CORRELATION BETWEEN PRO-INFLAMMATORY ALLELES AND CLINICAL AND LABORATORY MARKERS OF ALLERGY IN XHOSA SOUTH AFRICANS

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DECLARATION

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LIST OF COMMONLY USED ABBREVIATIONS

BHR	Bronchial hyper-responsiveness
Der f	*Dermatophagoides farinae*
Der p	*Dermatophagoides pteronyssinus*
GWAS	Genome wide association study
HWE	Hardy-Weinberg equilibrium
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ISAAC	The International Study of Asthma and Allergies in Childhood
LD	Linkage disequilibrium
OR	Odds ratio
PAMP	Pathogen-associated molecular pattern
PIA	Pro-inflammatory allele
SNP	Single nucleotide polymorphism
SPT	Skin prick test
STR	Short tandem repeat
T_H0	Naïve T helper lymphocytes
T_H1	Type 1 T helper lymphocytes (and their cytokines)
T_H2	Type 2 T helper lymphocytes (and their cytokines)
TLR	Toll-like receptor
TNF	Tumour necrosis factor

INTRODUCTION

As the burden of infectious diseases around the world has gradually decreased over the past 25 years (Lozano et al. 2012), increasing attention is being paid to non- communicable diseases. Asthma and allergies are being globally recognised as major public health problems (Baena-Cagnani 2001). Conservative estimates suggest that about 500 million people globally suffer from allergic rhinitis, and about 300 million people suffer from asthma (Masoli et al. 2004; Ozdoganoglu & Songu, 2012). Locally, the burden of allergic disease is thought to be increasing (Weinberg 2000; Zar et al. 2007), and there is concern locally and globally that regions and countries with poor socioeconomic conditions may have disproportionate morbidity and mortality from asthma and other allergies (Poyser et al. 2002). Of particular concern is the pattern of increased prevalence of allergic conditions associated with urbanization and adoption of a western lifestyle, in combination with increased morbidity and mortality associated with poor socioeconomic status (Poyser et al. 2002), meaning that the recently urbanized poor disproportionately bear the brunt of the allergy epidemic.

South Africa is acutely affected by asthma and allergic diseases. Our rates of urbanization (United Nations Department of Economic and Social Affairs Population Division (2014)) mean that our urban poor population is continuing to expand. Public health still faces the obstacles of poverty, systemic inequality and relatively poor access to health care. Data suggests that the prevalence of allergic disease is high, and still increasing (Zar et al. 2007), and that asthma mortality is disproportionately high (Masoli et al. 2004).

Globally, tremendous strides are being made in the study of allergic diseases. This progress has been largely spearheaded by a revolution in our understanding of the human genome, coupled with new technology that has made rapid and cost-effective genome sequencing a reality. Technological advances in the fields of immunology and histology have improved our ability

to elucidate clinical phenotypes, and improved statistical analysis has improved the quality of data obtained from research. Increasing understanding of the genetic, pathophysiologic and histologic bases of asthma and allergies may, in time, help us to improve the treatment (Barnes 2010), and possibly even prevention of allergic disease. Nonetheless, several important questions still remain, including the following. Why is the prevalence of allergy increasing in some countries, plateauing in some, and decreasing in others? What are the root genetic and environmental causes of asthma and allergies? What is the precise quantity and quality of the genetic contribution to the development of allergic disease? On what basis are the apparent differences in prevalence and severity of allergic disease on the basis of socioeconomic status, ethnicity and geographic location?

One of the hypotheses for the difference in prevalence of genetic markers associated with allergic disease is that of evolutionary adaptation of the immune system (Le Souëf et al. 2006). The assertion is that modern man's origins in a tropical environment conferred a survival advantage upon people who had an immune system primed to combat helminths and other parasitic illnesses (Le Souëf et al. 2000; Le Souëf et al. 2006). Our migration to more temperate climates has made the adverse effects (including allergic disease) of this priming outweigh the benefits (Le Souëf et al. 2000). To date, there has been no data published with the specific aim of interrogating this hypothesis.

Our study is primarily concerned with the relationship between genetics and allergic disease in a Black African (Xhosa) population. We feel that this is an important topic for many reasons. Firstly, we know from high quality studies (Van Niekerk et al. 1979; Ehrlich et al. 1995; Zar et al. 2007) that the prevalence of allergic disease is increasing in South Africa, specifically amongst Xhosa people. Secondly, there is very little data either locally or globally on the genetic determinants of asthma and allergies in populations who are not of Caucasian origin (Barnes et al. 2007). There is currently insufficient evidence to know whether the genetic determinants of allergic diseases are similar in people of different ethnicities, and there are examples in the local literature to suggest that allergic phenotypes (Abbott et al. 2013) and genotypes (Thawer-

Esmail et al. 2014) may not be the same as in the developed world.

Our study aims to examine the prevalence of alleles thought to promote allergic disease. We also aim to examine the relationship between these alleles and clinical and laboratory features of asthma and allergies. It is the first time that the relationship between many of the alleles and allergic disease is being studied in a Black African population. We therefore hope that our study will be a significant contribution to the local literature regarding the genetic aetiology of allergic disease. Until recently, the cost of performing genotyping was almost prohibitive in the developing world. This is rapidly changing, and the possibility of performing genotyping across hundreds of thousands of polymorphisms throughout the genome in the search for disease associations may become a reality even in resource constrained settings. This study currently represents the first in South Africa to examine the relationship between such a variety of genetic polymorphisms and allergic disease.

Our investigation involves a cross-sectional study of just over 200 Xhosa adolescents from a high school in Cape Town, South Africa. After informed consent was obtained, blood was drawn for genotyping, and further testing was performed including administering a questionnaire on personal history of asthma and allergies, skin prick testing for allergic sensitization to common aero- and food allergens, and blood total and *Ascaris*-specific IgE levels. This has enabled us to measure allele frequencies in this population, as well as to compare individual genotypes and allergic phenotypes in our sample. Several articles have been published by our group that report on the phenotypic characteristics of the sample population. These articles have focussed on the relationship between asthma and bronchial hyper-responsiveness (BHR) with allergy and atopy phenotypes (Levin et al. 2011), the influence of atopy and *Ascaris* sensitization on serum IgE levels (Levin et al. 2008) and the association between *Ascaris* sensitization and asthma and allergy phenotypes (Levin et al. 2012). However, until now, the prevalence of potential pro-inflammatory alleles (PIAs), as well as correlations between genotype and phenotype, has not been interrogated in this sample. This thesis represents the first, and only analysis of the genetic data obtained from this sample.

We hope that this study will be useful in guiding further research into the genetics of allergic disease in South Africa. In addition, our data forms part of a collaborative dataset from several countries around the world that aims to investigate the possibility of evolutionary adaptation of the immune system as an explanation for differences in allele prevalence between population groups (Le Souëf, personal correspondence). This data may contribute to the international literature in helping to better understand the impact of evolution and ethnicity on allergic disease.

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LITERATURE REVIEW

INTRODUCTION

The term ‘allergy’ was coined in the early 20th century by Viennese paediatrician Clemens von Pirquet Freiher (Igea 2013). At the time, the concept was meant to convey the idea that the first contact of the immune system with an antigen changes the way the body reacts to that antigen, such that on subsequent contacts, this change could induce a spectrum of responses, from protective to harmful (Igea 2013).

Much has changed since the term was proposed slightly over a century ago. Our understanding of the structure and function of the immune system has improved exponentially, we have a vastly more accurate idea of the basic pathophysiological mechanisms of allergy, and the contributions of genetics and environment and their impact on allergic disease have received significant recent attention. The last thirty years have witnessed explosive advances in the field of human genetics, and in conjunction with massive strides in immunology and statistical analysis, we are much closer than before to illuminating the root causes of allergic disease.

That being said, there are still many questions with regards to the pathogenesis, pathophysiological mechanisms, and contributions of genetic and environmental aetiologies in the causation of allergic disease. As with many other health care problems, there is still massive inequality in the field of allergy, both in terms of research and health outcomes. The magnitude of this inequality is beginning to be considered in South Africa (Green et al. 2007) and globally (Beran et al. 2015).

Our study forms the South African arm of a global study looking at the genetic

effects of ethnic origins on asthma and allergies. Our study population is a group of Xhosa teenagers, who as Black Africans, represent an understudied sector of the global society. We hope this study will contribute significantly to the local and, possibly, international literature in helping to identify genetic markers that are associated with allergic disease in this population group.

In reviewing the literature relevant to our study, I will therefore consider the pathogenesis and pathophysiology of allergic disease, the burden of allergic disease globally and in South Africa, before discussing the current state of genetic research in allergic disease. I will highlight the gaps in research that our study is hoping, in part, to fill, and give background in respect of the specific genes and polymorphisms of relevance to our investigation.

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PATHOGENESIS AND PATHOPHYSIOLOGY OF ALLERGIC DISEASE

Perhaps *the* key attribute of the immune system is the ability to discern self molecules from potentially harmful non-self molecules (Chinen et al. 2009). The immune system exists in a balance between self-tolerance and response to, or rejection of, non-self (Chinen et al. 2009). The typical allergic response involves an abnormal immune response to an inert molecule, usually an environmental protein (Eisenbarth 2006). Much research in allergy is devoted to answering why and how the immune system reacts to these essentially harmless triggers (Eisenbarth 2006).

The human immune system is made up of two complementary systems, the innate and adaptive immune systems (Chinen et al. 2009). The innate immune system provides rapid, non-specific responses based on recognition of microbial patterns not found in the host (Chinen et al. 2009), whereas the adaptive immune system enables recognition of specific pathogens and amplification of an appropriate and specific response to that pathogen (Chinen et al. 2009). Although much focus has been placed on the adaptive immune system (and T_H2 responses in particular), adaptive T lymphocyte responses require priming from the innate immune system (Eisenbarth 2006), meaning that alterations in both innate and adaptive immunity may play a role in the development of allergies.

Although allergic disease may present in many guises, a common pattern of inflammation is seen in most, and is often driven by IgE-dependent mechanisms (Barnes 2011). As this study is primarily interested in IgE-mediated allergic disease, the pathophysiology of IgE-mediated inflammation will form the focus of this review. However it is worthwhile to mention that a number of allergic responses are recognized as being either non IgE-mediated or mixed IgE- and non IgE-mediated reactions. It is hoped that an improved understanding of the mechanisms of causation of allergic disease will lead to improved methods of treatment or even prevention.

Mechanism of Allergic Inflammation

The allergic immune response has evolved from our inflammatory response to worm and parasite infestations (Barnes 2011). Central to most forms of allergic inflammation is T_H2 lymphocyte orchestrated activation of mast cells, with subsequent release of inflammatory mediators and influx of eosinophils and other inflammatory cells (Barnes 2011).

An allergic immune response occurs when B lymphocytes produce large quantities of allergen-specific IgE (Barnes 2011). This IgE then coats the surface of mast cells, the Fc portion of the IgE bonding to the high-affinity IgE receptor (FcεRI) on the mast cell membrane (Amin 2012). Subsequent exposure to the same allergen induces allergen binding to the Fab portion of the cell-bound IgE, and the resulting cross- linking triggers release of pre-formed mediators (prostaglandins, histamine and other mediators), in a process known as degranulation, as well as production of new mediators (leukotrienes, prostaglandin D_2, and interleukins 4, 5 and 13, amongst others) (Amin 2012). The cumulative effect of this mediator release includes increase in endothelial permeability, activation and chemoattraction of other inflammatory cells, induction of immunoglobulin class switching of B lymphocytes to produce more IgE, as well as potentially having a direct effect on airway damage and remodelling in asthma (Amin 2012).

Although the pathophysiology of the allergic response is fairly well studied, we still do not fully understand what primes the immune system to produce a vigorous T_H2 response to essentially inert molecules. Emerging evidence suggests that the innate and adaptive immune systems, working in concert, are able to induce three major types of response to pathogens (Annunziato et al. 2014). A type 1 immune response involves T_C1 cytotoxic lymphocytes, T_H1 lymphocytes and IFN-γ producing innate lymphoid cells (ILCs), and is largely concerned with protection against intracellular pathogens through phagocyte activation (Annunziato et al. 2014). Type 2 responses involve T_C2 cytotoxic lymphocytes, T_H2 lymphocytes and GATA-3 ILCs, and produce IL-4, IL-5 and IL-13, inducing mast cell, eosinophil and basophil activation (Annunziato et al. 2014). Type 2 immune responses protect against helminths and venoms,

largely through specific IgE antibody production (Annunziato et al. 2014).

Type 3 immune responses involve T_C17 and T_H17 lymphocytes as well as ILC3s, producing IL-17 and/or IL-22. This results in phagocyte activation, neutrophil recruitment and induction of epithelial antimicrobial responses, protecting against extracellular organisms like bacteria and fungi (Annunziato et al. 2014). Traditionally, it has been held that allergy results from an inadvertent activation of a type 2 immune response by an innocuous molecule, but recent research has highlighted the role of the type 2 immune response in response to toxins such as venom, and raised the possibility that allergens are not as innocuous as believed (Annunziato et al. 2014).

Not surprisingly, much attention has been directed towards the factors leading to the differentiation of the immune system towards the type 2 response. IL-4 is the main differentiation factor for T_H2 lymphocytes (Eisenbarth 2006), and the IL-4 pathway, including the molecule itself, its receptors, IL-13 (which shares many effects with IL-4 (Luzina et al. 2012)) and its signalling pathway (including STAT6) has been the focus of much interest. Recent research (Wan 2014) has suggested GATA3 as the 'master factor' in T_H2 differentiation, although no research has yet compellingly linked the GATA3 gene with allergic disease (Li et al. 2006; Suttner et al. 2009). Its signalling pathways, co-factors and other elements of its function remain to be fully discerned (Wan 2014; Yagi et al. 2011).

Microbial Influence

Since the recognition, over sixty years ago, that number of siblings (especially older siblings) is a protective factor in the development of hay fever (Strachan 1989), it has been plausible that exposure to microbial products can influence the character of the human immune response to antigen. Further studies have demonstrated that exposure to pets, microbes and dust from farm environments are also protective against the development of allergy, whilst exposures such as urban environments, small families, birth via caesarean section and maternal antibiotic use in pregnancy are promoting factors (Daley 2014). The 'hygiene

hypothesis' is a concept initially developed (Strachan 1989) as a means to explain why asthma and allergies were increasing rapidly in the industrialized world at the time (Daley 2014). The author theorized that early life exposure to viral and bacterial organisms influences how the immune system responses to further stimuli, and that elimination of these exposures results in a shift in immune response that promotes the formation of asthma and allergies (Daley 2014). The theory was advanced to suggest that the mechanism of this shift was through persistence of the T_H2 predominance in the immune system seen in perinatal cytokine profiles (Daley 2014).

Lipopolysaccharide (LPS), and endotoxin (its bioactive moiety), have been extensively studied in relation to their impact on immune responses (Simpson & Martinez 2010). LPS is a component of gram-negative bacterial outer membranes (Simpson & Martinez 2010), and is common to all gram-negative bacteria, making it an archetypal pathogen-associated molecular pattern (PAMP) (Simpson & Martinez 2010; Vercelli 2002). The evidence for the role of endotoxin in the formation of allergies has been contradictory. Exposure to endotoxin in early life has shown to be protective against allergy and asthma formation (von Mutius et al. 2000), but in certain settings, exposure to endotoxin has been shown to induce a potent inflammatory response that includes bronchospasm as one of its symptoms (Simpson & Martinez 2010). It is possible that the timing, level of exposure, environmental setting and genetic susceptibility all play a role in the response of the immune system to endotoxin (Simpson & Martinez 2010).

The role of viral infections, particularly rhinovirus-c (hRV-c) and respiratory syncytial virus (RSV) (Holt et al. 2012), in the development of asthma, has attracted much recent interest. There is accumulating evidence that viral infections in infancy and pre-school years is strongly associated with the development of asthma (Holt et al. 2012; Holt 2015). It is postulated that the combination of viral infection and susceptibility to atopy results in alternative activation of myeloid precursors in the bone marrow, with replacement of airway mucosal dendritic cells by cells primed towards T_H2 activation (Holt et al. 2012). This may, in part, be responsible for the incorporation of T_H2

inflammatory cells and cytokines into the inflammatory milieu of the airways (Holt et al. 2012).

Perinatal Factors

It has been hypothesised that a child's environmental surroundings in the first 1000 days post-conception greatly influence the development of chronic disease (Wegienka et al. 2015). There is a gradual accumulation of evidence to support this hypothesis with regards to asthma and allergic disease (Wegienka et al. 2015). In a recent review, Holt (2015) discusses the evidence for in-utero priming on the immune system, including the discovery of apparently aero-allergen specific T_H2 cells in cord blood, although he notes that this evidence has subsequently been called into question.

Much interest remains in the relationship between early life influences, and the subsequent development of allergies. The microbial organisms that we harbour and interact with on a daily basis are collectively referred to as the microbiome (Wegienka et al. 2015), and there is increasing interest in how the neonatal and infant microbiome impacts on the predisposition towards disease. Mode of delivery, choice of feed and duration of breastfeeding, exposure to household pets, exposure to environmental pollutants, and antibiotic use (either maternal use during pregnancy or infant use in the first two years of life) are all thought to influence the formation of the microbiome (Wegienka et al. 2015). The association of faecal composition at one month of age, and the subsequent development of atopy was highlighted in the KOALA study (Penders et al. 2007), which found that the presence of *E.coli* in the stool was associated with atopic eczema, whereas the presence of *C.difficile* was associated with atopic eczema as well as recurrent wheeze and allergic sensitisation. Recent evidence from the Canadian Healthy Infant Longitudinal Development (CHILD) study (Arrieta et al. 2015) has shown that infants at risk of asthma exhibit transient gut dysbiosis during the first one hundred days of life, and that the abundance of 4 bacteria (*Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia*) was significantly decreased in children at risk of asthma. This supports the hypothesis that dysbiosis in early life is associated with the development of allergic disease, and shows the potential for possible microbe-based

diagnostics and therapeutics in the future (Arrieta et al. 2015).

Oral Tolerance

The mucosa of the gastrointestinal tract is the largest body surface area exposed to the outside environment (van Wijk & Knippels 2007). Immediately after birth, it is confronted with a large amount of foreign antigen (van Wijk & Knippels 2007). The mucosal immune system, the largest component of the body's immune system (Wopereis et al. 2014), is faced with the task of distinguishing which of these antigens are potentially pathogenic, and which are not harmful (van Wijk & Knippels 2007). The fact that humans do not mount immune responses to many antigens, and that only a minority of people develop food allergies, is due to the induction of oral tolerance (Chehade & Mayer 2005). Oral tolerance refers to the process whereby immune responses to an antigen are actively inhibited by means of prior oral exposure to that antigen (Chehade & Mayer 2005).

The human gastrointestinal tract is colonized by approximately ten times more microbial cells than human body cells (Wopereis et al. 2014). In addition, these microbial cells contain about 150 times more microbial genes than the human genome (Wopereis et al. 2014). Although the human genome is relatively fixed, the combined genetic information of intestinal microbiota is highly variable depending on which organisms colonise the gastrointestinal tract (Wopereis et al. 2014). Improved understanding of the maintenance of this, generally symbiotic, relationship between the mucosal immune system and the intestinal microbiota has allowed for modification of the 'hygiene hypothesis' model of understanding allergy. It has become clearer that colonisation of the gastrointestinal tract with the right microbes of sufficient biodiversity is protective against allergies (Daley 2014), and conversely that dysregulation of this healthy symbiosis between the immune system and intestinal microorganisms may be a key factor in allergy formation (Daley 2014).

Factors that promote oral tolerance to an antigen include those related to both host and antigen (Chehade & Mayer 2005). Antigen factors include the dose and form of antigen, as well as the timing of exposure (Chehade & Mayer

2005), whereas host factors include age, genetic makeup and composition of intestinal flora (Chehade & Mayer 2005). Many cells play a role in the development of tolerance, including regulatory T lymphocytes and dendritic cells (Chehade & Mayer 2005; Wopereis et al. 2014), and although further research needs to be done in delineating the exact pathways of oral tolerance on the one hand, and allergic sensitisation on the other, it would seem that a breach of any of the normal mechanisms by which the immune system encounters allergens may be sufficient to cause sensitisation (Chehade & Mayer 2005).

Skin Barrier and Filaggrin

The phenomenon of the progression of allergic disease, from atopic eczema in infancy, to allergic rhinitis and asthma in childhood, is well described. One of the first clinicians to appreciate this progression was Lorn Shore, a South African, who described children as ‘standing in the queue for asthma’ (Weinberg 2014). The term ‘atopic march’ has now been coined to describe this phenomenon, but its underlying mechanisms remain to be fully established (Suzuki et al. 2011). Immunohistological evidence suggests that an intact epidermal barrier protects the immune system from exposure to allergens that could potentially lead to allergic reactions (Suzuki et al. 2011), but until recently the precise mechanisms by which allergens could penetrate into the sub-epidermal layer of the skin were not known (Suzuki et al. 2011). The discovery of the Epidermal Differentiation Complex, and in particular the filaggrin gene (Palmer et al. 2006), on chromosome 1 as a risk factor not only for the development of atopic eczema, but also for the development of asthma and allergic rhinitis, was an important piece in the puzzle of the ‘atopic march’.

Several studies (Palmer et al. 2006; Ekelund et al. 2008; Rice et al. 2008; Weidinger et al. 2008) have confirmed the importance of filaggrin gene mutations in the progression of allergic disease in Caucasian people, but this data has yet to be replicated in people of Black African origin (Thawer-Esmail et al. 2014).

Asthma

The World Allergy Organization defines asthma as: "… a chronic inflammatory disorder of the airways associated with airway hyperresponsiveness and airflow obstruction that is often reversible either spontaneously or with treatment." (Holgate et al. 2013). Although multiple clinical phenotypes exist, atopy (the predisposition to produce specific IgE antibodies to a potential allergen), remains the most common identifiable trigger (Holgate et al. 2013). Other identified triggers include genetic predisposition, environmental factors and likely alterations in the microbiome (Mims 2015), and are more fully described elsewhere in this review. Recently, increasing attention has been paid to the idea of asthma as a syndrome comprised of multiple clinical phenotypes (Agache et al. 2012; Haldar et al. 2008), rather than as a single disease entity.

Descriptions of different phenotypes is clinically useful in terms of disease presentation, triggers and response to treatment, but does not always provide information regarding precise pathophysiological mechanisms (Agache et al. 2012), and there has been a lack of long term studies to assess the stability of specific phenotypes (Agache et al. 2012). Significant progress is also being made in endotyping asthma. Whilst phenotypes refer to separate clinical entities, endotypes represent subtypes of disease based on specific pathophysiological mechanism (Skloot 2015). Improved understanding of asthma phenotypes and endotypes may assist in more accurate correlation with genetic susceptibility to disease (Giavina-Bianchi et al. 2013) and potentially allow for more personalised approaches to therapy (Skloot 2015).

The core clinical features of asthma are airway inflammation, bronchial hyper-responsiveness and reversible airflow obstruction (Holgate & Sly 2014). Recruitment and activation of multiple cell types, including mast cells, eosinophils, basophils, neutrophils, dendritic cells, macrophages and T lymphocytes contribute to cellular infiltration into the airways (Linzer 2007). Cytokine and pre-formed mediator release both contribute to the inflammatory infiltrate and perpetuate the cycle of inflammation through ongoing

recruitment and activation of inflammatory cells (Linzer 2007). T lymphocytes likely play an orchestrating role in the late allergy response, and contribute to airway remodelling (Mims 2015; Linzer 2007).

The abnormal T_H2 immune response, and in particular the increased expression of IL-13 within the lungs, initiates an aberrant airway repair process (Bonsignore et al. 2015). Some of the major effects of IL-13 include increased bronchial hyper- responsiveness (through the effects of inflammatory mediators on the local autonomic nervous system (Linzer 2007)), activation of fibroblasts and increased goblet cell differentiation (Bonsignore et al. 2015). The epithelium of the airway becomes fragile, and thickening of the basement membranes, in conjunction with increased production of mucous (as well as changes in mucous consistency) and endothelial leakage, lead to mucosal oedema (Linzer 2007). In the presence of chronic inflammation, these processes contribute to airway remodeling.

Allergic Rhinitis

Under normal conditions, the nasal mucosa efficiently warms, humidifies and cleans inspired air (Skoner 2001). In allergic rhinitis, the normal mechanisms that contribute to this efficient process are derailed, leading to symptoms of watery rhinorrhoea, itch, nasal congestion and sneezing (Min 2010).

The allergic rhinitis response can be divided into early and late phases. In the immediate phase, release of pre-formed and newly synthesised inflammatory mediators from mast cells cause mucosal oedema and watery rhinorrhoea (Skoner 2001). Glandular secretion of mucoglycoconjugates and antimicrobial compounds, in addition to blood vessel dilatation leading to sinusoidal filling, result in occlusion and congestion of the nasal passages (Skoner 2001). The inflammatory mediators additionally stimulate sensory nerves to cause symptoms of itch and nasal congestion, and stimulate reflex responses such as sneezing (Skoner 2001).

In contrast, the late response is characterised by eosinophil chemotaxis (Min

2010). Chemoattractants such as IL-5 contribute to the mucosal infiltration with eosinophils, neutrophils, basophils, T lymphocytes, and macrophages (Skoner 2001). These cells become activated, and release inflammatory mediators, which in turn reactivate the pro-inflammatory cytokines of the immediate phase (Skoner 2001), leading to a cycle of ongoing inflammation. T lymphocytes, again, are the likely orchestrators of the process (Skoner 2001). In addition, priming of the allergic response results in both a decreased threshold for the quantity of allergen required to result in a response (Skoner 2001), and in non-specific hyper-responsiveness, resulting in an 'allergic' response to non-allergen triggers in an IgE-independent manner (Min 2010).

Atopic Eczema

An intact epidermis is a prerequisite for skin to function as a normal physical and chemical barrier (Bieber 2008). Alteration of this barrier leads to transepidermal water loss, which is the hallmark of atopic eczema (Bieber 2008). The central pathophysiological features of atopic eczema are chronic, relapsing skin inflammation and disturbance of the epidermal barrier function (Bieber 2008). The causes of these features are not yet fully understood, but evidence suggests a broad range of environmental modifiers affecting predominantly genetically susceptible individuals (Lyons et al. 2015). The changes described above lead to dry skin and, in some atopic eczema sufferers, IgE-mediated sensitisation to food, environmental, bacterial or even self-antigens (Bieber 2008).

Two hypotheses predominate regarding eczema formation. The first is that a primary defect in the epithelium leads to skin barrier dysfunction, with skin immune dysfunction occurring as an epiphenomenon. Alternatively, a primary disturbance in the local immune system may cause IgE-mediated sensitisation and inflammation, which then leads to barrier dysfunction (Bieber 2008; Kang & Stevens 2003).

As a contribution to skin barrier dysfunction, mutations in the filaggrin gene have garnered much recent attention in the literature. Filaggrin contributes to the keratin cytoskeleton by acting as a template for the cornified envelope

(Bieber 2008; Lyons et al. 2015). In addition, filaggrin breakdown products contribute to the water binding capacity of the epidermis (Bieber 2008). Genetic mutations in the filaggrin gene cause various changes in the functional product, leading to barrier dysfunction (Kang & Stevens 2003). In addition, skin inflammation can affect filaggrin expression (Bieber 2008). However, it appears that filaggrin gene mutations alone are insufficient to explain the development of atopic eczema in all patients. Mutations are only present in about 30% of adult patients with atopic eczema in European studies (Bieber 2008), and have not consistently been found in Black African populations (Thawer-Esmail et al. 2014). Mutations in the genes for other structural skin proteins may also be relevant in skin barrier dysfunction (Lyons et al. 2015).

Epithelial cells exist at the interface of the skin and the environment, and thus are the first line of defence against environmental pathogens (Bieber 2008). They are equipped with several structures to detect microbes and their components, including toll-like receptors and C-type leptins (Bieber 2008), which, when activated, cause production of antimicrobial molecules such as defensins and cathelicidins (Bieber 2008). In atopic eczema, the inflammatory response initiated by, amongst others, IL- 4 and IL-13, downregulate these antimicrobial products (Bieber 2008; Lyons et al. 2015). This leads to increased susceptibility to colonisation and infection by pathogens. More than 90% of atopic eczema patients are colonised by *Staphylococcus aureus*, which promotes ongoing inflammation through a variety of mechanisms (Kang & Stevens 2003), and contributes to topical corticosteroid resistance (Bieber 2008).

In patients with early-onset atopic eczema, skin lesions appear before IgE-mediated sensitisation (Bieber 2008), leading us to believe that in these patients, the skin is the primary site of sensitisation. The skin contains double the number of T lymphocytes in comparison with the circulation (Bieber 2008), which shows that severe skin inflammation is capable of affecting systemic adaptive immunity (Bieber 2008). In severe atopic eczema, IgE antibodies are formed not only against allergens, but also against skin proteins (Kang & Stevens 2003), and their levels correlate with disease activity (Bieber

2008).

IgE-mediated Food Allergy

Whilst this review has previously discussed the topic of oral tolerance, it is worthwhile re-iterating that the physical and immunologic components of the gastro- intestinal mucosal barrier are faced with the daunting task of processing ingested food into an absorbable form, whilst simultaneously preventing pathogen penetration into the body (Sicherer & Sampson 2010). In addition, whilst the systemic immune system is generally confronted by small amounts of foreign antigens, and is able to mount a rapid inflammatory response, the mucosal immune system is regularly confronted with massive amounts of antigens, and must suppress or regulate immune responses to food and 'harmless' commensal organisms (Sicherer & Sampson 2010). The inability to develop oral tolerance is thought to result from a combination of host factors, the dose and timing of antigen exposure and the microbial micromilieu in which the exposure takes place, as well as certain specific properties of the allergens themselves (Vickery et al. 2011).

The increased prevalence of food allergies in young children relative to adults suggests that immaturity of the mucosal barrier may be a factor in the development of food allergies (Wang & Sampson 2009). Variation in the rates of susceptibility to allergens amongst different mouse strains also suggests that genetic predisposition to food allergy is likely (Wang & Sampson 2009). The intestinal immune system in young children is relatively immature, showing decreased expression of certain enzymes, and decreased functionality of the secretory IgA system (Sicherer & Sampson 2010) as examples. There is also some evidence that physical barrier properties may be deficient in children with food allergies. Studies have shown that decreases in gastric acidity are linked with higher sensitisation rates (Sicherer & Sampson 2010), and there is increasing evidence that any disturbance of the mucosal barrier may lead to increased susceptibility to food allergy (Wang & Sampson 2009).

Mucosal immune responses to soluble proteins in early life tend to be T_H2 biased in both humans and other mammals (Vickery et al. 2011). There is some

evidence that genetic predisposition leads to an exaggeration of this bias (Vickery et al. 2011), but evidence also suggests that IgE antibodies to egg, milk and peanut occur commonly, even in healthy infants (Vickery et al. 2011). In healthy infants, this T_H2 weighted response seems to be transient, with IgE levels falling over time, in contrast to the persistence seen in allergic children (Vickery et al. 2011). The immune response to foreign antigens also seems to differ, at least in part, on the dose of exposure. The response to high-dose exposure involves deletion of effector T lymphocytes, whereas low-dose exposure results in activation of regulatory T cells, with suppressor functions (Sicherer & Sampson 2010). Although gut immaturity is thought to be a factor in the development of food allergy, exposure in the correct context may be beneficial at an early age. In Israel, where children are commonly fed peanut proteins at an early age, the prevalence of peanut allergy appears to be much lower than in the western world (Du Toit et al. 2008). The number, type and variety of the enteric flora also seem to play a role in food allergy. In humans, specific differences in the composition of intestinal microbes have been noted between allergic and non- allergic children (Vickery et al. 2011). This area would benefit from further research.

Relatively few food proteins account for the vast majority of food allergy (Radauer & Breiteneder 2007; Sicherer & Sampson 2010), suggesting that major food allergens may share specific properties which render them more allergenic than other food proteins. These properties include the fact that they are all water-soluble glycoproteins, relatively similar in size (10-70kd in size), and relatively stable against denaturing by heat, acid or proteases (Sicherer & Sampson 2010). Processing of food proteins also seems to play a role in their allergenicity. Heating or baking of egg and milk render them tolerable to most egg- or milk-allergic patients (Wang & Sampson 2009), whereas roasting peanuts increases their allergenicity, likely through a Maillard reaction (Wang & Sampson 2009). The carbohydrate moiety attached to an allergen may also play a role in the immune response to the allergen (Sicherer & Sampson 2010; Wang & Sampson 2009).

Allergic disease is therefore the result of a complex interplay between host and environment. The various components of our immune system are highly evolved in their ability to encounter large quantities of foreign antigen on a daily basis, and differentiate pathological from harmless. Abnormalities in the way these antigens are detected, processed and responded to, result in the formation of allergy. Although an increasing variety of allergic conditions are known, many share common pathophysiological mechanisms. Genetic predisposition, in hand with environment exposure and possibly our interaction with our microbiome, all play a role in the formation of allergic disease. Although we know more about the aetiology of allergy than ever before, many questions still require answering before further impacts can be made in the treatment and prevention of allergic disease.

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BURDEN OF ALLERGIC DISEASE

Allergic disease represents one of the most common set of illnesses worldwide, and has increasingly become a global health problem (Mallol et al. 2013; Pawankar et al. 2008). The combined impact of allergic disease on morbidity (Mallol et al. 2013; Ait-Khaled et al. 2007; Lai et al. 2009; Williams et al. 1999) and mortality (Lozano et al. 2012) is profound, and increasing attention is being paid to the quality of life of people who suffer from allergic illnesses. As the burden of communicable diseases gradually decreases (Lozano et al. 2012), the impact of non-communicable diseases comprises a larger proportion of overall mortality and morbidity, and deserves more attention in efforts to promote and improve health.

Allergic disease affects people of all socioeconomic and ethnic backgrounds (Mallol et al. 2013), although there are still significant differences in prevalence and severity between countries (Partridge 2009). The prevalence of allergic disease is rising in many countries, whilst in others is is plateauing or even decreasing (Mallol et al. 2013; Asher et al. 2006). The reasons for these changes are not yet known, and are directions for further research.

In contrast to the developed world, the significant burden of infectious diseases, combined with the relative scarcity of resources, has meant that asthma and allergic disease has traditionally not been a priority for governments, public health officials or researchers in the developing world (Zar et al. 2007). Much of the prevalence data for allergic illnesses comes from the developed world, although there have been many admirable recent efforts in South Africa to rectify this situation (Zar et al. 2007; Gray et al. 2014; Gray et al. 2015; Kung et al. 2014; Basera et al. 2015).

Initially, data from South Africa suggested that the prevalence of asthma was much lower than in the developed world (Van Niekerk et al. 1979), and that there was a significant gradient in prevalence between urban and rural children. More recent data (Zar et al. 2007) has shown prevalence figures comparable

with the developed world, and there is some evidence that suggests that the urban-rural divide has narrowed (Steinman et al. 2003; Levin et al. 2011). The increase in prevalence of allergic disease, particularly in rural communities, is hypothesised to be related to the adoption of a more western lifestyle (Weinberg 2000).

Unfortunately, diagnosis and management of allergic conditions still face many challenges in South Africa. Despite the excellent quality of local guidelines and protocols, uptake of these guidelines remains poor (Reddel et al. 2015). Asthma, in particular, remains a serious threat to public health, with data suggesting not only that our prevalence is still increasing rapidly, but that our mortality rate is one of the highest in the world (Green et al. 2007). Research from the United States of America suggests that African Americans have not only a higher prevalence of asthma than their Caucasian counterparts, but unacceptable and disproportionately high rates of hospitalisation and mortality (Gupta et al. 2006). There is no recent South African literature that has looked at the influence of race, specifically, on allergy morbidity or mortality. Rectifying the morbidity and mortality associated with allergic disease will require a concerted effort from government, public health officials, health practitioners and researchers.

Asthma

Asthma remains the best studied disease within the spectrum of allergy. With a global prevalence of 14.1% in 13-14 year old children (Mallol et al. 2013) and 11.4% in 6-7 year old children (Mallol et al. 2013), it is one of the most common severe childhood illnesses worldwide. In 2010, almost 350 000 people worldwide (Lozano et al. 2012) were believed to have died from asthma, with an age- standardised death rate of 5.2 per 100 000 (Lozano et al. 2012). Apart from mortality, asthma sufferers experience significant morbidity, including hospital admission, absence from school or work, and significant limitation of activity (Green et al. 2007).

The prevalence of asthma amongst South African children is increasing (Zar et al. 2007), in contrast to the situation in some parts of the developed world,

where prevalence figures seem to be stabilising (Asher et al. 2006). Early South African data suggested that asthma was uncommon amongst Black African children. Van Niekerk et. al. (1979) found a prevalence of exercise-induced bronchospasm of 3.17% and 0.14% for urban and rural Xhosa children respectively, in their study. At the time, the prevalence of asthma in the urban population was not significantly different from other data published in the developed world, and the authors postulated the low prevalence of asthma amongst rural Xhosa children to be due to environmental determinants rather than any genetic variation due to ethnicity. However a study in 2003 found a prevalence of bronchial hyper-responsiveness (based on histamine challenge) of 34.4% and 17% for similar populations (Steinman et al. 2003). Additional data has confirmed increasing rates of asthma amongst urban and rural Black African individuals (Mashalane et al. 2006; Calvert & Burney 2010; Levin et al. 2011; Steinman et al. 2003), and shows a narrowing of the urban-rural gradient (Steinman et al. 2003; Calvert & Burney 2010; Levin et al. 2011). Unlike in the developed world, the relationship between atopy and asthma is not well established in South Africa, and the developing world in general (Abbott et al. 2013). This merits further research, as it is possible that different pathophysiological mechanisms are at play in comparison with those described in the literature originating from the developed world.

The increased prevalence of asthma in South Africa is theorized to have occurred through increased urbanisation and adoption of a western lifestyle (Zar et al. 2007). It is not yet clear what aspects of urban life lead to an increased susceptibility to asthma, but possibilities include parental smoking, house dust mite exposure, decline in exposure to infectious diseases, differences in the pattern of gut microbial colonisation, exposure to domestic fuels, exposure to industrial pollution, dietary changes and overcrowding, amongst others (Weinberg 2000; Zar et al. 2007). Of concern is the relationship between socioeconomic status and asthma. Recent evidence (Poyser et al. 2002) has shown that asthma prevalence is directly proportional to socioeconomic status in South Africa. Conversely, the prevalence of severe symptoms seems to have an inverse relationship with socioeconomic status (Poyser et al. 2002), implying that people with limited resources bear the brunt

of asthma morbidity.

In addition to the problem of increasing asthma prevalence, children in the developing world in general, and South Africa in specific, seem to have more severe asthma (Poyser, Nelson, et al. 2002), as well as higher mortality (Beran et al. 2015). In the Global Burden of Asthma report, South Africa was ranked 25th in terms of asthma prevalence, but fourth in asthma case fatalities and fifth in case fatality rates (18.5 per 100 000 asthmatics) (Zar et al. 2007). Local data (Green et al. 2007) suggests that a high proportion of asthmatics suffer regular activity limitation and night symptoms, with more than half missing a day of school or work in the past 12 months, and 16% being admitted to hospital in the past year. Possible explanations for the high case fatality and frequency of severe symptoms include inadequate diagnosis, inadequate and inequitable access to care, poor patient understanding of the mechanisms and severity of their illness and poor socioeconomic conditions leading to an overall poorer quality of health (Beran et al. 2015; Poyser et al. 2002). Genetic predisposition to asthma severity on a racial basis has been suggested, but requires further research (Barnes et al. 2007).

Many challenges will need to be met in order to improve these statistics. These include, but are not limited to, improved strategies for a coordinated approach to asthma as a public health problem, improved uptake of international and local guidelines in the diagnosis and management of asthma, better access to care, negotiation with pharmaceutical companies to provide cost-effective treatments to people who need them, and interrogation of some of the modifiable root causes of poor health (Bateman et al. 2008; Beran et al. 2015). These root causes include improvement of socioeconomic conditions and overcrowding, as well as perceptions of and attitudes towards health (Beran et al. 2015).

Allergic Rhinitis

Allergic rhinitis is perhaps the most common chronic respiratory illness globally (Pawankar et al. 2008). The prevalence of allergic rhinitis has been estimated at 14.6% and 8.5% amongst 13-14 year old children and 6-7 year old

children respectively (Mallol et al. 2013), and it is estimated to occur in about 500 million people (Ozdoganoglu & Songu 2012). Like many other allergic disorders, the prevalence of allergic rhinitis seems to be increasing (Asher et al. 2006), and there is wide variation in the prevalence data between countries, from 1% to 45% in 13-14 year old children in ISAAC (International Study of Asthma and Allergies in Childhood) (Aït-Khaled et al. 2009). Similarly to asthma, the prevalence of allergic rhinitis seems to be higher in higher income countries, but the prevalence of severe symptoms is higher in less affluent countries (Aït-Khaled et al. 2009). The reasons for the wide variation in prevalence are not yet known, and represent directions for further research. Although, in isolation, allergic rhinitis is not associated with mortality, sufferers experience significant morbidity and decreased quality of life (Ozdoganoglu & Songu 2012). People with allergic rhinitis frequently experience comorbidity with allergic conjunctivitis, asthma and eczema (Asher et al. 2012).

Until recently, large-scale estimates of prevalence and morbidity in South Africa were lacking. Phase I and phase III of ISAAC have shown a very high prevalence (30.4% in 1995 and 38.5% in 2002) and suggest that the prevalence in increasing (Zar et al. 2007). Further analysis of the ISAAC phase I data from South Africa suggests an increase in allergic rhinitis with increasing socioeconomic status (Mercer et al. 2004), but noted potential confounding due to difficulty in standardization of self-reports of allergic rhinitis. In addition, pupils from low socioeconomic backgrounds who attended traditionally higher socioeconomic schooling showed increased susceptibility to some rhinitis symptoms (Mercer et al. 2004). It is likely that similar factors play a role in the increasing prevalence and socioeconomic discriminators that are seen with regards to allergic rhinitis, when compared with asthma statistics (Zar et al. 2007). Further research is needed to better quantify the burden of disease, morbidity and cost of allergic rhinitis in a South African context.

IgE-mediated Food Allergy

The global prevalence of IgE-mediated food allergy ranges from 1-10% depending on the geographical area and patient age (Gray et al. 2014).

Gathering accurate prevalence data for food allergy has been difficult because many studies have relied on self- or parent-reported symptoms, and relatively few studies have used oral food challenge tests (which is considered to be the gold standard diagnostic test) (Prescott & Allen 2011). Even tests using oral food challenge as a diagnostic criterion have suffered from poor challenge participation rates (Prescott & Allen 2011). Prevalence of food allergy tends to lag behind other allergic diseases in the developed world (Kung et al. 2014), and evidence is emerging that suggests an increase in food allergies in these locations (Kung et al. 2014). This has led to the description of food allergy as "the second wave" of the allergy epidemic (Prescott & Allen 2011). There is good evidence from the developed world that food allergy causes significant morbidity, and severely impacts quality of life for patients and their families (Walkner et al. 2015). Of concern, children in the current generation seem to be less likely to outgrow food allergy than their predecessors (Prescott & Allen 2011).

Food allergy was unfortunately not included in ISAAC, and there is very little local literature regarding prevalence and morbidity. Recently, the South African Food sensitisation and Food Allergy (SAFFA) study has published its interim results (Basera et al. 2015). The trial utilises robust methodology, using SPTs and a history of reaction to major food allergens to assess sensitisation, and correlating the prevalence of food sensitisation with proven food allergy by means of a rigorous open incremental oral food challenge. In their interim results, they report a prevalence of sensitization to any food allergen of 9.6% (wheal size ≥ 3mm larger than control), and a prevalence of challenge-proven IgE-mediated food allergy of 2.5% (Basera et al. 2015). The authors note that the prevalence of sensitization to at least one food is higher than previously reported in Africa, but that the prevalence of challenge-proven IgE-mediated food allergy remains on the lower end of the international spectrum (Basera et al. 2015). Whilst the final results of the SAFFA study are yet to be published, it is expected to provide interesting and important insights into the burden of food allergy in South Africa. Local literature with regards to food-induced anaphylaxis is restricted to case reports, and accurate statistics regarding prevalence do not, as yet, exist.

Atopic Eczema

According to ISAAC, the global prevalence of atopic eczema is 7.3% and 7.9% for 13-14 year old children and 6-7 year old children respectively (Mallol et al. 2013). There is, once again, a wide variation in prevalence between countries (from <1% in Albania to over 17% in Nigeria in the 13-14 year old age group) (Williams et al. 1999), and even within countries (Mallol et al. 2013). Atopic eczema has been shown to have significant physical, psychosocial and emotional effects on children (Carroll et al. 2005), as well as significant emotional and financial effects on families (Carroll et al. 2005). Patients with atopic eczema frequently report lower quality of life than controls or people with other chronic skin conditions (Carroll et al. 2005; Lewis-Jones 2005).

ISAAC has, again, provided the most robust data regarding the prevalence of atopic eczema in South Africa. The trend towards increasing prevalence was consistent with those of asthma and allergic rhinitis (Zar et al. 2007). ISAAC showed an increase in prevalence from 11.8% to 19.4% from phase I to phase III (Zar et al. 2007), and the study also gave some estimate of impact on quality of life, showing an increase in the prevalence of sleep disturbance from 8.4% to 15.7%. Again, further research into the burden of disease of atopic eczema in South Africa is urgently awaited.

In summation, allergic disease is a global health problem of increasing proportions. Allergic conditions contribute significantly to global morbidity and mortality, and have significant effects on the quality of life of sufferers and their families. Recent large-scale international trials and collaborations have provided new insights into the magnitude of the burden of allergic disease on society, and provide insight into directions for further research.

The burden of allergic disease in South Africa has not received enough attention, despite significant recent efforts. The prevalence of most common allergic diseases seems to be increasing over the past thirty years, and our morbidity and mortality statistics are alarmingly high. Poverty and inequality

in both socioeconomic conditions and access to quality care likely contribute to the high morbidity and mortality rates of asthma, and it is reasonable to suggest that they impact morbidity and quality of life for patients suffering from other allergic conditions. There is an urgent need for further research, as well as for a coordinated approach towards caring for South Africans affected by allergic disease.

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GENETICS AND ALLERGY

Although the evidence of a genetic basis for asthma and allergic disease is well established (Los et al. 2001; Barnes 2010a; Dávila et al. 2009; Hong et al. 2009), much work remains to be done in both discovering which genes are involved and evaluating the nature of the relationship between genotype and phenotype (Vercelli 2008). As previously stated, improved understanding of disease phenotypes and endotypes will hopefully yield improved results in genotype-phenotype correlation research. Modern genetics has, over the past thirty years, allowed the complete location and characterisation of many monogenic illnesses (Lander & Schork 1994), however the reality is that most human illnesses occur as a result of a complex interplay between multiple genes as well as environmental factors (Lander & Schork 1994). In the study of polygenic illnesses, a thorough understanding of the nature of genetic inheritance, and the potential interactions between alleles, the genes on which they are found, and the environment, is essential to ensure that research is accurate and reproducible (Lander & Kruglyak 1995). Novel techniques, including genome-wide association studies (GWAS), work hand-in-hand with more robust methods of statistical analysis, as well as advances in immunopathology. These methods have allowed us not only an improved understanding of the genetics of complex illnesses, but also to better define clinical and laboratory phenotypes, allowing for more accurate characterization of patients and their illnesses, and guiding further research (Meyers 2010). Nonetheless, much work remains to be done in this rapidly evolving field.

Genetics and Inheritance

The most common form of genetic variation in the human genome is the single-nucleotide polymorphism (SNP) (International HapMap Consortium 2003), which refers to the variation, between individuals, of single alleles at any given locus. It is estimated that about 11 million SNPs with a frequency of both alleles of ≥ 1% occur in the human genome (Kruglyak & Nickerson 2001),

accounting for >90% of the genetic variation in the population (International HapMap Consortium 2003). As the mutation rate is very low relative to the number of generations since the most recent common ancestor of any two people, nearly every SNP site results from a single mutation event (International HapMap Consortium 2003). A few (generally uncommon) illnesses result from single mutations, and are referred to as monogenic (Rosenwasser & Borish 1997). This group of illnesses includes cystic fibrosis, Huntington's Chorea and Tuberous Sclerosis, amongst others. More commonly, disease results from variations in multiple genes, and the complex interactions that result between these genes and the environment (Rosenwasser & Borish 1997).

Meiosis, Haplotypes and Linkage Disequilibrium

During meiosis, pairs of homologous chromatids (derived, respectively, from paternal and maternal chromosomes) exchange genetic material in a process known as meiotic recombination (Collins 2009). This process results in the gamete (sperm or oocyte) receiving a recombined chromosome consisting of maternally and paternally derived genetic material (Collins 2009). By using certain informative polymorphisms as markers, it is possible to derive the location of meiotic chromosome breaks (Collins 2009). It is then possible to estimate the relative distance from the marker SNP to a potential disease-associated SNP by assessing the degree of recombination between the two SNPs (International HapMap Consortium 2003). The closer together the two polymorphisms, the less likely they are to recombine freely (International HapMap Consortium 2003).

A specific set of alleles observed on a single chromosome (or part of a chromosome) is called a haplotype (International HapMap Consortium 2003). New haplotypes can be formed by mutations, or by recombination of maternal and paternal genetic material during meiosis (International HapMap Consortium 2003). This recombination seems to occur more frequently in certain regions of any given chromosome than in other regions, leading to the creation of 'haplotype blocks' (Pääbo 2003).

Linkage disequilibrium refers to the coinheritance of alleles at different loci, leading to associations between these alleles in the population (International HapMap Consortium 2003). Although the strength of association (or degree of linkage disequilibrium) between the two alleles depends on several factors, the strongest determinant of this association is the physical distance between them (Collins 2009). Association studies between marker and disease SNPs can therefore be used as a powerful tool in mapping the physical location of potential disease-causing alleles (Collins 2009).

Conversely, a study which aims to investigate a relationship between a SNP believed to play a role in the formation of a specific illness may be affected by confounding if linkage is not adequately investigated (Aissani 2014). Fortunately, the impact of confounding by linkage disequilibrium on association studies of common conditions may be small if the gene under investigation has only a small to moderate effect on the disease in question (Aissani 2014), as is the case for many common polygenic diseases.

Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium can be defined as "…the state of the genotypic frequency of two alleles of one autosomal gene locus after one discrete generation of random mating in an indefinitely large population." (Mayo 2008). If the two alleles are *A* and *a*, with frequencies *p* and *q* (=1-*p*), then the frequencies for the genotypes *AA*, *Aa*, and *aa* are p^2, $2pq$ and q^2 respectively (Mayo 2008). The central tenet of Hardy-Weinberg equilibrium is that a population that is sufficiently large, and continues to mate at random, will maintain these allelic and genotypic frequencies in the absence of external forces, including natural and artificial selection, mutation, non-random mating, migration and inbreeding (Mayo 2008). In reference to association testing, evaluating SNPs for Hardy-Weinberg equilibrium assists us in determining that our sample population is in equilibrium for that specific SNP, and helps us to identify problems in sampling stratification or in genotyping (Wigginton et al. 2005). Testing SNPs for Hardy-Weinberg equilibrium is important, as deviations may indicate significant problems in the data set (Salanti et al.

2005). In contrast, in other genetic association studies, deviation from Hardy-Weinberg equilibrium may allow inferences that the SNP in question is associated with the disease under investigation (Salanti et al. 2005).

Methods of associating genes with disease

Much of our initial understanding of the genetic inheritance of asthma and allergic disease came from large family and twin studies (Los et al. 2001). The complexity of inheritance of asthma and allergy can be attributed in part to many factors, including: that their pathogenesis is determined by many different genes (polygenic), different sets of genes interact in different families and racial groups (genetic heterogeneity) and that the same gene or set of genes can cause multiple different traits (pleiotropy) (Los et al. 2001).

There has been a rapid increase in the amount of information on polymorphisms in human genes (Daly & Day 2001). The human genome is highly polymorphic, with polymorphisms occurring on average once every fifty to one hundred base pairs (Daly & Day 2001). Some polymorphisms have a significant functional effect on the gene product, whilst others are useful as markers (Daly & Day 2001).

Multiple approaches have been used in the investigation of the inheritance of allergic diseases, and a few bear specific mentioning. Candidate gene analysis involves identifying genes which have a plausible physiological basis for conferring susceptibility to the disease in question (Vercelli 2008). Allele or genotype frequencies can then be compared between unrelated cases and controls (Vercelli 2008). Linkage analysis focuses on families that contain individuals who have the disease in question. The analysis involves genotyping family members with evenly spaced genetic markers, looking for areas that show a higher than expected amount of shared alleles among affected individuals (Vercelli 2008). This can be followed by positional cloning to narrow down the area of interest to a specific polymorphism (Vercelli 2008; Binia & Kabesch 2012).

In the past ten to fifteen years, the ability to identify and map an enormous number of SNPs and the concurrent advances in genotyping technology has led to the adoption of GWAS as the method of choice for investigating genetic disorders with complex inheritance models (Lockett & Holloway 2013). This powerful tool involves the simultaneous examination of hundreds of thousands of known SNPs in unrelated cases and controls (Willis-Owen et al. 2009), and is an excellent test for polymorphisms that confer small or moderate risk toward the disease under investigation (Vercelli 2008; Zhang et al. 2012; Lockett & Holloway 2013). Advantages of GWAS include that they can assess the entire genome, and that they have the ability to detect polymorphisms with relatively small effect size (Willis-Owen et al. 2009). Using GWAS, researchers have been able to both confirm associations between previously implicated genes and allergic disease, and uncover previously undiscovered regions of interest (Zhang et al. 2012). An excellent example of this is the association of the transmembrane protein ORMDL3 with childhood asthma (Moffatt et al. 2007) in an early GWAS.

Gene-environment interactions

The rapid increase in the global prevalence of asthma and allergic disease in the past two to three decades is considered to have occurred too quickly to be explained by changes in the genome sequence alone (Tan et al. 2012; Lee et al. 2015). It is hypothesised, but not yet proven, that this increase is occurring mostly in people at genetic risk of allergic disease (Tan et al. 2012). The most likely causes of this rapid increase are environmental factors (Blumenthal 2012). There is increasing evidence that the interaction between genetic and environmental factors may play an important role in the pathogenesis of aetiologically complex disease such as asthma (Vercelli 2004). The relationship between these interactions and the pathogenesis of allergic disease is complex, and non-linear (Vercelli 2004), meaning that the same genotype may be associated with the opposite phenotype in different environments (Vercelli 2004).

Environmental exposures have been linked epidemiologically with the development of asthma and allergic disease (Martino 2011). Furthermore,

microbial burden, environmental pollution and dietary changes have all been shown to affect gene expression and immune function (Martino 2011). The study of epigenetics provides us with a mechanism by which to explain the impact of these exposures on gene expression. Variation in gene expression is the primary reason that cells of multicellular organisms differ significantly in structure and function, whilst being genetically identical (Vercelli 2004). Many of these differences occur during development, especially in-utero (Prescott & Saffery 2011), and are retained during mitosis (Vercelli 2004). Although they do not involve mutations of the DNA itself, and are therefore reversible, they are heritable in the short term (Vercelli 2004).

These changes are referred to as epigenetic, and include histone methylation and acetylation, as well as DNA methylation, amongst others (Martino 2011). Epigenetic activation of a silent gene, or conversely silencing an active gene, may cause differing phenotypic effects in individuals with an identical genotype (Blumenthal 2012).

Several studies have looked at epigenetic effects on immune regulation in allergic disease (Prescott & Saffery 2011). Of particular interest is the influence of epigenetic modification on the differentiation of naïve T-helper cells (T_H0) into either T_H1 or T_H2 cells (Harb & Renz 2015). Although evidence for the effect of epigenetic modification on the development of allergic disease is accumulating, much still needs to be understood about their exact mechanisms (Harb & Renz 2015).

Gene-gene interactions

Although the fact that genetic interaction plays a role in determining phenotype is beyond question (Steen 2012), quantifying the effect in specific disease states has proven to be highly complex. One of the major obstacles has been in the very definitions of ‘gene-gene interaction’ and ‘epistasis’, two often interchangeably-used terms in genetics (Wang et al. 2010). In addition, there is little agreement in the literature with regards to how to delineate genuine gene-gene interaction, which in itself is relatively uncommon, from interactions between gene products (Wang et al. 2010). The more complex a

specie's genome, the greater the number of gene-gene interactions. This means that possibly the major limitation of comprehensive analyses of genetic interactions is the sheer number of these potential interactions (Phillips 2008). The effects of a polymorphism at a specific locus may be obscured by interactions with other loci (Phillips 2008), making mapping very difficult.

Despite these obstacles, advances in genetics, high throughput functional genomics, statistical regression analysis as well as systems biology have made it possible for the first time for us to examine gene-gene interactions in a unified and functional manner (Phillips 2008; Cordell 2009). Several studies have looked at associations between different SNPs and asthma or allergic disease, with interesting results (Godava et al. 2013; Wang et al. 2013; Choi et al. 2012), however more work needs to be done to elucidate the exact nature of gene-gene interactions in allergic disease.

South African Literature on Genetics and Allergy

The burden of infectious disease, as well as the previously prohibitive cost of genotyping, has precluded thorough study of the association between genetic polymorphisms and allergic disease in South Africa. The local literature is restricted to very few studies, with most reporting very small sample sizes. One example involved the description and characterisation of a new SNP in a child with asthma, which was also found to exist in his mother. The subject SNP is on chromosome 14q in the gene coding for α_1-antitrypsin (Pillay et al. 2001; Mahadeva et al. 2001). Another small study investigated the association between two polymorphisms in the β-2 adrenoceptor and atopy (Potter et al. 1993). The study found no significant association, but was too limited in sample size to draw any firm conclusions.

A slightly larger study investigated the relationship between three polymorphisms in the beta chain of the high-affinity immunoglobulin E receptor (Fcε-RIβ) and asthma, with some interesting results (Green et al. 1998). An SNP at position 181, I181L, was significantly associated with asthma in White patients, whilst a different SNP (E237G) was significantly

associated with asthma amongst Black African patients. In other studies, this polymorphism was associated with bronchial hyper- responsiveness, even in the absence of atopy (Green et al. 1998). The authors suggested this polymorphism as a possible explanation for the increased severity of asthma and increased mortality amongst Black African people with asthma. However, their sample size was again a limiting factor.

Variations in Genetics and Allergy Based on Ethnicity

There is strong evidence in the international literature that suggests that asthmatics of Black African origin suffer much higher morbidity and mortality than their Caucasian counterparts (Barnes 2010c; Joseph et al. 2006; Leong et al. 2012). Although asthma and allergy symptom severity has also been shown to be related to socioeconomic status (Lai et al. 2009; Williams et al. 1999), there is increasing data to show that at least part of this variation may be due to genetic variation between ethnic groups (Barnes 2010c). It is not yet known how much of the variation in phenotype is due to genetic discrepancies at the level of ethnicity, and difficulties in unbundling ethnicity (or ancestry) from the social and economic constructs that accompany racial classification make accurate comparisons even more difficult (Leong et al. 2012).

Most of the GWAS data with regards to asthma and allergic disease comes from cohorts of people of European ancestry (Barnes 2010c). The limited GWAS data on people of African or Hispanic origin suggests that different genes may be at play in people of non-European ancestry (Mathias et al. 2010; Barnes 2010c), or even that the same polymorphisms may cause different or opposite effects in people of different ethnicities (Barnes 2010c). On one hand, genetic admixture makes studying the effect of a specific ethnicity on allergy risk more difficult and, on the other, helps us to assess the relative contributions of different ethnicities to allergy risk in a specific individual or population (Barnes 2010b). Although most of this data is related to asthma, similar results have been seen in studies of the filaggrin gene and atopic eczema (Thawer-Esmail et al. 2014). In addition to differences in severity of symptoms, there is some evidence that genetic variation in ethnicity may have therapeutic

implications. In specific, variations in the frequency of SNPs in the ®-2 adrenoceptor may imply a poorer response to β-agonist therapy in people of African or Chinese origin (Xie et al. 1999).

Recent studies from the Americas (Vergara et al. 2013; Flores et al. 2012) confirm African ancestry as an independent risk factor for asthma and raised serum IgE levels, and affirm the need for further research in order to qualify this and similar relationships more fully.

Evidence for Evolutionary Adaptation in Immune Responses

Insufficient research has been performed to quantify the genetic factors that predispose to asthma and allergy in populations other than Caucasians (Barnes 2010c). This notwithstanding, many studies have noted that populations of differing ethnicities have different allele frequencies for many different genes (Le Souëf et al. 2006). Some authors (Le Souëf et al. 2006; Le Souëf et al. 2000) propose evolutionary adaptation in human immune responses as a plausible mechanism for this phenomenon, and it is against this background that our study is being performed. The hypothesis put forward is that populations originating in tropical environments, where they would have frequently encountered helminths and other parasites (Le Souëf et al. 2000), develop an immune system predisposed to mounting a strong T_H2 response. This could conceivably confer a survival advantage in such climates (Le Souëf et al. 2006). Upon migration to colder climates, where parasites and helminths would be much less prevalent (as they rely on heat and humidity to thrive (Stromberg 1997)), the balance between the benefit of this inflammatory immune profile and the risk of having a strong immune response against 'harmless' environmental proteins, shifts significantly (Le Souëf et al. 2000).

This has led to the prediction that people of tropical origin will show a higher prevalence of pro-inflammatory alleles (Le Souëf et al. 2000), and will subsequently have an increased predisposition to inappropriate immune responses (including allergy) if rapidly relocated to temperate areas (Le Souëf et al. 2000). Evidence is thus far insufficient to confirm or refute this

hypothesis.

In conclusion, the genetic basis for allergic disease is undisputed, but the nature of the complex association between multiple genes, as well as environmental influences, remains to be fully explained. As complex illnesses, allergic diseases are likely caused by the relatively small effects of multiple genes. When acting in concert, and in the presence of specific environmental cues, the effect on phenotype can be profound.

Until recently, our ability to examine the genetic causes of allergic disease was hampered by an incomplete understanding of the immunopathology of allergic phenotypes, the inability to sequence large sections of the genome in parallel, and the lack of statistical tools to examine complex interactions with sufficient power and accuracy. Advances in multiple areas of research including genetics, immunology and statistics have enabled us, for the first time, to begin to unravel the nature of the pathway from genotype to phenotype, and the complex interactions that underpin this pathway.

As our ability to examine the genetics of asthma and allergy has increased, the discovery of interactions between genes and the environment, as well as between different genes, has made analysis more complicated. Epigenetic modification of DNA is an understudied but important phenomenon that will help to enhance our understanding of why people with the same genotype may have different allergic phenotypes. Novel techniques are also helping us to begin to understand how gene- gene interactions help relatively minor alterations in the genome to have profound phenotypic effects.

The study of the relationship between genetics and allergic diseases in South Africa, and particularly amongst Black African and mixed race people, remains in its infancy. Hopefully the falling costs of high-throughput genomics, and an increasing interest in novel polymorphisms will help to rectify this situation. The possible influence of evolution on the genetics of asthma and atopy is a relatively novel hypothesis. This study forms a small part of efforts to investigate this further, and of on going efforts to better

understand the genetic aetiology of asthma and allergies amongst people of Black African origin.

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GENETIC POLYMORPHISMS

CD 14

CD14 is a molecule expressed on and secreted by myeloid cells (Vercelli 2002). Membrane-bound and soluble CD14 bind a variety of bacterial products including lipopolysaccharide (LPS) from gram-negative bacteria and lipoteichoic acids from gram-positive bacteria (Vercelli 2002). At a molecular level, CD14 acts by transferring LPS and other ligands to the Toll-like receptor/MD-2 signalling complex. Activation of this complex results in the initiation of innate host defence mechanisms, including release of inflammatory cytokines that are key to promoting a T_H1 response (Vercelli 2002).

A C-T polymorphism at position 159 in the promoter of CD 14 (159T>C) has been postulated to modulate the cellular response to endotoxin (Sackesen et al. 2011). Several studies in different populations have sought to correlate polymorphisms at this position with in-vitro (Sackesen et al. 2011; Baldini et al. 1999; Leung et al. 2003) and in-vivo (Koppelman et al. 2001; Kedda et al. 2005; Lange et al. 2005) responses, many with conflicting results. Even recent meta-analyses (Zhao & Bracken 2011; Zhang et al. 2011; Xu & Wang 2014) have reached differing conclusions about the link between the polymorphism and allergic disease including asthma and allergic rhinitis. Another promoter polymorphism (550C>T) has been investigated in recent studies. The CC genotype has been found to correlate with allergic rhinitis (Zhang et al. 2011), and also with decreased IL-8 levels in workers exposed to dust that contained endotoxin (Rylander & Michel 2005). Although no prior studies have evaluated the prevalence or phenotypic effect of these polymorphisms in Black African populations, in a review of the international literature regarding the 159T>C polymorphism, Zhang et al. (2008) looked at the allele frequencies as well as phenotypic associations in various populations around the world. They found a discrepancy in allele frequencies between different population groups, with people of African descent having the highest prevalence of the C allele, followed by Caucasians and then Asians (Zhang et al. 2008). Their review

underlines the significant discrepancy between studies in terms of associations between either allele and phenotypic features of allergy, which they suggest may be due to methodological concerns, gene-gene or gene-environment interactions, amongst others (Zhang et al. 2008).

ADRB2

The β-2 adrenoceptor is widely distributed in human lung tissue, existing primarily in airway smooth muscle, but also on other cells including epithelial and endothelial cells, type II cells and mast cells(Johnson 1998). The principal effect of β-2 adrenoceptor activation is smooth muscle relaxation and alleviation of bronchoconstriction (Bertics et al. 2003). The β-2 adrenoceptor gene is situated on the long arm of chromosome 5, and codes for an intronless gene of approximately 1200 base pairs (Johnson 2006). The gene is highly polymorphic, containing eighteen SNPs, of which four code for amino acid substitution (Hall & Sayers 2007). Three polymorphisms have been described in detail: 46G>A(Arg16Gly), 79G>C(Gln27Glu) and 491C>T(Thr64Ile) (Hall 1996).

Clinical interest in the β-2 adrenoceptor gene is two-fold. Firstly, there has been much speculation as to whether polymorphisms in this gene alter susceptibility to or severity of asthma. Initially, an inverse relationship between FEV_1 and β-2 adrenoceptor density has been reported (Johnson 1998). However, it is still unclear (despite several studies) exactly what the phenotypic effects of these polymorphisms are (Holloway & Yang 2005). A meta-analysis in 2005 found that neither the Arg16Gly nor Gln27Glu polymorphisms were associated with overall asthma susceptibility or bronchial hyper-responsiveness, but that the Gly16 allele predisposes to nocturnal asthma, and may be associated with asthma severity (Contopoulos-Ioannidis et al. 2005).

Secondly, there is a growing body of evidence to suggest that polymorphisms in the β-2 adrenoceptor gene have important effects in modulating responses to β-agonist therapy for asthma (Johnson 2006), particularly that polymorphisms may affect receptor downregulation in the face of long-term

usage. Initial studies focussed on short-acting β_2 agonists, and a large-scale randomised cross-over study showed that individuals homozygous for the Arg16 allele failed to maintain responses to salbutamol (Israel et al. 2004). Similar large-scale trials were, until recently, lacking for longer-acting agents, with one study suggesting a protective effect against receptor downregulation when a long-acting agent is combined with an inhaled corticosteroid (Bleecker et al. 2006). A recent multi-centre, double-blind, randomised placebo-controlled trial in the USA showed improvement over placebo in peak expiratory flow (PEF) in subjects receiving salmeterol, irrespective of Arg16Gly genotype (Wechsler et al. 2009). There was, however, evidence that Arg/Arg subjects had a significantly higher response (when compared to placebo) to metacholine challenge relative to Gly/Gly subjects. Another question that remains to be answered is whether individuals homozygous for the Arg16 allele should be given an alternative bronchodilator (Hall & Sayers 2007).

There have been no prior studies on the prevalence or phenotypic effects of β-2 adrenoceptor polymorphisms in local populations, and very little published literature worldwide on people of African origin. Almost all the data on people of African origin comes from studies of African-American populations, and it is not yet clear how generalizable that data is to local populations, as they represent a genetically admixed population (Barnes 2010b).

CMA1

Mast cells and their various inflammatory mediators have been implicated in a variety of pathologic features of allergic disease (Hossny et al. 2008; Iwanaga et al. 2004). Multiple mediators are released from mast cells following activation, and among the most abundant is the protease chymase (Iwanaga et al. 2004). Chymase is a 226 amino-acid residue protein that is encoded by a single gene. The enzyme is stored in, and released in fully active form from, mast cell secretory granules (Iwanaga et al. 2004). Although the function of mast cells in allergic inflammation has been extensively described, the role of chymase is not yet fully understood, with research indicating both pro- and anti-inflammatory properties (Sharma et al. 2005). Evidence suggests that

chymase contributes to fibrosis and tissue remodelling by activating procollagenase, releasing transforming growth factor ®1(TGF-®1), and converting angiotensin I to angiotensin II (which it does more efficiently than angiotensin converting enzyme) (Weidinger et al. 2005). Several studies have explored the association between genetic polymorphisms in the CMA1 gene and atopic disease. To date, the evidence has been conflicting, and of insufficient quality to draw any firm conclusions. Two polymorphisms, an SNP in the promoter region of the gene, and a short tandem repeat (STR) downstream of the gene, have been reviewed. Results regarding associations between the SNP and asthma have been inconsistent (Hossny et al. 2008; Iwanaga et al. 2004). Investigation of a large cohort of adults in Switzerland revealed an association between a (TG)n(GA)m repeat and atopic asthma (Hersberger et al. 2010), which was confirmed in smaller studies (Sharma et al. 2005; Hossny et al. 2008).

IL-12

IL-12 has been shown to be an important cytokine in the development of a T_H1 immune response (Hossny et al. 2008). In the presence of IL-12, naïve T-cells differentiate into T_H1 cells, with production of T_H1 cytokines (IL-2, IFN-γ) (Iwanaga et al. 2004) and generation of T_H1 memory cells. IL-12 therefore plays an important role in antagonism of T_H2 differentiation, and in initiating host defence against viral particles (Weidinger et al. 2005). IL-12 is comprised of 2 polypeptide chains that are bound by disulphide bonds. The first, a 35-kd chain, is encoded by *IL12A* on chromosome 3p12-q13.2. The second, a 40-kd chain, is encoded by *IL12B* on chromosome 5q31-33 (Martinez & Holt 1999). The *IL12A* gene has not been noted to have any associations with asthma, allergic rhinitis or atopic dermatitis (Martinez & Holt 1999), however there are several studies which have looked at associations between polymorphisms in the *IL12B* gene and asthma in particular.

Morahan and colleagues (2002) found no association between a promoter polymorphism of *IL12B* and asthma, but their data did suggest a significant association with asthma severity. Heterozygotes were found to be 4.6 times more likely to develop severe asthma. Interestingly, the polymorphism was

associated with both atopic and non-atopic asthma, leading the authors to suggest *IL12B* polymorphisms as a "final common pathway" for progression to severe disease for both forms of asthma. Other studies (Khoo et al. 2004; Randolph et al. 2004) have failed to replicate this data.

IL-13

IL-13 is a T_H2 cytokine which is a critical mediator of the inflammatory process of atopy and asthma (Corren 2013). It shares many functions with IL-4 (Heinzmann et al. 2000), and has several unique functions in addition. Prominent functions of IL-13 include goblet cell differentiation, IgE class switching, fibroblast activation and facilitation of allergen-mediated bronchial hyper-responsiveness (Heinzmann et al. 2000; Corren 2013). The IL-13 gene is located on chromosome 5q31, a locus which has been linked to high serum IgE levels (Xu et al. 2000).

The IL-13 receptor is a heterodimer comprising of IL-4Rα and IL-13Rα1 (Chen et al. 2004). This receptor also acts as an alternate receptor for IL-4, which may partly explain the homologous effects of these two molecules (Chen et al. 2004). Many studies have looked at the effect of IL-13 polymorphisms on asthma and atopy, either in isolation or in conjunction with IL-4 and IL-4R polymorphisms. Two recent meta-analyses showed an association between the IL-13 1112C>T polymorphism and paediatric (Liu et al. 2014), and adult (Wang et al. 2013) asthma respectively. Another meta-analysis (Ying et al. 2012) with a large number of cases and controls was unable to demonstrate an association between the same polymorphism and allergic rhinitis risk. The same authors did, however, find an association between the IL-13 130A>G polymorphism and allergic rhinitis risk (Ying et al. 2013).

IFN-γ and IFN-γR1

Although much focus is given to the contribution of T_H2 cytokines to the development of allergy, an absence or decrease of certain T_H1 cytokines may also play a significant role (Teixeira et al. 2005). IFN-γ is a pleiotropic

cytokine with diverse functions including response to viral, bacterial, mycobacterial and fungal pathogens, modulation of the immune response and even tumour surveillance (Schroder et al. 2004; Young & Hardy 1995; Billiau & Matthys 2009). It is produced by multiple immune cells in response to predominantly IL-12 secretion but also microbial stimuli (Smith & Denning 2014). Initially called Macrophage Activating Factor (MAF), IFN-γ is an important cytokine in the innate and adaptive immune systems. Secretion by antigen presenting cells (APCs), such as those of the monocyte/macrophage lineage as well as dendritic cells, likely causes local effects including self-activation and activation of local cells (Schroder et al. 2004). Early secretion by natural killer (NK) cells and possibly APCs is probably of great importance in initial host defence against infection, whereas T_H2 lymphocytes are the most significant secretor of IFN-γ in the adaptive immune system(Schroder et al. 2004). The IFN-γ receptor (IFNGR) is made up of pairs of ligand-binding chains (IFNGR1) and signal-transduction chains (IFNGR2) (Schroder et al. 2004). Signal transduction is effected predominantly via the Jak-Stat pathway (Subramaniam et al. 2001).

Several authors have explored links between polymorphisms in the IFN-γ gene (located on chromosome 12) or the receptor genes (located on chromosome 6 and 21 respectively (Dorman & Holland 2000)) and allergic disease. A recent meta-analysis showed an association between the AA genotype, at position 874 (874T>A) in the IFN-γ gene, and asthma risk (Nie et al. 2014). This association was present in both Caucasian and Asian subjects.

The evidence for polymorphisms in the IFNGR complex and allergic disease is somewhat more circumstantial. Pathogenic mutations in the IFNGR complex have been associated with, amongst others, mycobacterial infections, and susceptibility to viral infections (Dorman et al. 1999). However, there is limited evidence to suggest an association even between these pathogenic mutations and allergic disease (Wood et al. 2005). Non-pathogenic mutations have been shown to have a modest effect on serum IgE levels in certain populations (Gao et al. 1999), whilst other authors have suggested an

association between the IFN-γR1 mutation IFNGR -56T>C and ocular atopic dermatitis (Matsuda et al. 2007). These associations require further interrogation to determine their reproducibility.

TGF-β

TGF-β was first named for its ability to induce tumour-like growth characteristics in normal cells (Salib & Howarth 2009), but it has subsequently been shown to have a wide array of functions, and to be secreted by a variety of cells (Bossé & Rola- Pleszczynski 2007; Makinde et al. 2007). It forms part of a superfamily of cytokines, and occurs in 3 isoforms, of which TGF-β1 is the most extensively studied (Bossé & Rola-Pleszczynski 2007). TGF-β1 is synthesized as a 390 amino acid prepropeptide (Bossé & Rola-Pleszczynski 2007). Although ubiquitously expressed, the level of expression is subject to extensive transcriptional and post- transcriptional regulation (Bossé & Rola-Pleszczynski 2007; Makinde et al. 2007). In addition, the maturation, expression and activation of the protein are also extensively regulated (Bossé & Rola-Pleszczynski 2007).

TGF-β is predominantly secreted by eosinophils, but is also secreted by many other cells, including macrophages, lymphocytes, fibroblasts, epithelial cells and mast cells (Makinde et al. 2007). It is secreted in an inactive form, and a latency associated peptide must be removed to release the active form (Makinde et al. 2007). Several cytokines, including IL-5, IL-13 and TGF-β itself can induce its production and release, whilst IFN-γ plays an inhibitory role (Makinde et al. 2007). TGF-β mediates its effects via three receptors, namely TGF-βR1, 2 and 3, causing phosphorylation of intracellular serine/threonine kinase domains (Makinde et al. 2007; Bossé & Rola-Pleszczynski 2007). The main pathway by which its effects are mediated is the Smad pathway (Bossé & Rola-Pleszczynski 2007), however other pathways (including MAPK and Jak-Stat) are also activated by TGF-β (Makinde et al. 2007).

TGF-β has many effects, of which several are relevant in allergic inflammation, most notably in the asthmatic airway and in the nasal mucosa in allergic rhinitis. It is a potent chemoattractant of leucocytes, especially mast cells (Salib & Howarth 2009). It also plays an immunoregulatory role, and is thought to impact the interaction between regulatory T cells (Tregs) and $CD4^+$ cells (Salib & Howarth 2009). TGF-β also plays a role in wound healing. At low levels of activation, it induces the deposition of extracellular matrix to facilitate wound healing, however with sustained activation, excessive matrix deposition can lead to scarring and fibrosis (Salib & Howarth 2009).

There is considerable controversy over the role of TGF-β1 in allergic rhinitis (Salib & Howarth 2009) and asthma (Bossé & Rola-Pleszczynski 2007). On balance, there is an accumulating level of evidence that TGF-β1 is upregulated in asthmatic airways, and that there is also upregulation of TGF- β1 signalling pathways (Bossé & Rola-Pleszczynski 2007). It would appear that TGF-β1 plays a role in the initial recruitment of antigen presenting cells (APCs) and mast cells to the airways, and that subsequently TGF-β1 is involved in matrix deposition and airway remodelling (Bossé & Rola-Pleszczynski 2007). Some authors have suggested a relationship between TGF-β1 expression and severe asthma (Al-Alawi et al. 2014). Its role in allergic rhinitis, however, remains unresolved, and the balance between its pro- inflammatory and anti-inflammatory effects has not received enough attention to warrant firm conclusions (Salib & Howarth 2009).

The TGF-β1 gene is located on chromosome 19q13, a region that has been associated with allergic asthma and mite sensitivity (Che et al. 2014). Several studies (Grainger et al. 1999; Hobbs et al. 1998) have suggested an SNP at position 509 (509A>G) as a functional polymorphism, which influences circulating levels of TGF- β1. Some studies (Salam et al. 2007; Pulleyn et al. 2001; Bandaru et al. 2015) have shown an association between the T allele and asthma, whilst others (Heinzmann et al. 2005; MAK et al. 2006) have not been able to establish any association. A recent meta-analysis (Che et al. 2014) suggested an association in people of Asian origin, but not in those of

Caucasian descent.

IL-10

IL-10 is a predominantly anti-inflammatory protein produced by a wide variety of cells (Moore et al. 2001; Sabat et al. 2010). Its primary targets appear to be monocytes and macrophages, although there is evidence of effects on T and B lymphocytes, mast cells, eosinophils and neutrophils, amongst others (Sabat et al. 2010). The gene for IL-10 resides on chromosome 1 (Sabat et al. 2010), and it encodes a 178 amino acid protein which is released by almost all leukocytes (Sabat et al. 2010). Human IL-10 is a homodimer that is comprised of 2 non-covalently bonded monomers (Sabat et al. 2010).

The IL-10 receptor comprises of 2 chains, IL-10R1 and IL-10R2 (Moore et al. 2001). IL-10 signalling is achieved mainly via the Jak-Stat pathway (Moore et al. 2001; Sabat et al. 2010). IL-10 suppresses all pro-inflammatory functions of the monocyte/macrophage system, as well as enhancing the inhibitory, tolerance- inducing, and 'scavenger' functions of these cells (Sabat et al. 2010). IL-10 is thought to play an important role in the development of oral tolerance (Battaglia et al. 2004). Its role in response to respiratory viral infections is rather more complicated. Decreased levels of IL-10 synthesis are thought to be beneficial in the early stages of infection, where high levels of IL-10 have been associated with higher levels of viral infiltration(Zdrenghea et al. 2015), however IL-10 is important in attenuating the inflammatory response in the late stages of infection(Zdrenghea et al. 2015). IL-10 production has been reported as being both increased and reduced in asthmatics, possibly representing different clinical phenotypes of disease (Heaton et al. 2005).

Several polymorphisms in the IL-10 gene have been associated with allergic disease. A recent meta-analysis (Zheng et al. 2014) found that the AA genotype at position 1082, as well as the presence of the A allele at position 592, were both significantly associated with asthma susceptibility. Another meta-analysis (Nie et al. 2012) found a 27% increased asthma risk in people with the AA genotype at position 1082, as well as a significant asthma risk in those with the

A allele at position 592. However, another meta-analysis (Hyun et al. 2013) produced evidence which contradicts the others, in that they found no association between the 592A>C polymorphism, and an increased asthma risk in carriers of the G allele in position 1082.

Associations between other atopic diseases and IL-10 polymorphisms are much less well studied. A small study (Sohn et al. 2007) suggested an association between the A allele at position 592 and blood eosinophil counts in children with atopic dermatitis. In addition, a recent Brazilian study (Jacob et al. 2013) suggested an association between children homozygous for the G allele at position 1082 and persistence of cow's milk protein allergy.

IL-4 and IL-4R

IL-4 is a pleiotropic cytokine, with critical importance in allergic sensitization as well as both the early and late phases of the allergic inflammatory response (Maes et al. 2012). It binds to high affinity receptors on many haematopoietic and non-haematopoietic cells, with wide ranging effects (Maes et al. 2012), the best studied of which include stimulating naïve $CD4^+$ T cells to become T_H2 lymphocytes (Paul 2015), and inducing B cells to undergo isotype class shifting to produce IgE (Paul 2015).

The IL-4 gene, which has been mapped to chromosome 5q31, comprises of 4 exons and can be spliced, resulting in at least 2 protein product isoforms. Variants include either a full-length isoform translated from all 4 exons, or an alternative, IL-4δ2, which is translated from exons 1, 3 and 4 (Luzina et al. 2012). Although their functions seem to overlap, the full significance of this variable splicing remain to be fully studied (Luzina et al. 2012). Two IL-4 receptors exist in man, type I and type II (Paul 2015). Type I receptors comprise of the IL-4Rα chain as well as the IL-2Rγc chain (Paul 2015), whilst type II receptors comprise the IL-4Rα chain as well as the IL-13Rα1 chain (Paul 2015). This receptor homology explains some of the commonality of function between IL-4 and IL-13 (Paul 2015; Luzina et al. 2012; Maes et al. 2012), however the two proteins utilize the type II receptors quite differently,

which explains how they both have unique functions (Paul 2015; Maes et al. 2012). Haematopoietic cells usually express between 20-300 type I receptors per cell (Luzina et al. 2012), however activation of lymphocytes (for example, by bacterial lipopolysaccharide) increases the number of type I receptors by 5-10 fold (Luzina et al. 2012). Binding of IL-4 to type I receptors induces phosphorylation of STAT6 (Paul 2015), which, when activated, translocates to the cell nucleus and influences synthesis of inflammatory mediators (Luzina et al. 2012).

Effects of IL-4 secretion are numerous, and include stimulation of naïve $CD4^+$ T cells to differentiate into T_H2 lymphocy$_t$es, induction of B cell isotype switching to produce IgE. In addition, in allergic airway disease, IL-4 induces recruitment of eosinophils and mast cells into the airway (Maes et al. 2012), and promotes bronchial hyper-responsiveness (Maes et al. 2012). In addition, and in conjunction with IL-13, IL-4 promotes structural airway remodelling (Paul 2015; Maes et al. 2012; Luzina et al. 2012). Various polymorphisms in the IL-4 and IL-4R⟨ gene have been studied in relation to allergic diseases. A recent meta-analysis found an association between the TT genotype at position 589 in the IL-4 gene and asthma in both Caucasian and Asian populations, but not in those of African-American descent (Nie et al. 2013). Similar results in both Asian and Caucasian people, and in adults as well as in children, were found in another meta-analysis (Yang 2013). This meta-analysis also looked at a polymorphism in position 33 of the IL-4 gene, and showed an association between the TT genotype and asthma risk (Yang 2013). Although there is much less literature regarding association between IL-4 polymorphisms and allergic rhinitis, one meta- analysis found weak associations between both of the above polymorphisms and allergic rhinitis risk (Li et al. 2014).

The IL-4R⟨ chain also has several polymorphisms of interest in relation to asthma and allergic disease. Two polymorphisms, 223A>G, which results in an amino acid substitution in position 50 (50Ile(A)/Val(G)) and 1727A>G, resulting in amino acid substitution in position 551 (551Gln(A)/Arg(G)), have been shown to be associated with asthma in a meta-analysis of Han Chinese people (Huang et al. 2015). These findings have been confirmed in another

meta-analysis (Nie et al. 2013), suggesting a dominant inheritance model for 223A>G and recessive for 1727A>G.

IL-8

Interleukin-8 is a potent pro-inflammatory cytokine. It possesses chemoattractant properties, mostly towards neutrophils, but also T-lymphocytes, monocytes and basophils (Harada et al. 1994). It also causes neutrophil activation and release of lysosomal enzymes (Harada et al. 1994) and adhere to endothelial cells. In response to lipopolysaccharide, live bacteria, and other early pro-inflammatory cytokines (such as TNF-〈 and IL-1), IL-8 is synthesized by a wide variety of cells, but principally by the monocyte/macrophage system (Remick 2005). Its effects are mediated via two specific IL-8 receptors, IL-8RA and IL-8RB, members of the "seven spanners" receptor family (so termed because they cross the cell membrane seven times) (Remick 2005). The IL-8 receptor is coupled to a g-protein and signals are transmitted through a g-protein second messenger system (Remick 2005; Cheong et al. 2006).

IL-8 is thought to be an important inflammatory cytokine and chemokine in asthma. Increased IL-8 levels have been found in the sputum of asthmatics prior to an exacerbation (Cheong et al. 2006), and levels in sputum and BAL fluid have been found to correspond to asthma severity (Nakagome et al. 2012; Hosoki et al. 2015). In addition, a recent study correlated IL-8 levels in BAL fluid and FEV_1 in asthmatic patients (Hosoki et al. 2015).

Despite strong evidence that IL-8 is an important cytokine in the inflammatory process of asthma, there is a paucity of literature confirming associations between genetic polymorphisms in the IL-8 gene and allergic disease. A polymorphism in position 781 has been associated with asthma in people of Caucasian origin (Heinzmann et al. 2004), however this data has not been replicated in other studies (Cheong et al. 2006).

STAT6

STAT6 is a member of the signal transducers and activators of transcription (STATs) family (Walford & Doherty 2013). STATs undergo tyrosine phosphorylation by Janus kinases (JAKs) in response to appropriate cytokine exposure (Walford & Doherty 2013). Interaction of IL-4 and IL-13 with the IL-4R⟨ sub-unit results in a common STAT6-mediated signalling pathway that is a central part of the T_H2 immune response (Walford & Doherty 2013). Once phosphorylated, STAT6 is transported to the cell nucleus, where it regulates expression of various genes of importance in the allergic and parasitic immune response (Walford & Doherty 2013; Wurster et al. 2000).

STAT6 is therefore responsible for mediating the many features of the T_H2 allergic inflammatory response initiated by IL-4 and IL-13, depending on the type of cell in which it is acting. In T-lymphocytes, STAT6 is responsible for T_H2 differentiation and proliferation (Goenka & Kaplan 2011). In B-lymphocytes, STAT6 influences IgE and IgG1 isotype switching, and expression of certain cell surface markers necessary for antigen presentation by B cells, thereby directly impacting circulating levels of IgE (Goenka & Kaplan 2011). STAT6 is also an important signaling molecule in other cells, including eosinophils, the monocyte/macrophage system, the mammary glands, lung epithelium and skin (Stokes et al. 2015; Goenka & Kaplan 2011). Evidence for STAT6 being a central driver of allergic inflammation comes from murine models that show that STAT6 deficient mice show greatly attenuated predisposition to developing, amongst others, allergic airway disease, food allergies, atopic dermatitis and eosinophilic oesophagitis (Goenka & Kaplan 2011).

Because of the likely contribution of STAT6 to allergic inflammation, several SNPs have been studied in order to establish an association with allergic disease. The results have been conflicting, with several studies (Amoli et al. 2002; Casaca et al. 2013; Qian et al. 2014; Zhu et al. 2013) showing quite different results. Thus far there is no consensus in the literature regarding STAT6 polymorphisms and allergic disease.

TLR2 and TLR4

Toll-like receptors (TLRs) are a type of pattern recognition receptors (PRRs) that recognize certain evolutionarily conserved molecular patterns common to pathogens, but not to multicellular organisms (Lin 2004; Yang et al. 2006). They are expressed in the cells of the innate immune system (Yang et al. 2006), and interact with certain pathogen associated molecular patterns (PAMPs) such as bacterial endotoxin, lipoteichoic acid, and viral associated double-stranded RNA (dsRNA) (Lin 2004). This interaction causes release of pro-inflammatory cytokines such as TNFα, IFNγ and IL-1,6 and 12 (Yang et al. 2006), causing activation of the innate immune system and direct microbial killing (Lin 2004), promote regulation of the adaptive immune system via (amongst other things) differentiation and proliferation of T_H1 lymphocytes (Yang et al. 2006), and assist in the induction of immune tolerance (Lin 2004).

Two of these TLRs, TLR2 and TLR4, are of particular interest in allergy and asthma. Although they both have similar actions, they recognize different PAMPs (Lin 2004). TLR2 recognizes bacterial peptidoglycan and bacterial lipoprotein, whereas TLR4 recognizes lipopolysaccharide and lipoteichoic acid (Lin 2004). Evidence for associations between TLR polymorphisms and allergic disease is still accumulating (Yang et al. 2006), and their effect may be environment dependent.

In keeping with the 'hygiene hypothesis', the finding of a decreased prevalence of asthma and allergic disease in children raised on animal farms has led to a theory that exposure to microbial products alters the immune response away from T_H2 mediated inflammation (Eder et al. 2004). TLRs, as a vital part of the innate immune response to microbes, have garnered much interest as playing a role in this immune modification. Various studies have suggested a role in, amongst other things, the development of oral tolerance (Tunis & Marshall 2014), the development of T_H1 cutaneous sensitization (Jin et al. 2009), as well as directly triggering T_H1 effector functions (Imanishi et al. 2007). Furthermore, altered expression of TLRs has been demonstrated in allergic and non-allergic rhinitis (Vanhinsbergh et al. 2007) as well as in atopic dermatitis and contact dermatitis (Panzer et al. 2014).

Although results from several studies have been heterogeneous, a recent study suggested an association for the TLR2 2258G>A (Arg753Gln) polymorphism with asthma and atopy severity (Hussein et al. 2012). In contrast, a Japanese study (Noguchi et al. 2004) found no link between TLR polymorphisms and asthma or atopy.

The evidence for an association between the TLR4 896 A>G (Asp299Gly) polymorphism and allergic disease is similarly conflicting. Although there is some evidence for an association between this polymorphism and severity of asthma and allergic rhinitis (Yang et al. 2004), several studies (Bahrami et al. 2014; Sinha et al. 2014) including a recent meta-analysis (Yao et al. 2014), have failed to show an association between the polymorphism and the prevalence of allergic disease.

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METHODS

Study Design

The design of this study has been previously published (Levin et al. 2008; Levin et al. 2011), but for the purposes of this report, an overview is presented. A convenience sample of two hundred and eleven Xhosa children, with a mean age of 17 years, was obtained from a high school in Mowbray, Cape Town, between the months of May and September 2005. Children were identified as Xhosa if they indicated in their written questionnaire that both parents spoke Xhosa as a first language. The children completed a questionnaire modified from that used by the International Study of Asthma and Allergies in Childhood (ISAAC) regarding symptoms of asthma, eczema and rhinitis. Skin prick tests (SPTs) to common aeroallergens, blood samples for total IgE and *Ascaris*- specific IgE, and a modified methacholine challenge for bronchial hyper- responsiveness (BHR), were performed. In addition, blood samples were taken for genotyping of twenty-seven SNPs on sixteen distinct genes known or theorised to be associated with allergy and/or asthma.

We aim to report on the prevalence of the alleles of these SNPs in our sample, and to describe associations between any of the alleles and the clinical and laboratory markers of allergy. In addition, several similar studies have been performed on populations around the world (Le Souëf, personal communication), which will make it possible to compare the frequency of these alleles in different populations.

Physical Methods

Prevalence of asthma and allergy symptoms were assessed through self-reporting. Questionnaires, modified from those used by ISAAC regarding asthma, eczema and rhinitis, were administered in either English or Xhosa. The questionnaires were administered on two separate occasions to minimize selection bias.

To evaluate BHR, a modified metacholine challenge was performed. The method for this challenge has been previously published (Levin et al. 2011), but in brief it involved the use of a handheld glass nebuliser rather than traditional dosimeter. This method was based on a protocol described in 1983 (Yan et al.). The protocol was borne out of the need to devise a simple and rapid method to evaluate bronchial hyper-responsiveness, thus enabling screening of large numbers of patients in much less time than using traditional methods. The original study (Yan et al. 1983) showed good correlation between results obtained using the rapid method and those obtained using a traditional dosimeter. This was confirmed by our group on a small subset of our sample (Levin et al. 2011), however the presence of bias precluded the development of a fixed cutoff point. Metacholine was administered in doubling doses from 0.0015mg to 0.5mg, and BHR was defined as a fall in FEV_1 of ≥ 20% at any dose below the maximum dose administered.

SPTs were performed to house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), grass mix, Bermuda grass, mould mix, cat, dog, German cockroach (all ALK-Abello®), egg white, milk, peanut and potato (all fresh extracts). SPT's were deemed positive if wheal size was ≥3mm larger than the negative control.

Blood was drawn for total serum IgE, as well as IgE to *Ascaris*. Blood was also drawn for genotyping, which was performed by the University of Western Australia. This involved isolating DNA from the blood, amplification of the region of interest by use of PCR primers, and then digestion of the amplified regions with restriction enzymes. The digested products were separated on a 2% agarose gel and visualised under a transilluminator. Genotyping accuracy was confirmed by DNA sequencing a randomly selected subset of the population.

The table below shows the SNPs genotyped in this study. As far as possible, we have kept to the Human Genome Variation Society (HGVS)

recommendations for SNP nomenclature (den Dunnen & Antonarakis 2000; den Dunnen et al. 2016). We used the Database of Short Genetic Variations (dbSNP) from the National Center for Biotechnology Information (NCBI) to verify SNP nomenclature and reference alleles for these SNPs (Sherry et al. 2001). Six of the SNPs genotyped are on the complementary strand to the reference SNP obtained from dbSNP. These are marked with an asterisk in the SNP table below.

Gene	IL-10		IL-8	TLR2	IL-4		
Chromosomal location	1q32.1		4q13-q21	4q32	5q31.1		
SNP	592A>C	1082A>G	781C>T	2258G>A	33C>T	589C>T	2979T>G*
RS number	rs1800872	rs1800896	rs2227306	rs5743708	rs2070874	rs2243250	rs2227284
Reference alleles	A/C	A/G	C/T	G/A	C/T	C/T	A/C
Reference ancestral allele	C	A	C	G	T	C	A
Pro-Th2 allele	A	A	C	A	T	T	T

Gene	CD14		IL-12B		IL-13		
Chromosomal location	5q31.1		5q33.3		5q31		
SNP	159T>C*	550C>T	1188A>C	6408delCTCTAAinsGC*	130A>G*	1112C>T	4738A>G
RS number	rs2569190	rs5744455	rs3212227	rs17860508	rs20541	rs1800925	rs1295685
Reference alleles	A/G	C/T	A/C	TTAGAG/GC	C/T	C/T	A/G
Reference ancestral allele	G	C	A	N/A	C	T	G
Pro-Th2 allele	C	T	N/A	CTCTAA	A	T	A

Gene	ADRB2		IFNGR1		TLR4	SCGB1A	STAT6
Chromosomal location	5q31-32		6q23-q24		9q32-q33	11q12.3-q13.1	12q13
SNP	46G>A	79G>C	56T>C	611C>T*	896A>G	38G>A	2964A>G
RS number	rs1042713	rs1042714	rs2234711	rs1327474	rs4986790	rs3741240	rs324015
Reference alleles	A/G	C/G	C/T	A/G	A/G	A/G	A/G
Reference ancestral allele	G	G	C	A	A	G	A
Pro-Th2 allele	A	C	C	T	G	A	G

Gene	IFNG	CMA1	IL-4RA			TGFB
Chromosomal location	12q14	14q11.2	16p12.1-p11.2			19q13.1
SNP	874T>A	1903G>A	223A>G	1507T>C	1727A>G	509A>G*
RS number	rs2430561	rs1800875	rs1805010	rs1805015	rs1801275	rs1800469
Reference alleles	A/T	A/G	A/G	C/T	A/G	C/T
Reference ancestral allele	T	G	G	T	G	C
Pro-Th2 allele	A	G	G	T	G	A

Fig 1.1. List of all SNPs and genes under investigation. Reference data obtained from NCBI SNP database (dbSNP). For several SNPs, the study alleles are located on the complementary DNA strand to the reference alleles, leading to discrepancies between the study SNP and the stated reference alleles. Where these discrepancies exist, the SNP is marked with a *.

Statistical Analysis

All statistical analysis was completed by using R (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org). In total, there were eighteen objective (skin prick or blood test) markers and ten clinical markers of allergy. In addition, we genotyped the sample for twenty-seven different known genetic polymorphisms on sixteen distinct genes.

We assessed genotype and allele distribution (using the *genetics* package from R), the number of individuals successfully genotyped and performed an exact test of Hardy-Weinberg equilibrium on all SNPs. We used the D' statistic to assess LD and we also estimated all possible haplotype frequencies (using the *haplo.stats* package from R).

We looked for association between the twenty-seven SNPs and clinical and laboratory markers of allergy. We elected to set our significance level to a *P*-value <0.05 and not to correct for multiple testing, for several reasons. Firstly, there is no single established method of correcting for multiple testing (Hsueh et al. 2003; Farcomeni 2008). In addition, because this study is the first to explore these polymorphisms in a Black African population, we felt that the error of not reporting a positive association (missing a true positive association) would be more serious than reporting a false positive association. All associations were corrected for several exposures (age, gender, cat, dog or farm animal exposure, cigarette or fire smoke exposure and type of accommodation), which were added to the association models as fixed effects.

We modelled genotype (three genotypes, conferring two degrees of freedom) and additive allelic (counting the number of minor alleles to determine a 'dose-response') predictor representations of each SNP. The results guided us to find the best fitting model (lowest *P*-value) from the five possibilities: genotype, additive allelic, dominant, recessive and heterozygote. All *P*-values, effect sizes and confidence intervals were derived from these models.

RESULTS

Phenotypic Characteristics

The phenotypic characteristics of the sample population have been published previously (Levin et al. 2012; Levin et al. 2011; Levin et al. 2008), but are summarised below. Binary and categorical variables are reported as a number and percentage of positive results, and continuous variables are reported as medians with an interquartile range. The only continuous variables were total IgE and IgE to *Ascaris lumbricoides*. Both variables were not normally distributed, hence we have used median rather than mean as a marker of central tendency.

Our sample comprised 211 individuals, of which 60% were female (n=127). The self-reported prevalence of asthma was 10% (n=22), whilst bronchial hyper- responsiveness was demonstrated in 16% (n=33). The self-reported prevalence of rhinitis was 49% (n=103), and the self-reported prevalence of eczema was 21% (n=44). As previously reported (Levin et al. 2011), there was poor overlap between a self-reported diagnosis of asthma, and demonstrated bronchial hyper- responsiveness, with only 40% (n=8) of individuals reporting symptoms of asthma demonstrating bronchial hyper-responsiveness. Asthma was strongly associated with eczema and positive aeroallergen skin prick tests, whereas bronchial hyper- responsiveness was strongly associated with positive aeroallergen skin prick tests, and weakly associated with rhinitis. Total serum IgE levels were associated with atopy and IgE to *Ascaris lumbricoides* (Levin et al. 2008). IgE to *Ascaris lumbricoides* was strongly associated to aeroallergen sensitization and bronchial hyper-responsiveness, but not to asthma, rhinitis or eczema (Levin et al. 2012).

Objective markers of allergic disease	n	Positive (%)
Bronchial hyper-responsiveness	206	33(16)

Blood markers of allergic disease	n	Median (IQR)
Total IgE	210	107 kU/l (51-294)
Ascaris IgE	208	0 kU/l (0-0.45)

Skin tests	n	Positive (%)
Any positive SPT	211	72(34)
derp	210	51(24)
derf	210	48(23)
Any positive house dust mite SPT	211	55(26)
European grass	210	11(5)
Bermuda grass	210	9(4)
Any positive grass SPT	211	13(6)
Mould	210	2(1)
Cat	210	5(2)
Dog	210	6(3)
Cockroach	210	37(18)
Any positive aeroallergen SPT	211	67(32)
Egg white	210	7(3)

Skin tests	n	Positive (%)
Milk	210	4(2)
Peanut	210	3(1)
Fresh potato	210	3(1)
Any positive food SPT	211	12(6)

Self-reported symptoms	n	Positive (%)
Ever wheezed	210	73(35)
Wheeze in last year	210	53(25)
Self-reported diagnosis of asthma	210	22(10)
Ever had rhinitis	210	97(46)
Rhinitis in last year	210	93(44)
Self-reported diagnosis of rhinitis	210	103(49)

Ever had eczema	210	55(26)
Eczema in last year	210	55(26)
Self-reported diagnosis of eczema	210	44(21)

Genotype and Allele Frequencies

The genotype and allele distribution frequencies, number successfully genotyped and *P*-values for the exact test of Hardy-Weinberg equilibrium, are reported below. Genotype and allele frequencies for these SNPs in the Xhosa population have not been reported before. These frequencies are necessary in order to understand and interpret the association results reported below. It also establishes a baseline in this population, and in order to assist further studies. It may be possible in the future for us to collaborate with international colleagues in order to compare the frequencies of PIAs in this population with those in other populations.

IL10

592A>C

Number of samples typed: 188 (82.5%)
HWE exact p-value = 0.8634

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	264	0.7
A	112	0.3

Genotype Frequency:

Genotype	n	Proportion
C/C	93	0.49
C/A	78	0.41
A/A	17	0.09

1082A>G

Number of samples typed: 187 (82%)
HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
A	254	0.68
G	120	0.32

Genotype Frequency:

Genotype	n	Proportion
A/A	86	0.46
A/G	82	0.44
G/G	19	0.10

IL8

781C>T

Number of samples typed: 188 (82.5%)
HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	348	0.93
T	28	0.07

Genotype Frequency:

Genotype	n	Proportion
C/C	161	0.86
C/T	26	0.14
T/T	1	0.01

TLR2

2258G>A

Number of samples typed: 188 (82.5%)

HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	375	1
A	1	0

Genotype Frequency:

Genotype	n	Proportion
G/G	187	0.99
G/A	1	0.01

IL4

33C>T

Number of samples typed: 188 (82.5%)

HWE exact p-value = 0.6632

Allele Frequency: (2 alleles)

Allele	n	Proportion
T	199	0.53
C	177	0.47

Genotype Frequency:

Genotype	n	Proportion
T/T	51	0.27
T/C	97	0.52
C/C	40	0.21

589C>T

Number of samples typed: 188 (82.5%)

HWE exact p-value = 0.2239

Allele Frequency: (2 alleles)

Allele	n	Proportion
T	263	0.7
C	113	0.3

Genotype Frequency:

Genotype	n	Proportion
T/T	88	0.47
T/C	87	0.46
C/C	13	0.07

2979T>G

Number of samples typed: 188 (82.5%)

HWE exact p-value = 0.4790

Allele Frequency: (2 alleles)

Allele	n	Proportion
T	354	0.94
G	22	0.06

Genotype Frequency:

Genotype	n	Proportion
T/T	167	0.89
T/G	20	0.11
G/G	1	0.01

CD14

159T>C

Number of samples typed: 211 (92.5%)
HWE exact p-value = 0.3434

Allele Frequency: (2 all

Allele	n	Proportion
C	322	0.76
T	100	0.24

Genotype Frequency:

Genotype	n	Proportion
C/C	120	0.57
C/T	82	0.39
T/T	9	0.04

550C>T

Number of samples typed: 184 (80.7%)
HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	366	0.99
T	2	0.01

Genotype Frequency:

Genotype	n	Proportion
C/C	182	0.99
C/T	2	0.01

IL12B

1188A>C

Number of samples typed: 213 (93.4%)
HWE exact p-value = 0.7466

Allele Frequency: (2 alleles)

Allele	n	Proportion
A	296	0.69
C	130	0.31

Genotype Frequency:

Genotype	n	Proportion
A/A	104	0.49
A/C	88	0.41
C/C	21	0.10

6408delCTCTAAinsGC

Number of samples typed: 187 (82%)
HWE exact p-value = 0.6137

Allele Frequency: (2 alleles)

Allele	n	Proportion
GC	255	0.68
TTAGAG	119	0.32

Genotype Frequency:

Genotype	n	Proportion
GC/GC	85	0.45
GC/TTAGAG	85	0.45
TTAGAG/TTAGAG	17	0.09

IL13

130A>G

Number of samples typed: 188 (82.5%)
HWE exact p-value = 0.0756

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	267	0.71
T	109	0.29

Genotype Frequency:

Genotype	n	Proportion
C/C	100	0.53
C/T	67	0.36
T/T	21	0.11

1112C>T

Number of samples typed: 187 (82%)
HWE exact p-value = 0.8816

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	215	0.57
T	159	0.43

Genotype Frequency:

Genotype	n	Allele
C/C	61	0.33
C/T	93	0.50
T/T	33	0.18

4738A>G

Number of samples typed: 188 (82.5%)
HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	370	0.98
A	6	0.02

Genotype Frequency:

Genotype	n	Proportion
G/G	182	0.97
G/A	6	0.03

TLR4

896A>G

Number of samples typed: 185 (81.1%)
HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
A	368	0.99
G	2	0.01

Genotype Frequency:

Genotype	n	Proportion
A/A	183	0.99
A/G	2	0.01

IFNGR

56T>C

Number of samples typed: 186 (81.6%)

HWE exact p-value = 0.6597

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	200	0.54
T	172	0.46

Genotype Frequency:

Genotype	n	Proportion
C/C	52	0.28
C/T	96	0.52
T/T	38	0.20

611C>T

Number of samples typed: 187 (82%)

HWE exact p-value = 0.2533

Allele Frequency: (2 alleles)

Allele	n	Proportion
T	359	0.96
C	15	0.04

Genotype Frequency:

Genotype	n	Proportion
T/T	173	0.93
T/C	13	0.07
C/C	1	0.01

ADRB2

46G>A

Number of samples typed: 211 (92.5%)

HWE exact p-value = 0.6776

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	227	0.54
A	195	0.46

Genotype Frequency:

Genotype	n	Proportion
G/G	59	0.28
G/A	109	0.52
A/A	43	0.20

79G>C

Number of samples typed: 210 (92.1%)

HWE exact p-value = 0.6131507

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	353	0.84
G	67	0.16

Genotype Frequency:

Genotype	n	Proportion
C/C	147	0.70
C/G	59	0.28
G/G	4	0.02

SCGB1A

38G>A

Number of samples typed: 211 (92.5%)
HWE exact p-value = 0.6933

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	329	0.78
A	93	0.22

Genotype Frequency:

Genotype	n	Proportion
G/G	127	0.60
G/A	75	0.36
A/A	9	0.04

STAT6

2964A>G

Number of samples typed: 188 (82.5%)
HWE exact p-value = 0.5264

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	327	0.87
A	49	0.13

Genotype Frequency:

Genotype	n	Proportion
G/G	143	0.76
G/A	41	0.22
A/A	4	0.02

IFNG

874T>A

Number of samples typed: 188 (82.5%)
HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
A	310	0.82
T	66	0.18

Genotype Frequency:

Genotype	n	Proportion
A/A	128	0.68
A/T	54	0.29
T/T	6	0.03

CMA1

1903G>A

Number of samples typed: 188 (82.5%)
HWE exact p-value = 1

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	327	0.87
A	49	0.13

Genotype Frequency:

Genotype	n	Proportion
G/G	142	0.76
G/A	43	0.23
A/A	3	0.02

IL4RA

223A>G

Number of samples typed: 186 (81.6%)
HWE exact p-value = 0.8817

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	208	0.56
A	164	0.44

Genotype Frequency:

Genotype	n	Proportion
G/G	59	0.32
G/A	90	0.48
A/A	37	0.20

1507T>C

Number of samples typed: 188 (82.5%)
HWE exact p-value = 0.0285

Allele Frequency: (2 alleles)

Allele	n	Proportion
T	201	0.53
C	175	0.47

Genotype Frequency:

Genotype	n	Proportion
T/T	46	0.24
T/C	109	0.58
C/C	33	0.18

1727A>G

Number of samples typed: 188 (82.5%)
HWE exact p-value = 0.6104

Allele Frequency: (2 alleles)

Allele	n	Proportion
G	313	0.83
A	63	0.17

Genotype Frequency:

Genotype	n	Frequency
G/G	129	0.69
G/A	55	0.29
A/A	4	0.02

TFGB

509A>G

Number of samples typed: 187 (82%)
HWE exact p-value = 0.6771

Allele Frequency: (2 alleles)

Allele	n	Proportion
C	291	0.78
T	83	0.22

Genotype Frequency:

Genotype	n	Proportion
C/C	114	0.61
C/T	63	0.34
T/T	10	0.05

Linkage Disequilibrium

Linkage disequilibrium is reported for each gene with multiple SNPs of interest for our study, followed by a linkage disequilibrium map of all investigated SNPs.

	SNP	D'
IL-10	592A>C	1.00
	1082A>G	

	SNP	D'
IL-4	33C>T	0.80
	589C>T	
	589C>T	0.82
	2979T>G	
	33C>T	0.91
	2979T>G	

	SNP	D'
CD14	159T>C	0.94
	550C>T	

	SNP	D'
IL-12I	1188A>C	0.61
	6408delCTCTAAinsGC	

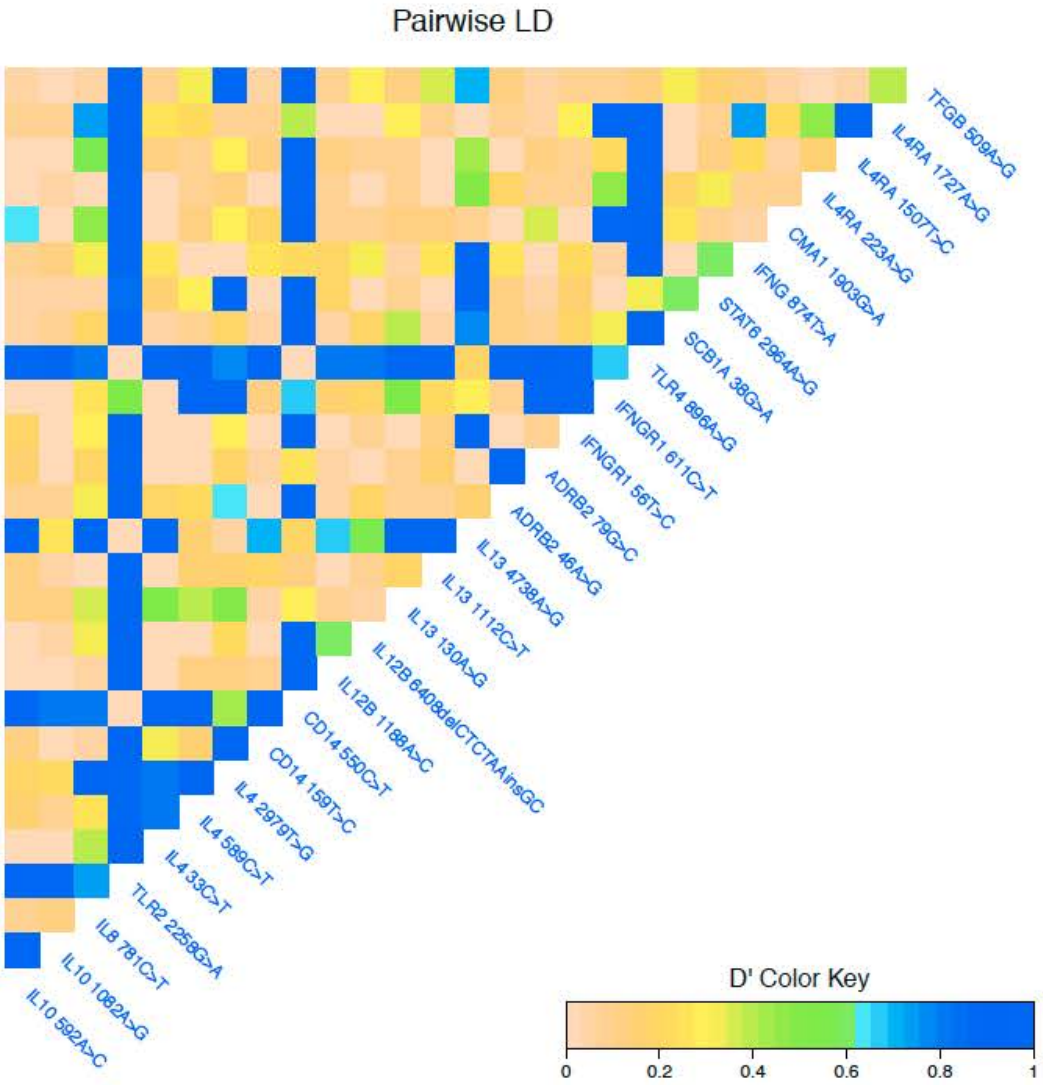
	SNP	D'
IL-13	130A>G	0.20
	1112C>T	
	1112C>T	0.99
	4738A>G	
	130A>G	0.99
	4738A>G	

	SNP	D'
ADBR2	46G>A	

	79G>C	1.00

	SNP	D'
IFNGR1	56T>A	1.00
	611C>T	

	SNP	D'
IL4R	223A>G	0.15
	1507T>C	
	1507T>C	1.00
	1727A>G	
	223A>G	0.48
	1727A>G	


Pairwise LD
TFGB 509A>G
IL4RA 1727A>G
IL4RA 1507T>C
IL4RA 223A>G
CMA1 1903G>A
IFNG 874T>A
STAT6 2964A>G
SCB1A 38G>A
TLR4 896A>G
IFNGR1 611C>T
IFNGR1 56T>C
ADRB2 79G>C
ADRB2 46A>G
IL13 4738A>G
IL13 1112C>T
IL13 130A>G
IL12B 6408delCTCTAAinsGC
IL12B 1188A>C
CD14 550C>T
CD14 159T>C
IL4 2979T>G
IL4 589C>T
IL4 33C>T
TLR2 2258G>A
IL8 781C>T
IL10 1082A>G
IL10 592A>C
D' Color Key
0
0.2
0.4
0.6
0.8
1

Exposures

We adjusted all association tests for the possible confounding effect of environmental exposures. We corrected for six exposures (cat, dog or farm animal exposure, smoking cigarettes, exposure to household or other smoke on a regular basis, and type of accommodation). The first five exposures are binary, whereas accommodation was divided into four categories. The distributions of the exposures are given below, followed by a summary of associations between the exposures and our phenotypic endpoints for reference purposes.

Exposures	**n**	**Positive (%)**
Cat exposure	209	107(51)
Dog exposure	209	153(73)
Farm animal exposure	209	24(11)
Smoker	209	34(16)
Any smoke exposure	209	123(59)
Type of accommodation	208	
House		129(62)
Flat		26(13)
Shack		45(22)
Other		8(4)

Association Between Clinical Phenotypes and Confounders

Phenotype	Confounders						
	Age	Gender	Cat Exposure	Dog Exposure	Farm Animal Exposure	Type of Accommodation	Any Smoke Exposure
SPT binary	0,3357	0,7395	0,2036	0,1937	0,4039	0,6582	0,8007
derp	0,0696	0,7625	0,0688	0,3467	0,8959	0,8960	0,4844
derf	0,0424	0,8021	0,0439	0,3741	0,8352	0,9710	0,7932
housedust mite	0,0454	0,4233	0,1415	0,5874	0,6954	0,8713	0,6058
euro grass	0,5457	0,4001	0,0198	0,9707	0,7920	0,8488	0,5944
bermuda	0,4639	0,2722	0,0683	0,7470	0,9713	0,6821	0,7758
any grass	0,7880	0,2073	0,0321	0,7511	0,6619	0,8533	0,5257
mould	0,8640	0,1581	0,9729	0,2627	0,4837	0,5886	0,1669
cat	0,5803	0,9648	0,6113	0,0753	0,2663	0,8976	0,0281
dog	0,2907	0,2266	0,9526	0,7197	0,7021	0,8864	0,0160
cockroach	0,1966	0,3466	0,2080	0,1757	0,9384	0,5342	0,6006
aero binary	0,2423	0,5105	0,0844	0,4396	0,5127	0,8323	0,7709
egg white	0,8689	0,8344	0,6531	0,3514	0,8187	0,0721	0,7805
milk	0,9384	0,6542	0,2802	0,9343	0,3207	0,0194	0,5831
peanut	0,8334	0,8365	0,0370	0,8012	0,3905	0,5886	0,3084
fresh potato	0,5706	0,3306	0,5852	0,1465	0,3130	0,1596	0,0899
foods binary	0,6669	0,4285	0,4957	0,2508	0,5822	0,2030	0,7772
ascaris2	0,2562	0,1538	0,7003	0,3791	0,8290	0,4056	0,1919
wheezeever	0,1680	0,0547	0,9137	0,8543	0,2668	0,3368	0,2800
wheezeinlastyear	0,8462	0,1636	0,4973	0,9424	0,9657	0,8104	0,3162
selfasthma	0,7533	0,4573	0,1394	0,2975	0,7017	0,6530	0,8830
rhinoever	0,1964	0,5943	0,7101	0,7520	0,7082	0,4507	0,0557
rhinolastyear	0,4078	0,3445	0,3453	0,7342	0,3127	0,2867	0,0762
selfrhino	0,1727	0,1392	0,2951	0,0947	0,0609	0,5528	0,8741
eczemaever	0,9619	0,2598	0,4977	0,9257	0,8758	0,8618	0,7978
eczemalastyear	0,7275	0,4233	0,1910	0,4265	0,8758	0,7426	0,5605
selfeczema	0,1059	0,2641	0,3911	0,0520	0,6206	0,2581	0,0631
hyper	0,0048	0,4384	0,0238	0,7119	0,5147	0,9649	0,3111

Associations Between Genotypes or Alleles and Clinical and Objective Markers of Allergy

We found several significant associations between SNPs and markers of allergic disease. Several phenotypes, specifically skin prick tests to cat, milk, peanut and fresh potato, were excluded from association testing because the scarcity of positive results precluded meaningful interrogation. We present below a tabulated summary of *P*-values as markers of association. All associations have been corrected for the identified exposures (or potential confounders). Those associations deemed to be possibly significant (as determined by having a *P*-value of < 0.05), were then further interrogated to determine the precise nature of the associations with odds ratios as a measure of effect sizes.

Genotypes and Clinical Phenotypes										
Genotype	**Wheeze ever**	**Wheeze in last year**	**Self reported asthma**	**Rhinitis ever**	**Rhinitis in last year**	**Self reported rhinitis**	**Eczema ever**	**Eczema in last year**	**Self reported eczema**	**Bronchial hyper-responsiveness**
IL10 592A>C	0.5114	0.7853	0.1679	0.8566	0.9993	0.2443	0.1071	0.0425	0.7448	0.6477
IL10 1082A>G	0.0107	0.0438	0.0494	0.5855	0.8293	0.0195	0.4657	0.7023	0.7268	0.0610
IL8 781C>T	0.6327	0.5336	0.9542	0.4196	0.5995	0.4289	0.7052	0.6157	0.7868	0.4474
TLR2 2258G>A	0.3584	0.3877	0.5365	0.1757	0.2091	0.2408	0.1267	0.1693	0.1018	0.5332
IL4 33C>T	0.3509	0.2526	0.6838	0.2713	0.4909	0.1105	0.7927	0.9497	0.5169	0.0823
IL4 589C>T	0.0056	0.0182	0.5162	0.8631	0.9304	0.3202	0.0877	0.3268	0.6047	0.4141
IL4 2979T>G	0.3383	0.4205	0.3590	0.4155	0.5047	0.2763	0.7033	0.5890	0.6159	0.0285
CD14 159T>C	0.9328	0.8059	0.4223	0.8563	0.8437	0.2134	0.6982	0.4811	0.5016	0.7352
CD14 550C>T	0.3743	0.2594	0.1360	0.8762	0.7722	0.7491	0.3838	0.3252	0.2609	0.2383
IL12B 1188A>C	0.3906	0.4271	0.6850	0.4773	0.2040	0.8978	0.7330	0.4976	0.4787	0.1336
IL12B 6408delCTCTAAinsGC	0.0258	0.0866	0.0589	0.1997	0.2298	0.1757	0.4070	0.1234	0.7217	0.6190
IL13 130A>G	0.8025	0.9978	0.5846	0.3291	0.5047	0.0428	0.7102	0.8358	0.0043	0.5272
IL13 1112C>T	0.9285	0.4246	0.7155	0.8460	0.7191	0.6246	0.1329	0.0840	0.1244	0.8498
IL13 4738A>G	0.3762	0.6983	0.7470	0.1378	0.1549	0.0056	0.5692	0.6244	0.1513	0.1151
ADRB2 46G>A	0.6039	0.6795	0.4430	0.1764	0.2518	0.9285	0.4197	0.8412	0.6719	0.4565
ADRB2 79G>C	0.8263	0.3765	0.6765	0.7205	0.5642	0.2037	0.1289	0.1284	0.9600	0.3585
IFNGR1 56T>C	0.1657	0.2691	0.7378	0.4674	0.7977	0.7972	0.6223	0.3306	0.8533	0.2202
IFNGR1 611C>T	0.5723	0.5786	0.6156	0.2938	0.3783	0.3790	0.7176	0.7669	0.7261	0.7652
TLR4 896A>G	0.6507	0.4636	0.5552	0.7422	0.6514	0.5495	0.4066	0.4286	0.1863	0.0600
SCGB1A 38G>A	0.6190	0.1934	0.3331	0.1755	0.4675	0.6888	0.1146	0.4242	0.1974	0.6255
STAT6 2964A>G	0.6433	0.9305	0.1293	0.5987	0.4665	0.9674	0.1948	0.6389	0.0586	0.6493
IFNG 874T>A	0.8283	0.8812	0.6621	0.3184	0.2068	0.2092	0.1717	0.1704	0.8838	0.9861
CMA1 1903G>A	0.6982	0.8958	0.5030	0.5242	0.3884	0.8232	0.2587	0.1982	0.8991	0.1057
IL4RA 223A>G	0.3606	0.7994	0.3899	0.4119	0.6480	0.4004	0.5445	0.8631	0.7538	0.8842
IL4RA 1507T>C	0.1290	0.2506	0.7847	0.7499	0.5469	0.4907	0.0647	0.2580	0.8515	0.5700
IL4RA 1727A>G	0.0353	0.0629	0.6677	0.9659	0.9908	0.4772	0.8254	0.8560	0.9043	0.2424
TGFB 509A>G	0.2387	0.2206	0.3015	0.9815	0.9303	0.5417	0.4018	0.3099	0.3928	0.9822

Alleles and Clinical Phenotypes										
Allele	**Wheeze ever**	**Wheeze in last year**	**Self reported asthma**	**Rhinitis ever**	**Rhinitis in last year**	**Self reported rhinitis**	**Eczema ever**	**Eczema in last year**	**Self reported eczema**	**Bronchial hyper-responsiveness**
IL10 592A>C	0.9735	0.9938	0.3302	0.8948	0.9909	0.6146	0.0387	0.0248	0.5702	0.3655
IL10 1082A>G	0.0711	0.1812	0.9040	0.8790	0.8504	0.0075	0.7583	0.8508	0.4402	0.0273
IL8 781C>T	0.6798	0.5932	0.7595	0.3115	0.8145	0.4414	0.6631	0.7273	0.5269	0.2069
TLR2 2258G>A	0.3584	0.3877	0.5365	0.1757	0.2091	0.2408	0.1267	0.1693	0.1018	0.5332
IL4 33C>T	0.5023	0.1265	0.3962	0.1063	0.2367	0.1563	0.5747	0.8733	0.3851	0.1253
IL4 589C>T	0.0883	0.0487	0.5092	0.6449	0.8763	0.8447	0.0392	0.2122	0.3265	0.5213
IL4 2979T>G	0.1601	0.2064	0.1620	0.9834	0.8249	0.2177	0.7169	0.4811	0.9785	0.8227
CD14 159T>C	0.7424	0.5642	0.2033	0.9051	0.8516	0.3454	0.4083	0.2819	0.2402	0.4331
CD14 550C>T	0.3743	0.2594	0.1360	0.8762	0.7722	0.7491	0.3838	0.3252	0.2609	0.2383
IL12B 1188A>C	0.2428	0.2068	0.4049	0.8595	0.9817	0.6612	0.4349	0.2499	0.3541	0.9688
IL12B 6408delCTCTAAinsGC	0.3627	0.4750	0.3625	0.3272	0.2261	0.7294	0.1800	0.0415	0.4912	0.6810
IL13 130A>G	0.7354	0.9791	0.3008	0.2027	0.5526	0.6044	0.4087	0.5510	0.8563	0.4156
IL13 1112C>T	0.7109	0.2712	0.5178	0.6403	0.4280	0.4389	0.2457	0.3944	0.2657	0.5766
IL13 4738A>G	0.3762	0.6983	0.7470	0.1378	0.1549	0.0056	0.5692	0.6244	0.1513	0.1151
ADRB2 46G>A	0.9668	0.9382	0.3029	0.5313	0.4580	0.7242	0.8024	0.9018	0.4889	0.6235
ADRB2 79G>C	0.5532	0.1859	0.6708	0.4181	0.2961	0.1110	0.5346	0.6822	0.8582	0.6713
IFNGR1 56T>C	0.8233	0.9480	0.9841	0.7260	0.7618	0.6208	0.7867	0.5973	0.7712	0.3786
IFNGR1 611C>T	0.9655	0.7043	0.5207	0.1310	0.1829	0.2090	0.6293	0.6149	0.5345	0.8756
TLR4 896A>G	0.6507	0.4636	0.5552	0.7422	0.6514	0.5495	0.4066	0.4286	0.1863	0.0600
SCGB1A 38G>A	0.4260	0.0751	0.3134	0.2259	0.2892	0.4659	0.3399	0.3868	0.1610	0.6650
STAT6 2964A>G	0.3486	0.7409	0.5001	0.4872	0.2387	0.8054	0.1405	0.5141	0.0201	0.9633
IFNG 874T>A	0.5883	0.9954	0.5229	0.5264	0.2556	0.1244	0.7917	0.9934	0.6753	0.8903
CMA1 1903G>A	0.7099	0.7821	0.3070	0.2680	0.1741	0.6949	0.1535	0.1065	0.6699	0.0497
IL4RA 223A>G	0.1547	0.5548	0.1713	0.2137	0.4589	0.7768	0.4613	0.6608	0.6942	0.9457
IL4RA 1507T>C	0.0446	0.1053	0.9639	0.6867	0.6899	0.2329	0.7742	0.8140	0.7053	0.2910
IL4RA 1727A>G	0.0161	0.0244	0.8032	0.8087	0.8920	0.4274	0.5658	0.6667	0.7680	0.4826
TGFB 509A>G	0.5229	0.1940	0.2562	0.9543	0.7747	0.3185	0.3553	0.5066	0.7507	0.9998

Genotypes and Objective Markers of Allergic Disease								
Genotype	**Any positive SPT**	**derp**	**derf**	**Any positive HDM SPT**	**cockroach**	**Any positive aeroallergen SPT**	**Any positive food SPT**	**Total serum IgE**
IL10 592A>C	0.2588	0.0303	0.0436	0.0715	0.3436	0.1815	0.4885	0.6714
IL10 1082A>G	0.0459	0.0528	0.0216	0.0110	0.2196	0.0257	0.2601	0.6663
IL8 781C>T	0.6203	0.5224	0.5675	0.6825	0.8131	0.6808	0.3661	0.9662
TLR2 2258G>A	0.3037	0.3639	0.3588	0.3345	0.5371	0.3194	0.7724	0.6329
IL4 33C>T	0.8304	0.8476	0.9843	0.9840	0.6036	0.8622	0.1776	0.3205
IL4 589C>T	0.0673	0.3028	0.2593	0.2403	0.0750	0.1043	0.8998	0.8618
IL4 2979T>G	0.2572	0.1730	0.2171	0.1994	0.6898	0.2910	0.9857	0.9966
CD14 159T>C	0.6451	0.6411	0.3216	0.4746	0.1804	0.6746	0.5745	0.2488
CD14 550C>T	0.4138	0.2578	0.2886	0.3415	0.1680	0.4516	0.8847	0.6766
IL12B 1188A>C	0.7783	0.8633	0.8928	0.8771	0.7695	0.6686	0.0656	0.6284
IL12B 6408delCTCTAAinsGC	0.7899	0.8458	0.8623	0.9092	0.8631	0.6528	0.0356	0.8539
IL13 130A>G	0.6446	0.9200	0.4856	0.7701	0.8056	0.5189	0.1046	0.9079
IL13 1112C>T	0.5292	0.4781	0.8718	0.7902	0.6244	0.7434	0.1566	0.4602
IL13 4738A>G	0.3570	0.5450	0.6166	0.5014	0.1169	0.3696	0.3983	0.0881
ADRB2 46G>A	0.0644	0.2444	0.5257	0.2418	0.1345	0.0411	0.8007	0.9320
ADRB2 79G>C	0.1787	0.4288	0.2014	0.4875	0.4667	0.3684	0.3361	0.1073
IFNGR1 56T>C	0.3188	0.2601	0.2076	0.2797	0.4044	0.2340	0.0212	0.0126
IFNGR1 611C>T	0.3166	0.1591	0.1264	0.1201	0.1631	0.3176	0.5943	0.5047
TLR4 896A>G	0.7695	0.2932	0.2610	0.2685	0.4756	0.5890	0.6851	0.9626
SCGB1A 38G>A	0.6748	0.6811	0.6274	0.7674	0.0689	0.6109	0.8364	0.6213
STAT6 2964A>G	0.5071	0.3111	0.6759	0.4186	0.5016	0.4684	0.7731	0.8226
IFNG 874T>A	0.1327	0.2453	0.4953	0.2348	0.9354	0.1966	0.0118	0.7384
CMA1 1903G>A	0.9930	0.2415	0.9836	0.9965	0.7529	0.9735	0.9435	0.2062
IL4RA 223A>G	0.2410	0.1678	0.1259	0.3095	0.5328	0.2664	0.8480	0.6499
IL4RA 1507T>C	0.9819	0.6957	0.9979	0.8277	0.7960	0.9926	0.6127	0.0822
IL4RA 1727A>G	0.4937	0.2919	0.1870	0.4176	0.6165	0.4903	0.1209	0.2666
TGFB 509A>G	0.5153	0.5100	0.7138	0.4941	0.6672	0.7656	0.2140	0.3932

Alleles and Objective Markers of Allergic Disease								
Allele	**Any positive SPT**	**derp**	**derf**	**Any positive HDM SPT**	**cockroach**	**Any positive aeroallergen SPT**	**Any positive food SPT**	**Total serum IgE**
IL10 592A>C	0.1025	0.0082	0.0144	0.0220	0.1512	0.0662	0.5167	0.3715
IL10 1082A>G	0.0324	0.0301	0.0074	0.0043	0.1662	0.0103	0.6457	0.6646
IL8 781C>T	0.3779	0.2662	0.3070	0.4121	0.6020	0.4602	0.1563	0.7931
TLR2 2258G>A	0.3037	0.3639	0.3588	0.3345	0.5371	0.3194	0.7724	0.6329
IL4 33C>T	0.9698	0.8486	0.8662	0.9694	0.8979	0.9584	0.0685	0.3115
IL4 589C>T	0.0231	0.1312	0.1048	0.1036	0.0262	0.0370	0.6555	0.7696
IL4 2979T>G	0.6849	0.6536	0.4882	0.4737	0.4333	0.6087	0.9104	0.9338
CD14 159T>C	0.9145	0.5634	0.1381	0.2956	0.3826	0.4693	0.6064	0.1718
CD14 550C>T	0.4138	0.2578	0.2886	0.3415	0.1680	0.4516	0.8847	0.6766
IL12B 1188A>C	0.5700	0.8850	0.9050	0.7477	0.6324	0.4647	0.0797	0.8748
IL12B 6408delCTCTAAinsGC	0.6348	0.9890	0.6082	0.8323	0.6261	0.9067	0.0146	0.6702
IL13 130A>G	0.4550	0.6833	0.2702	0.4823	0.6132	0.3049	0.0440	0.7076
IL13 1112C>T	0.2593	0.2374	0.6083	0.5148	0.4325	0.4443	0.0841	0.2261
IL13 4738A>G	0.3570	0.5450	0.6166	0.5014	0.1169	0.3696	0.3983	0.0881
ADRB2 46G>A	0.0995	0.5658	0.3486	0.3112	0.1203	0.0527	0.5080	0.7899
ADRB2 79G>C	0.9743	0.9485	0.4761	0.8169	0.4491	0.4564	0.4977	0.1874
IFNGR1 56T>C	0.8618	0.9753	0.9766	0.7385	0.3434	0.7873	0.0736	0.0198
IFNGR1 611C>T	0.6764	0.9472	0.9656	0.8325	0.6333	0.6604	0.3076	0.9640
TLR4 896A>G	0.7695	0.2932	0.2610	0.2685	0.4756	0.5890	0.6851	0.9626
SCGB1A 38G>A	0.4025	0.4031	0.3945	0.4701	0.4697	0.3380	0.9746	0.6329
STAT6 2964A>G	0.2731	0.6173	0.3791	0.4417	0.2402	0.2785	0.6766	0.5851
IFNG 874T>A	0.4282	0.4472	0.2519	0.8259	0.8563	0.6677	0.4287	0.7869
CMA1 1903G>A	0.9087	0.2165	0.8754	0.9498	0.7699	0.8414	0.9944	0.8859
IL4RA 223A>G	0.1575	0.1274	0.2216	0.2502	0.6115	0.2318	0.5708	0.4458
IL4RA 1507T>C	0.8684	0.6648	0.9488	0.5435	0.5426	0.9241	0.6615	0.3876
IL4RA 1727A>G	0.2834	0.2024	0.0994	0.2850	0.3820	0.2761	0.3127	0.6237
TGFB 509A>G	0.2498	0.9914	0.5987	0.8152	0.4203	0.4676	0.1073	0.7792

Summary of Effects of Genetic Associations

SNP	Phenotype	Inheritance Model	Effect Type	Magnitude of Effect
IL10 592A>C	Positive *Der p* SPT	additive allelelic	each A allele changes odds ratio %	109 (95% CI:21 to 267)
	Positive *Der f* SPT	additive allelelic	each A allele changes odds ratio %	101 (95% CI:15 to 256)
	Any positive HDM SPT	additive allelelic	each A allele changes odds ratio %	86 (95% CI:9 to 221)
	Eczema ever	additive allelelic	each A allele changes odds %	-43 (95% CI:-66 to -3)
	Eczema in past year	additive allelelic	each A allele changes odds %	-46 (95% CI:-69 to -8)
IL10 1082A>G	Any positive SPT	additive allelelic	each G allele changes odds ratio %	-43 (95% CI:-67 to -4)
	Positive *Der p* SPT	additive allelelic	each G allele changes odds ratio %	-47 (95% CI:-72 to -6)
	Positive *Der f* SPT	additive allelelic	each G allele changes odds ratio %	-56 (95% CI:-78 to -19)
	Any positive HDM SPT	additive allelelic	each G allele changes odds ratio %	-57 (95% CI:-77 to -22)
	Any positive aeroallergen SPT	additive allelelic	each G allele changes odds ratio %	-50 (95% CI:-71 to -15)
	Wheeze ever	recessive	GG versus AA+AG	-90 (95% CI:-99 to -50)
	Wheeze in past year	recessive	GG versus AA+AG	-87 (95% CI:-99 to -31)
	Self-reported rhinitis	additive allelelic	each G allele changes odds %	-48 (95% CI:-68 to -16)
	BHR	additive allelelic	each G allele changes odds %	-54 (95% CI:-79 to -8)
IL4 589C>T	Any positive SPT	additive allelelic	each C allele changes odds ratio %	-47 (95% CI:-70 to -8)
	Positive cockroach SPT	additive allelelic	each C allele changes odds ratio %	-54 (95% CI:-78 to -9)
	Any positive aeroallergen SPT	additive allelelic	each C allele changes odds ratio %	-44 (95% CI:-68 to -3)
	Wheeze ever	heterozygote	TC versus both homozygotes	-67 (95% CI:-84 to -34)
	Wheeze in past year	heterozygote	TC versus both homozygotes	-65 (95% CI:-84 to -27)
	Eczema ever	additive allelelic	each T allele changes odds %	79 (95% CI:3 to 215)
IL12B 6408delCTCTAAinsGC	Any positive food SPT	dominant model	any TTAGAG vs GC/GC	305 (95% CI:31 to 1374)
	Wheeze ever	recessive	TTAGAG/TTAGAG versus other two	-87 (95% CI:-99 to -34)
	Eczema in past year	additive allelelic	each TTAGAG allele changes odds %	-45 (95% CI:-70 to -2)
IL13 130A>G	Any positive food SPT	additive allelelic	each T allele changes odds ratio %	-74 (95% CI:-95 to -3)
	Self-reported rhinitis	recessive	TT versus CC+CT	192 (95% CI:6 to 807)
	Self-reported eczema	heterozygote	CT versus CC+TT	216 (95% CI:45 to 608)
ADRB2 46A>G	Any positive aeroallergen SPT	recessive	AA versus GG+GA	166 (95% CI:25 to 472)
IFNGR1 56T>C	IgE	additive allelic	% change in IgE per minor allele	-30 (95% CI:-48 to -6)
	Any positive food SPT	dominant model	All 52 with CC said had negative SPTs. So the effect of TT+CT (any T) is very strong. But could not be estimated, because the effect is indivisible by 0.	
STAT6 2964A>G	Self-reported eczema	dominant	GA+AA versus GG	-67 (95% CI:-90 to -12)
IL4RA 1507T>C	Wheeze ever	additive allelelic	each C allele changes odds %	69 (95% CI:1 to 188)
IL4RA 1727A>G	Wheeze ever	dominant	GA+AA versus GG	-55 (95% CI:-79 to -8)
	Wheeze in past year	dominant	GA+AA versus GG	-58 (95% CI:-83 to -6)

Polymorphisms in the IL-10 gene were associated with several clinical and objective markers of allergy. 592A>C was associated with ever having eczema (additive allelic model, each A allele reduced odds ratio by 43% [95% CI: 3% to 66%]), as well as with having symptoms of eczema in the preceding year (additive allelic model, each A allele reduced odds ratio by 46% [95% CI: 8% to 69%]). 592A>C was also associated with a positive *Dermatophagoides pteronyssinus* skin prick test (additive allelic model, each A allele increases odds ratio by 109% [95% CI: 21% to 267%]), with a positive *Dermatophagoides farinae* skin prick test (additive allelic model, each A allele increases odds ratio by 101% [95% CI: 15% to 256%]), as well as with any positive house dust mite skin prick test (additive allelic model, each A allele increases odds ratio by 86% [95% CI: 9% to 221%]).

The IL-10 SNP 1082A>G was associated with a history of ever having wheeze (recessive model, GG reduced odds ratio by 90% [95% CI: 50% to 99%] in comparison to AA and AG), with a history of wheeze in the preceding year (recessive model, GG reduced odds ratio by 87% [95% CI: 31% to 99%] in comparison to AA and AG), with self-reported diagnosis of rhinitis (additive allelic model, each G allele reducing odds ratio by 48% [95% CI: 16% to 68%]) and with bronchial hyper-responsiveness (additive allelic model, each G allele reduced odds ratio by 54% [95% CI: 8% to 79%]). 1082A>G was also associated with having any positive skin prick test (additive allelic model, each G allele decreases odds ratio by 43% [95% CI: 4% to 67%]), with a positive *Dermatophagoides pteronyssinus* skin prick test (additive allelic model, each G allele reduces odds ratio by 47% [95% CI: 6% to 72%]), with a positive *Dermatophagoides farinae* skin prick test (additive allelic model, each G allele reduces odds ratio by 56% [95% CI: 19% to 78%]), with having any positive house dust mite skin prick test (additive allelic model, each G allele reduces odds ratio by 57% [95% CI: 22% to 77%]) and with having any positive aeroallergen skin prick test (additive allelic model, each G allele reduces odds by 50% [95% CI: 15% to 71%]).

In addition, we found several associations between polymorphisms in genes that code for T_H2 mediators, and markers of allergic disease. The IL-4

polymorphism 589C>T was associated with ever having wheeze (heterozygote model, TC reduces odds ratio by 67% [95% CI: 34% to 84%] in comparison to both homozygotes), with a history of wheeze in the preceding year (heterozygote model, TC reduces odds ratio by 65% [95% CI: 27% to 84%] in comparison to both homozygotes), as well as with a history of ever having eczema (additive allelic model, each T allele increases odds ratio by 79% [95% CI: 215% to 3%]). 589C>T was also associated with having any positive skin prick test (additive allelic model, each C allele reduces odds ratio by 47% [95% CI: 8% to 70%]), with a positive cockroach skin prick test (additive allelic model, each C allele reduces odds ratio by 54% [95% CI: 9% to 78%]), and with having any positive aeroallergen skin prick test (additive allelic model, each C allele reduces odds ratio by 44% [95% CI: 3% to 68%]).

The IL-13 SNP 130A>G was associated with a self-diagnosis of allergic rhinitis (recessive model, TT increases odds ratio by 192% [95% CI: 6% to 807%] in comparison to CC and CT), as well as with a self-diagnosis of eczema (heterozygote model, CT increases odds ratio by 216% [95% CI: 45% to 608%] in comparison to CC and CT). In addition, 130A>G was associated with having any positive food allergen skin prick test (additive allelic model, each T allele decreases odds ratio by 74% [95% CI: 3% to 95%]).

The IL4RA polymorphism 478T>C was associated with ever having wheeze (additive allelic model, each C allele increases odds ratio by 69% [95% CI: 1% to 188%]). Another IL4RA SNP, 551A>G, was associated with a history of ever having wheeze (dominant model, GA and AA reduce odds ratio by 55% [95% CI: 8% to 79%] in comparison to GG), as well as with a history of wheeze in the preceding year (dominant model, GA and AA reduce odds ratio by 58% [95% CI: 6% to 83%] in comparison to GG). The STAT6 polymorphism 2964A>G was associated with a self-diagnosis of eczema (dominant model, GA and AA reduce odds ratio by 67% [95% CI: 12% to 90%] in comparison to GG).

There were, in addition, associations between genes that code for T_H1 mediators, and clinical and objective markers of allergic disease. The IL-12

polymorphism 6408delCTCTAAinsGC was associated with a history of ever having wheeze (recessive model, TTAGAG/TTAGAG reduces odds ratio by 87% [95% CI: 34% to 99%] in comparison to TTAGAG/TCTAAGC and TCTAAGC/TCTAAGC), and with a history of eczema in the preceding year (additive allelic model, each TCTAAGC reduces odds ratio by 45% [95% CI: 2% to 70%]).

6408delCTCTAAinsGC was also associated with having any positive food allergen skin prick test (dominant model, TTAGAG/TTAGAG and TTAGAG/GC increase odds ratio by 305% [95% CI: 31% to 1375%] in comparison to GC/GC). IFNGR 156T>C was associated with serum IgE levels (additive allelic model, each C allele increases serum IgE level by 30% [95% CI: 48% to 6%]), and with having any positive skin prick test (dominant model). In this specific instance, the effect size could not be estimated, as all 55 individuals homozygous for the C allele had a negative skin prick test, meaning that the effect of the T allele (either TT or TC) is likely to be very strong.

DISCUSSION OF RESULTS

Phenotypic Characteristics

For a full discussion of the phenotypic characteristics of our sample population, the reader is referred to the previously published literature from our group (Levin et al. 2011; Levin et al. 2012; Levin et al. 2008). However, a few pertinent characteristics bear specific mention.

The lack of overlap between bronchial hyper-responsiveness and self-reported symptoms of asthma may in part be explained by different phenotypes of asthma, but also highlights possible methodological concerns in the diagnosis of bronchial hyper-responsiveness (particularly the use of a handheld glass nebuliser rather than a dosimeter) and/or in the diagnosis of asthma. As previously mentioned, relying on self-reporting of a diagnosis of asthma rather than physician diagnosis may not accurately estimate the true prevalence of asthma (Zar et al. 2007). Additionally, language differences (most of our sample population do not speak English as a first language at home) and cultural differences in the terminology and understanding of health and disease may also call into question the prevalence data, as well as any significant associations, of our other self-reported clinical phenotypes. As these self-reported phenotypes represent the majority of the genetic interactions uncovered in our study, unfortunately many of our results are limited by the above concerns. Assessing children at only one school may limit the generalizability of our findings to Xhosa speaking children as a whole.

In addition, several objective phenotypes (specifically skin prick tests to European grass, Bermuda grass, mould, cat, dog, egg white, milk, peanut and fresh potato) showed too few positive results to perform meaningful interrogation of association with the SNPs. These phenotypic characteristics were therefore excluded from any association testing.

Genotype/Allele/Haplotype Frequencies

All of our SNPs were in Hardy-Weinberg Equilibrium. The genotype, allele and haplotype frequencies are published to establish a baseline for this population, and may help to guide further research. In the future it may be possible, as part of an international collaboration, to compare the PIA and haplotype frequencies against those of other populations. This may allow interrogation of the theory that evolutionary adaptation of the human immune system could influence a given population's genetic predisposition towards asthma and allergic disease. Further research is required in this regard.

Genotype/Phenotype Associations

We have reported several significant associations between SNPs and markers of allergic disease in this population. As this is essentially an exploratory study, two points require clarification from the outset. At this juncture, we have focussed solely on associations between SNPs and disease markers, and not on haplotypes, which would be possible in the future. As previously stated, we have elected not to correct for multiple testing, and we welcome further studies in this population to replicate or refute our results. In spite of several significant results, our sample size likely precludes definitive conclusions to be made from the associations, however they do provide an important guide for further research in this population group.

Anti-inflammatory/Tolerance Genes

IL-10 is a multifunctional cytokine that has potent anti-inflammatory activity on a variety of immune cells (Sabat et al. 2010). Specifically, IL-10 plays an important role in both the prevention and limitation of overwhelming immune reactions (Sabat et al. 2010). IL-10 also plays an important role in the biology of B- and T lymphocytes, perhaps most importantly in the differentiation and and function of regulatory T lymphocytes (Moore et al. 2001). Through this role, IL-10 is thought to play an important part in the attainment of immune tolerance (Moore et al. 2001).

592A>C is a promoter polymorphism in the IL-10 gene which is thought to play a role in the rate of IL-10 production (Sohn et al. 2007). We found a role

for the A allele in increasing the Odds Ratio (OR) for several skin prick tests, especially house dust mite skin prick tests. Sensitization to house dust mite is known to be commonly associated with allergic rhinitis and asthma, although we found no specific associations with either of these two phenotypes. Our results are in keeping with findings from several studies, including several meta-analyses. Zheng et. al. (2014) showed an association between the A allele and a higher incidence of asthma in a recent meta-analysis, but this association only reached statistical significance in adults and with atopic asthma in particular. Nie et. al. (2012) also demonstrated an association between the A allele and an increased risk of asthma, again with atopic asthma in particular. Sohn et. al. (2007) found that the A allele was observed at a higher rate in children with atopic eczema. In contrast, we found that the A allele was associated with a decreased OR of atopic eczema, specifically for a self-reported history of ever having eczema, and for a history of eczema occurring during the preceding year. We postulate that this discrepancy between allele effects for different clinical phenotypes may infer specific gene-gene or gene-environment interactions with respect to atopic eczema in this population group, or that this may be a false positive result.

The G allele in the SNP 1082A>G showed several significant associations, predominantly with decreased OR of several aeroallergen skin prick tests, with a personal history of wheezing as well as a personal history of rhinitis, and with bronchial hyper-responsiveness. These results are in agreement with several published studies. Two recent meta-analyses (Nie et al. 2012; Zheng et al. 2014) both demonstrated an association between AA homozygotes and an increased risk of asthma, and another meta-analysis (Hyun et al. 2013) showed an association between the G allele and a reduced OR for asthma (although this effect was only statistically significant in certain Asian populations, and only in adults). Our results indicate a strong association between the G allele and protection against aeroallergen sensitization, and it is possible that the G allele increases production of IL-10, resulting in a decreased risk of allergic disease.

T_H2 Genes

IgE-mediated sensitization is a necessary process in the development of many allergic diseases. IL-4 and IL-13 are central to the development and propagation of allergic inflammation, and share several core functions including the induction of IgE class-switching and goblet cell hyperplasia (Kau & Korenblat 2014). Both IL-4 and IL-13 signal through a common pathway, and enjoy significant receptor homology (Kabesch et al. 2006).

In this study, IL-4 589C>T showed significant associations with several markers of aeroallergen sensitization, as well as with a personal history of wheezing and of having eczema. The C allele was found to reduce the OR of having any positive skin prick test, with having any positive aeroallergen skin prick test, and with having a positive cockroach skin prick test. This is in keeping with several results from the literature, including two recent meta-analyses (Tang et al. 2014; Zhu et al. 2013), which suggested a recessive effect of the C allele being protective against the development of asthma. In our study, the T allele was found to increase the OR of a history of ever having eczema, and again several meta-analyses have suggested a role for the T allele in the formation of asthma (Yang 2013) and allergic rhinitis (Li et al. 2014) respectively. Our results also suggest a heterozygote effect on a personal history of ever having wheeze or having wheeze in the preceding year. To our knowledge, this specific association has not been previously described in the literature, however it is possible that different variations of polymorphisms may cause allergic disease in different populations, or result in different endotypes.

We found significant associations between two polymorphisms in the gene coding for the IL-4 Receptor (IL4RA), and allergic phenotypes. In this study, the C allele of IL4RA 478T>C was associated with an increased OR of ever having wheeze. This is in contrast to some of the published literature regarding this polymorphism. The T allele has previously been associated with higher serum IgE levels and a higher risk of bronchial hyper-responsiveness (Howard et al. 2002), whereas a recent meta- analysis (Michel et al. 2010) showed no association between this polymorphism and asthma risk. Again, this

discrepancy may represent unique features of this population, or may represent an example of the limitations of our study. The A allele of the SNP IL4RA 551A>G was, in our study, associated with a decreased OR of ever wheezing or having wheeze in the last year. Several previous studies have suggested an association between the G allele of this polymorphism and increased risk of allergic disease. Nie et. al. (2013) suggested, in a recent meta-analysis, that the G allele confers an increased risk of asthma in children, and that there was a specific association with an increase in atopic asthma risk. Three other meta-analyses have also suggested an association between the G allele and increased risk of allergic disease, specifically asthma (Huang et al. 2015; Loza & Chang 2007) and allergic rhinitis (Xu & Zhang 2014), although the association with allergic rhinitis was possibly dependent on ethnicity. Other trials, including a meta-analysis (Bunyavanich et al. 2011), a systematic review of the literature (Murk et al. 2011) and a GWAS (Michel et al. 2010) have found no association between this specific polymorphism and risk of allergic disease. The literature is thus conflicted, and further research will hopefully shed light on this matter.

An SNP in the IL-13 gene, 130A>G , was associated with several disease phenotypes. The T allele was weakly associated with a reduced OR of having any positive food skin prick test, although the small numbers of positive skin prick tests may make the significance of this result open to question. In addition, stronger associations were found between the T allele and an increased OR of self-reported allergic rhinitis, and heterozygotes were, interestingly, strongly associated with an increased OR of self-reported eczema. Although it is difficult to explain why different combinations of alleles had quite discrepant effects on various allergic diseases, again this could be due to inaccuracies in the self-reporting of allergic rhinitis and eczema respectively, or because of unexplained variation in gene-gene or gene-environment interactions between the two diseases. Several other authors have interrogated the relationship between this polymorphism and allergic disease. Many have found a relationship between the A allele (equivalent to the T allele in this study) and an increased risk of allergic disease (Heinzmann et al. 2000; Ying et al. 2013; Graves et al. 2000), whilst others found no relationship (Howard et al. 2001; Ziyab et al. 2013), although the latter suggested an

epistatic relationship between this SNP and one in the STAT6 gene. This study does not provide clarity to the literature regarding this specific polymorphism, but possibly suggests that the relationship between IL-13 and allergic disease in the Xhosa population is not binary. No other significant associations were found between any of our other IL-13 SNPs and allergic disease.

A polymorphism in the gene encoding for the STAT6 protein, 2964A>G, was significantly associated with a self-reported diagnosis of eczema. The A allele demonstrated a small, dominant, protective effect against eczema. This is in contrast to a recent article (Lee et al. 2015), which suggested no role for the polymorphism in the development of atopic eczema. Other meta-analyses (Zhu et al. 2013; Qian et al. 2014) have yielded varying results for the association of the polymorphism with asthma, with the former suggesting no role, and the latter suggesting a protective effect of the G allele. Further studies in this population would be required to replicate our results before this association could be confirmed.

T_H1 Genes

IL-12 and IFN-γ are both play important roles in the generation of a T_H1 immune response. IL-12 is one of the most important stimuli in directing the differentiation of naïve T cells into T_H1 lymphocytes (Leonard & Sur 2003). IFN-γ is produced by T_H1 lymphocytes (amongst others) and is a suppressor of T_H2 lymphocytes and their cytokines (Teixeira et al. 2005).

We found the polymorphism in the IL12B gene, 6408delCTCTAAinsGC, to be associated with having any positive skin prick test, although again the small number of children with positive food skin prick tests impacts the validity of this association. The TTAGAG allele was strongly associated, in a dominant model, with an increased OR of a positive food skin prick test. In contrast, TTAGAG decreased the OR of ever having wheeze (recessive model), or of self-reported eczema in the last year, although this particular association was comparatively weak. The results from the literature for this polymorphism have also been quite conflicting. A longitudinal study (Khoo et al. 2004) found

no association with asthma, but showed an association between the CTCTAA allele (equivalent to the TTAGAG allele in this study) and increased IgE in males, and decreased FEV_1 in females. Another study (Morahan et al. 2002) showed an increased prevalence of both atopic and non-atopic asthma in heterozygote children, whilst other studies (Tug et al. 2009; Noguchi et al. 2001) have showed no association between the polymorphism and asthma or allergic rhinitis.

We did not find any significant associations between polymorphisms in the gene for IFN-γ, but in terms of genes coding for the IFN-γ receptor, IFNGR 156T>C, showed some interesting associations. The C allele was found to be associated with reduced serum IgE levels, whilst the T allele showed a strong association with having any positive food skin prick test. The effect is not quantifiable because all individuals who were homozygous for the C allele had negative skin prick tests for all foods under investigation, but it appears to be highly significant.

Other genes

The gene for the β adrenoceptor showed no associations with any of our specific asthma phenotypes, including self-reported asthma or wheeze, serum IgE levels or bronchial hyper-responsiveness. However, ADBR2 16G>A was associated with having any positive aeroallergen skin prick test. The A allele showed a significant increase in the OR for this phenotype. There is no published literature on the effect of polymorphisms of the β adrenoceptor on aeroallergen sensitization or allergic rhinitis, however this polymorphism has been extensively studied in regard to asthma phenotypes and serum IgE levels. The results have been contradictory and, on the whole, rather disappointing. Only one study has previously looked at associations between polymorphisms in this gene and asthma in the Xhosa population, and also found no significant associations (Potter et al. 1993). One meta- analysis (Contopoulos-Ioannidis et al. 2005) showed no association between either of the two polymorphisms under investigation in this study and the prevalence of asthma or of bronchial hyper-responsiveness. The G allele of the ADBR2 16 G>A polymorphism was,

however, found to be associated with nocturnal asthma and an increased prevalence of severe asthma. Another meta-analysis (Migita et al. 2004) showed no association between either of the polymorphisms and asthma prevalence or severity, and these results were largely replicated in the most recent meta-analysis (Liang et al. 2014) of these polymorphisms to date. More recently, attention has been turned to the effect of β adrenoceptor polymorphisms and response to β agonist therapy (Israel et al. 2004). This, however, was not an endpoint of our study.

Conclusion

This is the first trial of its kind in the Xhosa population, and indeed in South Africa. Our self-reported symptoms may not have sufficient validity to be reliably compared to physician diagnosed allergic illnesses, but our prevalence data show general agreement with recent published South African literature. As a pilot study of these polymorphisms in this population, our results cannot be reliably generalized. Furthermore, the limitations described above render interpretation of some of the associations very difficult. Of particular interest are the association results involving SNPs in which the self-reported markers correlate with more objective end points of allergic sensitization (for example IL-10 1082A>G and IL-4 589C>T). With regards to these specific polymorphisms, further research in this population would be useful to see whether these results can be replicated. With respect to other polymorphisms, where our self-reported end-points were inconsistent with more objective allergic markers, or out-of-keeping with other international literature, it is very difficult to postulate whether these associations are of any relevance. It is possible that different polymorphisms contribute to allergic disease in different population groups, and because this particular group is so under-studied, it may be many years before further light is shed on the clinical relevance of our findings. In terms of the broader question of the relevance of race and ethnicity on the genetic susceptibility to asthma and allergic disease, our findings are unlikely to provide significant concrete evidence. However, we believe that this study represents a valuable first step in the evaluation of the relationship between genotype and phenotype in the development of allergic disease in the

Xhosa population. We eagerly anticipate collaboration with colleagues who have performed similar studies in other population groups, who may be able to help us ascertain whether there are significant discrepancies in prevelance of these PIAs between the Xhosa population and other populations around the world.

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