatment after experiencing a severe CADR together with the longer duration of treatment required to achieve cure and the higher side effect profile in comparison to first-line agents (Rezakovic et al. 2014).

As a consequence of significant adverse health effects, suboptimal treatment and poor outcome associated with the use of the second line treatment, there is a need for re-challenge to identify the offending drug and remove it from the primary treatment regimen. Despite the associated risks of a recurrence of a severe CADR, first line treatment is necessary as it provides better infection control. Ultimately the balance between the benefit of re-exposure and risk of severe CADR and suboptimal treatment has to be weighed before re-challenge (Lehloenya & Dheda 2012; Lehloenya et al. 2011).

4. Drug causality and re-challenge

Clinical assessment is commonly used to indicate the causality of ADRs if there is a history of a recent new drug having been administered. Establishing drug causality becomes very difficult when there is more than one drug administered at the same time such as with TB drugs or when there is polypharmacy in cases of multiple co-morbidities such as HIV-TB co-infection. In these settings there is a need to test for drug causality.

There are various methods of determining drug causality. These modalities could either be performed *in vivo* or *in vitro*. *In vitro* tests includes lymphocyte transformation test/assay (LTT/LTA), CD69 up-regulation flow cytometry test and cytokine production assays. LTT/LTA is one of the most widely used laboratory tests for the identification of the culprit drug following ADR/CADR. The sensitivity and specificity of LTT/LTA varies

with the drug being evaluated, experience of the laboratory technicians performing the test, type of CADR being investigated and the subject (Pichler & Tilch 2004).

Lochmatter and colleagues reported that, LTT/LTA has a low specificity of 85% and sensitivity (60-70%) in comparison to other methods such as oral provocation test (Lochmatter et al. 2009). Suzuki *et al* evaluated the usefulness of LTT/LTA for determining the offending anti-tuberculosis drugs causing side effects in comparison to the gold standard (oral provocation test). They reported that the sensitivity of LTT/LTA was only 14.9% in identifying the offending anti-tuberculosis drugs. (INH 14.3%, RIF 13.6%, EMB 14.3%, PZA 0%) (Suzuki et al. 2008). Despite being used for more than 30 years LTT/LTA is still considered experimental due to its limitations.

In vivo modalities are the oral provocation test and skin testing (patch, prick and intradermal). These are re challenge procedures where the patient is re-exposed or re-introduced to the drug which previously caused the adverse drug reaction.

Oral provocation test also known as controlled challenge, re-exposure test, and drug re-challenge test is the controlled administration of a drug in order to diagnose drug reaction causality. It could entail either the administration of the offending drug *per se* or a structurally or pharmacologically related compound (Aberer et al. 2003). Oral provocation testing remains the “gold standard” in establishing drug causality relative to a specific ADR (Kurniadhi et al. 2006).

An oral provocation test has its own limitations and a negative provocation does not completely prove tolerance in the future or exclude the drug as the culprit if other associated co-factors and comorbidities are absent during the oral challenge or if it is done at too low a dose and not over a long enough period of time. A positive provocation test similarly might not indicate lifelong hypersensitivity for similar reasons. The severity of CADR recurrence is also not predictable (Aberer et al. 2003).

In our cases and controls re-challenge followed a specific protocol in which the patients underwent serial testing (patch, skin prick and then oral) over a 2 week period. If any

test caused a reaction re-challenge was halted. If there was no reaction the patient was maintained on the drug to which they had been re-challenged for the period of the TB treatment. This re-challenge was not done for academic reasons but to re-introduce the best medication for controlling TB especially in immunosuppressed patients.

Skin testing with the suspected offending drug may be useful in determining the cause of a CADR. This could either be in the form of a patch, prick or intradermal test. These tests also pose a risk of eliciting the initial CADR. It is postulated that the severity of the re-challenge reaction should be less severe compared to oral re-challenge as there is exposure to lower concentrations of the offending drug. This is not proven to be the case in clinical practice. Shebe and colleagues reported recurrence of drug reaction with eosinophilia and systemic symptoms syndrome secondary to rifampicin patch testing in a human immunodeficiency virus-infected man (Shebe et al. 2014).

Skin test sensitivity (70%) and specificity is still low especially in non-immediate reactions even when used in its maximum concentration compared to oral provocation testing (Rerkpattanapipat et al. 2011).

Genes that control the immune response in humans, especially those within the major histocompatibility complex, have been shown to modulate the expression of diseases induced by exogenous triggers (infections, foreign protein, environmental allergens or medication) (Miura et al. 2009; O'Connell et al. 2009; Louie et al. 2004). The human genome, mainly HLA, has been well documented to have a role in modulating infectious diseases such as TB and HIV (Louie et al. 2004). HLA class I and II (HLA-B8, HLA-B15 and HLA-DR2) have been shown to be associated with a risk of active TB while a HLA-DR6 association decreases the risk of TB (Louie et al. 2004). HLA class I has been associated with HIV control and progression. HLA-B* 58:01 reduces viral load replication and induces cytotoxic T cell lymphocyte response in HIV-infected persons and is associated with good prognosis. HLA-B*58:02 is associated with rapid progression of the disease and poor prognosis (Miura et al. 2009; O'Connell et al. 2009).

5. Summary

At present there is no practical screening test to identify those susceptible to tuberculosis drug-associated reactions and the susceptibility genes remain unknown. Various studies have shown immunogenetics to be population and drug specific (Chung & Hung 2012). We have a well characterized confirmed severe CADR population in Cape Town. The culprit drug has been confirmed through patch and/or prick testing and/or oral drug re-challenge. They are an ideal population cohort to investigate the immunogenetics of anti-tuberculosis drug-associated CADR.

Challenges to this study will be to tease out the HLA contributions of HIV and TB to our findings. The use of a group of drug tolerant patients who also have HIV and TB we hope will resolve their contribution.

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Chapter 2: Publication-ready manuscript

GENOMICS STUDY OF ANTI-TUBERCULOSIS DRUG-INDUCED HYPERSENSITIVITY REACTIONS

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Abbreviations: CADR, cutaneous adverse drug reactions; HLA, human leukocyte antigen; TB, tuberculosis; HIV, human immunodeficiency virus; ADR, adverse drug reaction; DRESS, drug reactions include drug reaction with eosinophilia and systemic symptom; DIHS, drug induced hypersensitivity syndrome; SJS, Stevens Johnson syndrome; TEN, toxic epidermal necrolysis; RIF, rifampicin ; INH, isoniazid

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ABSTRACT

All first-line anti-tuberculosis drugs can be associated with all phenotypes of cutaneous adverse drug reactions (CADR). Second-line drugs are associated with much poorer outcomes. Thus, identifying the offending drug in poly-pharmacy is difficult. Re-challenge with the drug is the gold standard in identifying the offender, however poses unacceptably high risk of CADR recurrence. Population and drug-specific genomics help identify those susceptible to adverse reactions to a drug facilitating avoidance of the drug. We aimed to investigate the genomic susceptibility in patients with confirmed rifampicin and or isoniazid-associated hypersensitivity reactions using both genome-wide association studies and candidate gene approaches. There were no significant candidate genes between our cases and study controls. However, when compared to the general population of black South Africans, HLA-B*58:02 (OR=3.6; 95% CI: 1.4-8.99) and HLA-DRB1*09:01 (OR=15.3; 95% CI: 2.1-113.1) were significantly more prevalent among our cases. HLA-B*58:02 and HLA-DRB1*09:01 may be associated with rifampicin and isoniazid-associated CADR. Alternately HLA-B*58:02 may be associated with HIV status rather than CADR. A sufficiently powered study is needed to confirm this association.

INTRODUCTION

Tuberculosis (TB) is a common worldwide infectious disease. In 2013, 9 million people globally developed TB. South Africa had the 6th highest estimated incidence rate of TB (410000–520000 per 100 000 population). TB is a leading cause of mortality in the developing world, particularly in populations with a high HIV burden. In 2013 approximately 13% of the 9 million cases worldwide were HIV co-infected (World Health Organization 2014). The pandemic of HIV and TB co-infection has resulted in multiple new problems, including a higher incidence of cutaneous adverse drug reactions (CADR). The offending drugs often include anti-retroviral drugs, anti-tuberculosis drugs and other drugs used for management of HIV-associated opportunistic infections (Mouton et al. 2014; Marks et al. 2009; Mehta et al. 2008; Pozniak et al. 1992).

TB-associated CADRs range from mild, self-resolving to severe and life threatening reactions. Some are severe enough to warrant interruption of therapy, such as drug reaction with eosinophilia and systemic symptoms also called drug induced hypersensitivity syndrome (DRESS/DIHS); Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (Lehloenya & Dheda 2012).

Recent studies have shown that drugs, presented by specific human leukocyte antigen (HLA) alleles, are recognized by specific T cell receptors leading to activation of cytotoxic T lymphocytes and cytotoxic signal expression (Chung et al. 2008). These HLA genetic associations with immune-mediated cutaneous drug reactions are complex, drug specific and ethnicity specific. HLA-B*15:02 is found in 100% of Han Chinese who develop carbamazepine (CBZ) -induced SJS/TEN (Hung et al. 2006; Chung et al. 2004). It is also found in 75% of Malay patients with CBZ-induced SJS/TEN (Chang et al. 2011). HLA-B*58:01 is found in 100% of Han Chinese with allopurinol cutaneous adverse drug reaction including SJS/TEN and DRESS (Hung et al. 2005). HLA-B*5701 is found in 78% of the Western Australian cohort with abacavir hypersensitivity (Mallal et al. 2002). HLA-B*13:01 is a predictor of dapsone hypersensitivity in Chinese persons with leprosy (Zhang et al. 2013).

Sharma and colleagues have demonstrated the association of HLA class II haplotypes associated with anti-tuberculosis drug induced liver injury. These include HLA-DRB1*03 for isoniazid, HLA-DQA1*01:02 for rifampicin, and HLA-DQB1*02:01 for ethambutol (Sharma et al. 2005). Other studies show the association between isoniazid-induced hepatitis and genetic polymorphisms of drug metabolism enzymes, including cytochrome P450 2E1, N-acetyltransferase 2 (NAT2), and glutathione-S-transferase M1 (GSTM1) (Huang et al. 2002). Fukino and colleagues included NAT2 slow acetylator phenotype, high concentration of serum rifampicin, and GST M1 null genotype as risk factors for anti-tuberculosis-induced hepatitis (Fukino et al. 2008).

First line anti-tuberculosis therapies, while effective; are associated with CADR_s (Rezakovic et al. 2014). Lehloenya and colleagues reported that the spectrum of TB-associated severe CADR_s which required admission in South Africa included DIHS/DRESS (38%), SJS (26%), TEN (20%), SJS/TEN overlap (8%), and lichenoid drug reactions (5%) (Lehloenya et al. 2011). Among these patients, rifampicin was the most commonly implicated drug, followed by isoniazid, pyrazinamide and lastly ethambutol. Respectively these drugs were responsible for 57%, 22%, 13% and 4 % of CADR_s (Lehloenya et al. 2011).

A study done in a primary care clinic in South Africa reported that CADR_s to anti-tuberculosis drugs occurred in 13% of non-HIV-infected patients and 27% of those who were HIV-infected (Marks et al. 2009). Lehloenya and colleagues also reported a high association, 92% of severe TB-associated CADR_s admitted between January 2001 and April 2009 were HIV co-infected patients (Lehloenya et al. 2011).

Adverse drug reactions caused by anti-tuberculosis therapy can lead to interruption of therapy, treatment failure and/or the risk of drug-resistance (Rezakovic et al. 2014). The consequences of TB-associated CADR_s are thus significant and include increased mortality and morbidity and hence the need for second line anti-tuberculosis therapy.

Second line anti-tuberculosis therapy is less effective, has a higher incidence of toxicities (Rezakovic et al. 2014) and their own range of severe CADR_s. As a consequence of significant adverse health effects, suboptimal treatment and poor

outcome associated with the use of the second line therapy, there is a need for re-challenge to identify the offending first line drug and remove it from the primary treatment regimen. Despite the associated risks of a recurrence of a severe CADR, first line treatment is necessary as it provides better infection control. Ultimately the balance between the benefit of re-exposure and risk of severe CADR and suboptimal treatment has to be weighed before re-challenge (Lehloenya & Dheda 2012; Lehloenya et al. 2011).

At present there is no practical screening test to identify those susceptible to tuberculosis drug-associated reactions and the susceptibility genes remain unknown. Various studies have shown immunogenetics to be population and drug specific (Chung & Hung 2012). We have a well characterized, confirmed severe CADR population in Cape Town. The culprit drug has been confirmed through patch and/or prick testing and/or oral drug re-challenge. They provided an ideal population cohort to investigate the immunogenetics of anti-tuberculosis drug-associated CADRs.

RESULTS

Demographic characteristics (Table 1)

We recruited a total of 44 participants for this study. The 14 cases all had re-challenge reactions to either RIF and/or INH. The 26 controls tolerated these drugs and comprised 12 patients with anti-tuberculosis drug-associated CADRs tolerant to RIF and INH on re-challenge (Group1a) and 18 cases with no CADRs but on TB medication (Group 1b).

The median age of our study population was 35 years with cases 32 years and controls 36years (Group1a, 35 years and Group1b 36 years). There were more females amongst the cases (57%) compared to the controls (43% overall; Group1a, 50%; Group1b 39%). The majority of the patients were black, 31/44 (70%) and the remaining 13/44 (30%) were of mixed race origin. This ratio was similar for cases and both control groups.

Most of the study cohort was HIV positive accounting for 82% of patients. Eleven out of 14 (79%) cases were HIV-infected as were 25/30 (83%) of the controls. All 12 (100%) of

control Group1a were infected compared to only 13/18 (72%) of control Group1b. The median CD4 count of those cases who were HIV-infected was 149cells/mm³ (range: 74-329) and it was 171cells/mm³ (range: 76-323) among controls although Group1b, 300 cells/mm³ (range 124-333) had a higher median CD4 count compared to Group 1a, 108 cells/mm³ (range 39-141). Less than 50% of those who experienced CADR were on ARVs (42% cases and 43% Group 1a controls). Seventy-two percent of those on TB medication who had not experienced CADR (Group 1b) were on ARVs.

Clinical characteristics:

None of the cases or controls had a history of ADRs to any previous medication. No cases or controls that experienced CADR to anti-tuberculosis drugs had been previously treated for TB. In contrast seven of the 18 controls who had not experienced CADR had been previously treated for different forms of TB. All the cases were admitted to hospital for management of their drug reactions compared to 11/30 (37%) of the controls. The reasons for the admission of the controls ranged from other diseases to CADR due to either TB and/or other medications. Thirteen of the fourteen cases (93%) had DRESS on initial presentation and a single case of SJS. Amongst the controls 7/12 (58%) had DRESS, the rest had SJS (1), SJS/TEN overlap (1), TEN (1), and pseudoporphyria (1), while one CADR was unknown.

All cases and 12/30 (40%) controls (Group1a) were exposed to cover drugs during the process of re-challenge. Among those re-challenged, some had reaction to at least one of the cover drugs. The implicated drugs were moxifloxacin and ofloxacin among the cases and ethionamide, moxifloxacin and amikacin among the controls.

Offending first-line drugs:

Of the 14 cases that developed re-challenge reactions, 7 were due to RIF, 5 reacted to INH and 2 reacted to both RIF and INH independently. Only one control developed a re-challenge reaction, attributed to PZA on oral re-challenge. Three of the RIF-associated re-challenge reactions were on patch testing and 5 were on oral re-challenge. All 4 cases who developed INH-associated re-challenge reactions and the 2 who developed re-challenge reactions to both INH and RIF occurred on oral re-challenge. None of the

re-challenge reactions occurred following prick testing (Table S1). None of the re-challenge reactions was life threatening. The skin changes resembled DRESS when present. Itch (10/14), erythema (13/14) and rash (13/14) were the most frequent re-challenge reactions amongst the 14 cases. Fever (8/14), hepatitis (6/14) oedema (5/14), sore eyes (2/14) and eosinophilia (2/14) occurred less frequently.

HLA genotyping:

HLA-B genotyping was successfully done in most of our participants (Table 2& 3). Due to technical problems, breaks in the cold chain during sample transportation and collection method used, most of the controls had poor quality and low DNA yields and thus limited PCR product for analysis.

Cases compared to RIF and INH tolerant controls.

There were no significant associations observed between HLA genotype and CADR when the cases were compared to the RIF and INH tolerant control population (Table3). Some genotypes did have an odds ratio that was greater than 1 when cases were compared to tolerant controls; however, this positive association was not statistically significant (Table 3). There were no HLA genotypes that showed a significant negative association i.e. a protective effect (Table 3).

Cases compared to the general South African black population

HLA-B*58:02 (OR=3.6; 95% CI: 1.4-8.99) and HLA-DRB1*09:01 (OR=15.3; 95% CI: 2.1-113.1) showed significant positive associations with CADR when the cases were compared to the general South African black population (Table 3 and 4). A positive association (OR>1) that did not reach statistical significance emerged with other HLA genotypes when comparing the cases and the general South African black population (Table5 and 6). There were no HLA genotypes that showed a significant negative association i.e. a protective effect (Table 3).

HLA-B genotype and the effect of HIV status

Out of the 44 participants there were 36 who were HIV infected, 7 were HIV negative and the status of one was unknown. HLA-B *58.02 was found in 11 of the HIV infected cohort, it was found in 1 of the HIV negative cohort and for one HIV positive patient the HLA typing had failed and was not available. HLA-B *58.02 was not found to be statistically associated with HIV positive status ($p=0.42$). As there was no documentation of HIV status amongst the 200 general population historic controls the HIV association with HLA-B *58.02 could not be assessed. All study cases and controls had TB so this association with HLA-B *58.02 could not be computed.

DISCUSSION

To our knowledge, this is the first study to show an association between HLA-B*58:02 and HLA-DRB1*09:01 alleles and severe cutaneous adverse drugs reactions secondary to rifampicin and isoniazid in an African population. We found HLA-B*58:02 and HLA-DRB1*09:01 to be significantly more prevalent in patients who developed rifampicin and isoniazid-associated severe CADR as compared to a historic general South African black population. We found no significant associations between HLA genotype and rifampicin/isoniazid-associated severe CADR when we compared the cases to our study controls that had tolerated rifampicin and isoniazid. Considering the small sample size, the statistical significance for both alleles was associated with relatively tight confidence intervals.

It has been convincingly shown that HLA genotype can serve as a predictor of CADR. The best-known example is the 100% association of HLA-B*15:02 in carbamazepine-induced SJS/TEN in the Han Chinese populations. The same HLA-B*15:02 has been shown to have weaker associations in non-Asian populations (Ferrell & McLeod 2008). This association has led to a recommendation by the Food and Drug Administration (FDA) in the United States recommending that all patient of Asian descent should be screened for HLA-B*15:02 before initiating carbamazepine (Ferrell & McLeod 2008). This allele was not present in our patients or controls.

Other significant population and drug-specific HLA associations include HLA-B*58:01 in allopurinol-induced SJS/TEN in Han Chinese and Thai population (Tassaneeyakul et al. 2009; Hung et al. 2005); dapsone-induced DRESS/DIHS and HLA-A*31:01 in Chinese population (Zhang et al. 2013); abacavir-induced hypersensitivity syndrome and HLA-B*57:01 in western Australia (Mallal et al. 2002) and HLA-B*35:05 and nevirapine-associated CADR in Thailand population (Chantarangsu et al. 2009). None of these HLA-B alleles were detected or strongly associated with CADR, if present, in our study. Specific HLA alleles have also been identified for other forms of ADR not involving the skin. None of these HLA alleles were detected with CADR in our study. These studies highlight that HLA-ADR associations are population, drug and ADR phenotype specific.

Before the current study, to our knowledge, there was no data on HLA genotypes that are associated with CADR and anti-TB drugs in Africans. We identify 2 candidate HLA alleles that need confirmation of their association in African patients who develop rifampicin or isoniazid-associated CADR in larger studies. The value of identifying candidate alleles could lead to CADR preventative screening prior to initiating anti-tuberculosis therapy in black South Africans.

HLA-B*40:01 in Taiwan Han Chinese and HLA-B*07:02 in Caucasians have been found to be protective against CMZ-induced SJS/TEN (Hung et al. 2006; Alfirevic et al. 2006). Unfortunately we found no significantly protective HLA genotype for rifampicin/isoniazid-associated CADR in our study.

We found that HLA-B*58:02 cases were more associated with clinical features suggestive of DRESS/DIHS. DRESS/DIHS was the prevalent phenotype of CADR, accounting for 93% of cases and only 53% of controls who experienced CADR (Group 1a). This association is worth exploring in more detail to determine CADR subtypes and their relationship to HLA genotypes in the black South African population. Interestingly most of the re-challenge reactions were suggestive of DRESS/DIHS. No firm conclusion could be drawn as the reactions were not allowed to develop and the patient treated as soon as an adverse effect was noted during the re-challenge.

Three of the cases developed re-challenge reactions to rifampicin patch test and 10 were either due to oral rifampicin and/or isoniazid. Seven of our cases developed re-challenge reactions to rifampicin, 5 reacted to isoniazid and two to both drugs. HLA-B*58:02 was associated with both rifampicin and isoniazid-related cases. However, HLA-DRB1*09:01 was found in isoniazid related cases (one case due to INH and one case due to both rifampicin and isoniazid). We cannot draw a definitive conclusion that HLA-B*58:02 or HLA-DRB1*09:01 are specific to either isoniazid and/or rifampicin-related CADR. Larger studies are needed to determine if HLA genotypes are TB-drug specific.

HLA-B*58:02 is associated with rapid progression of HIV in a person who expresses the allele (Ngumbela et al. 2008). The HLA-B*58:02 noted in our cases and controls tolerant of the drugs might not be associated with CADR but could be a reflection of the HIV status and control in HIV-TB co-infected persons. Although our numbers were small there was no association between HIV infection and the presence of the HLA-B*58:02 in our study co-hort. HLA-B* 58:01 has been shown to be protective in HIV infected persons by reducing viral load replication and inducing cytotoxic T cell lymphocyte responses (Miura et al. 2009; O'Connell et al. 2009). Although present amongst the study and general population controls the numbers were too small to allow for meaningful analysis of a contribution of this effect on or results. A bigger study is needed to explore the association between HLA-B*58:02 and HLA-DRB1*09:01 in HIV-TB co-infected persons with severe CADR and relate it to HIV disease progression. This would establish more firmly whether HIV is a confounder in the HLA-B*58:02 allele CADR association.

The possible explanations for the large number of HIV infected individuals in our study population includes the high prevalence of HIV in the general South African population, higher rates of TB infection among HIV infected individuals and low social economic status (Department of Health Republic of South Africa 2014). We also postulate theoretically the presence of HLA-B* 58:02 vs HLA-B*58:01 may play a role in the progression of HIV predisposing them to TB.

Study limitations are as follows:

- The lack of a significant association between HLA genotype and CADR when cases were compared to our study controls could be explained by the small sample size. Alternatively other factors such as TB or viruses such as HIV or HHV6 present in both groups may be acting as confounders.
- Our population was not homogenous comprising patients of black and mixed descend.
- Due to the small study population and the rarity of the disease, we used only isoniazid and rifampicin associated CADR for HLA genotyping as they are the most important first line anti-tuberculosis drugs even though they are not the same calls of drug and do not share the same metabolism. A comparison of all first line anti-tuberculosis drugs for specific HLA associations in a larger study is needed.
- Our preliminary hypothesis was that HLA may present the drug to T cells to induce hypersensitivity reactions, and therefore, we checked the HLA association. The sample size of cases was too small, and the p value became non-significant after correction for multiple testing. An increased sample size of cases as well as the drug-tolerant controls is necessary to investigate whether the corrected p value is still significant.

In summary, HLA-B*58:02 and HLA-DRB1*09:01 may be associated with rifampicin and isoniazid-associated CADR .Alternately HLA-B*58:02 may be associated with HIV status rather than CADR. A sufficiently powered study is needed to confirm this association.

MATERIALS AND METHODS

Study participants

A case-control study was conducted in, Cape Town, South Africa. The cases were patients with previous tuberculosis-associated severe CADR who were re-challenged with first-line anti-tuberculosis drugs and subsequently developed re-challenge

reactions to either isoniazid and/or rifampicin. Two groups of controls were selected. Control group 1 (30 patients), were patients that tolerated first line anti-tuberculosis drugs, either isoniazid and rifampicin tolerant during re-challenge following severe CADR (12 patients Group 1a) or isoniazid and rifampicin tolerant throughout a course of treatment (18 patients Group 1b). Control group 2 comprised 200 black South Africans from the general population whose HLA allele frequencies had previously been published (Paximadis et al. 2012). This study was conducted for one year (December 2013-December 2014) but the cases and control group 1a were part of a larger study evaluating the best method for TB medication re-challenge/reintroduction after CADR. The Seventy four patients made up the cohort who were re-challenged prospectively over 8 years. The cases and controls which were recruited from this cohort to participate in our study according to protocol were 44. The remaining 30 who had all been re-challenged were not contactable or available for various reasons (9 dead, 5 refused to participate, 16 not reachable). Of the 44 recruited, 14 were rifampicin re-challenge positive cases, 7 were isoniazid re-challenge positive, 3 were pyrazinamide re-challenge positive, 2 were ethambutol re-challenge positive and 18 were full first line TB medication re-challenge negative controls. Some of the cases and controls recruited did not have blood samples taken for genotyping (5) while the rest (14) had failed genotyping. Finally genotyping data was available for 26 cases and control group 1a for analysis (Table S2 Figure 1). The control patients who experienced no ADRs to anti-tuberculosis first-line were consecutive cases of patients on TB treatment admitted to hospital for unrelated reasons (Group 1b). Cases and controls were recruited in a ratio of 1:2

Inclusion criteria of the study participants

Cases:

1. Participants willing and able to sign an informed consent
2. Adults who had serious CADR and had positive oral provocation test, patch test or skin prick test to anti-tuberculosis drugs with either rifampicin and/or isoniazid identified as the offending drugs

Controls:

1. Adult patients who had serious CADR but tolerated rifampicin and isoniazid after re-challenge
2. Adult patients who tolerated anti-tuberculosis drugs without developing any CADR

Exclusion criteria

1. Patients who are unwilling to sign an informed consent
2. Patients who had serious CADR to multiple anti-TB drugs

Management of participants before and during re-challenge

Cases

The anti-tuberculosis drugs were stopped when severe CADR to anti-TB drugs was suspected. Before re-challenge all patients were investigated to confirm active TB. Three second line anti-TB drugs (streptomycin, terizidone, ofloxacin, moxifloxacin, ethionamide, amikacin, kanamycin or para-amino-salicylic acid) to which the patient had not been previously exposed, were introduced to control active TB while waiting for the severe CADR and blood parameters to normalize. Depending on the sensitivities of the patient's strain of TB, isoniazid followed by rifampicin, pyrazinamide and ethambutol were re-introduced consecutively and additively in accordance with a strict protocol. Once the patients had fully recovered the patients were re-challenged in hospital. A patch test was performed first, if negative, it was followed by a prick test and if this was also negative oral re-challenge was initiated. This re-challenge protocol was part of larger study which is still underway evaluating the best method to re-introduce medication after CADRs. For some cases only oral re-challenge was undertaken (Table S3) because of time constraints dictated by multiple co-morbidities and significantly ill patients. A positive re-challenge reaction following any of these re-challenge modalities lead to immediate withdrawal of the drug from the treatment regimen. Cases with a re-

challenge reaction to either isoniazid and/or rifampicin following any of the three re-challenges assessments constituted the cases (Figure 1). A re-challenge reaction was defined previously and fully described (Lehloenya et al. 2011). The patient demographics and CADR and re-challenge reactions are summarized in (Table S3 and S4).

Controls

Controls were consecutive patients who had tolerated rifampicin and isoniazid during the re-challenge process (12 patients, Group 1a) and consecutive patients who had been on TB treatment for at least 12 weeks without developing any ADR reaction but were admitted for unrelated medical problems (Group1b) (Figure 1). The causes of CADR in the control group 1a patients who had negative re-challenge to INH and Rif after CADR were due to drugs listed (Table S5).

HLA genotyping

Ten millilitres of a participant's whole blood were collected in ethylenediaminetetraacetic acid (EDTA) tubes and stored at -80 degree Celsius within an hour of sample collection. Genomic DNA was isolated from the whole blood using the Gentra Puregene DNA purification kit (QIAGEN, Hilden, Germany). Each sample was typed for HLA-A, HLA-B, HLA-C, HLA-DRB1 alleles at high resolution by polymerase chain reaction with a Group Specific Sequencing Primers (GSSPs) typing method using the SeCore® HLA Sequence-Based Typing kits (Invitrogen by Life Technologies, Wisconsin, USA) according to the manufacturer's protocols. Briefly, purified DNA was prepared to a working solution at 15-30 ng/μL in DNA hydration solution. For each amplification reaction, 50ng prepared DNA was combined 9.9 μl of Amp Mix with 0.1 μl of FastStart™ Taq DNA Polymerase. We confirmed the presence of PCR products (~1100 and ~1300bp at A locus, or ~950 and ~1400bp at B locus, respectively) by 2% agarose gel electrophoresis. The resulting product was treated with 2μl ExoSAP-IT™ to degrade the unincorporated primers and hydrolyze the free nucleotides. For each positive PCR reaction, we set up forward and reverse sequencing reactions for exon 2, 3 and 4 at A, B, C or DRB loci. After cycle sequencing, an ethanol precipitation with PPT buffer was performed to remove excess terminators. The HLA type results were determined by

multicolor, fluorescence-based, BigDye® Terminator sequencing (Hung et al. 2010; Hung et al. 2005).

Ethics

The study was conducted in accordance with ethical principles contained in the Helsinki declaration. The study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 582/2012) (Appendix 1). Informed written consent (Appendix 2) was translated into isiXhosa and Afrikaans, the other two major languages in the catchment area. Informed written consents were obtained from all study participants. Participant's details were collected from their hospital medical records and recorded in a clinical record form (Appendix 3). Anonymity was assured by allocating consecutive numbers to patients and recruited control patients.

Statistical analysis

This is a pilot hypothesis generating study. As such the issue of multiplicity was deliberately not taken into account. Prior to this study, there was no available data to guide any sample power calculations.

The data was analyzed using GraphPad Prism software. Comparisons of the frequencies of the HLA allele or carriers between the subgroups were performed using Fisher's exact tests. As the pre-defined hypothesis based test was applied for the HLA association analysis, no correction of the p value for multiple testing was used in this study. Odds ratios (ORs) were calculated using Haldane's modification, which added 0.5 to all cells to accommodate possible zero counts. The statistical significance was defined as a two-tailed $p < 0.05$

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Table 1: Demographic and clinical characteristics of 14 cases that developed a re-challenge reaction to rifampicin and/or isoniazid and 30 controls who tolerated rifampicin and/or isoniazid

	Cases; n=14	Controls; n=30		
		Group 1a; n=12	Group 1b; n=18	Total; n=30
Age(years), median [IQR]	32 (28-37)	36 (33-39)	36(32-48)	36 (33-41)
Gender: n (%)				
Female	8 (57%)	6 (50%)	7 (39%)	13 (43%)
Male	6 (43%)	6(50%)	11 (61%)	17 (57%)
Race: n (%)				
Black	10 (71%)	8 (67%)	13 (72%)	21 (70%)
Mixed race	4 (29%)	4 (33%)	5 (28%)	9 (30%)
HIV status: n (%)				
Positive	11 (79%)	12 (100%)	13 (72%)	25 (83%)
Negative	3 (21%)	0 (0%)	4 (22%)	4 (13%)
Unknown	0 (0%)	0 (0%)	1 (6%)	1 (4%)
CD4 count cells/mm ³ , median [IQR]	149 (74-329)	108 (39-141)	300 (124-333)	141 (76-323)
On ARVs	6 (43%)	5 (42%)	13 (72%)	18 (60%)
PREVIOUS TB: n	0 (0%)	0 (0%)	7 (39%)	7 (23%)
CADR to first-line TB treatment	14 (100%)	12 (100%)	Na	12 (40%)
Types of initial CADR				
SJS	1 (7%)	1 (8%)	Na	1 (3%)
TEN	0 (0%)	1 (8%)	Na	1 (3%)
SJS/TEN overlap	0 (0%)	1 (8%)	Na	1 (3%)
DRESS/DIHS	13 (93%)	7	Na	1 (3%)
Pseudo porphyria	0 (0%)	1 (8%)	Na	1 (3%)
Unknown	0 (0%)	1 (7%)	Na	1(3%)
CADR to second-line TB treatment	3 (21%)	1 (8%)	Na	Na
Moxifloxacin	2	0	Na	Na
Ofloxacin	1	0	Na	Na
moxifloxacin/ethionamide/amikacin	0	1	Na	Na

CADR = cutaneous adverse drug reaction; IQR = interquartile range; HIV = human immunodeficiency virus; TB =tuberculosis; ARVs= antiretroviral drugs; DRESS drug rash with eosinophilia and systemic symptoms; SJS = Stevens Johnson syndrome; TEN = toxic epidermal necrolysis; SJS/TEN = Stevens Johnson syndrome and toxic epidermal necrolysis overlap; na=not applicable

Table 2: The association between HLA- genotype and the HIV status

SORT NO	HIV	CASE/ CONTROL	CD4 count cells/mm ³	HLA-A Allele 1	HLA- A Allele 2	HLA- B Allele 1	HLA-B Allele 2	HLA- C Allele 1	HLA-C Allele 2	HLA- DRB Allele 1	HLA- DRB Allele 2
KS13	1	Case	58	*29:02	*66:01	*13:02	*58:02	*06:02	*06:02	*03:01	*08:04
KS20	1	Case	329	*68:01	*68:02	*15:10	*82:02	*03:02	*03:04	*03:02	*10:01
KS22	0	Case		*30:09	*68:02	*44:03	*58:02	*16:01	*18:01/02	*13:02	*15:03
KS24	1	Case	320	*03:01	*30:02	FR	FR	*04:01	*06:02	*01:02	*03:02
KS25	1	Case	80	*03:01	*30:01	*15:10	*58:02	*03:04	*06:02	*10:01	*15:03
KS26	1	Case	223	*03:01	*23:01	*07:02	*58:02	*02:10	*06:02	*03:02	*13:01
KS29	1	Case	457	*02:05	*30:02	*45:01	*58:01	*07:01	*16:01	*01:02	*13:01
KS32	0	Case		FR	FR	*18:02	*44:02	FR	FR	*04:01	*12:02
KS33	1	Case	145	*23:01	*30:04	*15:03	*58:02	FR	FR	*03:01	*08:04
KS35	1	Case	63	*30:01	*33:01	*42:01	*42:01	*17:01	*17:01	*03:02	*03:02
KS37	1	Case	401	*24:02	*30:01	*13:02	*18:01	*05:01	*06:02	*03:01	*07:01
KS38	0	Case		*26:01	*32:01	*07:02	*27:05	*02:02	*02:10	*04:01	*09:01
KS44	1	Case	74	FR	FR	*42:01	*58:02	FR	FR	*09:01	*13:01
KS72	1	Case	149	FR	FR	*15:10	*58:01	FR	FR	*04:03	*14:01/54
KS 7	1	control 1a	24	FR	FR	*18:01	*42:02	FR	FR	FR	FR
KS 8	1	contro1a	91	FR	FR	*42:01	*57:03	FR	FR	FR	FR
KS14	1	control 1a	22	FR	FR	*07:02	*58:02	FR	FR	FR	FR
KS16	1	control 1a	168	FR	FR	*57:03	*58:01	FR	FR	FR	FR
KS19	1	control 1a	39	FR	FR	*35:01	*53:01	FR	FR	FR	FR
KS21	1	control 1a	Unk	FR	FR	*08:01	*58:02	FR	FR	FR	FR
KS23	1	control 1a	395	FR	FR	*07:05	*13:02	FR	FR	FR	FR
KS45	1	control 1a	Unk	FR	FR	*15:01	*15:03	FR	FR	FR	FR
KS54	1	control 1a	44	FR	FR	*15:03	*27:05	FR	FR	FR	FR
KS57	1	control 1a	131	FR	FR	*15:01	*47:01	FR	FR	FR	FR
KS58	1	control 1a	125	FR	FR	*18:01	*44:03	FR	FR	FR	FR
KS66	1	control 1a	141	FR	FR	*57:02	*58:02	FR	FR	FR	FR
KS 1	0	control 1b		FR	FR	*07:02	*15:03	FR	FR	FR	FR
KS 2	1	control 1b	5	FR	FR	*39:10	*42:01	FR	FR	FR	FR
KS 9	1	control 1b	314	FR	FR	*15:10	*42:01	FR	FR	FR	FR
KS10	1	control 1b	323	FR	FR	*58:02	*58:02	FR	FR	FR	FR
KS12	1	control 1b	101	FR	FR	*08:01	*13:02	FR	FR	FR	FR
KS17	1	control 1b	236	FR	FR	*42:01	*44:03	FR	FR	FR	FR
KS18	1	control 1b	333	FR	FR	*42:02	*58:02	FR	FR	FR	FR
KS30	1	control 1b	124	FR	FR	*15:10	*42:01	FR	FR	FR	FR
KS31	0	control 1b		FR	FR	*15:10	*42:01	FR	FR	FR	FR
KS34	1	control 1b	76	FR	FR	*44:03	*58:02	FR	FR	FR	FR
KS36	unk	control 1b		FR	FR	*13:02	*58:02	FR	FR	FR	FR
KS39	1	control 1b	274	FR	FR	*42:01	*58:01	FR	FR	FR	FR
KS42	1	control 1b	720	FR	FR	*13:02	*15:03	FR	FR	FR	FR
KS43	0	control 1b		FR	FR	*15:10	*44:03	FR	FR	FR	FR
KS53	1	control 1b	548	FR	FR	*42:01	*58:02	FR	FR	FR	FR
KS55	0	control 1b		FR	FR	*45:01	*58:02	FR	FR	FR	FR
KS56	1	control 1b	300	FR	FR	*15:03	*27:05	FR	FR	FR	FR
KS74	1	control 1b	724	FR	FR	*07:02	*08:01	FR	FR	FR	FR

1=HIV infected; 0= HIV uninfected; unk=unknown; FR =failed typing

Table 3: Comparison of the HLA-B allele frequencies among the cases that developed a re-challenge reaction to rifampicin and/or isoniazid and the two control groups

HLA	Cases n=13	Tuberculosis controls n=30			Population controls = 200		
	n	n	OR (95% CI)	p value	n	OR (95% CI)	p value
B*07:02	2	3	1.6(0.2 - 10.1)	0.636	18	1.7(0.4 - 7.87)	0.349
B*07:05	0	1	0.1(0 - 860.4)	1	na	na	na
B*08:01	0	3	0(0 - 250.2)	0.55	na	na	na
B*13:02	2	4	1.2(0.2 - 6.8)	1	8	4.1(0.8 - 20.29)	0.119
B*15:01	0	2	0.1(0 - 392)	1	na	na	na
B*15:03	1	5	0.4(0 - 4)	0.663	34	0.4(0.1 - 3.32)	0.711
B*15:10	3	4	1.8(0.4 - 8.8)	0.427	32	1.5(0.4 - 5.34)	0.462
B*18:01	1	2	1.2(0.1 - 13.4)	1	12	1.3(0.2 - 9.98)	0.564
B*18:02	1	0	50.3(0 - 401350)	0.302	0	320(0 - 2569260.31)	0.061
B*27:05	1	2	1.2(0.1 - 13.4)	1	0	320(0 - 2569260.31)	0.061
B*35:01	0	1	0.1(0 - 860.4)	1	na	na	na
B*39:10	0	1	0.1(0 - 860.4)	1	na	na	na
B*42:01	3	8	0.8(0.2 - 3.5)	1	36	1.3(0.4 - 4.67)	0.721
B*42:02	0	2	0.1(0 - 392)	1	0	320(0 - 2569260.31)	0.061
B*44:02	1	0	50.3(0 - 401350)	0.302	na	na	na
B*44:03	1	4	0.6(0.1 - 5.3)	1	23	0.6(0.1 - 5.01)	1
B*45:01	1	1	2.4(0.1 - 39.1)	0.516	26	0.6(0.1 - 4.49)	1
B*47:01	0	1	0.1(0 - 860.4)	1	na	na	na
B*53:01	0	1	0.1(0 - 860.4)	1	na	na	na
B*57:02	0	1	0.1(0 - 860.4)	1	na	na	na
B*57:03	0	2	0.1(0 - 392)	1	na	na	na
B*58:01	2	2	0.1(0 - 392)	0.581	32	0.9(0.2 - 4.18)	1
B*58:02	7	10	1.8(0.6 - 5.5)	0.376	38	3.6(1.4 - 8.99)	0.013

13 cases out of 14 cases had successful HLA-B genotyping and 1 failed.

Controls are: 30 patients that tolerated rifampicin and/or isoniazid) and 200 black South African from the general population.

na: not applicable

Table 4: Comparison of the HLA-DRB1 allele frequencies among the cases that developed a re-challenge reaction to rifampicin and/or isoniazid and black South African from the general population

HLA	Cases n=14	Population controls= 200		
	N	n	OR (95% CI)	p value
DRB1*01:02	2	19	1.6(0.3-7.07)	0.639
DRB1*03:01	3	30	1.5(0.4 -5.11)	0.466
DRB1*03:02	5	39	2.0 (0.7-5.62)	0.191
DRB1*04:01	2	10	2.9 (0.6-13.78)	0.182
DRB1*04:03	1	0	43.7(1.7-1097.78)	0.065
DRB1*07:01	1	28	0.5 (0.1-3.7)	0.710
DRB1*08:04	2	17	1.8 (0.4 -8.0)	0.357
DRB1*09:01	2	2	15.3(2.1-113.1)	0.023
DRB1*10:01	2	10	2.9(0.6 -13.8)	0.182
DRB1*12:02	1	0	43.7(1.7-1097.8)	0.065
DRB1*13:01	3	50	0.8(0.2-2.9)	1.000
DRB1*13:02	1	23	0.6(0.1-4.6)	1.000
DRB1*14:01	1	3	4.6(0.5-44.9)	0.238
DRB1*15:03	2	34	0.8(0.2-3.7)	1.000

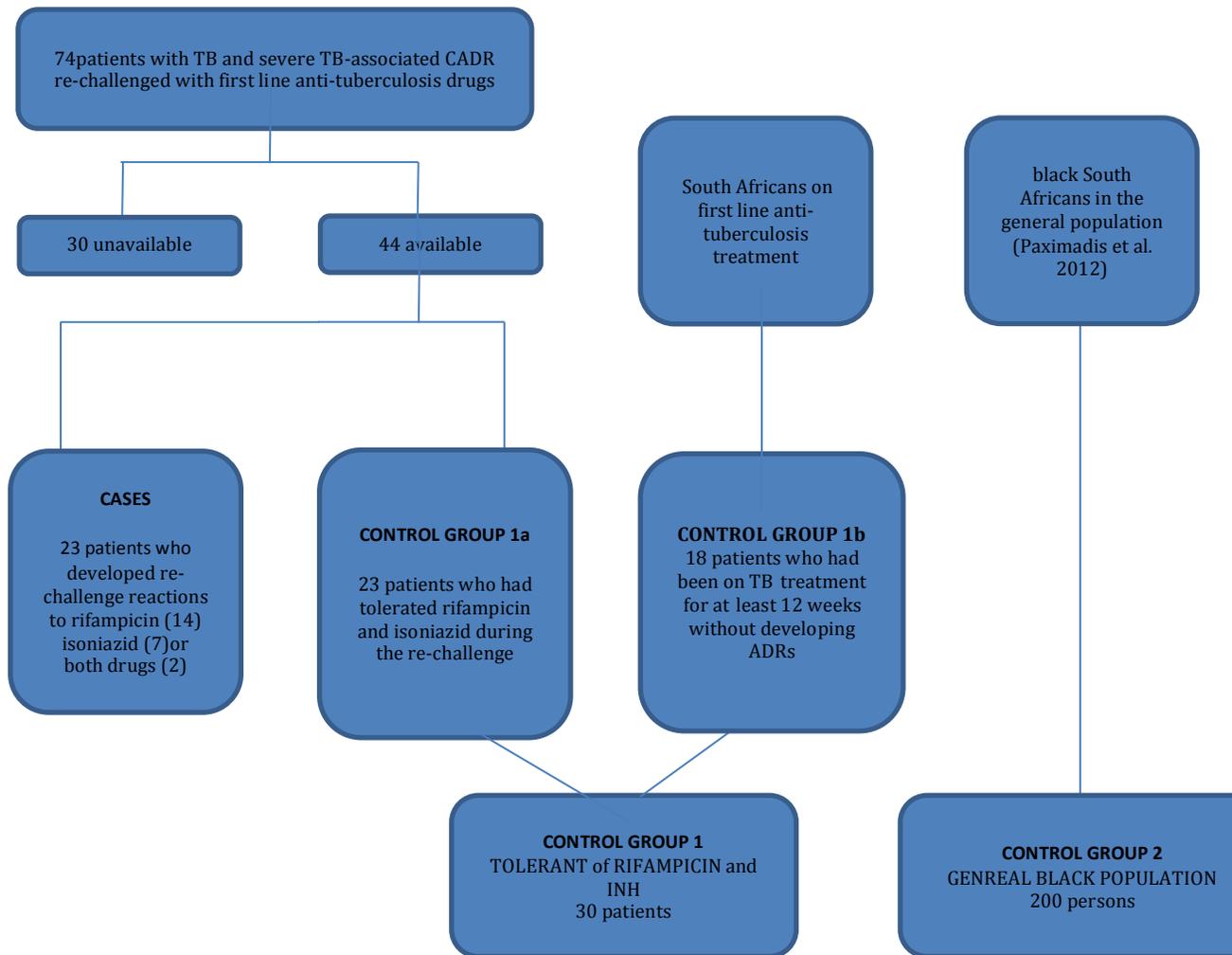
Table 5: Comparison of the HLA-C allele frequencies among the cases that developed a re-challenge reaction to rifampicin and/or isoniazid and black South African from the general population

HLA	Cases n=10	Population controls= 200		
	n	n	OR (95% CI)	p value
C*02:02	1	36	0.5(0.1-4.0)	1.000
C*02:10	2	0	888.8(0.1-6431264.5)	0.002
C*03:02	1	6	3.5(0.4-30.2)	0.291
C*03:04	2	20	2.1(0.4-9.5)	0.282
C*04:01	1	48	0.4(0.1-3.0)	0.492
C*05:01	1	3	6.5(0.7-64.6)	0.178
C*06:02	5	60	1.9(0.7-5.4)	0.214
C*07:01	1	30	0.6(0.1-4.9)	1.000
C*16:01	2	26	1.6(0.3-7.1)	0.635
C*17:01	2	44	0.9(0.2-4.0)	1.000
C*18:02	1	13	1.5(0.2-12.4)	0.500

Table 6: Comparison of the HLA-A allele frequencies among the cases that developed a re-challenge reaction to rifampicin and/or isoniazid and black South African from the general population

HLA	Cases n=11	Population controls= 200		
	n	n	OR (95% CI)	p value
A*02:05	3	27	2.2(0.6-7.8)	0.199
A*03:01	1	26	0.7(0.1-5.3)	1.000
A*23:01	2	32	1.2(0.3-5.1)	0.694
A*24:02	1	8	2.3(0.3-19.5)	0.385
A*26:01	1	3	5.9(0.6-58.2)	0.193
A*29:02	1	25	0.7(0.1-5.5)	1.000
A*30:01	3	40	1.4(0.4-5.0)	0.481
A*30:02	2	38	0.9(0.2-4.2)	1.000
A*30:04	1	7	2.6(0.3-22.0)	0.351
A*30:09	1	0	380.9(0.0-3063625.4)	0.052
A*32:01	1	5	3.6(0.4-32.1)	0.276
A*33:01	1	1	15.8(1.1-233.1)	0.102
A*66:01	1	3	5.9(0.6-58.2)	0.193
A*68:01	1	13	1.4(0.2-11.2)	0.533
A*68:02	2	34	1.1(0.2-4.8)	1.000

Figure 1: Summary of cases and two control groups of the study.



Supplemental materials

Table S1: Association between CADR and modalities of re-challenge among the of 14 cases that developed a re-challenge reaction to rifampicin and/or isoniazid

Mode of re-challenge	Number
RIF patch testing reaction	3
INH oral re-challenge reaction	4
RIF oral re-challenge reaction	5
INH & RIF oral re-challenge reaction	2

INH= isoniazid and RIF= rifampicin

INH &RIF: Had a reaction to isoniazid after normalized, was re-challenged with rifampicin and developed another reaction

Table S2: The distribution of the cases, controls and HLA genotyping

Potential positive cases for inclusion	Total	Included in the study	Included in analysis	Excluded from analysis	Reason for exclusion
*RIFAMPICIN	14	14	8	6	5 failed genotyping 1 blood not sent
*ISONIAZID	7	7	6	1	1 failed genotyping
PYRAZINAMIDE	3	3	1	2	2 failed genotyping
ETHAMBUTOL	2	2	0	2	2 failed genotyping
SUCCESSFUL RHZE RECHALLENGE	18	18	11	7	3 blood not sent 4 failed genotyping
TOTAL	44	44	26	18	18

2 cases positive to rifampicin re-challenge were also positive on isoniazid re-challenge.
RHZE=RIF INH, PZA, ETH treatment

Table S3: Re-challenge methods and outcome reactions in the cases (bold) and control group 1a

Method used to induce re-challenge reaction and outcome				RE-challenge reaction features																
PT	SPT	Oral	Reaction	nausea	vomiting	Itch	skin pain	sore throat	sore eye	erythema	rash	swelling	rigors	abdominal pain	Diarrhoea	fever	hepatitis	eos		
No	No	No	0																	
No	No	No	0																	
ND	ND	Yes	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1		
No	No	No	0																	
ND	ND	No	0																	
ND	ND	No	0																	
Yes	ND	ND	1	0	0	1	0	0	1	1	1	1	0	0	0	1	1	0		
			0																	
Yes	ND	ND	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1		
No	No	No	0																	
ND	ND	Yes	1	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0		
ND	ND	Yes	1	0	0	1	0	0	0	1	1	1	0	0	0	1	0	0		
Yes	ND	ND	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0		
ND	ND	Yes	1	0	0	1	0	0	1	1	1	1	0	0	0	1	0	0		
No	No	Yes	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0		
No	No	Yes	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0		
ND	ND	Yes	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0		
ND	ND	Yes	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0		
ND	ND	Yes	1	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0		
ND	ND	Yes	1	0	0	0	0	0	0	1	1	1	0	1	1	1	1	0		
ND	ND	ND	0																	
ND	ND	No	0																	
ND	ND	No	0																	
ND	ND	No	0																	
ND	ND	Yes	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
0	0	Yes	1	0	0	0	0	0	0	1	1	1	1	0	0	1	1	0		
3	9	12	15	1	1	10	0	0	2	13	13	5	1	1	1	8	7	2		

PT=patch test; SPT skin prick test; oral= oral re-challenge; Yes=PT/SPT/oral re-challenge was done and there was a reaction; No= PT/SPT/oral re-challenge was done but there was no a reaction; ND = PT/SPT/oral re-challenge was not done at all; CADR=cutaneous adverse drug reaction; DRESS=Drug reaction with eosinophilia and systemic symptoms; RIF=rifampicin, INH=isoniazid, PZA=pyrazinamide; moxiflox=moxifloxacin, oflox=ofloxacin, ethion=ethionamide, ami=amikacin

Table S4: Blood parameters on admission CADR

	WCC	Eos	Max Eos	Lymphs	ALT	Max ALT	AST	Max AST	Tot bili	Conj bili	Urea
Normal range	4.0-10 × 10 ⁹ /l	0.00-0.45× 10 ⁹ /l	0.00-0.45× 10 ⁹ /l	1.00-4.00× 10 ⁹ /l	5-40U/l	5-40U/l	5-40U/l	5-40U/l	0-21µmol/l	0-6µmol/l	2.6-7.0mmol/l
KS7	4.29	0.97	0.97	0.34	12	13	48	90	3	1	8.6
KS8	6.22	0.03	0.08	0.49	38	38	37	41	3	1	3.2
KS13	5.66	0.5	0.5	0.5	19	29	29	30	2	1	6.9
KS14	3.88	0.1	0.17	0.67	26	33	53	126	6	3	7.3
KS16	unk	unk	unk	unk	unk	unk	unk	unk	unk	Unk	unk
KS19	4.9	0.11	0.11	0.4	22	24	36	36	2	3	3.3
KS20	6.52	0.05	0.05	0.65	179	179	79	79	19	11	3.7
KS21	unk	unk	unk	unk	unk	unk	unk	unk	unk	Unk	unk
KS22	4.58	0.9	0.9	0.94	27	27	41	41	5	2	1.1
KS23	3.53	0.14	0.25	ND	52	52	31	31	4	ND	3.8
KS24	11.1	0.05	0.05	0.25	132	132	187	187	4	1	8.7
KS25	8.14	0.01	0.01	ND	58	329	106	821	8	2	3.5
KS26	8.07	0.08	0.08	0.67	53	53	32	57	4	ND	5.5
KS29	5.99	0.2	0.2	2.48	54	54	80	80	4	1	2.2
KS32	4.71	0.16	0.25	1.77	19	19	30	46	5	1	5.2
KS33	7.16	0.04	0.22	ND	168	168	118	118	6	2	2.7
KS35	4.94	0.72	1.88	0.43	40	653	49	727	11	8	3.7
KS37	5.39	0.15	0.35	1.02	52	140	47	148	4	3	2
KS38	8.49	0.43	0.43	0.77	63	219	100	288	18	10	6.1
KS44	1.52	0.07	0.07	0.29	19	31	31	32	3	2	4.2
KS45	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
KS54	3.05	0.06	0.22	ND	13	33	35	83	16	15	3.8
KS57	3.74	0.65	0.65	0.84	51	91	89	169	5	3	9
KS58	10.53	0.12	0.59	5.08	9	9	7	21	27	ND	3.7
KS66	2.85	0.18	0.27	ND	25	47	57	59	ND	ND	ND
KS72	4.43	0.12	0.22	1.18	16	16	38	41	5	2	2.6

WCC=white cell count; Eos= eosinophils; Lymphs= lymphocytes; AST=aspartate transaminase; ALT=alanine transaminase; Tot bili=total bilirubin; Conj bili= conjugated bilirubin; unk=unknown; nd=not done

Table S5: The causes of CADR in the control group 1a patients who had negative re-challenge to INH and Rif after CADR

SORT #	BACTRIM	ARVS	ANTI TB 1st line	ANTI TB 2nd line	ANTIBIOTICS	OTHER MEDICATION
7	1#	0	0	0	0	0
8	1#	0	0	0	0	0
14	1#	0	0	0	0	0
16	0	1+	0	0	0	0
19	1#	1+	0	0	0	Phenytoin
21	0	1+	0	0	0	0
23	0	1+	0	0	0	vit B complex
45	0	1+	0	0	0	0
54	1#	0	0	0	0	0
57	1#	0	0	1 amikacin (moxifloxacin, ethionamide,)	0	Pyridoxine
58	0	0	0	0	0	0
66	0	0	PZA*	0	0	0

*Positive re-challenge

#Bactrim withdrawn – not re-challenged

+ARVs changed to exclude nevirapine and efavirenz

Case 58: No cause for the drug reaction found and patient had full first line TB treatment successfully re-introduced.

APPENDICES

Appendix 1a: Ethics approval letter from the Faculty Reserch Ethics Comittee



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • Facsimile [021] 406 6411
Email: Sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

29 May 2013

HREC/REF: 582/2012

Dr R Lehloeny
Dermatology
Ward G-23
NGSH

Dear Dr Lehloeny

**Project Title: GENOMICS STUDIES OF ANTI-TUBERCULOSIS DRUGS INDUCED
HYPERSENSITIVITY REACTIONS (MMed-candidate-Dr K Shebe)**

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 15 June 2014.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student:-Dr K Shebe is also involved in this project.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

Hrec/ref:582/2012

Appendix 1b: Annual Progress Report/Renewal



UNIVERSITY OF CAPE TOWN

HUMAN RESEARCH
ETHICS COMMITTEE

18 JUN 2014

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB0001938)

This serves as notification of annual approval, including any documentation described below.

<input checked="" type="checkbox"/> Approved	Annual progress report	Approved until/renewal date	15/6/2015
<input type="checkbox"/> Not approved	See attached comments		
Signature Chairperson of the HREC			Date Signed 17/6/2014

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	06/05/13		
HREC REF Number	582/2012	Current Ethics Approval was granted until	15/06/2014
Protocol title	GENOMICS STUDIES OF ANTI-TUBERCULOSIS DRUGS INDUCED HYPERSENSITIVITY REACTIONS		
Principal Investigator	DR RANNAKOE LEHLOENYA		
Department / Office Internal Mail Address	G23 DERMATOLOGY WARD, GROOTE SCHUUR HOSPITAL, OBSERVATORY, CAPE TOWN 7925		
1.1 Does this protocol receive US Federal funding?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	

2. Protocol status (tick ✓)

<input checked="" type="checkbox"/>	Research-related activities are ongoing
<input type="checkbox"/>	Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	85
Total number of records or specimens collected, reviewed or stored since last progress report	N/A
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

4. Signature

Signature of PI		Date	20/6/13
Signature of Supervisor (if PI is a student)		Date	

GENOMICS STUDY OF ANTI-TUBERCULOSIS DRUG-INDUCED HYPERSENSITIVITY REACTIONS

I, _____ agree to take part in this research on my own free will. The reasons, benefits and the risks of the research have been explained to me in the language I understand by Doctor.

I have been told that the reason for this research is to find out which of my genes contributed to the reaction I had to my treatment for tuberculosis.

Two blood samples of 5ml each will be collected from me. One will be sent to a laboratory overseas that has the ability to do the tests and the other sample will be stored here in Cape Town for future studies related only to this research. The samples sent overseas will be destroyed immediately after the experiments. My expressed permission will be obtained for these future studies. I understand that the research may not benefit me directly, but may help others in the future including my own children or family.

The records of the research will be kept safely and only the team doing the research will be able to use it. The findings of the study may be published in scientific journals and meetings, but I will not be identified. I understand that I will not get any payment for the work related to the research.

Would you like to know the finding of the research yes _ no _

If yes at the following address and telephone number: _____ .

If you have any further questions you can contact Dr Shebe or Dr Lehloenyia at 021 404 5269

Permission of this research was given on 29th May, 2013 by the Human Research Ethics Committee of the University of Cape Town. If you have any complaints about the conduct of this research please contact Professor Marc Blockman on Tel: 0214066492

I agree to take part of my own free will and I can stop at any time without having to give a reason for it.

_____	_____
Printed name of participant	Signature, Mark, or Thumb Print
_____	_____
Investigator's name (Print)	Signature
Date _____	
_____	_____
Witness's name (Print)	Signature
Date _____	

CONSENT TO OBTAIN A SPECIMEN FOR GENETIC TESTING SAMPLE TYPE: BLOOD

GENETIC TESTING REQUESTED FOR:

Susceptibility to adverse reactions to TB drugs

The intended purpose of this test is predictive

- 1. I have been informed about the purpose of this genetic test.
- 2. I have received an explanation of the limitations of this genetic test.
- 2. Dr _____ has discusses the benefits and risks of this genetic test with me.
- 4. I understand some genetic tests can involve possible medical, psychological or insurance issues of my family
- 5. I have been informed how I will receive the results
- 6. I have been informed who may have access to my blood sample, and that any leftover sample may be retained by researchers at the University of Cape Town.
- 7. I have been informed who may have access to my genetic test results, which is part of my confidential medical records.
- 8. My questions have been answered to my satisfaction.

Permission of this research was given on 29th May, 2013 by the Human Research Ethics Committee of the University of Cape Town. If you have any complaints about the conduct of this research please contact Professor Marc Blocman on Tel: 0214066492

I consent to have samples taken from genetic testing for the condition listed above and I am aware that I can withdraw this consent at any time without having to give a reason for it

Printed name of participant

Signature, Mark, or Thumb Print

Investigator's name (Print)
Date _____

Signature

Witness's name (Print)

Signature

Appendix 2b: IsiXhosa consent form

GENOMICS STUDY OF ANTI-TUBERCULOSIS DRUG-INDUCED HYPERSENSITIVITY REACTIONS

(Iincukacha ngegenes ezichaphazelekayo ekulweni komzimba namachiza esifo semiphunga)

Mna Ndiyavuma ukuthata inxaxheba kolu phando ngaphandle kokugunyaziswa. Injogo, inzuzo kunye namakhwiniba zichaziwe nguGqirha..... esebenzisa ulwimi olucacileyo.

Ndixelelwe ukuba iinjongo zoluphando kukuzama ukufumana ukuba iigenes zam ziyachaphazeleka kusini na ekulweni komzimba wam namachiza esifo semiphunga.

Kuya kuthathwa kum igazi elilinganiselwa kwisihlanu samamiliitha (tisipuni) kabini kuphela. Elinye igazi liyakuthunyelwa kumaziko okuhlola igazi akumazwe angaphesheya kolwandle, ukuze lihlolwe khona. Elinye igazi liyakugcinwa kumaziko okuhlola igazi alapha eKapa ukuze lihlolwe kwixesha elizayo lihlolwelwa kwaolu phando kuphela. Igazi elithunyelwe kumazwe angaphesheya liya kulahlwa kwangoko kwakuqitywa uhlolo. Imvume yam iyakuphinde icelwe xa kufuneka olunye uhlolo kwixesha elizayo. Ndiyazi ukuba kungenzeka mna ngokwam ndingazuzi nto kolu phando koko kuzuze abanye abantu ekuquka kubo abantwana nosapho lwam.

Amacwecwe oluphando ayakugcinwa eluvalelweni ukuze afumaneke kuphela kubantu abenza olu phando kuphela. Iziphumo zophando kungenzeka zipapashwe emaphepheni enzuluwazi kunye nasezintlanganisweni kodwa igama lam alisayi kungqanyaniswa nezo ziphuma. Ndiyazi andisayi kuhlawula ngokuthatha inxaxheba kolu phando.

Ungathanda na ukuzazi iziphumo zophando? Ewe noba hayi

Ukuba uthi ewe nceda ubhale idilesi nenombolo yakho yemfonomfono.....

Ukuba unemibuzo ngophando tsalela umnxeba uGqirha Shebe okanye uGqirha Lehloenya ku 021 404 5269.

Imvume yokwenza uphando ifumaneke ngomhla ka.....inikezwa licandelo leHuman Research ethics committee kwi Univesiti yaseKapa (University of Cape town). Ukuba zikhona izikhalazo mayela nolu phando nceda utsalele umnxeba uProfesa Marc Blockman ku 021 406 6492.Ndivumile ukuthabatha inxaxheba kolu phando kwaye ndiyazi ndingasirhoxisa esi sivumelwano nangaliphi ixesha ngaphandle kokunika izizathu.

Igama lophandwayo	utyikityo, uphawu okanye umnwe
.....
Igama lomphandi	utyikityo
.....
Umhla.....	
Igama lengqina	utyikityo
.....

IMVUME YOKUNIKEZELA NGEGAZI KUHLULO LWEGENES

Uhlobo lwesample: Igazi

Uhlobo lohlobo lwegenes elizakwenziwa:

Kungaba zikhona na izintsolo zokuthi umzimba wam ungahle ulwe namachiza esifo semiphunga.

1. Ndixelelwe ngeenjongo zolu hlobo lwegenes
2. Ndichazelwe ngamakhwiniba anxulumene nolu hlobo lwegenes
3. UGqirha.....undichazele ngendzuzo nokungahle kungahabi kakuhle kolu hlobo lwegenes.
4. Niyazi ukuba ukuhlolwa kwe genes zam kungahle kudale ingxaki emzimbeni, egqondeni nomshwalense (insurance) kusapho lwam.
5. Ndichazelwe ukuba ziyakufumaneka njani iziphumo zophando
6. Ndichazelwe ukuba ngubani oyakukwazi ukufumana igazi endinikezele ngalo, nokuba igazi eliseleyo liyakugcinwa ngabaphandi kwiUnivesiti yase Kapa (Unuversity of cape Town)
7. Ndichazelwe ukuba ngubani oyakufumana iziphumo zophando eziyingxenye zeemfihlo eziqulathwe kwingcombolo zonyango.
8. Imibuzo yam iphendulwe ngokwanelisayo

Imvume yokwenza uphando ifumaneka ngomhla ka.....inikezwa licandelo leHuman Research ethics committee kwi Univesiti yaseKapa (University of Cape town) Ukuba zikhona izikhalazo mayela nolu phando nceda utsalele umnxeba uProfesa Marc Blockman ku 021 406 6492

Ndivumi ukuba kuthathwe igazi kuhlolo lwegenes phantsi kwezi meko zingentla kwaye ndiyazi ndingasirhoxisa esi sivumelwano nangaliphi ixesha ngaphandle kokunika izizathu.

Igama lophandwayo	utyikityo, uphawu okanye umnwe
.....
Igama lomphandi	utyikityo
.....
Umhla.....	
Igama lengqina	utyikityo
.....
Umhla.....	

Appendix 2c: Afrikaans consent form

TOESTEMMINGSVORM

GENOMIKA VAN ANTI-TUBERKULOSE MIDDEL GEINDUSEERDE HIPERSENSITIWITEITS REAKSIES

Ek, _____ stem in om deel te neem aan hierdie navorsing uit vrye wil. Die redes, voordele en risikos hieraan verbonde is aan my meegedeel deur dokter _____ in n taal wat ek verstaan.

Ek is meegedeel dat die rede vir hierdie navorsing is om te bepaal of my gene bygedra het tot die reaskie wat ek agv my anti-TB medikasie gehad het.

Twee bloed monsters van 5ml elk sal van my geneem word. Een sal na n laboratorium in die buiteland gestuur word wat oor die nodige toetse beskik en die ander monster sal in Kaapstad geberg word vir moontlike verdere toetse wat slegs met hierdie navorsing verband sal hou. Eersgenoemde monster sal vernietig word sodra die toetse afgehandel is. My toestemming sal verkry word alvorens verdere toetse op die ander monster verrig word. Ek verstaan dat die navorsing my nie direk sal bevoordeel nie, maar dat dit vir andere tot voordeel mag wees in die toekoms, insluitende my kinders en familie.

Die rekords van die navorsing sal veilig bewaar word en slegs die persone wie die navorsing gedoen het sal toegang daartoe he. Die resultate van die navorsing mag gepubliseer word in wetenskaplike joernale en by wetenskaplike vergaderings voorgedra word, maar ek sal nie geidentifiseer word nie. Ek verstaan dat ek nie vir die werk verbonde aan die navorsing betaal sal word nie.

Wil u ingelig word aangaande die bevindinge van die navorsing? Ja nee

Indien wel, wat is u adres en telefoon nommer? _____

Indien u enige verdere vrae het kan u drs Shebe of Lehloeny kontak by 021 404 5269

Toestemming vir hierdie navorsing is gegee op _____ deur die Menslike Navorsings Etiese Komitee van Kaapstad. Indien u enige klagtes aangaande die uitvoering van die navorsing het kontak asb Prof Marc Blockman by 021 406 6492.

Ek stem in om deel te neem uit vrye wil en kan ter enige tyd onttrek sonder om n rede daarvoor te verskaf.

Geskrewe naam van deelnemer:

Handtekening of duim vingerafdruk

Ondersoeker se naam (skryf)

Handtekening

Datum _____

Datum _____

Getuie se naam (skryf)

Handtekening

Datum _____

TOESTEMMING VIR DIE VERKRYGING VAN N MONSTER VIR GENETIESE TOETSE

MONSTER TIPE: BLOED

GENETIESE TOETS AANGEVRA VIR:

Vatbaarheid vir nuwe effekte van TB medikasie
Die veronderstelde doelstelling van die toets is voorspellend.

1. Ek is ingelig aangaande die doelstelling van die genetiese toets.
2. Aan my is die tekortkominge van die toets verduidelik
3. Dr. _____ het met my die voordele en risikos van die genetiese toets bespreek.
4. Ek verstaan dat party genetiese toetse moontlike mediese, psigologiese of versekerings implikasies vir my en my familie kan inhou.
5. Aan my is meegedeel hoe ek die resultate kan bekom.
6. Aan my is meegedeel wie toegang tot my bloed monster het en dat wat daarvan oor is deur die navorsers van die Universiteit van Kaapstad gehou kan word .
7. Aan my is meegedeel wie toegang het tot my genetiese toets resultaat en dat dit deel uitmaak van my konfidesieel mediese rekord.
8. My vrae is beantwoord na my bevrediging.

Toestemming vir die navorsing was gegee op _____ deur die Menslike Navorsings Etiese Komitee van die Universiteit van Kaapstad. Indien ek enige klagtes aangaande die uitvoering van die navorsing het kan ek Prof Marc Blockman kontak by 021 404 6492.
Ek gee toestemming dat monsters van my geneem mag word vir genetiese toetse vir die toestand vermeld hierbo en ek is bewus dat ek hierdie toestemming ter enige tyd kan onttrek sonder om n rede daarvoor te verskaf.

Geskrewe naam van deelnemer:

Handtekening of duim vingerafdruk

Ondersoeker se naam (skryf)

Handtekening

Datum _____

Datum _____

Getuie se naam (skryf)

Handtekening

Appendix 3: Clinical research form

A. Patient Demographics

A1: Surname: _____ A2: Name _____

A3: Folder No: _____ A4: Date of birth: _____

A5: Sex: Male (1) Female (2)

A6: Ethnicity: Asian Yes (1) No (0) Black Yes (1) No (0)

Coloured Yes (1) No (0) Indian Yes (1) No (0) White Yes (1) No (0)

Other capture text _____

B: Comorbidities:

Hypertension Yes (1) No (0) Diabetic (Yes) No (0) Epilepsy (Yes) No (0)

Others capture text _____

C: HIV status:

C1: HIV status known Yes (1) No (0)

C2: HIV infected Yes (1) No (0)

C3: CD4 count known: Yes (1) No (0)

C4: If known value of CD4 count: _____ cells/mm³

C5: On HAART Yes (1) No (0)

D: Previous TB history

D1: Yes (1) No (0)

D2: Date of diagnosis _____

D3: How was the TB diagnosed Sputum Yes (1) No (0) CXR Yes (1) No (0)

Abd USS Yes (1) No (0) FNAB Yes (1) No (0) CSF Yes (1) No (0)

Other capture text _____

D4: Sensitivity testing Yes (1) No (0)

D5: If Yes, Was it MDR Yes (1) No (0) Monoresistance Yes (1) No (0)

Nonresistance Yes (1) No (0)

E: Previous completion Tb treatment

E1: Yes (1) No (0)

E2: If yes, how long was the TB treatment _____?

E3: If No, how long was the TB treatment _____?

F: ADR in previous TB treatment? Yes (1) No (0)

F1: Specify type of drug reaction

Stevens Johnson syndrome (SJS) Yes (1) No (0) Toxic epidermal necrolysis (TEN)

Yes (1) No (0) SJS/TEN overlap Yes (1) No (0)

Drug hypersensitivity syndrome (DHS)/ drug reaction with eosinophilia and
systemic symptoms (DRESS) Yes (1) No (0)

Lichenoid drug reaction Yes (1) No (0) Photo induced drug reaction Yes (1)

No (0) Fixed drug eruption Yes (1) No (0)

Vasculitis Yes (1) No (0) AGEP Yes (1) No (0) Angioedema Yes

(1) No (0) Morbilliform/exanthematous Yes (1) No (0)

Other (capture text)

G: Previous causative TB drugs known:

G1: Yes (1) No (0)

G2: If yes specify drug(s):

Rifampicin Yes (1) No (0) Isoniazid Yes (1) No (0) Pyrazinamide Yes (1) No

(0) Ethambutol Yes (1) No (0) streptomycin Yes (1) No (0)

Ethionamide Yes (1) No (0) Ofloxacin Yes (1) No (0) Ciprofloxacin Yes (1)

No (0) Azithromycin Yes (1) No (0) Amikacin Yes (1) No (0)

Thiacetazone Yes (1) No (0) Clofazimine Yes (1) No (0) Clarithromycin
Yes (1) No (0) kanamycin Yes (1) No (0) Terizidone Yes (1) No (0) PAS Yes
(1) No (0) Cycloserine Yes (1) No (0) Rifabutin Yes (1) No (0)
Other (capture text) _____

H: Previous re-challenge of the TB medication:

Yes (1) No (0)

H1: If Yes, Date of Re-challenge _____

H2: Date of completion of re-challenge _____

H3: If No, Reasons capture text _____

I: Outcome of previous re- challenge

I1: Successful re-challenge with the patient on at least 4 anti Tb drugs

Yes (1) No (0)

I2: If yes, specify anti Tb drugs

Rifampicin Yes (1) No (0) Isoniazid Yes (1) No (0) Pyrazinamide Yes (1) No
(0) Ethambutol Yes (1) No (0) streptomycin Yes (1) No (0)
Ethionamide Yes (1) No (0) Ofloxacin Yes (1) No (0) Ciprofloxacin Yes (1) No
(0) azithromycin Yes (1) No (0) amikacin Yes (1) No (0)
Thiacetazone Yes (1) No (0) Clofazimine Yes (1) No (0) Clarithromycin
Yes (1) No (0) kanamycin Yes (1) No (0) Terizidone Yes (1) No (0) PAS Yes
(1) No (0) Cycloserine Yes (1) No (0) rifabutin Yes (1) No (0)
Other (capture text) _____

I3: Date of discharge _____.

I4: Completed treatment Yes (1) No (0)

I5: If yes, how long _____

I6: If No, reason capture text _____

J: Date of current TB diagnosis _____

J1: How was the TB diagnosed Sputum Yes (1) No (0) CXR Yes (1) No (0)

Abd USS Yes (1) No (0) FNAB Yes (1) No (0) CSF Yes (1) No (0) Other capture

text _____

J2: Sensitivity testing Yes (1) No (0)

J3: If Yes, Was it MDR Yes (1) No (0) Monoresistance Yes (1) No (0)

Nonresistance Yes (1) No (0)

K: History of developing an IRIS

K1: Yes (1) No (0)

K2: If yes, specify capture text _____

L: History of ADRS with current TB treatment

L1: Yes (1) No (0)

L2: Date of the first symptom of ADR _____

L3: Symptoms: Nausea Yes (1) No (0) Vomiting Yes (1) No (0) Itch Yes (1) No (0)

General pain Yes (1) No (0) Sore throat Yes (1) No (0) Sore eyes Yes (1) No (0)

Erythema Yes (1) No (0) Headache Yes (1) No (0) Swelling Yes (1) No (0) Rigors

Yes (1) No (0) Muscle pain Yes (1) No (0) Abdominal pain Yes (1) No (0)

Diarrhea Yes (1) No (0) Palpitation Yes (1) No (0) Fever Yes (1) No (0)

Others capture text _____

L4: History of taking medication 8weeks before the reaction Yes (1) No (0)

L5: If Yes, Bactrim Yes (1) No (0) ARV'S Yes (1) No (0) anti TB Yes (1) No (0)

Antibiotics Yes (1) No (0) others capture text _____

L6: Date of admission _____

L7: Date of stopping the medication _____

L8: Blood results on admission

EOS _____ LYMPHOCTE _____ ALT _____ AST _____

LIPASE _____ WCC _____ HB _____ TOT BILI _____

CONJ BILI _____ PLT _____ UREA _____

CREATININE _____ HEP A _____ HEP B _____

HEP C _____ EBV _____ VZV _____ HSV _____ -

HHV6 _____ HHV7 _____ TOXO _____

L9: Type of current ADR

Stevens Johnson syndrome (SJS) Yes (1) No (0) Toxic epidermal necrolysis

(TEN) Yes (1) No (0) SJS/TEN overlap Yes (1) No (0)

Drug hypersensitivity syndrome (DHS)/ drug reaction with eosinophilia and

systemic symptoms (DRESS) Yes (1) No (0)

Lichenoid drug reaction Yes (1) No (0) Photo induced drug reaction Yes

(1) No (0) Fixed drug eruption Yes (1) No (0)

Vasculitis Yes (1) No (0) AGEP Yes (1) No (0) Angioedema

Yes (1) No (0) Morbilliform/exanthematous Yes (1) No (0)

Other (capture text)

M: Current causative TB drugs known:

M1: Yes (1) No (0)

M2: If yes specify drug(s):

Rifampicin Yes (1) No (0) Isoniazid Yes (1) No (0) Pyrazinamide Yes (1) No

(0) Ethambutol Yes (1) No (0) streptomycin Yes (1) No (0)

Ethionamide Yes (1) No (0) Ofloxacin Yes (1) No (0) Ciprofloxacin Yes (1)

No (0) Azithromycin Yes (1) No (0) Amikacin Yes (1) No (0)

Thiacetazone Yes (1) No (0) Clofazimine Yes (1) No (0) Clarithromycin Yes

(1) No (0) kanamycin Yes (1) No (0) Terizidone Yes (1) No (0) PAS Yes (1) No

(0) Cycloserine Yes (1) No (0) Rifabutin Yes (1) No (0)

Other (capture text) _____

M3: History of taking backbone drugs Yes (1) No (0)

M4: Duration of backbone drugs _____

N: Re-challenge of TB medication

N1: Yes (1) No (0)

N2: Date of re challenge _____

N3: Successful re-challenge with the patient on at least anti TB drugs

Yes (1) No (0)

N4: If yes, specify anti Tb drugs

Rifampicin Yes (1) No (0) Isoniazid Yes (1) No (0) Pyrazinamide Yes (1) No

(0) Ethambutol Yes (1) No (0) streptomycin Yes (1) No (0)

Ethionamide Yes (1) No (0) Ofloxacin Yes (1) No (0) Ciprofloxacin Yes (1) No

(0) Azithromycin Yes (1) No (0) Amikacin Yes (1) No (0)

Thiacetazone Yes (1) No (0) Clofazimine Yes (1) No (0) Clarithromycin Yes

(1) No (0) kanamycin Yes (1) No (0) Terizidone Yes (1) No (0) PAS Yes (1) No

(0) Cycloserine Yes (1) No (0) Rifabutin Yes (1) No (0)

Other (capture text) _____

N5: If No, History of ADRS

N6: Yes (1) No (0)

N7: Symptoms and signs of the re-challenge reactions

Nausea Yes (1) No (0) Vomiting Yes (1) No (0) Itch Yes (1) No (0)

General pain Yes (1) No (0) Sore throat Yes (1) No (0) Sore eyes Yes (1) No (0)

Erythema Yes (1) No (0) Headache Yes (1) No (0) Swelling Yes (1) No (0)

Rigors Yes (1) No (0) Muscle pain Yes (1) No (0) Abdominal pain Yes (1) No (0)

Diarrhea Yes (1) No (0) Palpitation Yes (1) No (0) Fever Yes (1) No (0) Others

capture text _____

N8: Types of the drug reactions on re-challenge

Stevens Johnson syndrome (SJS) Yes (1) No (0) Toxic epidermal necrolysis

(TEN) Yes (1) No (0) SJS/TEN overlap Yes (1) No (0)

Drug hypersensitivity syndrome (DHS)/ drug reaction with eosinophilia and

systemic symptoms (DRESS) Yes (1) No (0)

Lichenoid drug reaction Yes (1) No (0) Photoinduced drug reaction Yes

(1) No (0) Fixed drug eruption Yes (1) No (0)

Vasculitis Yes (1) No (0) AGEP Yes (1) No (0) Angioedema

Yes (1) No (0) Morbilliform/exanthematous Yes (1) No (0)

Other (capture text)

N9: Causative drug known yes (1) no (0)

N10: Specify causative drugs on re challenge

Rifampicin Yes (1) No (0) Isoniazid Yes (1) No (0) Pyrazinamide Yes (1) No
(0) Ethambutol Yes (1) No (0) streptomycin Yes (1) No (0)
Ethionamide Yes (1) No (0) Ofloxacin Yes (1) No (0) Ciprofloxacin Yes (1)
No (0) Azithromycin Yes (1) No (0) Amikacin Yes (1) No (0)
Thiacetazone Yes (1) No (0) Clofazimine Yes (1) No (0) Clarithromycin
Yes (1) No (0) kanamycin Yes (1) No (0) Terizidone Yes (1) No (0) PAS
Yes (1) No (0) Cycloserine Yes (1) No (0) Rifabutin Yes (1) No (0)
Other (capture text) _____

O: Duration before normalization after re challenge ADRS _____

P1: Last date of re challenge _____

P2: Specify anti Tb drugs the patient was discharge with

Rifampicin Yes (1) No (0) Isoniazid Yes (1) No (0) Pyrazinamide Yes (1) No
(0) Ethambutol Yes (1) No (0) streptomycin Yes (1) No (0)
Ethionamide Yes (1) No (0) Ofloxacin Yes (1) No (0) Ciprofloxacin Yes (1)
No (0) Azithromycin Yes (1) No (0) Amikacin Yes (1) No (0)
Thiacetazone Yes (1) No (0) Clofazimine Yes (1) No (0) Clarithromycin
Yes (1) No (0) kanamycin Yes (1) No (0) Terizidone Yes (1) No (0) PAS Yes
(1) No (0) Cycloserine Yes (1) No (0) Rifabutin Yes (1) No (0)
Other (capture text) _____

R: Final outcome

R1: Completed treatment Yes (1) No (0)

R2: If Yes, how long _____

R3: If No,

R3:1: still on medications Yes (1) No (0) and for how long _____

Appendix 4: Instructions to authors

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INSTRUCTIONS TO AUTHORS

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Aims and Scope

The *Journal of Investigative Dermatology (JID)* publishes reports describing original research on all aspects of cutaneous biology and skin disease. Topics include biochemistry, biophysics, carcinogenesis, cell regulation, development, skin structure, extracellular matrix, genetics, immunology, melanocyte biology, microbiology, molecular and cell biology, pathology, physiology, pharmacology, photobiology, percutaneous absorption, clinical research, epidemiology and other population-based research.

Case reports or case series, unless they provide new biologic insights, are rarely appropriate for the Journal.

Mutation reports of mutations in known genes with no new mechanistic data will not be considered.

Original Articles, Review Articles, and Letters to the Editor are standard features. Perspectives and Commentaries are invited by the Editorial Board. Online features now under development seek to make *JID* content more relevant and accessible to trainees and clinician-educators.

Reports that primarily or exclusively concern a methodology, with the data documenting utility or feasibility, rather than providing new biologic insights, are discouraged, although exceptions to our policy of rejection may occasionally be made. Submissions reporting new methods in combination with mechanistic insights into the problem being investigated are, in contrast, most welcome.

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Article Types

Original Articles

Original articles should not exceed 3,500 words and 6 figures or tables - not to exceed 7 printed journal pages. (For details on word and figure limits, see sections B and C below).

Original articles should be organized as follows: Title page, Abstract, Introduction, Results, Discussion, Materials & Methods, Conflict of Interest, Acknowledgments, References, Tables, Figure Legends, Supplementary Material.

Review Articles

A review article is expected to be comprehensive, scholarly, and balanced, presenting an expert curation of the literature in the topic of interest. Reviews are limited to: 100-word abstract; 3,000-word text, excluding references; and 2 figures. Authors are encouraged to consult the Editor before submitting a review for consideration.

Letters to the Editor

Letters to the Editor may report original data or discuss published articles. Letters are not to exceed 1,000 words and 2 figures or tables, and 15 references - not to exceed 3 printed journal pages. (For details on word and figure limits, see sections B and C below). Letters should not have an abstract. The Editor may solicit a response from the authors if a letter refers to an article published in *JID*. Letters that report original data will be fully peer-reviewed. All Letters to the Editor are subject to editing and possible abridgment.

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Manuscript Submission

JID requires electronic submission of manuscripts. Detailed instructions are at our [Manuscript submission website](#). For assistance with the site, contact ScholarOne Manuscripts at +1 434-964-4100. For questions regarding your submission, contact the Editorial Office at +1 919-932-0140.

Complete submissions contain all items below, and submissions are dated according to receipt of all items. No editorial decision will be communicated to the authors until the submission is complete. Authors are encouraged to read [JID's Editorial Policies](#) before submitting their work.

All Submissions MUST Include:

- Cover letter stating:
 1. The data in the manuscript is original and the manuscript is not under consideration elsewhere;
 2. None of the manuscript contents have been previously published except in abstract form;
 3. All authors have read and approved all versions of the manuscript, its content, and its submission to the *JID*;
 4. The authors should state their willingness to pay page charges (\$150/page, inclusive of color, for articles accepted after January 1, 2013), should the manuscript be accepted for publication;
 5. If the submission contains supplemental files, the authors should state their willingness to pay online fees (\$125/file). This is payable at proof stage;
 6. The corresponding author's address, telephone, fax, email (email address required).
- License to Publish, signed by the corresponding author.
- Declaration of Conflict of Interest, signed by all authors.
- Manuscript Submission Fee of \$50 (payable at time of submission)

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Manuscript Preparation

A. General

The manuscript should be double-spaced throughout with wide (1" or larger) margins. Set your word processing software to 8.5 x 11 inch paper. Number pages consecutively (with the title page as page 1). Begin a new page for reference lists, tables, and figure legends. The file should use the wrap-around end-of-line feature, e.g., returns at the end of paragraphs only. Place two returns after every element, such as title, headings, paragraphs, figure and table callouts. The paper should be concise, economical of references, figures and tables, and formatted as described below. Reports of investigative studies should be organized as follows, within the stated word limits: Title page, Abstract, Introduction, Results, Discussion, Materials & Methods, Conflict of Interest, Acknowledgments, References, Tables, Figure Legends, Supplementary Material.

Manuscripts that do not conform to these specifications will be returned to the authors for correction prior to review.

B. Word Limits

Word limits will be instituted for all manuscripts accepted after January 1, 2010.

- Original articles should not exceed 3,500 words and 6 figures or tables—not to exceed 7 printed journal pages.
- Letters to the Editor should not exceed 1,000 words, 2 figures or tables, and 15 references—not to exceed 3 printed pages.
- Word counts include text only; the abstract, references, figure legends, tables, and supplemental data are excluded (note that figure legends are limited to 125 words each, and 500 words of article text may be exchanged for 1 figure or vice versa).
- There may be exceptions, which will be handled at the discretion of the Editor.
- Submissions that do not comply with these guidelines will be returned to authors for revision.

C. Figure/Table Size Limits

Beginning January 1, 2013, illustrative elements (figure/tables) are limited to $\frac{1}{4}$ page each (approx 87.5 mm x 115 mm). Authors may supply fewer, larger elements (i.e., for an original article three $\frac{1}{2}$ -page figures may be presented rather than six $\frac{1}{4}$ -page figures — or any combination that results in no more than $1\frac{1}{2}$ pages of figures and tables). In addition, 500 words of text may be substituted for one figure, or vice versa. Submissions that do not comply with these specifications will be returned to the authors for correction prior to review.

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The title page should include the following:

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- Abbreviations used (see item Q below for detail on abbreviations)

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Begin with a brief introductory statement that places the work to follow in historical perspective and explains its intent and significance.

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Briefly present the experimental data in text, tables or figures (for details, see items N and O below). The Discussion should focus on the interpretation and significance of the findings with concise objective comments that describe their relation to other work in the area. Do not repeat information in Results. *Results and Discussion may be presented separately or combined into a single section.*

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Book chapter:

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Q. Abbreviations

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